AN INVESTIGATION INTO THE USE OF BIOLOGICAL CONTROL AGENTS AS A SUSTAINABLE ALTERNATIVE TO SYNTHETIC FUNGICIDES IN TREATING POWDERY MILDEW IN TUNNEL CUCUMBERS

by

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I declare that "An investigation into the use of biological control agents as a sustainable alternative to synthetic fungicides in treating powdery mildew in tunnel cucumbers" is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references. This dissertation is submitted to the Faculty of Applied Natural Sciences, University of South Africa, in partial fulfilment of the requirements for the degree of Magister of Technologiae in the Department of Nature Conservation.

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ABSTRACT

The use of biological control agents (BCAs) in the past has shown limited success as its application has often been done incorrectly, and in addition, management practices are rarely altered to incorporate BCAs. Criteria for the correct application of BCAs have been devised as part of the research, and companies selling these products may use the said criteria. Such application will ensure the correct BCAs are used and, more specifically, used under the correct conditions. The powdery mildew (PM) fungus is often seen to develop resistance to synthetic fungicides and, therefore, alternative control measures are required. BCAs as an alternative pose less risk to the environment, workers and the consumer.

A pre-trial has been conducted with a range of BCAs to see if they can control powdery mildew (PM) in a greenhouse environment on hydroponically grown cucumber (*Cucumis sativus* L.) plants using the variety Baccara that has only a moderate tolerance to PM. The BCAs have been compared to the control (synthetic fungicide: Bravo). Comparative work includes Coyier's model, which has been modified and adapted for these trials to determine the percentage of leaf area covered by the PM infection. Furthermore, the number of fruit harvested per treatment, kilogram yield, total mass of yield and average fruit mass is also used to determine the efficacy of the BCAs as these factors have economic significance to commercial growers. The pre-trial showed promise until the fertigation computer failed, resulting in a nutrient shortage and imbalance, confirming that BCAs alone cannot control PM. Synthetic fungicides were applied until control of PM and plant nutrition was regained. BCAs were re-introduced and used until the end of crop production.

The confirmation from the pre-trial has led to the inclusion of silicon in conjunction with the BCAs in the two subsequent trials (Trials 1 & 2). Silicon was applied with the BCAs as a foliar spray on a weekly basis. In trials 1 and 2, the cucumber variety, Palladium, with a high genetic tolerance to PM is used, as this variety is suited to form part of the holistic approach used for trials 1 and 2.

Trial 1 showed that treatment A, containing *Streptomyces griseovirdis* and *Streptomyces aureofaciens,* had the highest yield. Both of these are bacterial BCAs and demonstrated their adaptability to varied climatic conditions, notably when low humidity was experienced.

In treatment B, *Trichoderma harzianum* strains, Rifai and Uppington, show the slowest rate of PM development.

In trials 1 and 2, the best actual PM control was obtained by two fungal based BCAs (Trial 1, treatment C was *Ampelomyces quisqualis)* and (Trial 2, treatment B was *Trichoderma harzianum* strains, Rifai and Uppington), showing that fungal BCAs have a place for this application, but the growth-enhancing properties of bacterial based BCAs make economic sense and would make them attractive to growers. Treatment A (*Streptomyces spp*.) had the most number of fruit for the entire growing period and the best overall yield (kg yield) again. Two of the BCA / silicon treatments have marginally better PM control compared to that of the control (E) treatment, although not statistically significant. Treatment E (control) has the highest average fruit mass in this instance but does not have the highest yield (kg yield) when compared to treatments A and B, possibly due to the growth-enhancing properties of most of these BCAs.

Therefore, most of these BCA treatments give fairly inconsistent results that vary possibly according to season, humidity and temperature, making it difficult to predict their efficacy. Using combinations or weekly alternations of these BCAs with extremes of climatic adaptation will probably be the most reliable method of obtaining consistent results. Bacterial BCAs are shown to have lower humidity requirements and produce the most consistent results in terms of fruit number, yield and fruit mass and a combination of bacterial and fungal based BCAs would possibly be the best as this would control PM and yet still have the growth enhancing properties from the bacterial based BCAs. From the research, it can be said that some BCAs in trials 1 and 2 produce results similar to that of the control in terms of percentage leaf area covered by PM and some are shown to have improved yields. Results produced from certain BCA treatments are thus equal to the control; yet provide an environmentally friendly alternative to synthetic fungicides.

Silicon is listed as a beneficial element rather than an essential element; however, literature claims it to be highly effective in treating PM in cucurbits. Results from trials 1 and 2 show that control of PM is possible in most cases, when a holistic approach is used. This approach includes a cucumber variety with a high PM tolerance, optimum nutrition, cultural practices and silicon in combination with the BCAs. A complete change of management practices is necessary to implement such a BCA program.

KEY WORDS

- Biological control agents
- Biocontrol
- Cucumber
- *Cucumis sativus*
- *Erysiphe cichoracearum*
- *Golovinomyces cichoracearum*
- Induced resistance
- *Podosphaera xanthii*
- Powdery mildew
- Silicon
- *Sphaerotheca fuliginea*

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GLOSSARY OF TERMS

Actinomycetes: gram-positive bacteria that can form branching filaments. They may form true mycelia or produce conidiospores.

Acylation: addition of acyl. Acylation-stabilization is achieved by the introduction of an acid radical from acids, such as coumaric, ferulic, or caffeic acids into for example cylated anthocyanins.

Aliphatic: with open-chain structure.

Antibiosis: inhibition of growth of a microorganism by substances (antibiotics) produced by other microorganisms.

Ascospores: the sexually produced spores of the Ascomycotina contained within an ascus.

Axenic: describes the culture of single species in the absence of all others, pure culture.

Bacillus: rod-shaped bacterium.

BCAs: biological control agents which include fungi, bacillus or protozoa.

Biological: concerning living things.

Biosynthesis: biological production of chemical.

Carbohydrates: a large group of sugars, starches, celluloses, and gums that contains carbon, hydrogen and oxygen in similar proportions.

Chelation: compound of metal and non-metal.

Chlorotic: loss of greenness through chlorophyll.

Cleistothecium: (pl. cleistothecia), an enclosed ascocarp containing randomly dispersed asci.

Conidia: a specialized fungal hypha that produces conidia. Conidia are asexual spores.

Cucurbits: plant of gourd family.

ECM: ectomycorrhizas

Endophytic: plant living inside another.

Entomopathogenic: causing disease to insects.

Epidemiology: science that involves the study of the incidence and distribution of diseases in large populations, and the conditions of the disease.

Epinasty: downward bending of a petiole, so that the angle between its base and the stem becomes obtuse; the orientation of the lamina may be vertical, with the apex hanging downwards, or it may continue the curve of the petiole so that its upper surface faces inwards towards the stem. The petiole and lamina remain turgid, and the condition must be distinguished from wilting, in which the tissues become flaccid.

Exudate: material that has passed from within a plant structure to the outer surface or into the surrounding medium, e.g. by diffusion and not usually through an aperture, as in root exudates or leaf exudates.

FA: fulvic acid.

Glycosylation: addition of carbohydrate.

Gliotoxin: a fungal toxin produced by various species of *Trichoderma spp., Gladiocladium fimbriatum, Aspergillus fumigatus* and *Penicillium*. Gliotoxin is used as an immunosuppressive agent.

Holistic: adjective of holism, a general label applied to any philosophical approach that focuses on the whole living organism. (Reber, A.S. 1984. The Penguin Dictionary of Psychology. Pengiun Books, London)

In vitro: within a glass; observable in a test tube; in an artificial environment.

In vivo: within a living organism or body, under normal environmental conditions.

Inoculum: substance used in inoculation.

IPM: Integrated Pest Management is the use of techniques, technology, information, and communication to reduce pesticide residue exposure in sustainable agriculture.

ISR: Induced Systemic Resistance.

L-phenylalanine: a non-protein amino acid from which e.g. isoquinoline alkaloids are derived via decarboxylation.

Lignan: colourless, crystalline, solid, dimeric compounds derived from precursors related to those involved in the formation of lignin, i.e. the union of two units of phenyl propane, cinnamic acid, or their derivatives, through their aliphatic side-chains. They occur in chiral forms.

Lignin: a class of polymers formed from mostly C6-C3 monomeric units (cinnamic acid, coumarin), occurring in all plants; lignification, the deposition of lignin e.g. in secondary cell walls,

Microbe: a microscopic organism.

Mircoorganism: general term used for protozoa, bacteria and viruses.

Myco: fungus or fungi.

Mycorrhiza: the symbiotic association of the mycelium of a fungus with roots of seeds or plants.

Necrosis: death of tissue.

Osmoticum: particles that cause osmosis.

p-coumaric acid: a hydroxy acid derived from L-phenylalanine, involved in formation of phenylpropanoids, readily convertible into salicylic acid.

Pathogen: cause of disease.

PGPR:- Plant Growth–promoting Rhizobacteria.

Phenol: an acidic aromatic compound, a common constituent of organic compounds, such as cinnamic acid.

Phenolics: see tannins.

Physicochemical: of physics and chemistry.

Phytoalexins: a general term referring to substances that inhibit further development of a fungus in hypersensitive host tissue (also antimicrobial). The first one isolated was an isoflavan; others are sesquiterpenes.

PM: powdery mildew.

Polycyclic: with several whorls or cycles.

PR: pathogenesis related.

Proteoid roots: masses of very fine rootlets, which have a high surface area

Pyochelin: the siderophore pyochelin and its precursor salicylic are produced by Pseudomonads.

Pyoverdine: siderophore produced by many *Pseudomonas.*

RH: relative humidity.

Rhizosphere: soil around plant roots.

Salicylic acid: ($C_7H_60_3$).

Salicylic acid: orthohydroxybenzoic acid, obtained from the bark of the white willow and wintergreen leaves, its salts are the **salicylates**.

SAPB2: salicylic acid- binding protein 2

SAR: systemic acquired resistance.

Sclerotium: (pl. Sclerotia) a small, compact, hardened mass of hyphae that may bear fruiting bodies. Sclerotium can help fungus survive in adverse environments.

Siderophores: diverse group of low molecular weight, iron-chelating compounds produced by microorganisms to help them absorb iron, an important step in virulence. Under iron-limiting conditions, bacteria produce a range of iron chelating compounds or siderophores which have a very high affinity for ferric iron.

Spermosphere: the spermosphere is the volume of soil that surrounds a seed.

Sporulation: produce spores.

Sterols: terpenoids, solid, unsaturated steroid alcohols with an -OH group at the C3 position (bottom left below) that occur both free and as esters or glycosides and are classified according to the organism in which they are found as mycosterols and phytosterols. Phytosterol ($C^{29}H^{50}O$).

Substrate: the material forming the growth medium for an organism or the substance or object on which an organism lives and from which it obtains nourishment.

Sucrose: a common disaccharide made up of glucose and fructose.

Synthetic: produced artificially by the synthesis of simpler materials or substances rather than occurring naturally.

Synthesize: to unite or produce by synthesis.

Tannins: complex, aromatic compounds (phenolics) that precipitate proteins and occur especially in the bark of many shrubs and trees, varying considerably in chemical composition and with different biosynthetic pathways and so a term of little use, see rather pro-anthocyanidins, hydrolysable tannins; tanniniferous, producing tannins.

Trichome: an epidermal outgrowth, e.g. a hair (branched or unbranched), a papilla.

µS: micro siemens. Value conversion: 1 mS/cm = 1000 µS/cm.

VAM: an endomycorrhizal association between a fungus and a plant root where the fungal hyphae form vesicles and arbuscules within the plant cell.

Xylans: a polysaccharide component of hemi cellulose and contains only xylose molecules, such as galactomannans, glucurono-arabinoxylans, xyloglucans.

Xylanase: a carbohydrase enzyme.

Glossary source: [Angiosperm Phylogeny Website] Retrieved 7 February 2004, from http://www.mobot.org/MOBOT/Research/APweb/welcome.html. Unless otherwise stated.

CHAPTER 1

LITERATURE REVIEW

1.1 POWDERY MILDEW

1.1.1 Classification of cucumber powdery mildew

Powdery mildew on English cucumbers (*Cucumis sativus* L.) is caused by the fungi *Podosphaera xanthii* (Castagne) (Shishkoff, 2000) and *Golovinomyces cichoracearum* (DC) Gelyuta, V.P. (Matsuda & Takamatsu, 2003; Keinath & DuBose, 2004; Suzuki *et al*., 2004). *Podosphaera xanthii* was formerly known as *Sphaerotheca fuliginea* Schlecht. Ex Fr. Pollacci (Braun, 1987), and often is referred to incorrectly as *Sphaerotheca fusca* (Sztejnberg, 1998; Elad *et al*., 1998; Romero *et al*., 2003). There are five races, some of which attack all cucurbits, while others have a host range restricted to certain types of cucurbits (McGrath, 1997).

Golovinomyces cichoracearum was formerly known as *Erysiphe cichoracearum* DC. Ex Mérat (Braun, 1987) and, currently, two races are known, according to the American Phytopathological Society (Matsuda & Takamatsu, 2003; Suzuki *et al*., 2004). *P. xanthii* is known to infect about 60 plant genera and *G. cichoracearum* about 160 genera, with all cucurbits being susceptible (Watt, 1994). According to Kuepper & Thomas (2002), no practical need exists to distinguish between these two fungi since strategies for their control are identical.

1.1.2 Epidemiology and economic loss caused by powdery mildew

Powdery mildew (PM) of cucurbits is one of the most destructive foliar diseases in both temperate and subtropical climates (Sitterly, 1978). Both *P. xanthii* and *G. cichoracearum* appear on leaves, petioles and stems as a white, powdery mass composed of mycelium and countless spores (Hansen, 2000; Mercure, 1998) and are brown only in the case of *P. xanthii* (Watt, 1994; Hazelrigg, 2003, Figure 1.1). Dorsal sides of cucumber leaves may show infection (Mercure, 1998) in severe cases, leaves may turn yellow (chlorotic), brown and shrivel. PM causes premature senescence of leaves, reducing the photosynthetic area (Cheah & Falloon, 2001; Keinath *et al*., 2000) but generally does not cause leaf spotting (Mercure, 1998) as it is superficially separated by the fungal haustorium. Cucurbit fruit are not directly attacked by PM, but fruit may be malformed or sunburned due to loss of foliage. In severe infection cases,

both the yield and fruit size may be reduced (Hansen, 2000). PM is a polycyclic disease, and thus many infection cycles occur throughout the season (Latin, 2001). Powdery mildew infection also predisposes plants to other diseases, such as *Didymella bryoniae*, often referred to as black rot or gummy stem blight (McGrath, 1997; Ferreira & Boley, 1992; Bergstrom *et al*., 1982).

Figures 1.1A & B. *Golovinomyces cichoracearum* as seen in winter (left) and the brownish appearance of *Podosphaera xanthii* (right) as seen in summer.

The economic impact of PM on the cucumber industry in South Africa is difficult to establish. According to de Vries (*personal communication, October 2005), an estimated 110 ha of English tunnel cucumbers are planted in South Africa annually. Powdery mildew may cause an estimated loss of 5 to 8% of the total yield of cucumbers. The average planting density for tunnel cucumbers is 1.6 plants per square metre.

Therefore, 110 hectares provides 1 100 000 m^2 of production area. This 1 100 000 m^2 has approximately 1.6 plants per m^2 and two planting cycles per year translates into 3 520 000 plants planted annually in South Africa. Based on an approximate yield of 20 fruits per plant, 70 400 000 fruits per year could be produced.

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The monetary value of such volumes (using R2-00 per fruit as an average price) could mean a crop value of R140 800 000 per annum. Based on a 5 to 8% loss due to powdery mildew, the economic loss could be between R7 and R11 million p.a.

1.1.3 Climatic requirements of powdery mildew

High temperatures and low relative humidity on the leaf surface are prerequisites for PM development, making powdery mildew far more destructive and prevalent in greenhouses and tunnels (Kristková & Lebeda, 1999a). Greenhouse environments are conducive to several diseases, among these diseases are PM and *Botrytis* (Elad *et al*., 1996). In greenhouse studies, particularly in the warmer months, research found that *P. xanthii* is more common on cucurbits than *Golovinomyces cichoracearum. G. cichoracearum* may have a lower temperature requirement and is thus found mainly in spring and early summer (Kristková & Lebeda, 1999b). Under favourable conditions powdery mildew develops quickly on cucurbits because the length of time between infection and the appearance of symptoms is usually only three to seven days, and a large number of conidia can be produced in a short time (McGrath, 1997).

Other favourable conditions include dense plant growth and low light intensity. High relative humidity (RH) is favourable for infection and conidial survival, but infection can also take place at RH levels as low as 50%. Dry conditions are favourable for colonization, sporulation, and dispersal. Rain and free moisture on the plant surface are unfavourable for PM development but is often a catalyst for other fungal diseases (McGrath, 1997). In general, the occurrence, distribution and severity of PM infection are correlated with temperature, relative humidity, light and wind (Coyier, 1985). Cucurbit plants are generally not affected until or after fruit initiation, and leaves are most susceptible 16 to 23 days after unfolding (McGrath, 1997). In contrast to Roberts and Boothroyd (1972), McGrath and Thomas (1996) stated that the optimum temperature for disease development was 20° and 27ºC, and infection might occur between 10° and 32ºC. Development ceases at 38ºC and above. Therefore, the conclusion could be drawn that high relative humidity was favourable for infection and conidial survival, while dry conditions favoured colonization, sporulation and dispersal (McGrath & Thomas, 1996).

1.1.4 Reproduction of powdery mildew

PM fungi reproduce by means of asexual spores, known as conidia, or by means of sexual spores, known as ascospores. The conidia of *P. xanthii* and *G. cichoracearum* are difficult to distinguish between, and cleistothecia, the sexual fruiting bodies, are rarely observed. As a result, these fungi are often confused. The name of the fungus has frequently been reported without valid confirmation (McGrath, 1997). The primary initial inoculum is believed to be airborne conidia from other cucurbit crops or from conidia from greenhouse-grown cucurbits; conidia remain viable for seven to eight days. Because the PM fungus is an obligate parasite (Hausbeck *et al*., 1994:2), it cannot survive in the absence of living host plants, except as cleistothecia. Once conidia germinate, mycelium develops in 24 to 48 hours, and maturation of conidia occurs in as little as 72 hours under optimal environmental conditions (Coyier, 1985). Conidia grow superficially on the leaf surface where their specialised hyphae, called haustoria absorb nutrients from the host plant. McNally *et al*. (2003) stated that glucose in the host plant was the preferred substrate of PM.

Although *P. xanthii* and *G. cichoracearum* are described as being host-specific, the range of host plants is broad, and the role of non-cucurbit hosts as sources of inoculum cannot be established without further research (McGrath, 1997).

1.2 CONTROL OF POWDERY MILDEW

1.2.1 Chemical control

The vast majority of cucumber growers are still reliant on synthetic fungicides to control powdery mildew; these synthetics comprise both systemic and contact fungicides. The use of numerous synthetics over the decades has resulted in PM developing resistance to many fungicides; therefore, many fungicide resistance management programmes have been developed (Keinath & DuBose, 2004; Zitter, 2004; Matherson & Porchas, 1998; McGrath & Zitter, 1996). As the PM fungus developed resistance to many synthetic chemicals, growers relied on cocktails of fungicides and heavier dosages to maintain some degree of PM control.

The increasing concerns for public health, the environment as well as the expanding competition in the agricultural market motivates growers to seek disease control strategies that use reduced amounts of synthetic fungicides. For these reasons, a need has arisen for new and effective means of disease control that poses less risk to human health and the environment (Cheah and Cox, 1995).

1.2.2 Integrated control methods

Integrated pest management (IPM) is a number of practices or inputs used in disease and pest suppression. An IPM approach can be achieved by manipulation of the physicochemical and microbiological environment through management practices, such as soil amendments, crop rotation, fumigants or solarization (Whipps, 1997a). IPM often incorporates synthetic chemicals as part of the control measures, but a holistic approach as implied here includes all beneficial inputs, but excludes synthetic chemicals. A holistic approach may include the following:

1. The use of resistant varieties (Watt, 1994; Davis *et al*., 2001; Mercure, 1998). Vigorous plants, capable of withstanding or repelling disease, should be grown (Kuepper & Thomas, 2002).

2. Anti-transpirant sprays (polymers), such as Wilt-Pruf or Vapor Gard, and oils, such as neem oil or JMS Stylet Oil mixed with water, also give protection against certain fungi. Foliar sprays with *Bacillus laterosporus* and Wilt-Pruf (polymer) seem most effective and could be an area for further research (Quarles, 2004). Oils, such as JMS Stylet Oil, neem oil and baking soda, also show promise in treating PM or could improve the efficacy of biological agents (Davis *et al*., 2001; Kuepper & Thomas, 2002). Oils should not be used when temperatures are above 32˚C (Quarles, 2004; Davis *et al*., 2001; Kuepper & Thomas, 2002).

3. Cultural practices include removal of infected plant debris, practising good sanitation (Cheah & Falloon, 2001; Davis *et al*., 2001), removal of alternative hosts (Cheah & Falloon, 2001; Mercure, 1998) and crop rotations, where possible (Watt, 1994). New plantings should also be separated from old plantings (Mercure, 1998). Low light situations should be avoided and the plant

density corrected (Hansen, 2000), ensuring adequate ventilation and the correct plant spacing is used (Mercure, 1998). The climatic requirements of PM should be known and the environment adapted, if possible, to disfavour PM. Overhead irrigation should be avoided, and the plants watered early in the day to allow leaves to dry thoroughly before nightfall (Mercure, 1998).

4. Management of plant fertility and irrigation scheduling is of major importance (Kuepper & Thomas, 2002). Excessive use of nitrogen fertilization should be avoided (Hansen, 2000; Cheah & Falloon, 2001) as such fertilization often predisposes plants to disease.

1.2.3 Influence of plant nutrition and media in managing disease levels

Graham & Webb (1991) described resistance in the host-pathogen relationship as the ability of plants to limit the penetration, development and/or reproduction of invading pathogens. Tolerance of host plants is measured in terms of the ability to maintain growth and yield production in spite of infection or invasion of pathogens. Although both factors are genetically controlled, the environment and thus nutrition of the host plant could influence, to a certain extent, the expression, especially in moderately susceptible or resistant genotypes (varieties). Nutrition of plants has a direct impact on the predisposition of plants to be attacked or to be affected by pests and diseases. By affecting the growth pattern, the anatomy and morphology and, particularly, the chemical composition, the nutrition of plants could contribute either to an increase or decrease of the resistance or tolerance to pests and diseases (Krauss, 1969; Graham, 1983; Perrenoud, 1990; Marschner, 1995).

In principle, three major mechanisms were involved in the host-pathogen relationship, namely the host's metabolism and the chemical composition, the host's anatomy and morphology, and the host and pathogen life cycles coinciding.

The ratio between nitrogen and potassium plays an important role in the host and pathogen relationship. Perrenoud (1990) reviewed this subject and concluded that the use of potassium (K) decreased the incidence of fungal diseases in 70% of the cases.

The observation by Last (1962) that nitrogen (N) increased the level of infection of barley powdery mildew as well as the grain yield of the infested plant was a good example of changes in tolerance. With N, the more vigorous the growth of the plant, the greater the role of assimilates in reducing the competitive effect of the pathogen. The concentration of soluble assimilates in a plant cell is an important factor for the development of invading pathogens especially for obligate parasites, such as powdery mildew or rust. This group of pathogens requires living plant cells to complete their life cycle.

Sufficient N levels are required for the longevity of cells, rapid turnover of assimilates and high content of low molecular mass compounds. Vigorous plant growth stimulated by ample N would suppress infestation by this group of pathogens. The differences in the N levels may explain differences in the expression of plant diseases in relation to the nutrition of the host. Up to 10% of the nitrogen in the ammonium form is recommended; depending on stage of growth over 20% is very risky (Donnan, 2004). Tisdale *et al*., 1993 recommended NH_4+, as energy would be saved since NO_3- uses two NADH molecules for each NO_3- ion reduced in protein synthesis. Therefore, NH_4+ supplied plants have higher carbohydrate and protein levels. An ammonium cation is also less subject to leaching and denitrification; uptake is best at neutral pHs but depressed under acid conditions. High levels of NH_4+ retard growth and restrict potassium uptake and create deficiencies. Calcium (Ca) nitrate is the main source to consider for cucurbits, and uptake is usually favoured by low pH. Calcium is indispensable for the integrity and stability of cell walls. With insufficient Ca, cell walls leak low molecular mass organic compounds (Tisdale *et al*., 1993).

Phenolic compounds play an important role in the host-pathogen relationship, since they are the basis for many defence mechanisms. They act as phytoalexins or as precursors of lignin and suberin, which act as mechanical barriers (Graham & Webb, 1991).

Table 1.1. Common antagonisms occurring in crops in general (Nelson, 1985)

Nutrient in Excess	Induced Deficiency
N	K
K	N, Ca, Mg, B
Na	K, Ca, Mg
Ca	Mg
Mg	Ca
Ca	B
Fe	Mn
Mn	Fe

The fungicidal effect of the trace nutrients manganese (Mn), copper (Cu) and zinc (Zn) as constituents of fungicides is well established. However, Mn, in particular, has an important function in the synthesis of lignin and phenols and, therefore, in controlling disease and pathogens. Graham & Webb (1991) described the role of Mn in disease resistance; with lignification being the obvious basis of resistance to powdery mildew. Both Mn and Cu are involved in activating the metabolic pathway to synthesize the precursors and lignin. Acute deficiencies of trace nutrients, like Mn, inhibit photosynthesis, which weakens the competitiveness of the host plant.

In South Africa, sawdust has become the standard medium in tunnel cucumber and tomato production. This medium has poor qualities that hinders root development, nutrient uptake and predisposes plants to disease. The benefit of sawdust is merely the low cost. Poor attributes of sawdust are well documented (Table 1.2.), and therefore, an alternative to this medium is required. Substitution of sawdust will take a complete mind shift, but the cost of alternative mediums or substrates is often a deterrent.

Sawdust, inherently, has very low levels of nitrogen, which would reduce the soluble nitrogen supply considerably to the plants growing in the medium. Even with constant liquid feeding and high levels of slow release fertilizers, it is difficult to keep up with the supply of nitrogen to plants in medium containing fresh sawdust. Sawdust has a C/N ratio between 250 and 800, with 500 being the average. Sawdust must be composted,

the higher the C/N ratio (carbon/nitrogen) the longer it takes to produce a usable end product. Finer sawdust needs amending to improve aeration and allow oxygen into the root zone (Handreck & Black, 1984).

Table 1.2. Approximate nitrogen contents of some dried organic materials (Handreck & Black, 1984)

Material	**N in dry material (%)**	**C / N ratio**
Pinus radiata bark	0.1	500
Pinus radiata sawdust	0.09	550
Peat	1.5	30
Eucalypt sawdust	0.1	500

Uptake of nutrients, especially potassium, phosphorous and iron is almost stopped and the ability of water to move into the roots is reduced almost threefold in the absence of oxygen in the root zone, mainly under waterlogged conditions, such as in poorly ameliorated sawdust. Oxygen diffuses through air 10 000 times the rate at which it diffuses through water, thus waterlogged soils and medium are undesirable (Handreck & Black, 1984). Oxygen content of a fully aerated solution at 10˚C is 13 ppm, and at 30˚C, it is 7ppm, which may not seem significant, but at higher temperatures the oxygen requirement increases proportionately with respiration (Morgan, 2003). Alcohol, hydrogen sulphide and ethylene are produced in the roots and hormone production is upset in waterlogged soils and medium. Toxic levels of iron and manganese are also associated with waterlogged mediums. The combined effects of these changes are seen as stunted or wilted tops and root death; roots will often be at the surface of the medium where oxygen is available (Handreck & Black, 1984).

Cucumber roots have a high oxygen requirement, and sudden temporary wilt, epinasty, of greenhouse cucumber plants is often noted (Morgan, 2003). High solution

temperatures also reduce the amount of dissolved oxygen in the solution (Hochmuth, 2001).

The role of plant nutrition becomes apparent when investigating essential and non-essential elements. Although not essential for growth and reproduction, some elements are beneficial for the health and growth of certain plants. Some of these beneficial elements include aluminium (Al), cobalt (Co), chromium (Cr), fluorine (F), iodine (I), nickel (Ni), selenium (Se), silicon (Si), sodium (Na) and vanadium (V). Besides the essential elements required for plant growth, the beneficial elements mentioned above, notably silicon, are beneficial to plant growth and resistance. The role of silicon in controlling PM is well established and as such is used for trials 1 and 2.

1.2.4 Role of silicon in managing disease levels

Research revealed the following roles and modes of action, which could be attributed to silicon (Si). Although silicon has not been recognized as an essential element for higher plants, the beneficial effects have been demonstrated in many plants. Silicon is abundant in all field-grown (soil) plants, and concentrations in plant tissues sometimes exceed that of nitrogen and potassium (Epstein, 1994). Silicon and calcium are responsible for cell strength, but both require boron to deliver the full benefit (Sait, 2003). Hydroponically grown cucumbers are often grown in nutrient solutions without added Si (Belanger *et al*., 1995). The beneficial effects of silicon are threefold:

1. It protects against insects, fungal attack, increases growth and amelioration of abiotic stresses that are well documented in rice and greenhouse cucumbers (Cherif *et al*., 1994b; Winslow, 1992; Samuels, 1991; Marschner 1995; Epstein, 1994). Fungal disease resistance in greenhouse cucumbers has been proven to increase substantially in response to Si fertilization (Belanger *et al*., 1995; Menzies & Belanger, 1996).

2. It protects against toxicity of metals (Valamis & Williams, 1967; Baylis *et al*., 1994). Silicon has also been shown to ameliorate certain mineral imbalances and other diseases caused by abiotic stresses in plants (Marschner, 1995; Epstein, 1994). Several studies have found that Si can reduce or prevent manganese (Mn) and

iron (Fe) toxicity and could also have beneficial effects on aluminium (Al) toxicity (Marschner, 1995; Tisdale *et al.,* 1993). It seems that Si does not affect Mn uptake but rather improves Mn distribution in plant tissues when Si levels in tissue are low or improves distribution when Mn is at toxic levels in leaves (Marschner, 1995). Furthermore, Si has been shown to alleviate an otherwise detrimental nutrient imbalance between zinc and phosphorus (Marschner, 1995; Epstein, 1994).

3. It can reduce salinity stress and reduce transpiration in plants (Epstein, 1994; Marschner, 1995; Tisdale *et al*., 1993).

Cucumbers are known to take up Si actively or passively (Miyake & Takahashi, 1983). Menzies, *et al.* (1991a) investigated the different rates of Si fertilization (potassium silicate) on powdery mildew severity after cucumber leaves were inoculated with PM conidia. Results revealed that leaf area covered by powdery mildew reduced by as much as 98%, with concentrations of 100 ppm or more of SiO_2 giving best results. Cherif and Belanger (1992) found that nutrient concentrations of 100 to 200 ppm SiO_2 significantly reduced root mortality, root decay and yield losses on plants inoculated with *Pythium ultimum.* In addition, treated plants were more productive than those not treated with Si. In trials carried out by Dik *et al*. (1998), silicon was added to the nutrient solution at a concentration of 0.75 millimol, resulting in a PM disease reduction of 10- to16% averaged over all the trials.

Research has shown that soluble Si taken up by plants tended to accumulate in the apoplast, particularly in epidermal cell walls (Epstein, 1994; Marschner, 1995; Tisdale *et al*., 1993; Samuels *et al*., 1993). Consequently, many investigators hypothesized that Si inhibited fungal disease by physically inhibiting fungal germ tube penetration of the epidermis (Datnoff *et al*., 1997; Belanger *et al.,* 1995). Subsequently, investigators have found that only the trichome bases on the cucumber epidermis have a tendency to become silicified (Belanger *et al*., 1995; Samuels *et al*., 1993).

Si has been observed to accumulate around fungal hyphae and infection pegs in infected host plant cells (Datnoff *et al*., 1997; Belanger *et al*., 1995) and investigators have now shown that phenolic materials and chitinases also rapidly accumulate in these infected host cells (Menzies *et al*., 1991b; Cherif *et al*., 1994a; Belanger *et al.,* 1995). Infected cells of silicon-amended cucumber plants accumulated phenolic materials

much quicker than infected cells of non-amended plants (Cherif *et al*., 1992; Menzies *et al*., 1991b) and were shown to have a significantly higher percentage of infected cells, which accumulated phenolics (Cherif *et al*., 1992; Menzies *et al*., 1991b). These phenolics were also conclusively shown to be fungitoxic (Cherif *et al*., 1994a) as fungal hyphae penetrating the phenolic-laden cells of Si amended plants were found to be seriously damaged by the accumulation of phenolics (Cherif *et al*., 1992). It therefore seems likely that Si fertilization reduced disease susceptibility primarily by stimulating the host plant's defences (Belanger *et al*., 1995; Datnoff *et al.,* 1997), although it could be possible that already silicified epidermal cells could play a role in disease inhibition (Belanger *et al*., 1995; Datnoff *et al*., 1997).

McNally *et al*. (2003) explained the role of phenolic acids in PM resistance in cucumber. This role was further noted in microscopic observations by Fawe *et al*., (1998) in which epidermal cells of Si-treated cucumber plants accumulated phenolic-like material that was detrimental to fungal cells. Therefore, it could be assumed that there was conclusive evidence that Si played an active role in disease resistance by inducing and increasing the production and accumulation of antifungal low-molecular-weight metabolites (flavonoid phytoalexins) during pathogenesis (Fawe *et al*., 1998).

In trials by Dik *et al.* (1998), the addition of silicon was done in conjunction with BCAs, and no interaction between silicon and bio-control agents was noted. Combinations of multiple small effect treatments were tested in six replications to establish the magnitude of the combined effects of an ISR–inducing bio-control treatment, called BioYield®, in the transplant medium, a calcium silicate fertilizer, a phosphorous acid foliar fertilizer (ISR inducer) and antagonistic leaf inhabiting *Bacillus cereus.* Combinations of treatments in cucumber increased yields above those of individual treatments, and disease was kept at low levels (Backman & Dorman, 2003).

1.2.5 Biological control agents for powdery mildew control

BCAs are biotic organisms, which include bacterium, fungi and protozoa. BCAs have certain criteria that need to be met to ensure their survival and aid their proliferation. An understanding of the mode of action and environmental requirements of each of these BCAs is needed to understand some of the complexities happening at cellular level and

would assist in enhancing BCA efficacy. *Bacillus spp*. and fungal-based BCAs were used in this research. The criteria required by the BCAs included the following:

- High humidity is favoured by most BCAs and is also beneficial for the uptake of inorganic nutrients. Spraying should be done in the evening to take advantage of high humidity at night (Schönherr, 2004). Low light intensity at the time of spraying was preferable; spraying between 10 a.m. and 3 p.m. should be avoided as UV light could kill microbes as they fly through the air (Sait, 2003). The environment should be manipulated, if possible, to favour the BCA and disfavour the pathogen, Raupach & Kloepper (1998). Bacteria are less moisture dependent and have a wider range of use (Quarles, 2004). The minimum and maximum temperature range should be considered for that particular BCA, for example PlantShield ® HC (*Trichoderma harzianum* Rifai strain KRL-AG2) that is not effective below 10˚C (50˚F).
- The pH of the media and surrounding environment should be considered as beneficial fungi are suited to more acidic conditions and beneficial bacteria to neutral and alkaline conditions (Table 1.3). The pH of foliar sprays should ideally be between 6 and 7 (Zimmer *et al*., 2003)

Table 1.3. Ranges of soil pH preferred by various beneficial soil microorganisms (Handreck & Black, 1984)

MICROORGANISMS	PREFERRED pH RANGE	COMMENTS
Rhizobium bacteria	Above 5	H+ is toxic to them; inoculation of legume roots requires Ca
Ectomycorrhizal fungi	4 to 6, some to 7	Higher pH inhibits their spread to seedlings and inhibits seedling infection/inoculation
Endomycorrhizal fungi	4.5 to 8	Some species still effective at higher pH levels
Decomposers of organic matter	5 to 9	Bacteria and Actinomycetes become less numerous as pH declines; fungi, which are slow decomposers, dominate at a low pH
Bacteria that convert ammonium to nitrate	Above 6	Ammonium from fertilizers is slowly converted only to nitrate in medium below about pH 6
Bacteria that attack fungi	6.5 to 7.5	Some are active at higher or lower pH Levels; greatest effectiveness is near neutrality

- Non-ionic wetting agents should always be used as they increase retention of spray liquids on leaf surfaces (Schönherr, 2004). The use of standard ionic wetting agents or buffer agents on most BCAs is not recommended. The spray equipment should be able to reach the undersides of leaves and reach plant tops.

- Co-inoculants (multiple BCAs) when used are to be compatible (Raupach & Kloepper 1998). When for example *Trichoderma spp*. is used, it should be ensured that the correct strain had been used for the application, such as *Trichoderma virens* that comprised either a P or Q strain, based on antibiotic profiles. Strain P produced glioviren that is effective against *Pythium ultimum* but not on *Rhizoctonia solani.* Strain Q produces an antibiotic called gliotoxin that is highly effective against *R. solani* but less so against *P. ultimum* (Howell, 1991). It should be established whether the particular BCA has preventative or curative qualities and is suited for the application/purpose.

- If BCAs are used as part of an IPM programme or holistic approach, the grower should ensure that the holding period of the particular synthetic chemical has elapsed prior to application of BCAs. Applications of fungicides should be avoided at least one week prior to application for *T. harzianum* and at least a week thereafter (Pundt & Smith, 2001).

- All life has the same basic nutritional requirements, which includes a source of energy. A source of carbon from carbon dioxide or monoxide, methane, carbon monoxide, complex organic materials (Hurlbert, 1999) can be stimulated with household sugar (sucrose) or fulvic acid. Fungi can be stimulated with the use of complex carbohydrates as well as composts, kelp, humic acid and *Aloe vera*. However, bacteria and fungi should always be fed simultaneously to maintain a good balance (Sait, 2003; Hurlbert, 1999). Fungi and bacteria contain high levels of nitrogen, and it would possibly make sense to add a balanced organic fertilizer as a nitrogen source to aid their proliferation, which could include nitrogen as ammonia, nitrate, nitrite or a nitrogenous organic compound-like protein or nucleic acid (Hurlbert, 1999).

- A source of oxygen is essential as all life uses oxygen in a bound form and may require gaseous oxygen (air). Oxygen could, however, be fatal to certain microbes, and consequently, microbes are often termed aerobic or anaerobic. Water is vital, as all life requires liquid water to grow and reproduce. Water should be free of chlorine and should, therefore, be aerated for 30 minutes to remove this micro-biocide (Sait, 2003). Some resting stages of cells, such as bacterial spores, could exist for long periods without free water although they do not grow or metabolise (Hurlbert, 1999).

- The expiry date should be checked, and storage instructions of all BCAs should be adhered to prior to application (Pundt & Smith, 2001). Existing disease might require two treatments per week to regain control (Sait, 2003). BCAs should be used at the correct stage, usually before plants are four weeks old (Abd-El-Moity *et al*., 2003).

1.3 BIOLOGICAL CONTROL AGENT ENVIRONMENTS

The efficacy and survival of BCAs is dependent on both biotic and abiotic factors (Elad *et al*., 1996). Successful and reproducible biological control requires the knowledge of the ecological interactions taking place in the soil and root environments and being able to predict the environment under which bio-control can best be achieved (Deacon, 1994; Whipps, 1997a). As such, BCA success is not only the biological agent *per se,* but there are also the interactions with the crop, the existing micro-biota as well as the environment. Within the root zone, the roots exude organic material, which provides the energy for the development of active microbial populations in the rhizosphere around the root.

The rhizosphere is the root surface itself together with the region of soil surrounding this root, where the microbial population is affected by the roots presence. The loss of organic material around the root is the driving force for the development of active microbial populations, and such development is known as the rhizosphere effect (Whipps, 1990). Oxygen is part of the respiration process where carbohydrates are broken down to provide energy for the plant: this process is the reverse process of photosynthesis. Products of respiration are carbon dioxide and a wide range of carbon compounds, some of which are exuded from the roots into the rhizosphere and the balance of the energy for the plant development (Jarvis, 2003).

BCAs with the appropriate ecological rhizosphere competence and adaptation could be crucial for reproducible biological control activity in the spermosphere (the volume of soil that surrounds a seed) and rhizosphere (soil around plant roots). Therefore, the environmental and nutritional needs of these BCAs must be understood and met to obtain consistent results and good performances by BCAs. Saproptrophs or biotrophs reside in the rhizosphere, but sometimes, a plant pathogen could also infect the host and cause disease.

Multiple microbial (co-inoculants) interactions involving bacteria and fungi in the rhizosphere are shown to provide enhanced bio-control in most cases when compared to BCAs used singly. Antagonistic symbioses could exist between pathogens and roots, resulting in disease (Whipps & Davies, 2000).

1.3.1 The use of beneficial fungi as biological control agents

Fungi used for bio-control have a greater potential than bacteria due to fungi's ability to spread through soil and in the rhizosphere by means of hyphal growth (Bailey & Lumsden, 1998). *Trichoderma spp*. dominates the fungi-based BCAs, perhaps due to ease of growth and adaptability to the host. *Trichoderma* species produce a 22 Kda xylanase, which induces K^+, H^+ and Ca^{2+} channelling responses when injected into plant tissues as well as PR protein synthesis, ethylene biosynthesis and glycosylation and fatty acylation of phytosterols (Bailey & Lumsden, 1998). These PR proteins lead to systemic acquired resistance.

Ingham (2003) revealed that fungi-dominated compost had 100% calcium retention, and bacteria-dominated compost retained only 2% of the calcium. Fungi are known to reduce the pH of their surrounding environment through the release of organic acids and have been proved to prefer a more acidic environment. Fungi can be stimulated with the use of complex carbohydrates as well as kelp, humic acid and *Aloe vera*. Fungi prefer a solid substrate to proliferate and will always be out-competed by bacteria in a liquid medium (Sait, 2003). Beneficial fungi are generally only effective when humidity is high (60 to 80%) ideally, which is often the case inside a greenhouse or plastic tunnel or within the root zone (Quarles, 2004).

Trichoderma harzianum Rifai strain KRL-AG2 (active ingredient- 1.5%) is marketed as a biological foliar and root fungicide and contains 1.0×10^7 colony forming units per gram dry mass. The recommendation is 85 to 142g per 380 litres on cucumbers. It was clearly stated that *Trichoderma harzianum* Rifai strain KRL-AG2 was not effective below 10˚C (50˚F). Applications of fungicides should be avoided at least one week prior to application of *T. harzianum* and at least a week thereafter (Pundt & Smith, 2001). Trials by Elad *et al*. (1998) showed that *Trichoderma harzianum* T39 applied to the soil instead of as a spray resulted in 75 to 90% less mildew coverage on the leaves. The conclusion was the mode of action was mainly induced resistance. The efficacy of *Trichoderma harzianum* T39 was improved with the use of two aliphatic petroleum distillate oil products.

Ampelomyces quisqualis Ces. (AQ 10)® is referred to as a biofungicide for powdery mildew control. *A. quisqualis* is a hyper parasite and attacks a wide range of PM species and genera. It spreads through the air and acts quickly, although it has been shown to be more effective when mixed and sprayed with horticultural oil (Quarles, 2004; Elad *et al*., 1998). *A. quisqualis* (1 x 10^8) is recommended using 100g of *A. quisqualis* per 100 litres water with 200ml of neem oil. The recommendation was that *A. quisqualis* (Sutton 1980; Falk *et al*., 1995a, b) should be used as a preventative for PM and should be applied when humidity is high and a minimum of two sequential sprays was needed. Spray screens smaller than 100 meshes were not advised (Pundt & Smith, 2001). Germination of *A. quisqualis* conidia requires free water, which requires a relative humidity of 90% or higher. The optimum temperature for growth and infection by *A. quisqualis* is 20˚C to 25˚C. Temperatures above 30 ˚C are tolerated, but neither growth nor infection took place. Parasitation of PM mycelium was seen as a brown discolouration within 10 to 14 days (Crüger, 1984).

Verticillium lecanii had been advised for PM control in cucurbits and for the control of aphids and whitefly (Verhaar & Hijwegen, 1993; Verhaar *et al.* 1996). In trials, Verhaar *et al.* (1996) managed to keep PM below 15% with the use of *V. lecanii* and a cucumber variety partially resistant to PM.

1.3.2 Plant growth-promoting Rhizobacteria (PGPR) as biological control agents

PGPR bacteria enhance growth by suppressing either the disease or the pathogen directly. Most bacteria are considered PGPRs and could also increase growth by N² fixation involvement (Hong *et al*., 1991), solubilizing nutrients, such as P (Whitelaw, 2000), promoting mycorrhizal function (Garbaye, 1994), regulating ethylene production in roots (Glick, 1995), releasing plant hormones (Arshad & Frankenberger, 1991) and decreasing heavy metal toxicity (Burd *et al*., 1998; Whipps, 2000).

The key feature of all PGPR is that they all colonize roots to some extent. Colonization could involve simple root surface development or could be endophytic colonization, depending on bacterial strain and plant type (Whipps, 2001). Endophytic bacteria may have an ecologically beneficial position as they could grow and compete

on the root surface and, perhaps, develop within the root where they are relatively safe from competition and environmental stresses (McInroy & Kloepper, 1995). Bacterial genetics play a role in colonization and nutrient uptake and could play a role in bio-control by improving uptake and metabolic function (Malony *et al*., 1994).

Bacillus subtilis (Ehrenberg) Cohn strain QST 713 is a biological bactericide (BCA) sold as Serenade® SC. Serenade® control was recommended for the control of deuteromycetes, oomycetes, ascomycetes and bacterial plant pathogens. *B. subtilis* is known to release cell contents during growth to eliminate or reduce competitors in its immediate environment, thus protecting its niche. *B. subtilis* has also been proven to induce the plant's natural systemic resistance (Ohio Extension, 2003) against bacterial and fungal pathogens, inhibit pathogen spore germination and disrupt germ tube and mycelial development. Serenade® has a shelf life of two years under dry ambient conditions. *Bacillus subtilis* is compatible with many fungicides, bactericides, insecticides, foliar nutrients and adjuvants used in the control of crop diseases. Serenade® is incompatible with strong oxidisers, acids, bases and chlorinated water. Serenade® is said to be non-toxic to beneficials when used at the labelled rates. This benign profile makes it compatible in an IPM programme (Copping, [n.d]), or as part of a holistic approach. Trials by Olsen *et al*. (2001) using Serenade® at 1% w/w contained PM infection to 2.4% of the leaf area compared to the non-treated control that was 20.3% covered by PM.

In trials by Taguchi *et al.*, (2003), *B. subtilis* IK1080© was applied through hot air ducting at rate of 10g/1000m² daily in a cucumber greenhouse and it completely controlled gray mould, which could pave the way for broader disease control. Concentrated metabolites of *B. subtilis* (CMBS) isolate AP-3 and wettable powder of *B. subtilis* (WPBS) isolate AP-3 were used in trials and sprayed twice a week till runoff, which provided 100% PM control and also significantly increased plant fresh weight (Bettiol *et al*., 1997).

Bacillus subtilis and azoxystrobin were the most effective fungicide combinations in PM prevention trials. An additional benefit in their trials was an increase in the mass of marketable quality fruit (Keinath & DuBose, 2004).

Two products, Kodiak® and Epic®, are marketed overseas and are a mixture of two *Bacillus subtilis* strains combined with a fungicide (carboxin-PCNB-metalaxyl), which is further evidence that there is room for IPM (Raupach & Kloepper, 1998) should a holistic approach fail. The above shows that BCAs and synthetic chemicals can be used together in an IPM program; however, the use of synthetics should be a last resort, as BCAs should ultimately reduce the dependency on these harsh chemicals.

Streptomyces griseovirdis is extremely toxic to mammals, aquatic vertebrates, fish, bees and beneficial insects and microbes, but the antibiotics can control blights and other fungal pathogens (Quarles, 2004; Pundt & Smith, 2001). *Streptomyces* is soil-dwelling bacteria, which bacteria include disease-causing and disease-suppressing strains, sometimes within the same species (Peet, [n.d.]).

Bacteria are less moisture dependent than beneficial fungi and consequently have a wider range of use (Quarles, 2004) but they prefer a higher pH. Powell (2003) stated that acid impaired the mobility of bacteria (Table 1.3). Bacteria can be stimulated with simple household sugar or fulvic acid, but bacteria and fungi used as co-inoculants should always be stimulated or fed simultaneously to maintain a good balance between the two (Sait, 2003).

1.3.3 Co-inoculants and biological control

Improved bio-control could be achieved by adding mixtures or combinations of BCAs, especially if they have different or complementary modes of action or root-colonizing abilities. Such multiple interactions would resemble a more balanced diversity, such as that found in nature. Co-inoculants of three PGPR, *Bacillus pumilus* (Meyer & Gotheil), *Bacillus subtilis* (Ehrenberg & Cohn) and *Curtobacterium flaccumfaciens* (Hedges, Collins & Jones) provided the best control for several pathogens on cucumber than when used singly (Raupach & Kloepper, 1998). Combinations of fungi and bacteria also provided enhanced bio-control (Duffy *et al*., 1996).

Elad *et al*. (1998) found that *Trichoderma harzianum* T39 reduced powdery mildew severity by 97%, but its efficacy declined to between 18 to 55% control as the epidemic progressed, and the control on older leaves was poor; *Ampelomyces*

quisqualis (AQ10)® achieved up to 98% control and retained significant control on older leaves. The co-application of these two BCAs did not improve control in this case.

Pseudomonas fluorescens and a mixture of *Trichoderma spp.* isolates were the most effective bio-control agents in trials by Abd-El-Moity *et al*. (2003) in controlling powdery and downy mildew. The highest yields were obtained with *P. fluorescens, Bacillus subtilis* and a mixture of *Trichoderma spp.* in the trials. These antagonists gave best results when plants were treated from four weeks of age; these plants were healthier than those treated from five and six weeks of age. Co-inoculants should not contain members that are inhibitory to another or interfere with non-pathogens.

Raupach & Kloepper (1998) stated that a mixture of introduced BCAs would closer mimic the natural situation and might broaden the spectrum of biological activity. The following list contains the three main goals in achieving control of a wide spectrum of pathogens by applied antagonists / BCAs:

- BCA strain mixtures with superior bio-control activity should be developed. This development could include mixtures of organisms with broader colonization patterns, mixtures of BCAs that control different pathogens, mixtures of BCAs with different mechanisms of disease suppression or antagonists with different optimum pH, temperature or moisture requirements.

- The genetics of the BCA should be modified to add mechanisms of disease suppression that are operable against more than one pathogen.

- The environment should be altered to favour the BCA and to disfavour pathogens.

1.3.4 BCAs and their mode of actions

BCAs have various modes of action, which need to be understood so that each of them can be used for the correct purpose. This understanding would ensure greater efficacy and so encourage its use. Modes of action include:

- Inhibition of the pathogen by antimicrobial compounds (antibiosis).
- Competition for iron through the production of siderophores.
- Competition for colonization sites and nutrients (space).
- Induction of plant resistance mechanisms (IR: ISR & SAR).
- Inactivation of pathogen (mycoparasitism).
- Parasitism and extra-cellular enzymes.

Some BCAs have significant effects on plant growth promotion and rhizosphere competence; certain BCAs have a single mode of action, and others exhibit several modes. Each of these modes of action is described below.

1.3.4.1 Antibiosis

Antibiosis is the production of antifungal metabolites (excluding metal chelators and enzymes) produced by bacteria *in vitro* (cultured) but may also do so *in vivo.* The role of antibiotics in bio-control has been demonstrated by using mutants that fail to produce antibiotics (Nowak –Thompson *et al*., 1999).

Antibiotic production by fungi used as BCAs has been reported for isolates of *Trichoderma sp*. (Howell, 1998). *Trichoderma virens* comprises either a P or Q strain, based on abiotic profiles. Strain P produces glioviren that is effective against *Pythium ultimum* but not on *Rhizoctonia solani.* Strain Q produces an antibiotic called gliotoxin that is highly effective against *R. solani* but less so against *P. ultimum* (Howell, 1991). *Trichoderma virens*, formerly known as *Gliocladium virens,* is used for damping off and root rot pathogens but not on PM; consequently, they are pathogen specific (Pundt & Smith, 2001).

Streptomycin is the only true antibiotic labelled for vegetable production, so it is a key tool in bacterial disease control as a foliar spray or seed treatment. Slow drying conditions favour its systemic action. It is sold as Agri-Strep®, Agrimycin 17®, and Streptomycin 17® (Bessin *et al*., 2004).

1.3.4.2 Competition for iron and siderophores

Bacteria can produce a range of iron chelating compounds, or siderophores, which have a high affinity for ferric iron (O'Sullivan & O'Gara, 1992; Loper & Henkels, 1999). Siderophores from azobacter are low molecular mass organic molecules that have an affinity for iron. Azobacter is also implicated in inducing systemic resistance in some plants (Zimmer *et al.,* 2003). These bacterial iron chelators are thought to harness and limit the supply of iron in the rhizosphere, making it unavailable to pathogenic fungi, thereby restricting their growth, (O'Sullivan & O'Gara, 1992; Loper & Henkels, 1999). Recent studies have shown that the iron nutrition of the plant influences the rhizosphere microbial community structure (Yang & Crowley, 2000).

Pseudomonads also produce two other siderophores: pyochelin and its precursor salicylic acid. Pyochelin is thought to contribute to the protection of tomato plants from *Pythium* by *Pseudomonas aeruginosa* (Schroeter) Migula 7NSK2© (Buysens *et al.,* 1996). Dynamics of iron competition in the rhizosphere is often complex, influenced by environmental factors and further complicated by pyoverdine and salicyclate that could act as elicitors for inducing systemic resistance against pathogens in some plants (Métraux *et al.*, 1990; Leeman *et al*., 1996b).

1.3.4.3 Competition for colonization sites (space) and nutrients

Competition for carbon, nitrogen and iron was demonstrated to be the mechanism for suppression of *Fusarium* wilt when non-pathogenic *Fusarium* and *Trichoderma spp.* were used as BCAs (Mandeel & Baker, 1991). Mycorrhiza (fungi) was also considered a strong contender for space in this category due to their obligate association with roots.

Entomopathogenic fungi, such as *Meterhizium anisopliae, Beauvaria bassiana* and *Verticillium lecanii* that cause Muscadine disease (Zimmer *et al*., 2003), would control insect problems and would also occupy space that pathogens would occupy. *Verticillium lecanii* is also described for PM control in cucurbits (Verhaar & Hijwegen, 1993).

1.3.4.4 Induced resistance (IR), induced systemic resistance (ISR) and systemic acquired resistance (SAR)

In the early part of the 20th century, evidence began to accumulate that plants could be protected against infection by prior infection of the plant with another pathogen. This phenomenon became known as induced or acquired resistance to disease (Hammerschmidt & Becker, 1997). One of the characteristics of acquired resistance was that it was effective against a broad spectrum of pathogens.

Perhaps the greatest development and growth in bio-control was induced resistance, or otherwise defined as “the process of active resistance dependent on the host plant’s physical or chemical barriers, activated by biotic or abiotic agents” (Kloepper *et al*., 1992). Most work on IR has focused on non-pathogenic *Bacillus* that colonizes the rhizosphere on *Pseudomonas.* Although they are spatially separated, the noted effect must be IR (Sticher *et al.*, 1997; van Loon, 1997). Bacteria differ in ability to induce resistance; some bacteria are more active on certain plant species and have varying results within the same species; in some cases they have no effect on other species, (van Loon, 1997).

The definition of IR (Kloepper *et al*., 1992) covers biotic (living) and abiotic (non-living) inducers, but the phenotypic (visual) effects of root inoculation with bacteria to abiotic agents appear subtly different, with microorganisms causing localised damage. Pieterse *et al*. (1996) distinguished these two.

Firstly, Induced systemic resistance (ISR) was the original term used for all bacterially induced resistance; however, this term now refers mainly to chemically induced resistance, such as that induced by phosphate and potassium salts. Pathogenesis-related (PR) proteins, such as chitinases, ß -1, 3-glucanases, proteinase inhibitors and a rare type or two, are not universally associated with bacterial IR, (Hoffland *et al*.,

1995). Ethylene stimulation caused by inducing bacteria at inoculation point could also be required for ISR to occur, (Knoester *et al*., 1999). Non-pathogenic organisms or bio-chemical stimuli induce ISR, where jasmonic acid (JA) is used as the signalling molecule. *Bacillus subtilis* is a PGPR that releases antagonistic substances into the root zone that fend off or destroy several species of pathogenic fungi. It is also a growth stimulant and can induce ISR in some plants (Zimmer *et al*., 2003). Plants exhibiting ISR show the following:

- Strengthening of epidermal and cortical cell walls and formation of barriers beyond the infection area, (Benhamou *et al.,* 1996a, b and c).
- Increased levels of enzymes, such as chitinase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, (M'Piga *et al*., 1997; Chen *et al*., 2000).
- Enhanced phytoalexin production (van Peer *et al*., 1991; Ongena *et al*., 1999).
- Enhanced expression of stress-related genes (Timmusk & Wagner, 1999).
- Increased root and shoot development and earlier fruit development as the plant reacts as if under attack, which requires more energy and greater nutrient uptake and enhanced photosynthesis (Zimmer *et al.*, 2003).

Biochemical changes are not found in all bacterial–plant associations, but the ability of bacteria to colonize the rhizosphere is considered a key feature in most bacterial-root interactions concerning ISR; however, this may not always be the rule (Steijl *et al*., 1999). A phosphate (P) containing nutrient solution of 20ppm applied through a hydroponic system induced ISR against PM as well as the 1% foliar solution of mono-potassium phosphate (MKP) effectively protect the foliage against PM (Reuveni *et al*., 2000).

Secondly, systemic acquired resistance (SAR) is a pathogen or perceived pathogen mechanism induced in plants; this resistant state is normally dependent on endogenous accumulation of salicylic acid (Pieterse *et al*., 1996; Whipps, 2001).

Pathogen-induced necrosis on the leaves of many plant species, including cucumber (*Cucumis sativus*), tobacco (*Nicotiana tabacum*) and *Arabidopsis*, results in the production of a phloem mobile signal that triggers systemic resistance to subsequent pathogen attack (Kuc, 1982; Ryals *et al*., 1994). The development of SAR depends on

the rate at which the inducing pathogen causes necrosis on the inoculated leaf. In cucumber, an incompatible bacterial pathogen causes a rapid hypersensitive response (HR) induced systemic resistance within two days. A compatible pathogen that causes a slow, spreading necrosis requires one week or more to induce systemic resistance (Smith *et al*., 1991). Pathogen-induced necrosis on the inoculated leaf is accompanied by the accumulation of salicylic acid at the site of inoculation, in phloem fluids, and in healthy non-inoculated leaves (Malamy *et al*., 1990; Métraux *et al*., 1990; Enyedi *et al*., 1992; Summermatter *et al*., 1995).

Scientists Dhirendra Kumar and Daniel F. Klessig of Boyce Thompson Institute of Plant Research (BTI) at Cornell University discovered the presence of the SABP2 protein in tobacco in 1997. Many plants produce these pathogen–resistant proteins (PR); however, the exact trigger for these defences was not known until now. The salicylic acid-binding protein 2 (SAPB2) gene sends a warning through plant immune systems signalling attack. Salicylic acid is a naturally occurring compound or plant hormone produced in most plants in response to attack by microbial pathogens. Salicylic acid is also found in aspirin ($C_7H_60_3$) and even *Aloe vera* contains high quantities of salicylic acid and is a proven systemic resistance activator (Paul, 2004; Zimmer *et al*., 2003).

In contrast to cucumber inoculated with HR causing bacteria, 60 to 70% of systemically accumulating salicylic acid in tobacco mosaic virus-inoculated tobacco was found to originate from the inoculated leaf. In addition, removal of the inoculated tobacco leaf prior to salicylic acid accumulation prevented SAR. Research still needs to determine whether the difference in the pattern of salicylic acid accumulation in the two systems is a result of differences in SAR mechanisms between the two plant species or perhaps due to the different rates of necrosis induced by incompatible bacteria (Shulaev *et al*., 1995). Cucumber SAR is correlated with the systemic accumulation of extracellular peroxidase, β -1, 3 glucanase and chitinase (Hammerschmidt *et al*., 1982; Boller & Métraux, 1988; Ji & Kuc, 1995).

The latest research by McNally *et al*. (2003) showed the production of phytoalexin compounds, *C*-glycosyl flavonoids, was clearly linked with induced resistance against powdery mildew. Phytochemical analyses of leaf tissues harbouring fungal colonies led to the identification of six structurally related *C*-glycosyl flavonoid phytoalexins, namely, vitexin, isovitexin, orientin, isoorientin, cucumerin A and B. Phenolic acids involved in

lignification, such as sinapinic acid, were detected in equal concentrations in PM resistant and susceptible cucumber plants, showing this to be an important form of resistance in cucumber. However, the discovery of *C*-glycosyl flavonoids as phytoalexins and *p*-coumaric acid and its methyl ester within disease-resistant plants supported previous studies that associated these compounds with induced resistance against PM. P-coumaric acid is a hydroxy acid derived from L-phenylalanine, involved in formation of phenylpropanoids and is readily convertible into salicylic acid. L-phenylalanine is a non-protein amino acid from which, for example, isoquinoline alkaloids are derived via decarboxylation.

McNally *et al*. (2003) found phytoalexin synthesis was triggered by a combination of an eliciting treatment and fungal penetration and seemed closely synchronised with the germination of fungal conidia. Hours preceding the collapse of conidial chains, phytoalexins permeate the extrahaustorial matrix and saturate the haustorial lumen of the PM colonies, inhibiting the epidermal cells.

The accumulation of salicylic acid was claimed to be a requirement for SAR; however, it does not appear to be the only mobile signal transported from the inoculated leaf, since salicylate hydroxylase (NahG) rootstocks, which do not accumulate salicylic acid, were still able to transmit a signal for SAR to wild-type scions (Vernooj *et al*., 1994). Cucumber plants inoculated on one leaf with the SAR-causing pathogen *Pseudomonas syringae* pv. *Syringae* accumulated high levels of salicylic acid in phloem exudates even when the inoculated leaf remained on the plant for only six hours (Rasmussen *et al*., 1991). Maximum levels of salicylic acid were measured 18 hours after inoculation in this system, so the inoculated leaf did not synthesize the majority of systemically accumulating salicylic acid.

As SAR is a pathogen or perceived pathogen mechanism induced in plants; this resistant state is normally dependent on endogenous accumulation of salicylic acid and is characterized by the activation of genes encoding PR proteins. In addition to peroxidase, many plants also systemically accumulate a group of proteins collectively known as the Pathogenesis-Related (PR) proteins. These proteins have been classified into several major groups that have been given names PR1 through to PR5. Chitinase was a particularly useful marker for SAR in cucumber, since levels of the enzyme in control leaves were extremely low (Métraux *et al*., 1988).

Trial results indicated that *Pseudomonas fluorescens* WCS417r induced a pathway different from the one that controls classic systemic acquired resistance and that this pathway led to a form of systemic resistance independent of salicylic acid accumulation and PR gene expression (Pieterse *et al*., 1996; Whipps, 2001). Application of exogenous salicylic acid or its derivative acetyl salicylic acid, blocks and binds the key compound, jasmonic acid, which plants produce in response to physical injury. Salicylic acid antagonizes the production of JA; this antagonisation may mean that the production of PR proteins could override the production of proteinase inhibitors when the plant is under pathogenic attack and hence produces salicylic acid.

Salicylic acid is a critical hormone for signalling innate immunity that is then perceived by the SAPB2 protein, and a message is transmitted via a lipid-based signal to activate the plant's immune systems. SAR can last throughout the life of an annual plant (Paul, 2004).

Not all other forms of induced resistance, such as with salicylic acid (known chemical SAR inducer), are involved in ISR expression; however they are rather dependent on the bacterial strain and concomitant host (Pieterse *et al*., 1996; de Meyer *et al*., 1999; Chen *et al*., 1999). Acquired resistance developed in the inoculated leaf and in the leaves on the same plant that did not receive inoculation. A mechanism similar to SAR may exist to confer resistance to heat stress. In trials, salicylic acid, the chemical inducer of SAR and SAR gene expression, were induced by high temperature treatments, providing evidence of the existence of SAR by heat stress (Kubo & Sato, 2002). Therefore, it could be stated that resistance of greenhouse-grown cucumbers could be initiated by exposure or inoculation.

1.3.4.5 Inactivation of pathogen (Mycoparasitism)

Observations revealed the ability of fungi to parasitize spores, sclerotia, hyphae and other fungal structures (Jeffries & Young, 1994). A mycoparasite may also be detrimental to mycorrhiza, such as *T. harzianum* T-203©, which is known to have attacked the mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in an axenic system (Rousseau *et al*., 1996).

1.3.4.6 Parasitism and extra-cellular enzymes

The ability of some bacteria, especially actinomycetes, to parasitize and degrade spores of fungal plant pathogen is well documented (El-Tarabily *et al*., 1997). If fungal cells are lysed and cell walls are degraded, then it is generally assumed that cell wall-degrading enzymes produced by the bacteria are responsible, even though antibiotics are produced at the same time. Gliotoxin from Q strain *Trichoderma* is thought to be responsible for cytoplasmic leakage from *Rhizoctonia solani* (Harris & Lumsden, 1997).

1.3.4.7 Conclusion

Biological control agents (BCAs) have definite requirements, which include pH, humidity and nutritional needs to name but a few. The modes of action for each BCA needs to be understood as it is apparent that BCAs have been used in the past with insufficient guidelines and training to realise the full benefits or potential. The list of BCA requirements above (sections 1.2.5 and 1.3) is fairly extensive and people would need substantial training to implement the criteria, but the merits are numerous as can be seen in Table 1.4. However, such training would lead to a complete change in farming practices and management.

Table 1.4. Comparison of synthetic fungicides versus biological control agents

Attributes	Synthetic fungicide	Biological control agent
Environmentally sustainable	No	Yes
Holding period before harvest (Holding period)	Yes –variable	No
Worker safety	Dangerous- hazardous	No or low risk
Phytotoxicity risk	Possible	None reported
Enhance growth	No	Some e.g. PGPR

From the literature review it is apparent, there were a number of inputs to manage PM, but an alternative to synthetic fungicides is required that is environmentally sustainable and would help reduce resistance to fungicides. BCAs offered promise, but their use is limited, as they had often not been used for the correct purpose or under the correct environmental conditions. BCAs would be investigated specifically for PM control on tunnel cucumbers to establish whether they are a sustainable alternative and a replacement for synthetic fungicides.

CHAPTER 2

MATERIALS AND METHODOLOGY

2.1 RESEARCH PROBLEM STATEMENT AND HYPOTHESES

Problem statement:

The use and study of biological control agents (BCAs) has not gained momentum in South Africa. Literature indicates definite environmental and nutritional requirements concerning BCAs effectiveness as control agents. This investigation is aimed at determining whether BCAs were capable of controlling PM if used under the correct conditions and for the particular application. If the efficacy of the BCAs is unsatisfactory on its own, then a holistic approach could prove to be a solution and would be a softer approach compared to synthetics. The programme could help in reducing fungicide dependency or it could prove to be a sustainable alternative to fungicides. Knowledge of the mode of action used by these BCAs and their environmental requirements are vital to their success, since BCAs often showed promise in laboratories but fail in commercial agriculture.

This research proposes to investigate the use of biological control agents (BCAs) as a sustainable alternative to synthetic fungicides in treating powdery mildew in tunnel cucumbers. The question is, however, are the BCAs as good as the synthetic control, Bravo (chlorothalonil, 720g/litre) used in the trials?

Research Hypotheses

The research hypotheses are:

$H_{1:}$ $M_{BCA} > M_{Control}$ (where median number of fruit produced by BCA treatment is more than the control)

H_2: $M_{BCA} > M_{Control}$ (where median fruit mass of BCA treatment produced is greater than the control)

H_3: $M_{BCA} > M_{Control}$ (where median crop yield of BCA treatment is greater than the control)

$H_{4:}$ $M_{BCA} > M_{Control}$ (where median PM control of BCA treatment is better than the control)

Tested against the hypothesis:

H_0: There are no significant differences between the median values for the different treatments and the control.

If the null hypothesis was rejected then the research hypothesis was not rejected.

Possible sub-hypotheses that were considered were:

- Biological control agents are an economically sustainable alternative to synthetic fungicides in treating powdery mildew in tunnel cucumbers.
- Biological control agents are a scientifically sustainable alternative to synthetic fungicides in reducing powdery mildew in tunnel cucumbers.
- Biological control agents are an environmentally sustainable alternative to synthetic fungicides in treating powdery mildew in tunnel cucumbers.
- Different BCAs differ in their effectiveness in controlling powdery mildew.

Other considerations with this research were that certain BCAs are multi-modal and could enhance growth as well as reduce or control PM possibly through various modes of action.

Various biological control agents (BCAs), consisting of beneficial fungi (myco) and *Bacillus spp*., which are commercially available, and new potentially beneficial biological control agents in the experimental stage were tested. The efficacy of BCAs was investigated and the potential of BCAs was established for replacing synthetic fungicides in the control of PM on cucumber plants in tunnel or greenhouse production.

2.2 MATERIALS AND METHODS

2.2.1 Trial layout

The study site is located near Elandsfontein (S26°23.962 min / E 27°55.240 min), Johannesburg, South Africa and is situated at an altitude of 1639 metres. Trials were conducted in a plastic, film-type greenhouse (30 x 40M=1200m²) that had a double layer of plastic (polyethylene- 200 micron x 2); air was pumped between these two layers so that inflation could help with insulation. The greenhouse was heated overnight in winter with a coal-fired air exchanger. During the day the greenhouse was force ventilated to cool the greenhouse by means of five large electric extraction fans controlled by a thermostat control. No artificial lighting was used during the trials.

The roof of this structure is divided into five spans, each being 30 x 8m; consequently, allowing one treatment per span possible (Figure 2.1). A planting density of 1.66 plants per square metre was used, with a total population of 2000 plants in the greenhouse. Each span of the greenhouse accommodated 400 plants, of which 80 plants were to be used for data collection.

From these 80 plants, random numbers were selected and used in each treatment row for visual rating. Buffer/guard rows were allowed for between the different treatments to ensure that sprays did not drift across and distort the results as shown in Figures 2.2.

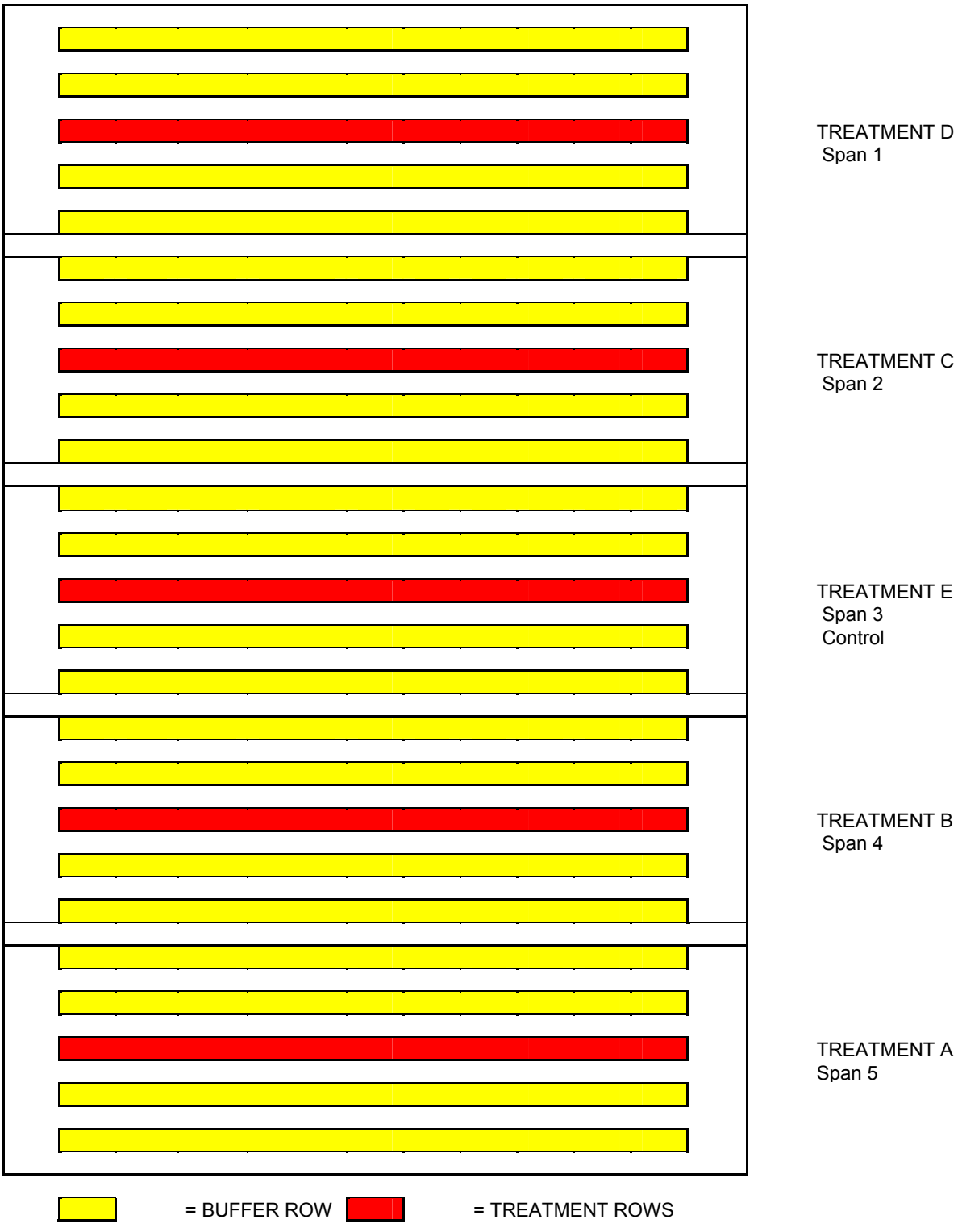


Figure 2.1. Schematic layout of trials.

Figure 2.2.A & B: (L-R) Photographic example of trial layout, 5 spans with 5 rows per span and 80 plants per row.

2.2.2 Experimental design

Trials were arranged in a random block design. The researcher's trial model is a combination of the work of Krishna *et al*. (1998), Raupach & Kloepper (1998) and Dik *et al.* (1998). Trials by Krishna *et al.* (1988) also used the Coyier model for PM evaluation. Raupach & Kloepper (1998) advised using mixtures of plant growth-promoting Rhizobacteria to enhance biological control on cucumber pathogens and their work also included Bravo as a control.

2.2.2.1 Trials

Cucumber seeds were sown directly into new 20 litre bags and fresh pine sawdust used as the growing medium for each of the trials. Plants were trellised to a height of 2.1m. All crooked fruit were removed at an immature stage; these fruit were not accounted for in the yield or mass data. Cucumber fruit were picked every two to three days depending on the growth rate. Only marketable fruit between 30 and 36 cm in length were used in the data collection. The yield and fruit mass data was collected from the 80 plants in each treatment. Ten plants were chosen from a randomised table of numbers and this data was used to determine the efficacy (statistically and

graphically) of the BCAs in controlling PM for each treatment. The balance of the plants in the greenhouse merely acted as guard rows between the different treatments and the control.

A pre-trial was done to determine the viability of the study. The pre-trial is independent to trials 1 and 2, which were conducted from spring through to autumn. Trials 1 and 2 differed from the pre-trial as silicon was also sprayed in conjunction with the BCAs (Tables 2.1, 2.2 & 2.3) at a rate of 12.5ml/100ℓ. As standard practice, the greenhouse was completely cleaned and sterilized after the pre-trial and trial 1, then left empty for a number of weeks to break the life cycle of pests and disease before further trials commenced.

Table 2.1. Biological agents and their corresponding trial code numbers

CM 1012 - *Streptomyces griseovirdis*
CM 1086 - *Streptomyces aureofaciens*
CM 1075 - *Trichoderma harzianum* Rifai
CM 1103 - *Trichoderma harzianum* Uppington
CM 1054 - *Ampelomyces quisqualis*
CM 1117 - *Ampelomyces quisqualis*
CM 1118 - *Ampelomyces quisqualis*

NOTE: Only *Bacillus subtilis* and *Streptomyces spp.* are bacterial; the balance of the BCAs is fungal-based.

Table 2.2. Names of biological agents used in each span/treatment in the greenhouse

Span 5 = Treatment A = CM 1012 +CM 1086
Span 4 = Treatment B = CM 1075 + CM 1103
Span 3 = Treatment E = Bravo (synthetic fungicide - control)
Span 2 = Treatment C = CM1054 + CM1117 + CM1118
Span 1 = Treatment D = *Bacillus subtilis* + activator - (carbon / energy source)

Table 2.3. Table of extraneous / confounding variables for the pre-trial, trials 1 and 2

	Pre-trial	**Trial 1**	**Trial 2**
Sowing date Season	2-4-2004 till 30-8-2004 (Winter)	27-9-2004 till 28-12-2004 (Spring to summer)	26-1-2005 till 9-5-2005 (Summer to autumn)
Cucumber variety name	Baccara (Winter variety)	Palladium (High PM tolerance)	Palladium (High PM tolerance)
Varietal tolerance to PM	Medium	High	High
Were biological control agents (BCAs) used as listed in Chapter 2 and Table 2.3 & 2.4?	Yes	Yes	Yes
Was silicon added to biological control agents spray solution?	No	Yes	Yes
Was fulvic acid added to BCA spray solution?	Yes	Yes	Yes
Is trial independent?	Yes	No	No
Comparative study BCAs vs. Synthetic fungicide?	Yes, BCAs compared to the control, Bravo	Yes, BCAs + **silicon** compared to the control, Bravo	Yes, BCAs + **silicon** compared to the control, Bravo
Day length – Start of trial Day length – End of trial (Sunrise/sunset)	06:18 / 18:05 06:23 / 17:55	05:52 / 18:07 05:16 / 19:04	05:38 / 19:03 06:37 / 17:33
Total day length – Start of trial Total day length – End of trial	11hrs 47 min 11 hrs 32 min	12 hrs 15 min 13 hrs 48 min	13 hrs 25 min 10 hrs 56 min
Ave. of day lengths	11 hrs 39 min	13 hrs 02 min	12 hrs 11 min
Number of plants per treatment	80	80	80

2.2.2.2 Trial nutrient solution

The nutrient hydroponic solution pH was maintained at 5.6 to 6.3 as this ensured that all elements were available to the plant, and the EC (electrical conductivity) was maintained at 1.6 to 2.0, as this ensured a high enough concentration of nutrients without causing any osmotic stress. Hydrogro (Table 2.4.), water-soluble hydroponic fertilizer, was used with calcium nitrate from Hortica which contains 19.5% Ca and 15.5% N. Hydrogro and calcium nitrate have been the most popular standard products used for cucumber hydroponic production in South Africa. The fertilizer and calcium nitrate were used in equal proportions: that is 1kg of each per 1000 litres of water.

Table 2.4. Hydrogro water-soluble hydroponic fertilizer contains the following grams (g) of element per kg

N	65g / Kg		**Fe**	1680 g / Kg
P	45g / Kg		**Mn**	400 mg / Kg
K	240g / Kg		**B**	500 mg / Kg
Mg	30g / Kg		**Zn**	200 mg / Kg
S	60g/ Kg		**Cu**	30 mg / Kg
			Mo	50 mg / Kg

2.2.2.3 Spray materials

Plants were sprayed till runoff (high volume) with the various BCAs on a seven-day spray interval program, and more often when disease pressure was noted. The spray dates were recorded as well as the volume and concentrations of the sprays. Since the greenhouse was divided into five spans, the trial was suited for four treatments and a control.

Bravo (Chlorothalonil, 720g/litre) was used as the control in the trials; this synthetic was also used as a control in Krishna *et al*. (1998); McGrath & Shishkoff (1999); Keinath &

DuBose (2004). The benefits of Bravo are its short holding period of only three days (Nel *et al*., 2003) and the fact that it is a contact fungicide. Chlorothalonil is registered in the USA as a fungicide for controlling PM.

Plants were sprayed with *Bacillus subtilis, Streptomyces griseovirdis, Streptomyces aureofaciens, Trichoderma harzianum* Rifai, *Trichoderma harzianum* Uppington and *Ampelomyces quisqualis.* The same BCAs were used in the pre-trial, trials 1 and 2. *Bacillus subtilis* is marketed as ^{D}Press© from Microbial solutions (Tables 2.1 & 2.2.).

All BCAs were applied in conjunction with fulvic acid (FA), the low molecular mass acid radical found in humic matter. Fulvic acid is an extremely complex bioactive yellow organic substance which provides multiple and natural chemical reactions in soil, helping to stimulate unique and positive organic processes. FA is the ultimate aerobic decomposition product for all living matter, with exceptional abilities to change, alter, molecularly combine with, or act upon virtually all other organic and inorganic matter. FA has a cation exchange capacity (CEC) of 1400, which ensures a good nutrient storage capability. Application of fulvic acid is recommended at 0.3% in water on a weekly basis. Compost, humic acid and fulvic acid are considered to be the best natural chelators (Sait, 2003).

2.2.2.4 Cucumber varieties

An English cucumber *Cucumis sativus* L. var. Baccara, was used in the pre-trial as it was a winter variety and had a relatively low tolerance to PM. An all year round variety, Palladium, was used in trials 1 and 2. A key feature of this variety is that it has a high genetic tolerance to PM.

2.2.3 Data collection for trials 1 and 2

A rating system had been devised to compare infection rates between the treatments. This rating system was adapted from Coyier (1986). In Coyier's model, zero/no PM infection was given a 1 rating. His system extended from a 1 to a 6 rating. The researcher's rating was identical to Coyier's model except that no PM infection was given a 0 rating and the rating was extended to a maximum of 5 (Table 2.5). The rating

model used was a visual rating system where the lowest five leaves (excluding the oldest two leaves) on each treatment were assessed for powdery mildew infection.

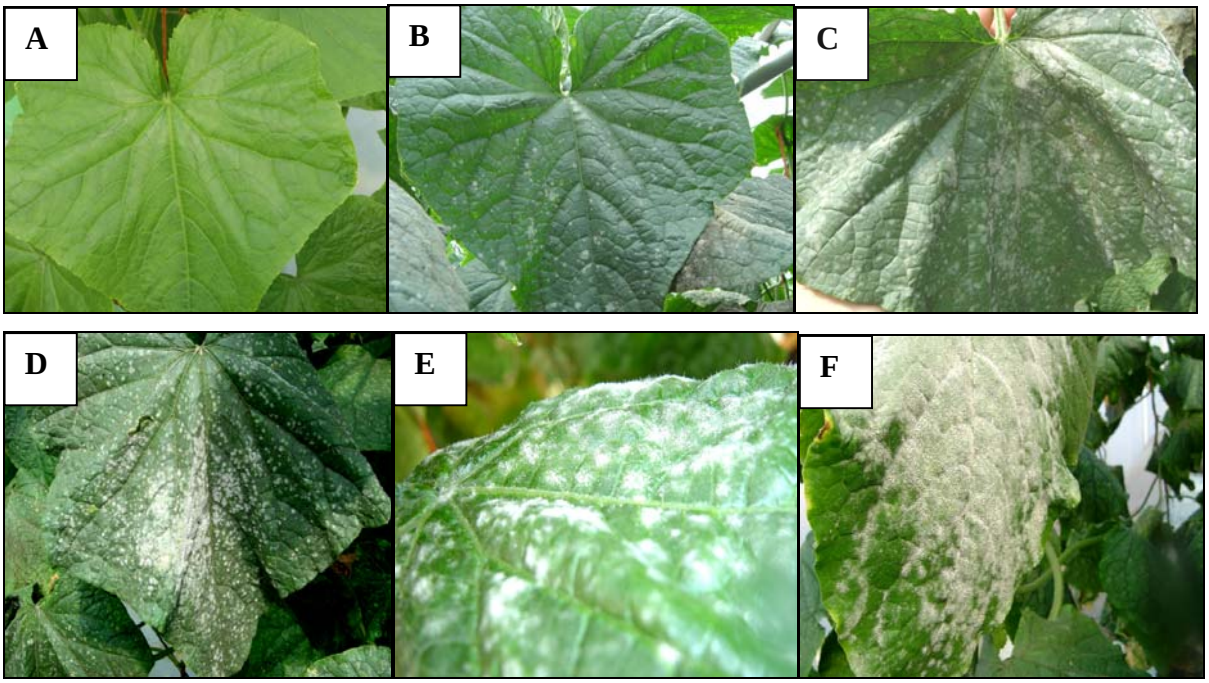
Mr S Vorster, commercial hydroponic farmer with 15 years experience, collected data daily. The researcher collected and logged data on a weekly basis after which the PM infection rating was done on a weekly basis by this researcher, according to the visual rating system method outlined in Table 2.5 and Figures 2.3 A-F. The hydroponic farmer collected the following data: harvest data with quantity and mass as a whole as well as spray dates, concentrations and volumes, temperature and humidity. The research required that fruit yield, average fruit mass and total yield mass, combined with the visual rating system be recorded as measurements of efficacy. The numbers of fruit per treatment (yield) were recorded as such numbers are of economic value to growers and determine the economic viability of BCAs.

A farm worker determined fruit mass on an Avery scale, which had a 150kg capacity. The scale was regularly calibrated (7-12-2004 / certificate number 2067), as the establishment is Eurep-gap compliant.

The average of the five leaves for each of the ten randomly selected plants were combined to obtain an average of the ten plants to rate PM infection. This average was used to determine variance between the treatments. The rating of PM infection was without the use of any lenses or magnification.

Table 2.5. Adapted Coyier (1986) powdery mildew severity rating model

0= NO COLONIES VISIBLE TO THE UNAIDED EYE
1= FEW SCATTERED, DISCRETE COLONIES
2= LARGE BUT STILL DISCRETE COLONIES
3= COLONIES MERGING TO FORM LARGE MILDEW COLONIES
4= MILDEW COVERING ABOUT HALF THE TOTAL LEAF AREA
5= MOST LEAF AREA COVERED BY THE FUNGUS



Figures 2.3. A-F: Photographic representation of Coyier (1986) model. Figure 2.3 A has no visible colonies and has a 0 rating, B represents a rating of 1 and C would be a rating of 2. Figure 2.3 D is typical of a 3 rating, E rates as 4 and F a 5. See corresponding Table 2.5 above.

2.2.4 Experimental process

The pre-trial allowed for viability studies and for improvements of the study process and inputs. Figure 2.4 shows these processes and changes, which were used in the pre-trial and trials 1 and 2. These processes are also stated in Table 2.3.

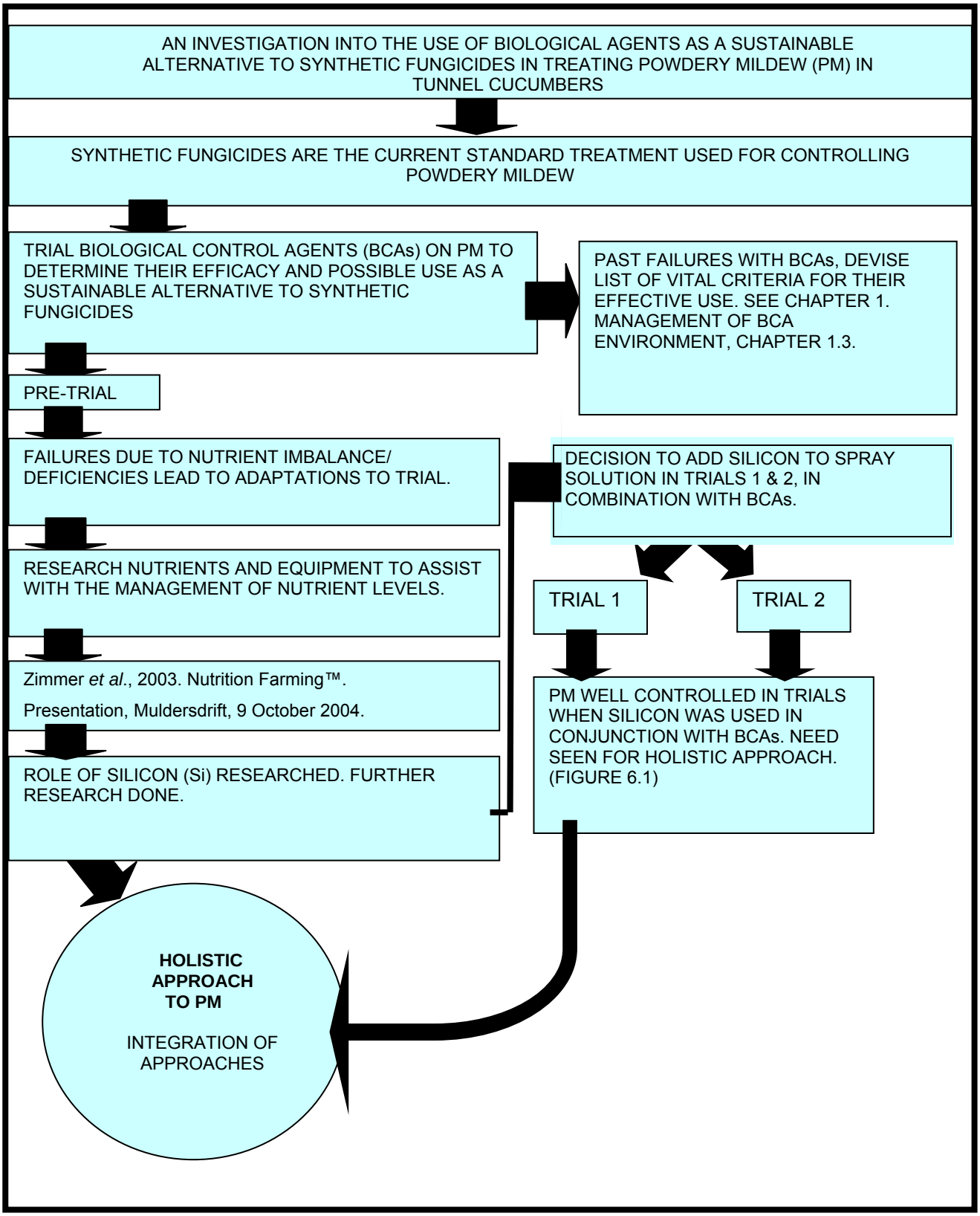


Figure 2.4. Flow chart showing course of research and final outcome.

CHAPTER 3

PRE-TRIAL RESULTS

3.1 PRE-TRIAL

Seeds were directly sown on into the 20 litre bags on 2 April 2004; a final population of 2000 plants was obtained in the greenhouse. The first fruit was picked on the 23 May 2004 and extended until the 30 August 2004. Trials were conducted as explained in chapter 2.2.1 to 2.2.3 and shown in figure 2.1. A winter cucumber variety, called Baccara, was sown. A comparative study between BCAs and the synthetic fungicide, Bravo, was conducted.

3.1.1 Dosage rate of pre-trial BCAs

The dosage of each biological used is listed in table 3.1 below with the corresponding span number. Silicon was not used in the pre-trial, only in trials 1 and 2.

Table 3.1. Optimum dosage rate of pre-trial BCAs

OPTIMUM DOSAGE RATE	SPAN NUMBER	TREATMENT	BIOLOGICAL AGENT NAME
10ml / 40L water	5	(A)	*Streptomyces griseovirdis & Streptomyces aureofaciens*
20 drops / 40L water	4	(B)	*Trichoderma harzianum* Rifai & *Trichoderma harzianum* Uppington
20 drops / 40L water	2	(C)	*Ampelomyces quisqualis*
80ml+240g/40L water	1	(D)	*Bacillus subtilis*
90ml / 40L water	3	(E)	Bravo (synthetic fungicide)

3.1.2 Spray dates for pre-trial

02/05/2004, 09/05/2004, 16/05/2004, 22/5/2004, 25/5/2004, 31/05/2004, 01/06/2004, 06/06/2004, 12/06/2004, 17/06/2004, 24/06/2004, 01/07/2004, 08/07/2004, 15/07/2004, 22/07/2004, 29/07/2004, 05/08/2004, 12/08/2004, 19/08/2004

Dates in red were treatments of a synthetic fungicide listed below that was used to regain / restore control when PM was severe and BCAs could not reduce the infection.

Table 3.2. Synthetic chemicals and dosage rates

Product	**Dosage Rate**	**Active Ingredient**	**Active Ingredient Concentration**	**Action**
Wuxal®	40ml /20ℓ	Amino Acids		Reduce plant stress
Agral 90®	2ml /20ℓ	Alkylated phenol-ethylene oxide condensate	940 g / ℓ	Spreader / Sticker
Oscar®	20g /20ℓ	Bupirimate & Hexaconazole	250 g / 30 g per ℓ respectively	Systemic fungicide (3-day hold)

3.2 RESULTS

3.2.1 Climatic data

The climatic data in the greenhouse was collected daily (Figures 3.1, 3.2 & 3.3) for maximum and minimum temperature; the minimum humidity was also recorded, as it was critical to fungal development and survival. High humidity is less critical to PGPR (Quarles, 2004). Sudden drops in temperature, as seen around 7 July 2004 (Figures 3.1 & 3.2), showed a corresponding rise in humidity levels. Fungal BCAs are sensitive to low humidity (Quarles, 2004) and as stated in chapter 1. This low minimum temperature experienced was below the optimum temperature for cucumbers. The heating system of the greenhouse could not compensate for the extreme cold.

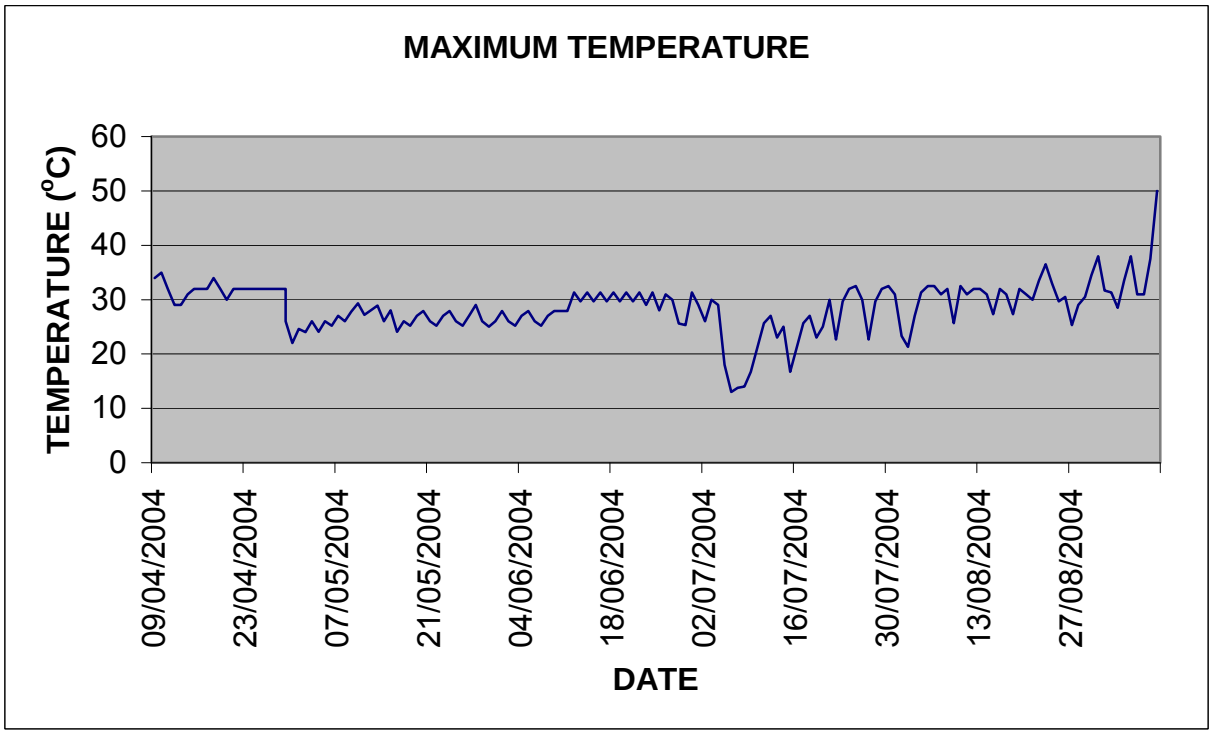


Figure 3.1. Maximum daily temperature in the greenhouse for the pre-trial.

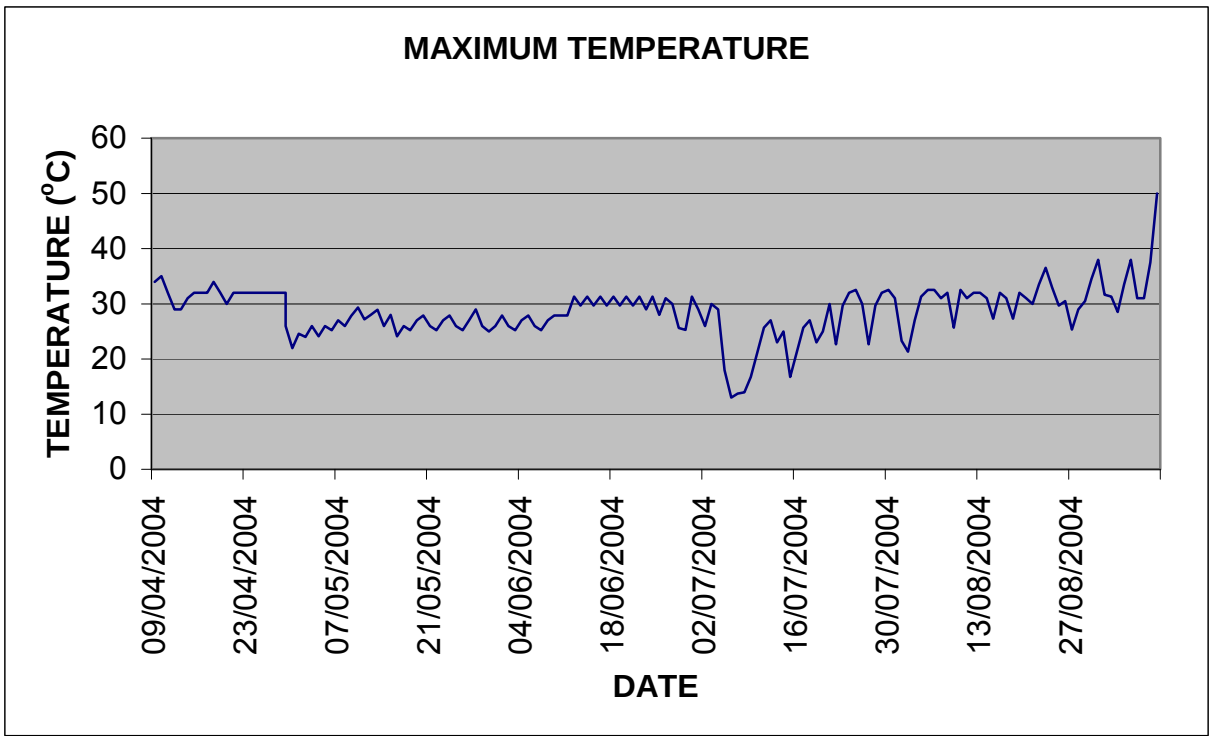


Figure 3.2. Minimum daily temperature in the greenhouse for the pre-trial.

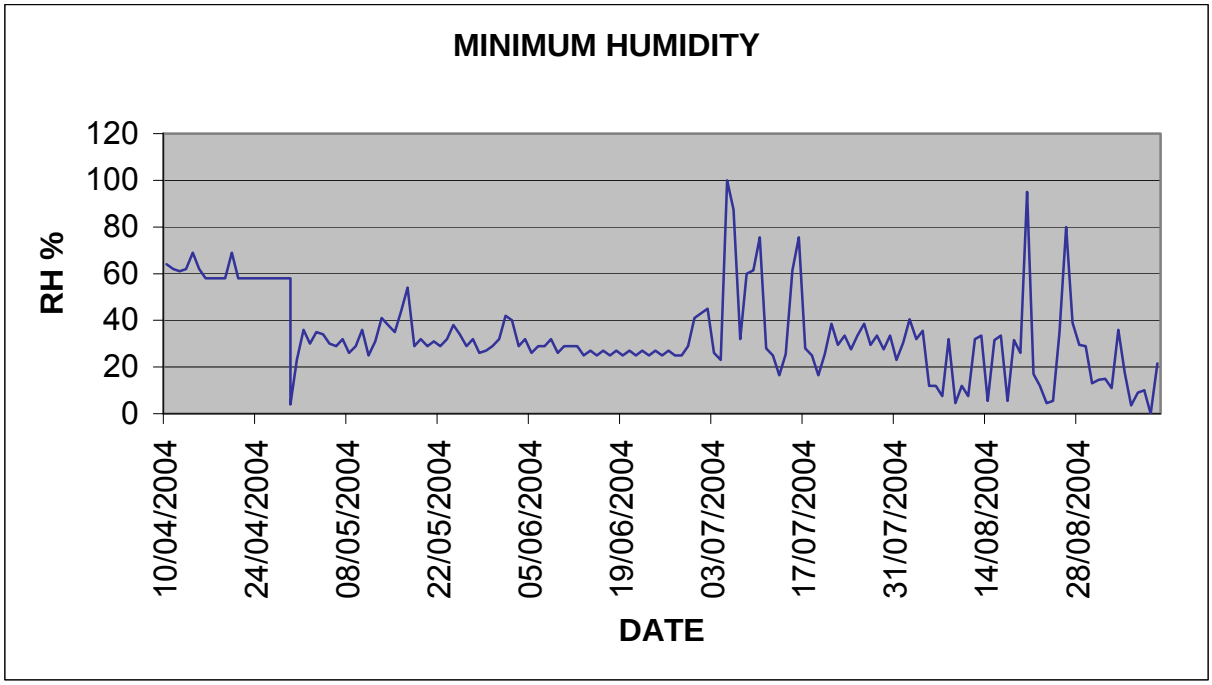


Figure 3.3. Minimum humidity in the greenhouse on a daily basis for the pre-trial.

3.2.2 Harvest data

Figure 3.4 shows the number of fruit harvested on each picking date for each of the treatments in the pre-trial. Using 80 plants per treatment, a total of 35 picks (harvests) were done over the production period, and the number of fruit harvested at each pick was recorded for each of the treatments. Treatment E had a large number of fruit produced around the 1 July 2004 but almost all of the BCAs had a better number of fruit in the early production stages when compared to the control.

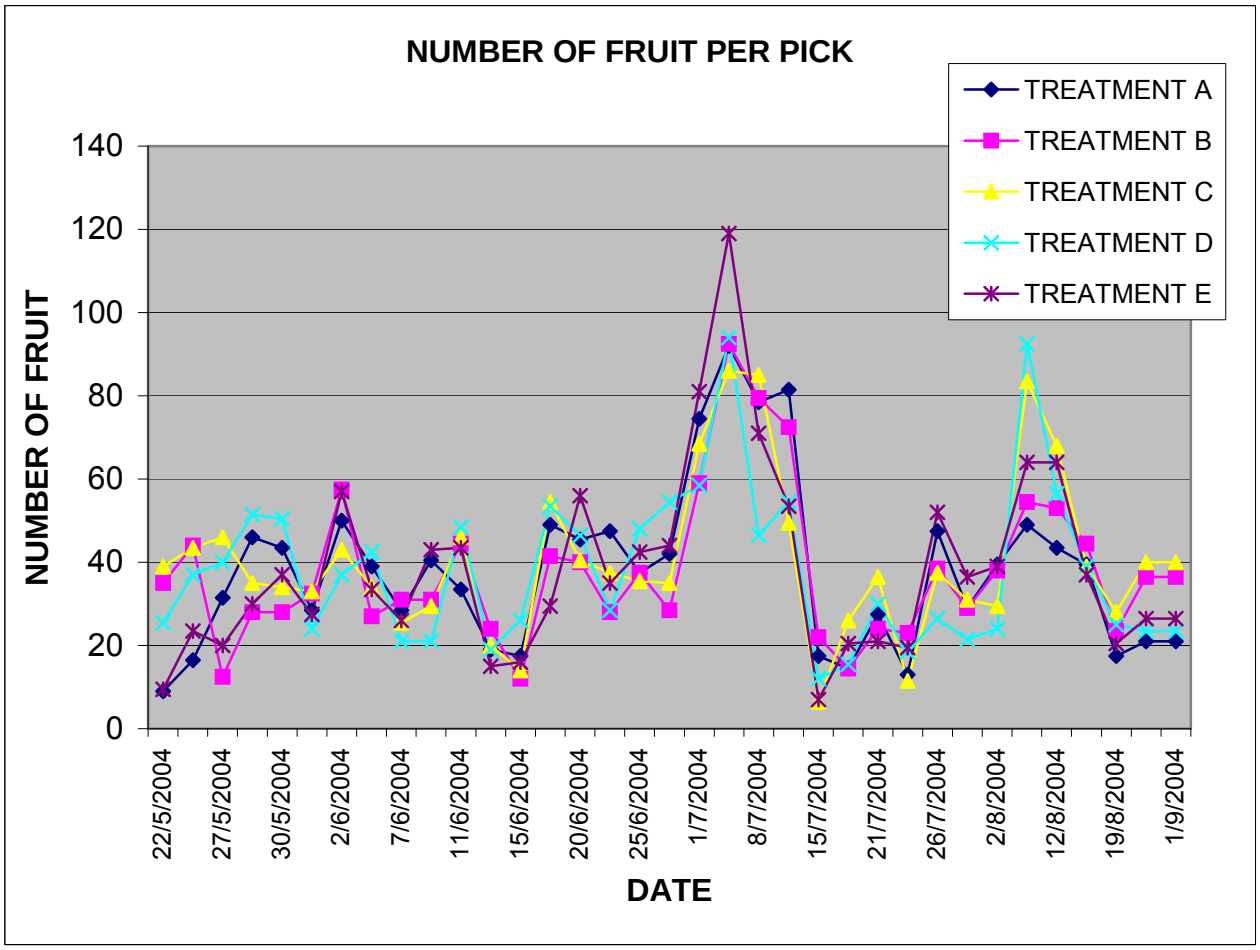


Figure 3.4. Number of fruit picked at each harvest date for each of the treatments in the pre-trial.

Figure 3.5 shows the average fruit mass per treatment for every pick. Treatment C showed the best fruit mass over the period. Treatment C had peaks in average fruit mass around the 15 June, 15 July and 24 July again. Treatment A however produced flushes in initial and final production stages. All fruit were of commercial grade and marketable.

See appendix (Table 7.1) with average fruit mass per treatment over the harvest period and total combined average per treatment during the pre-trial.

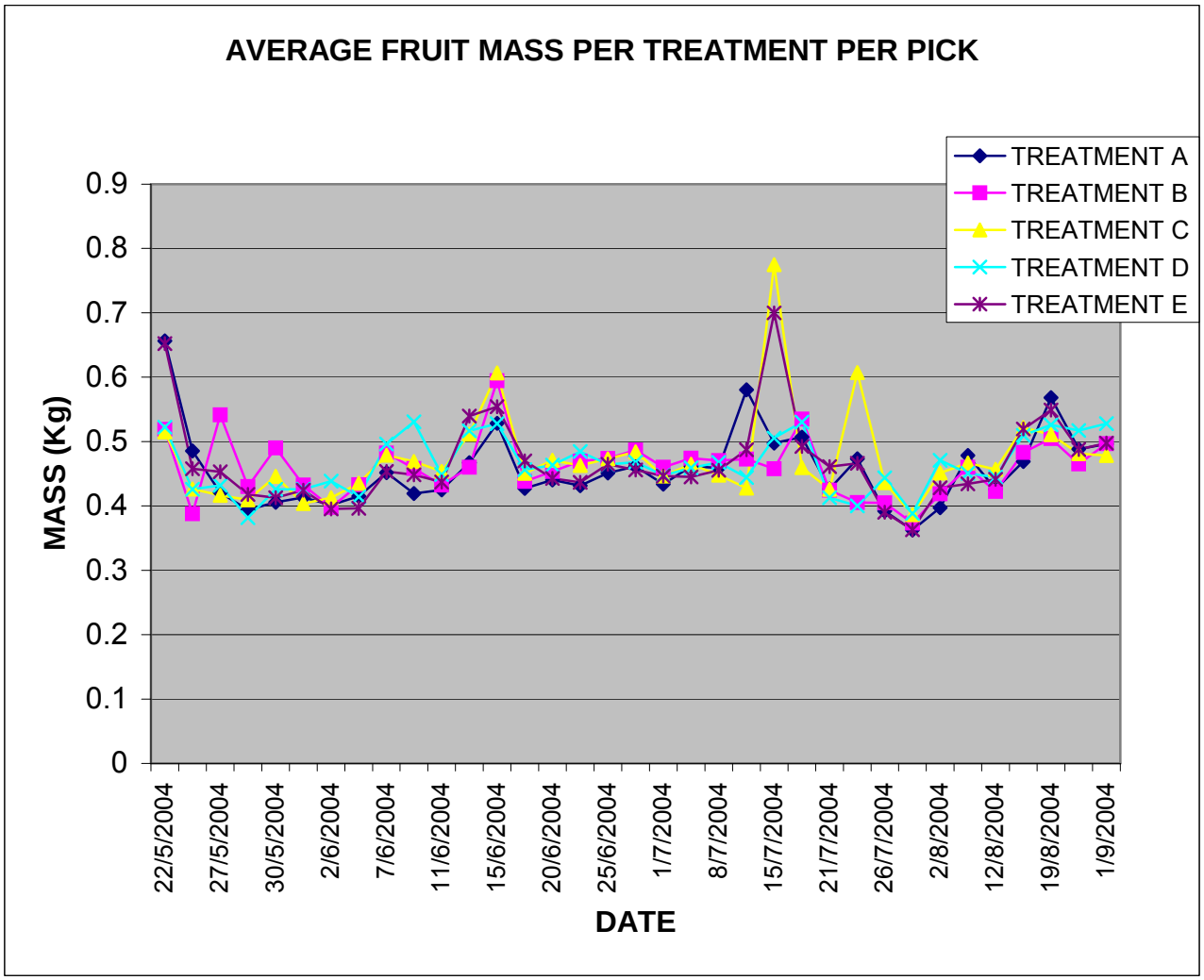


Figure 3.5. Average fruit mass per treatment over the harvest period, during the pre-trial.

Treatment C also had the greatest number of fruit (Figure 3.6) and highest average fruit mass as seen in Figure 3.7. The highest yield in terms of kilograms yielded per treatment over the production cycle is shown in Figure 3.8.

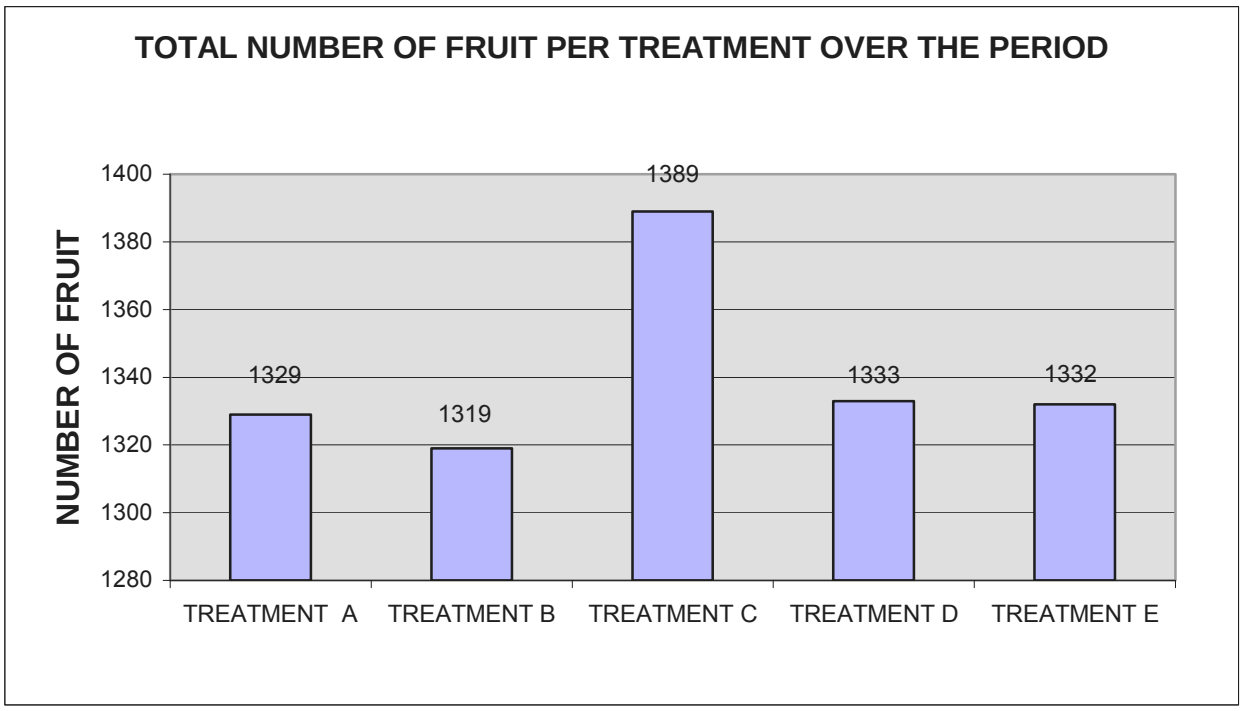


Figure 3.6. Total number of fruit per treatment over the harvest period.

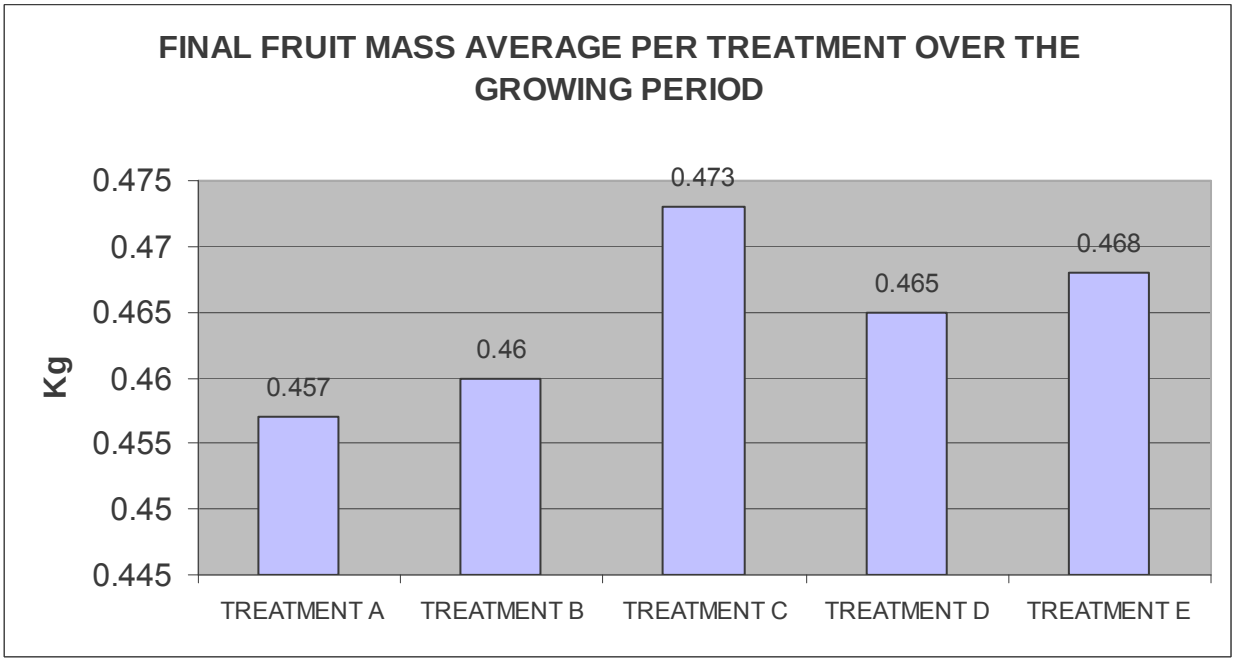


Figure 3.7. Average fruit mass per treatment over the full harvest period.

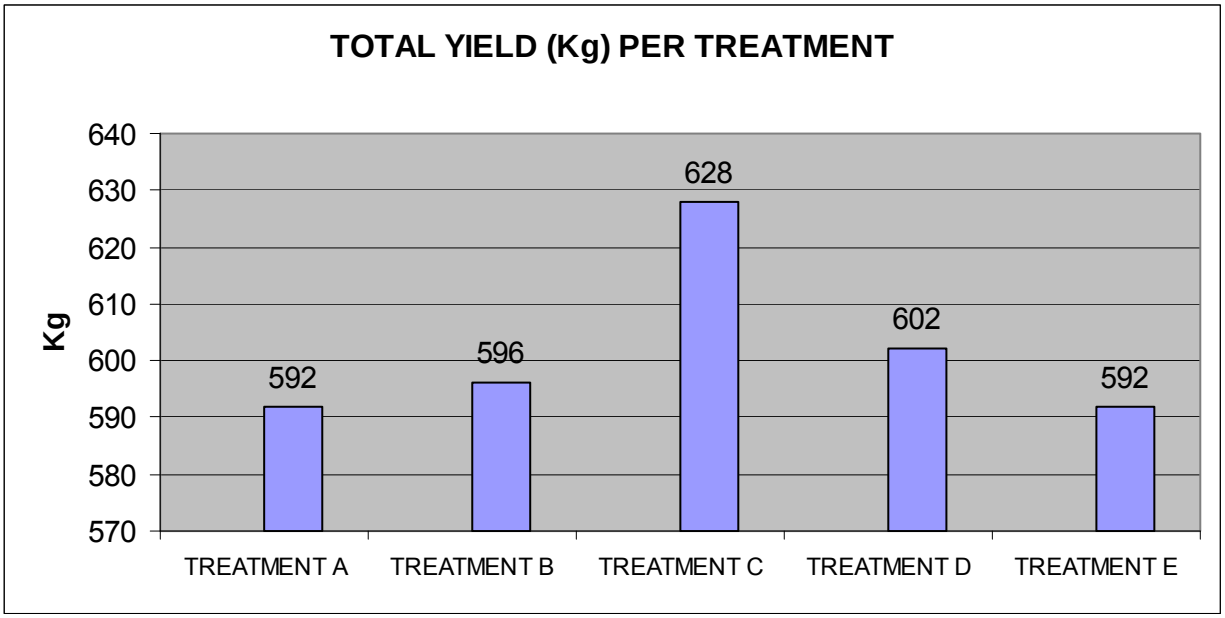


Figure 3.8. Total yield (Kg) per treatment, over the full harvest period.

The visual assessment ratings were carried out on the lowest five leaves used in the PM rating and results for each of the treatments can be seen in Figure 3.9. Despite Treatment C having the highest degree of PM, this treatment produced the greatest number of fruit, the best average fruit mass as well as the highest total yield in kilograms. Treatment C, although being fungal based seemed to give yield-enhancing properties similar to that as explained for bacterial based BCAs and as stated in section 1.3.2.

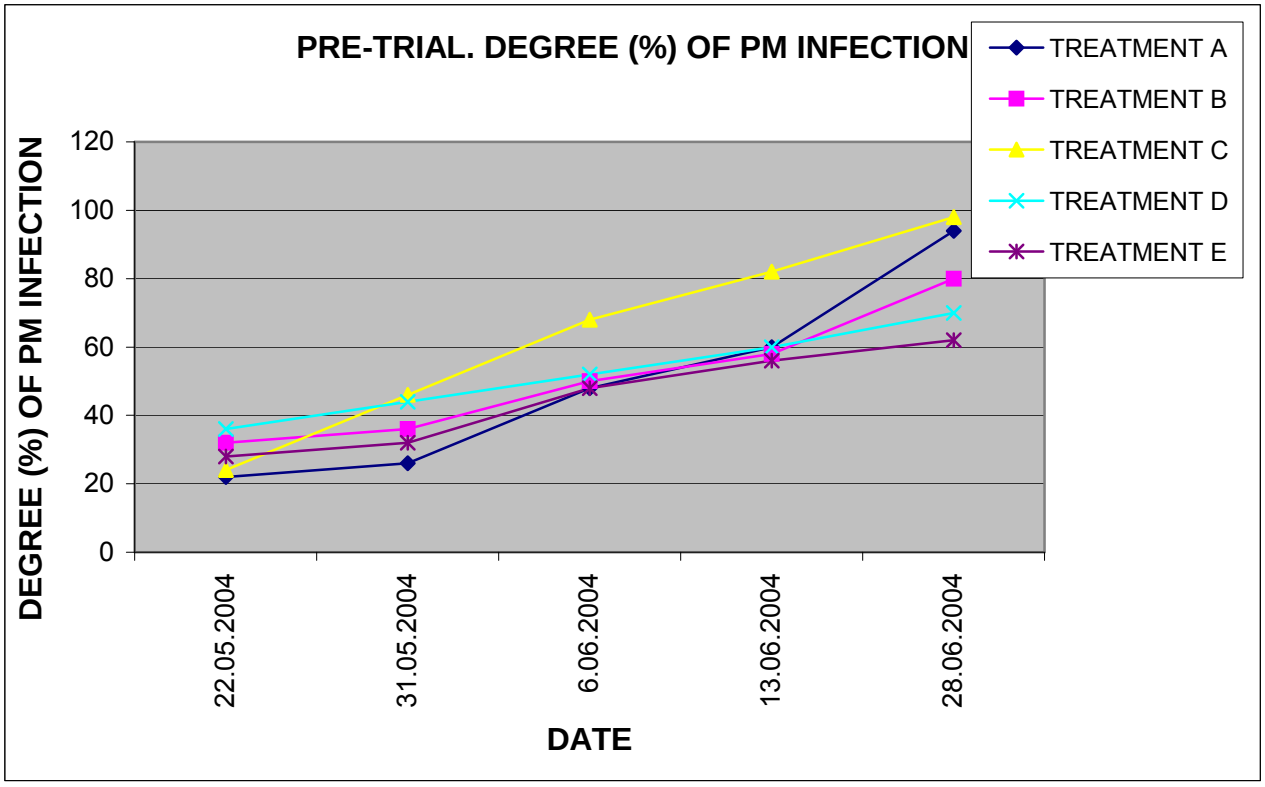


Figure 3.9. Degree (%) of PM infection per treatment over the period. An average of 10 plants per treatment was used.

3.3 PRE-TRIAL DISCUSSION

Treatment D displayed the best late vigour as seen on 9 August 2004 (Figure 3.4); this period also had the lowest humidity (Figure 3.3), which confirmed the statement by Quarles (2004) that bacteria are less moisture dependent than fungal BCAs.

Treatment C produced the greatest number of fruit (Figure 3.6. and Table 7.2.), the best average fruit mass as well as the highest total yield in kilograms, as depicted in figures 3.7 and 3.8.

Hansen (2000) stated that in severe PM infection cases, both the yield and fruit size might be reduced (Hansen, 2000). Such reduction is usually true as PM reduces the photosynthetic leaf area. However, treatment C produced the best results (Figures 3.6; 3.7 and 3.8) despite having the highest PM (%) infection as noted in figure 3.9. This growth or yield enhancement could have been due to the various modes of action

as explained in chapter 1.3.4 and according to this it seems the modes of action protects them against infection but also enhances growth. As can be seen from the control, despite having the lowest PM infection rate, the yield was one of the lowest, so showing the growth enhancing properties that is normally only associated with PGPR.

The nutrition for the cucumbers was compromised when the EC sensor failed and plants were nutrient deficient and stressed. As PM is opportunistic, the stressed plants were overpowered with PM, and the BCAs, on their own, could not control this disease. This entire crop was then sprayed with all the products stated in Table 3.2, which contained a systemic fungicide (Oscar) on the following dates:

17/06/2004, 24/06/2004, 01/07/2004, 08/07/2004, 15/07/2004

Once the control of the PM and plant nutrition was regained, use of BCAs resumed from the 22 July 2004 until the end of production. This pre-trial set the standard for trials 1 and 2 but was a failure as synthetic fungicides were used to manage the PM infection. From the pre-trial, it became apparent that nutrition was vital in maintaining cucumber plant health and resistance. Based on this, the pre-trial, the role of plant nutrition and media are evident as explained in section 1.2.3.

CHAPTER 4

RESULTS: TRIAL 1

4.1 TRIAL 1

Trial 1 was planted out on 30 September 2004 and plants grew until the 28 December 2004. Trials were conducted as explained in section 2.2.1, 2.2.2 and 2.2.2.1 and shown in Figure 2.1. An adaptable cucumber variety different to the pre-trial, called Palladium was used as this particular variety has a high genetic tolerance to PM. A comparative study between BCAs sprayed in combination with silicon and the synthetic fungicide, Bravo, was conducted.

Table 2.3, shows extraneous variables and differences between the pre-trial and trials 1 and 2. Trial 1 was planted in the same configuration as the pre-trial and sprayed with BCAs and the synthetic control at concentrations listed in Table 4.1. In addition to the BCAs, trial 1 had silicon (Si) added to the BCA mixture for reasons as discussed in section 1.2.4.

4.1.1 Dosage rate of trial 1 BCAs

Table 4.1. Optimum dosage rate of trial 1 BCAs

OPTIMUM DOSAGE RATE	SPAN NUMBER	TREATMENT	BIOLOGICAL AGENT NAME
10ml / 40L water	5	(A)	*Streptomyces griseovirdis & Streptomyces aureofaciens*
20 drops / 40L water	4	(B)	*Trichoderma harzianum* Rifai & *Trichoderma harzianum* Uppington
20 drops / 40L water	2	(C)	*Ampelomyces quisqualis*
80ml+240g/40L water	1	(D)	*Bacillus subtilis*
90ml / 40L water	3	(E)	Bravo (synthetic fungicide)
Silicon (Silicum) 12.50 ml/100L water		All treatments except control	(Active ingredient- Potassium silicate)

4.1.2 Spray dates for trial 1

BCAs and silicon were sprayed from 6 October 2004 until 19 December 2004 on a weekly basis.

4.2 RESULTS

4.2.1 Climatic data

The climatic data (Figures 4.1. & 4.2.) was collected for maximum and minimum temperature on a daily basis; the minimum humidity (Figure 4.3.) was also recorded, as it is critical to fungal development and survival and to a lesser extent, with bacterial BCAs.

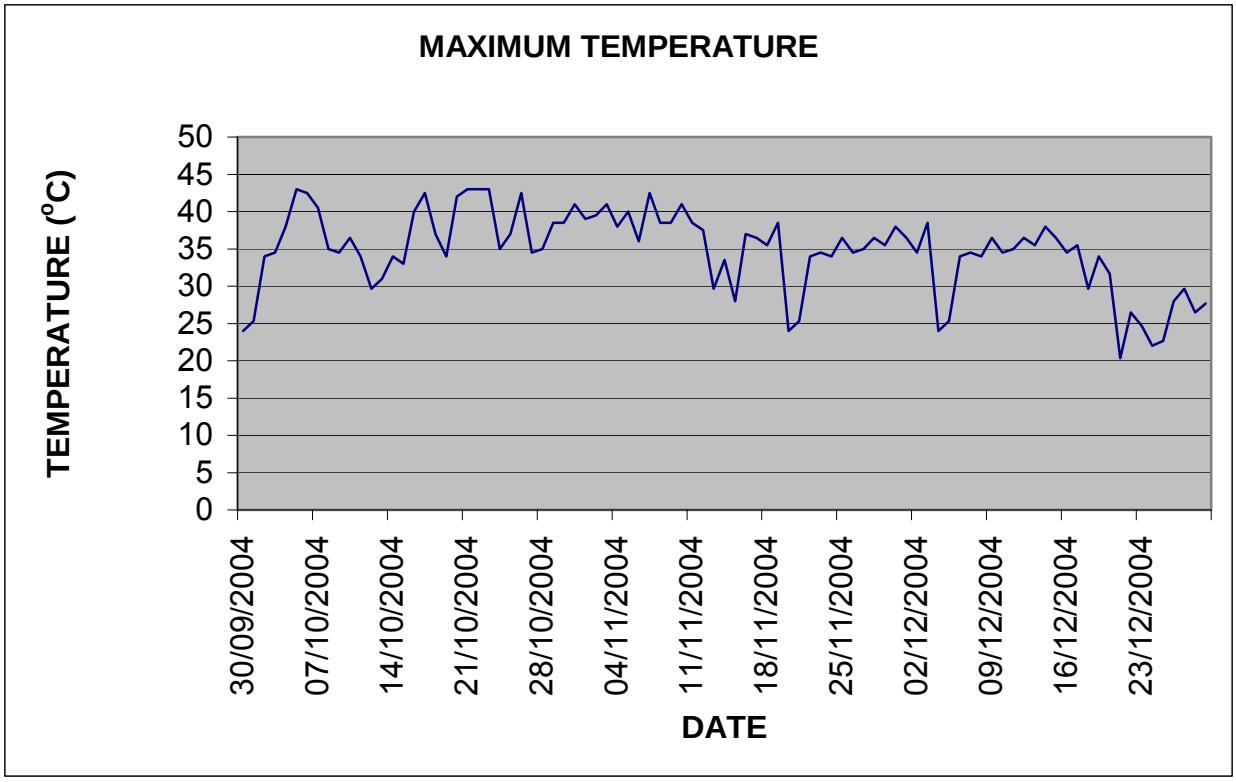


Figure 4.1. Maximum daily temperature in the greenhouse for trial 1.

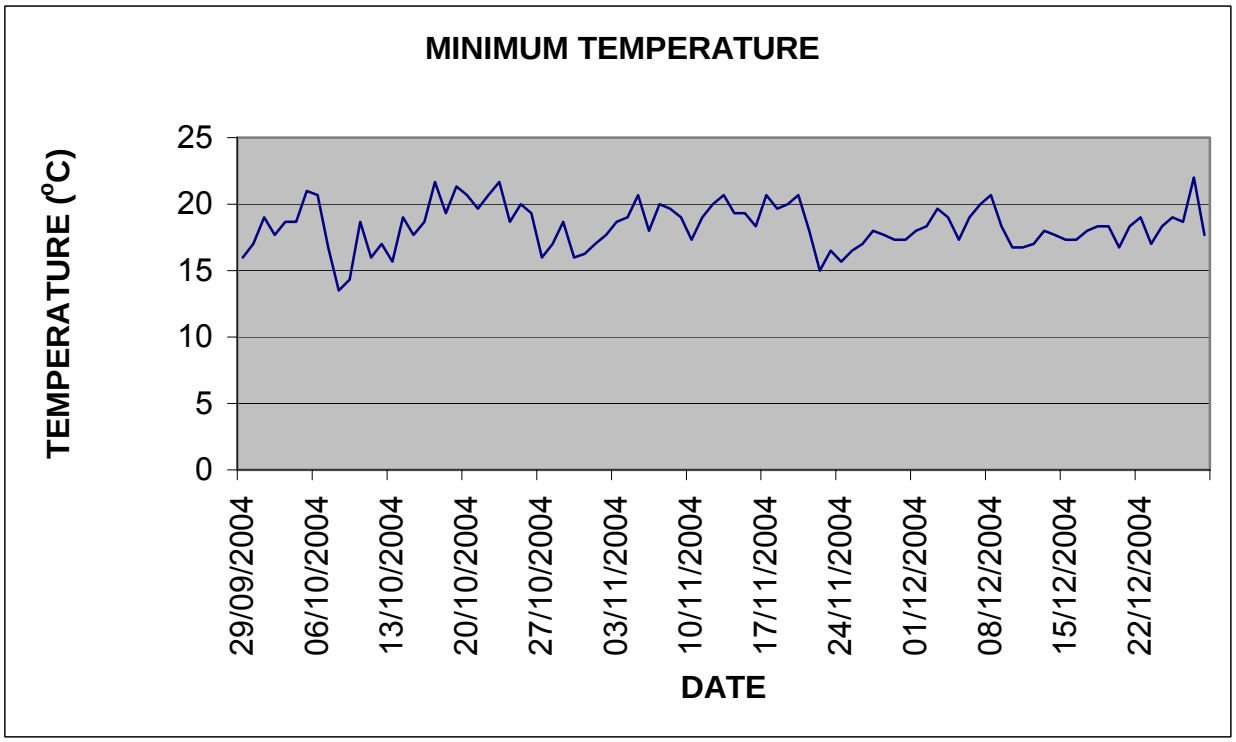


Figure 4.2. Minimum daily temperature in the greenhouse for trial 1.

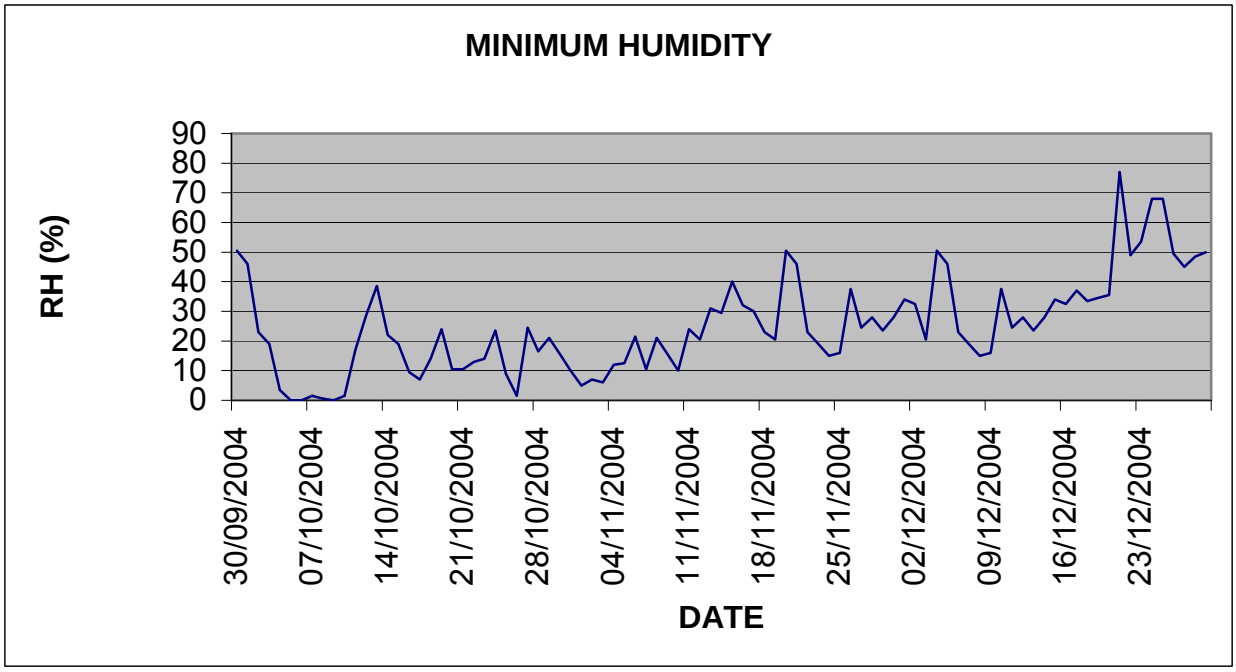


Figure 4.3. Minimum daily humidity in the greenhouse on a daily basis for trial 1.

4.2.2 Harvest data

In Figure 4.4, number of fruit per pick is shown for each of the treatments in trial 1. A total number of eight picks/harvests were done over the period, and the number of fruit at each pick/harvest date was recorded for each of the treatments. All fruit were of commercial grade and marketable. Treatment A (*Streptomyces spp*.), a bacterial-based BCA, had the greatest initial yield as seen in Figure 4.4 around the 21 November 2004.

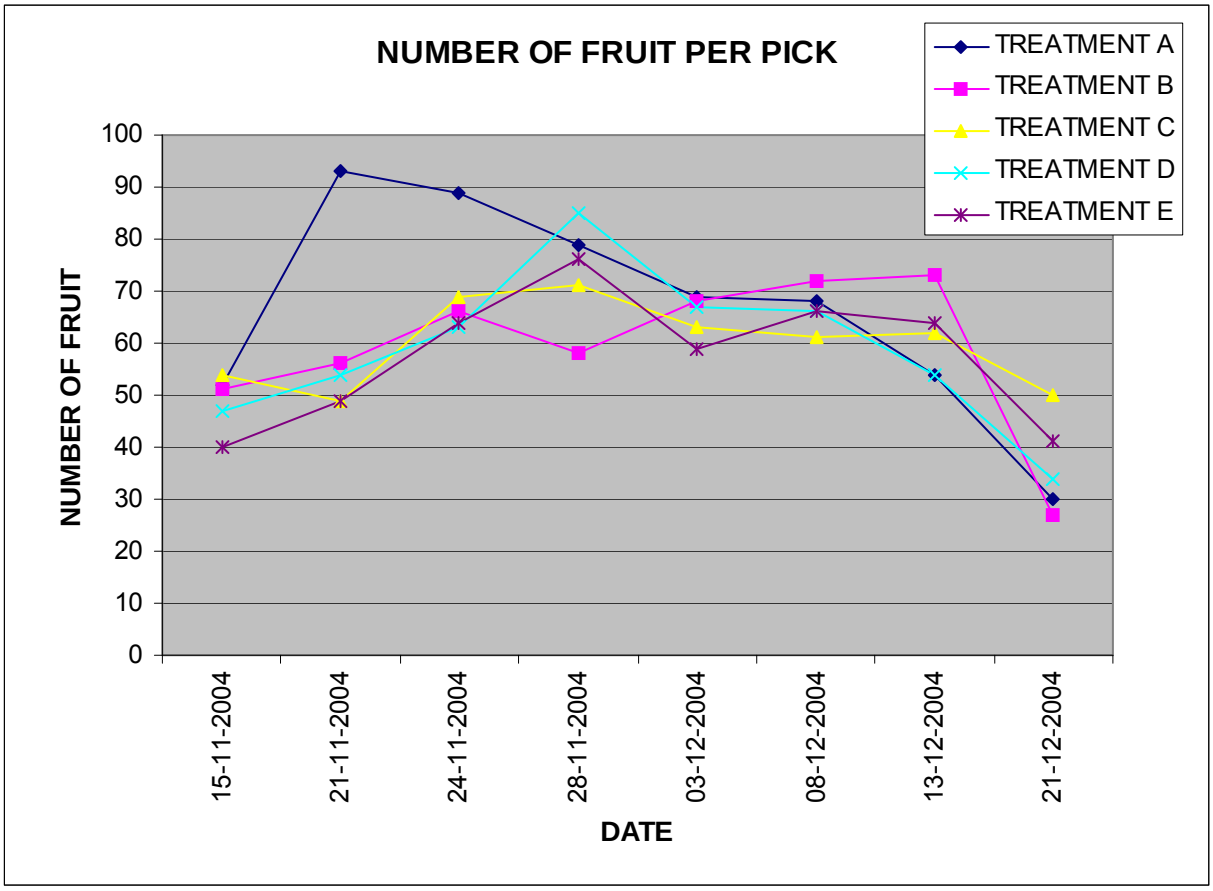


Figure 4.4. Number of fruit harvested at each picking date for each of the treatments in trial 1.

Figure 4.5 shows the average fruit mass per treatment for every pick. Treatment D (*Bacillus subtilis*) showed the best average fruit mass around the 21 November 2004. Treatment D had yet another late (8 to 20 December 2004) surge in average fruit mass towards the end of the production cycle, unlike any of the other treatments.

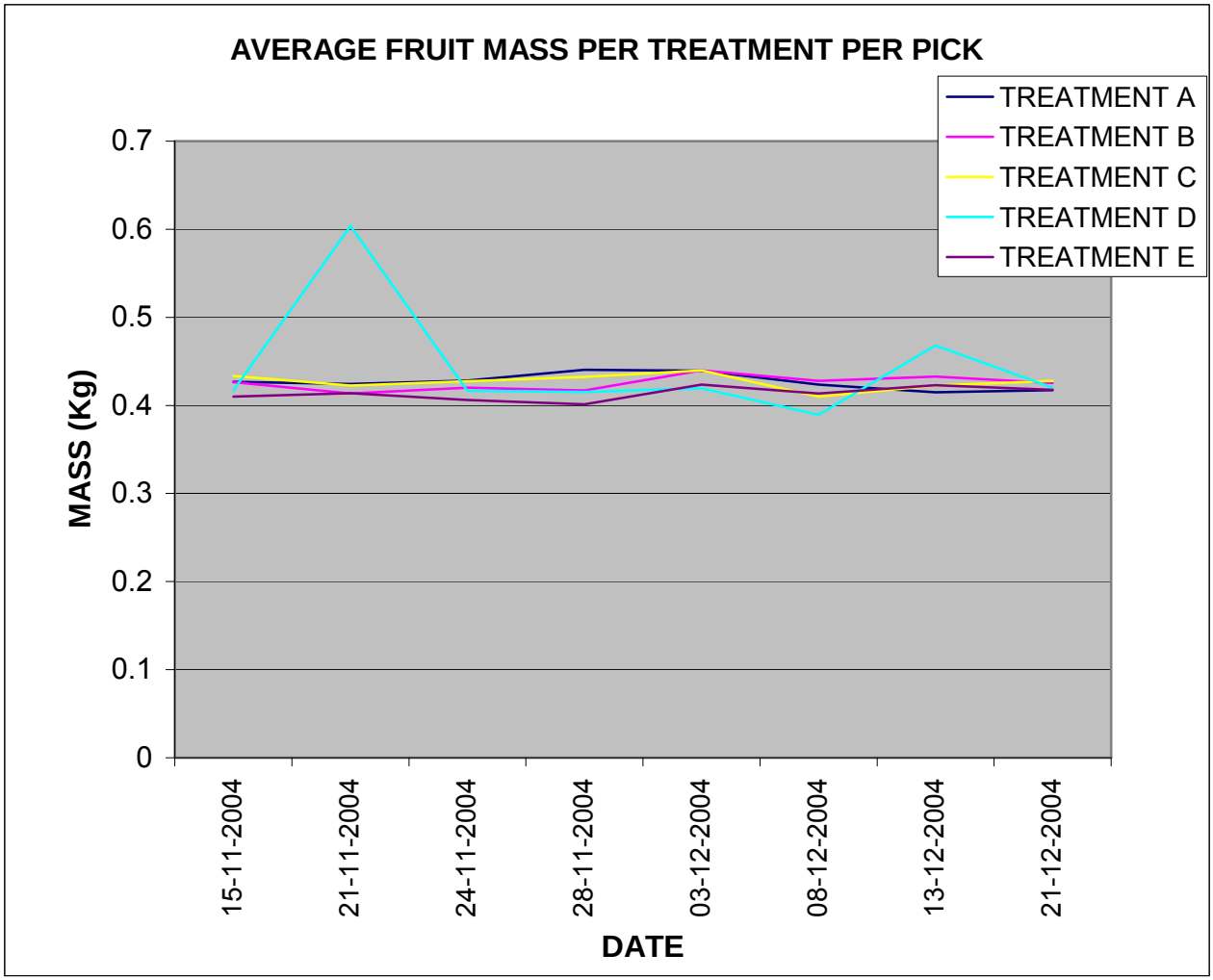


Figure 4.5. Average fruit mass per treatment for each pick over the period, trial 1.

Figure 4.6 depicts the total number of fruit per treatment over the entire harvest period for trial 1. Treatment A had the most number of fruit total and the highest yield in terms of kilograms (Figure 4.8.) and the highest number of fruit (Figure 4.6.) over the entire growing period. Treatment C had the second highest number of fruit over the growing period. All of the BCA treatments had higher yields than the control (Treatment E).

Figures 4.5 and 4.7 would be relevant to Spain, which grows the majority of the cucumbers for Europe, as they supply shops by the kilogram, in South Africa cucumbers are sold per fruit (Figure 4.4).

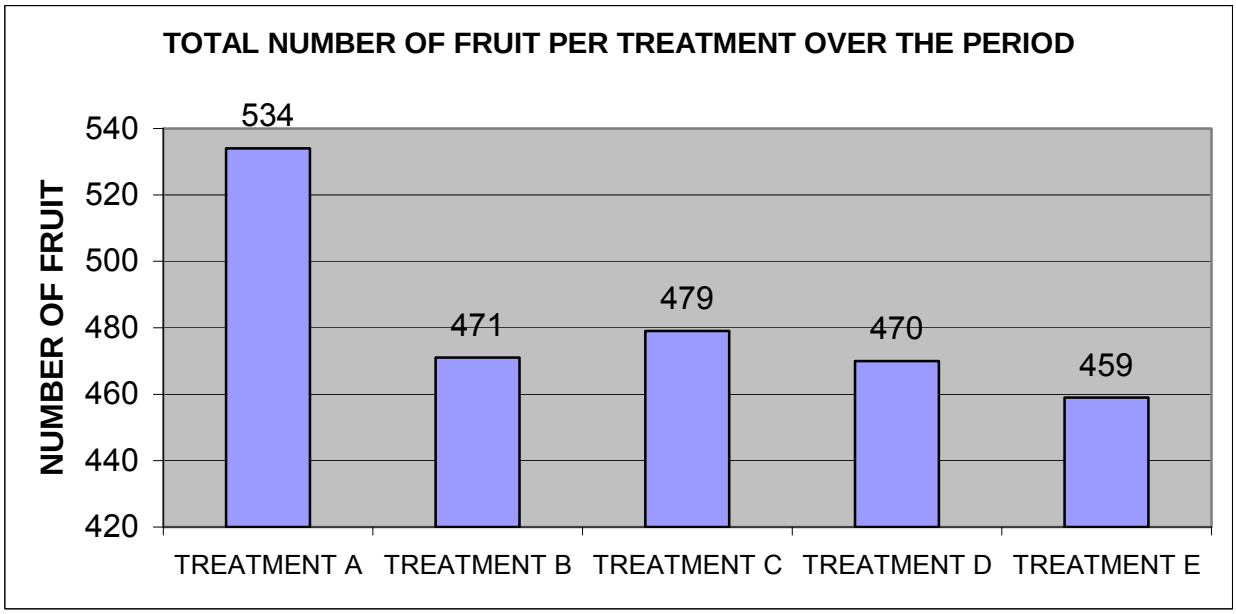


Figure 4.6. Total number of fruit per treatment over the harvest period, trial 1.

Fruit mass for each of the treatments at each picking date can be seen in Figure 4.7. Treatment A had an incredible growth phase in the beginning, which gave it an overall advantage despite having the lowest yields towards the end of the production cycle.

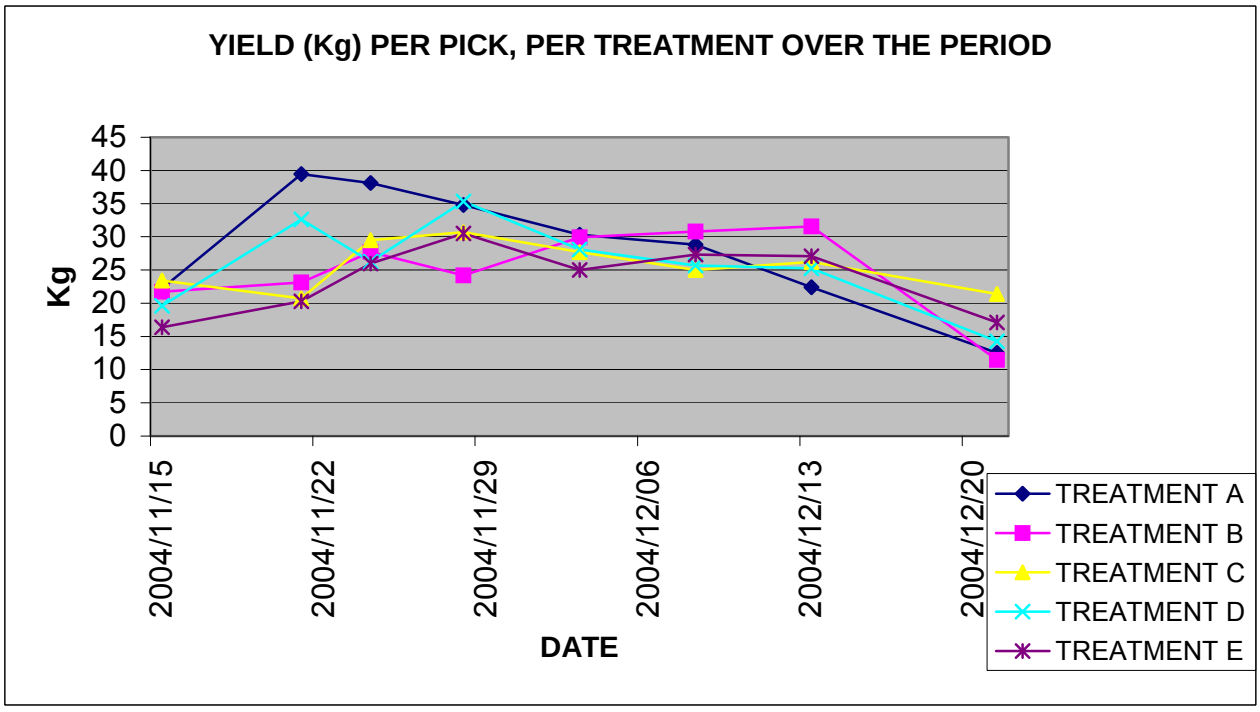


Figure 4.7. Yield mass per pick, per treatment over the entire harvest period.

Treatment D had the highest average fruit mass (Figure 4.9), but Treatment A had the best overall yield (kg yield) as shown in Figure 4.8 which is important in South Africa as cucumbers are sold per fruit (Figure 4.6.). As cucumbers are sold in kilograms in Spain, Figure 4.8 could be the most option for their particular marketing scenario. Treatment E (control) had the lowest yield mass (kg) and was surpassed by all of the BCA / silicon treatments.

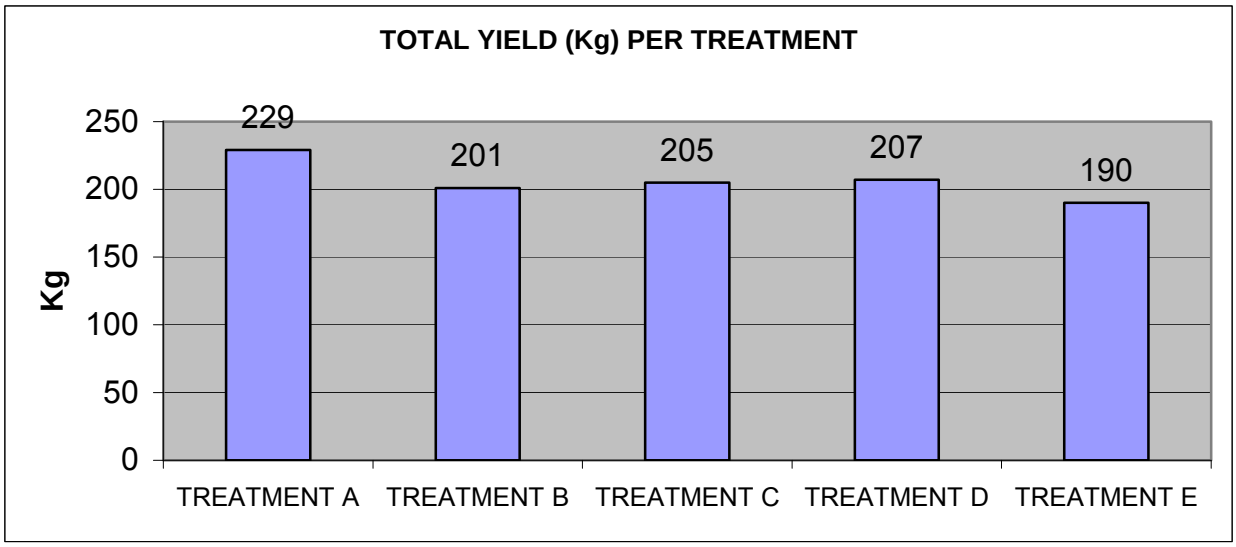


Figure 4.8. Total yield mass per treatment, over the full harvest period for trial 1.

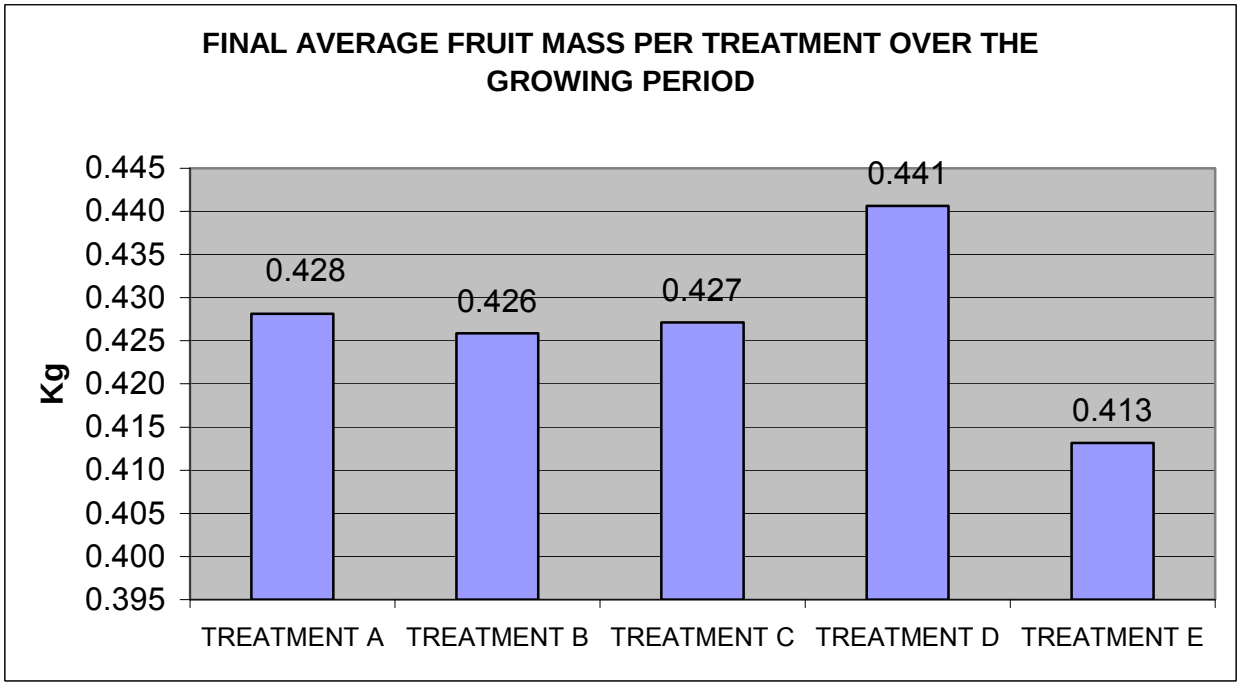


Figure 4.9. Final average fruit mass per treatment over the entire growing period.

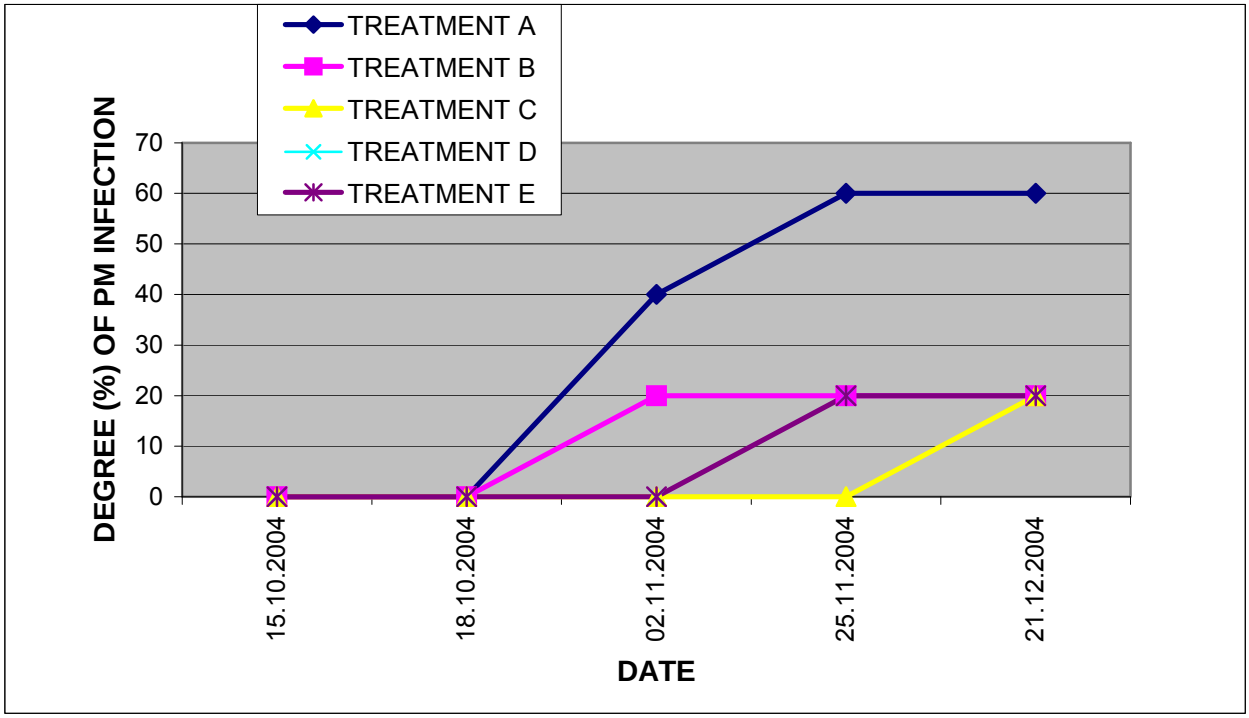


Figure 4.10. Degree (%) of PM infection per treatment over the period.

The graph does not reflect that both treatment D and E both have 20% PM infection on the 25 November until the 21 December 4004, the lines are actually hidden behind each other. Treatment C had the lowest degree of PM infection (Figure 4.10); therefore having the lowest PM covered leaf area, which appeared to correlate with the high average fruit mass (Figure 4.9.). Treatment D also had a relatively low PM percentage, but produced a greater average fruit mass over the growing period compared to Treatment C. The performance of Treatment D is most likely a result of growth promoting properties associated with bacterial biological control agents (section 1.3.2).

4.3 TRIAL 1: DISCUSSION

All of the treatments (Treatments A, B, C, D) consistently outperformed the control (Treatment E -synthetic treatment) in terms of the total number of fruit, total yield and highest average fruit (Figures 4.6; 4.8; 4.9). For most of the time the different BCA treatments also achieved a higher number of fruits per pick (Figure 4.4), a higher average weight per pick (Figure 4.5) and a higher yield (kg) per pick (Figure 4.7).

Of the different treatments, Treatment A produced the highest number of fruit (Figure 4.6.), the highest yield (kg/ treatment) (Figure 4.8.) and the second highest average fruit mass (Figure 4.9.) during the trial. The highest yield produced by Treatment A can be ascribed to the high number of fruit and mass of fruit obtained during the early stages of the trial (Figures 4.4 and 4.7 respectively). What is interesting is that during this time (15-24 November) Treatment A had the highest PM infection (Figure 4.10.).

Treatments C and D were the other treatments that also performed relatively well in terms of the total number of fruit (Figure 4.6), total yield (Figure 4.8) and average fruit mass (Figure 4.9). It is interesting to note that both of these treatments had the lowest PM infections recorded (Figure 4.10).

All the treatments except Treatment B decreased in yield from 28 November onwards (Figure 4.7) and also in the number of fruits produced (Figure 4.4). Treatment B was the only treatment to increase in the above categories and only started decreasing from 13 December onwards (Figures 4.4 and 4.7).

The highest and earliest PM infection was encountered by Treatments A and B from 25 November, Treatment B experienced the highest PM infection from 2 November onwards while Treatment C had a 0% infection until 21 December.

Although the results and Figure 4.10 seems to indicate that PM infection does not affect production and yield it is clear from Figures 4.4 and 4.7 that PM infection has a major influence on production and yield with all the treatments decreasing in these aspects from these dates from where the highest percentage infections occurred. Similar results were obtained by Hansen (2000) who found that both the yield and fruit size was reduced with severe PM infection.

The BCAs also showed the BCAs to have flushes or peaks in their yields (Figures 4.4, 4.5. & 4.7.), possibly due to the growth-enhancing properties of many of the BCAs, namely PGPR. Since the pre-trial failed when the BCAs were used alone, a large part of the success of trial 1 could possibly be attributed to the use of silicon (Section 1.2.4). The research topic investigated PM control as depicted in Figure 4.10, but the number of marketable fruit to a grower is possibly of more significance due to the economic value, as noted in Figure 4.6.

CHAPTER 5

RESULTS: TRIAL 2

5.1 TRIAL 2

Trial 2 was planted out on 26 January 2005, the first pick was on the 21 March 2005 and plants grew until 29 May 2005. Trials were conducted as explained in chapter 2.2.1 to 2.2.3 and shown in Figure 2.1. An adaptable cucumber variety, called Palladium, from the seed company Nunhems was used as in trial 1. This variety has a high genetic tolerance to PM. A comparative study between BCAs sprayed in combination with silicon and the synthetic fungicide, Bravo, was conducted.

Table 2.3 shows extraneous variables and differences between the pre-trial and trials 1 and 2. Trial 2 was planted in the same configuration as the pre-trial and trial 1. Plants were sprayed with BCAs, silicon and compared to the control (synthetic fungicide) at concentrations listed in Table 5.1.

5.1.1 Dosage rate of trial 2 BCAs

Table 5.1. Optimum dosage rate of trial 2 BCAs

OPTIMUM DOSAGE RATE	SPAN NUMBER	TREATMENT	BIOLOGICAL AGENT NAME
10ml / 40L water	5	(A)	*Streptomyces griseovirdis & Streptomyces aureofaciens*
20 drops / 40L water	4	(B)	*Trichoderma harzianum* Rifai & *Trichoderma harzianum* Uppington
20 drops / 40L water	2	(C)	*Ampelomyces quisqualis*
80ml+240g/40L water	1	(D)	*Bacillus subtilis*
90ml / 40L water	3	(E)	Bravo (synthetic fungicide)
Silicon (Silicum) 12.50 ml/100L water		All treatments except control	(Active ingredient- Potassium silicate)

5.1.2 Spray dates for trial 2

BCAs and silicon were sprayed from 8 February 2005 until 24 May 2005 on a weekly basis.

5.2 RESULTS

5.2.1 Climatic data

The climatic data was collected daily for maximum and minimum temperature (Figures 5.1 and 5.2); the minimum humidity was also recorded (Figure 5.3). The humidity in this trial seemed to be generally higher than in the pre-trial and trial 1, when comparing Figures 3.3, 4.3 and 5.3.

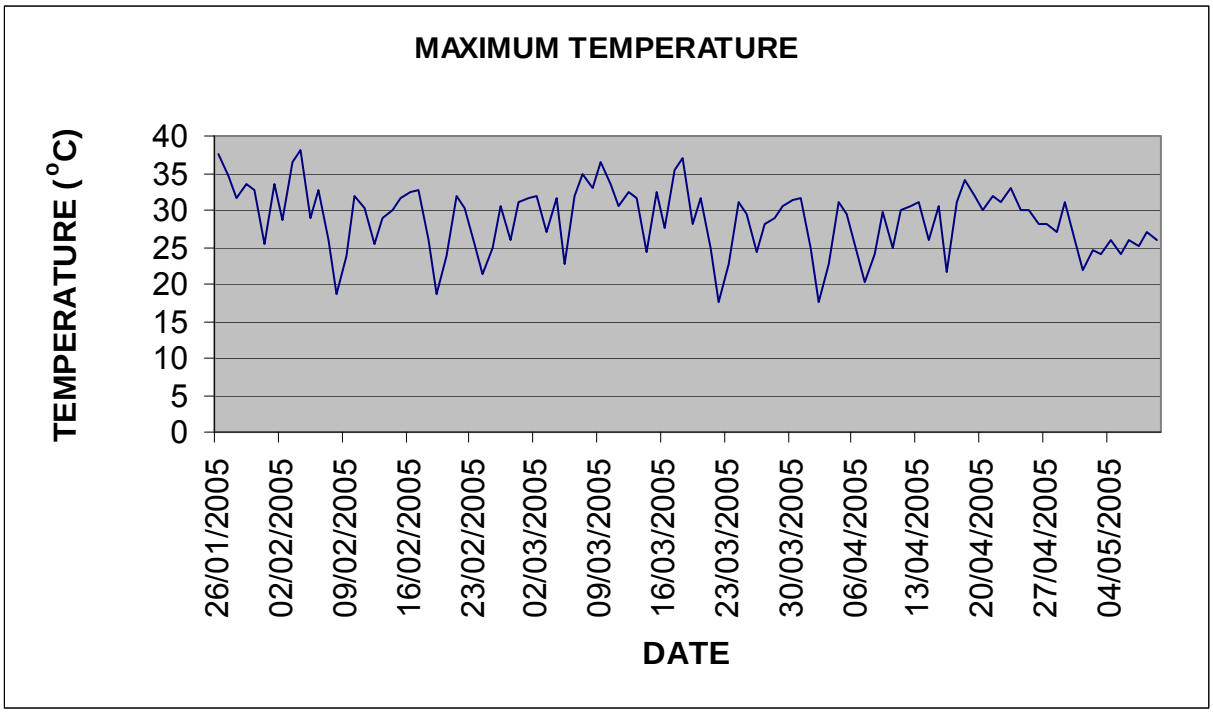


Figure 5.1. Maximum daily temperature in the greenhouse for trial 2.

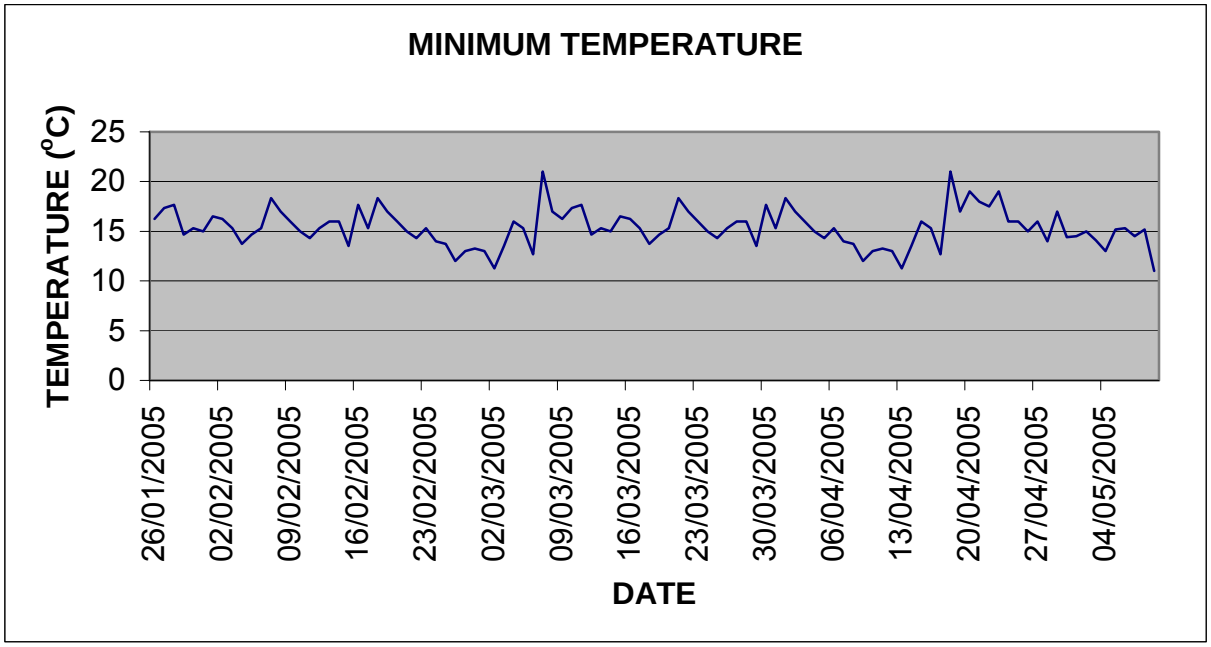


Figure 5.2. Minimum daily temperature in the greenhouse for trial 2.

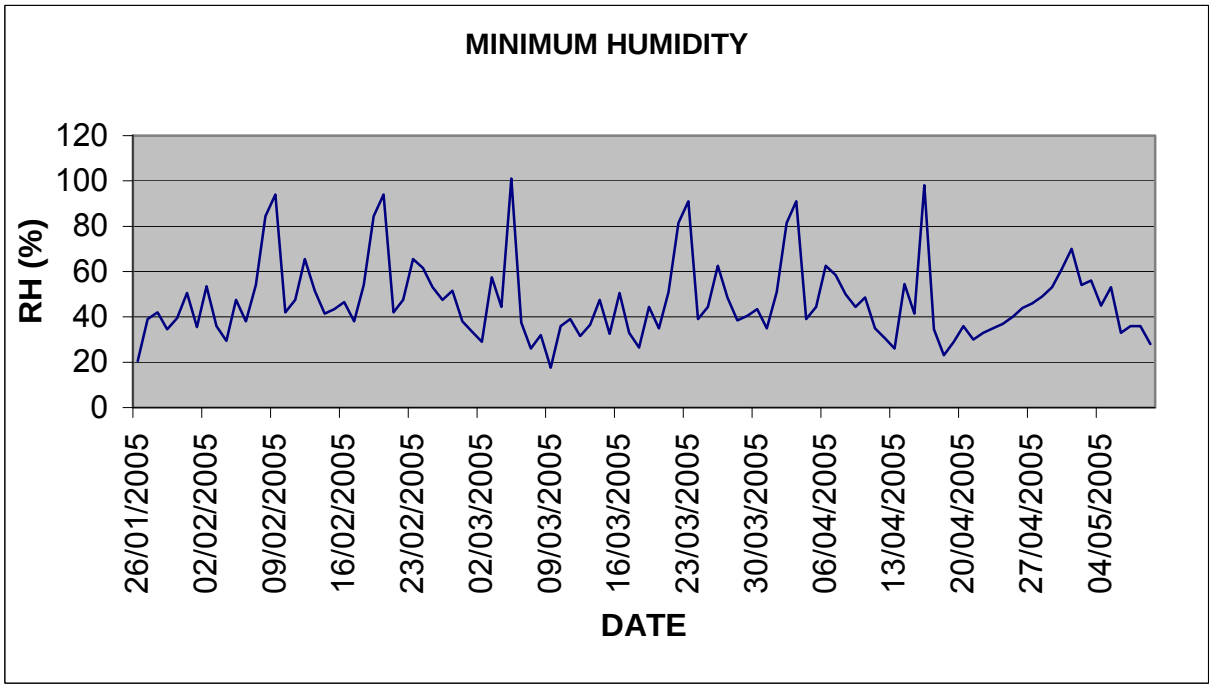


Figure 5.3. Minimum daily humidity in the greenhouse on a daily basis for trial 2.

5.2.2 Harvest data

In Figure 5.4, number of fruit harvested on each picking date is shown for each of the treatments in trial 2. A total number of nine picks were done over the period (Table 7.4), and the number of fruit at each pick was recorded for each of the treatments. Treatment C had a large initial yield, but Treatment B was possibly the most consistent in terms of yield over the growing period, which was particularly noticeable on the final few picks. All the yields showed a decrease in yield as the PM infection intensified (Figure 5.10).

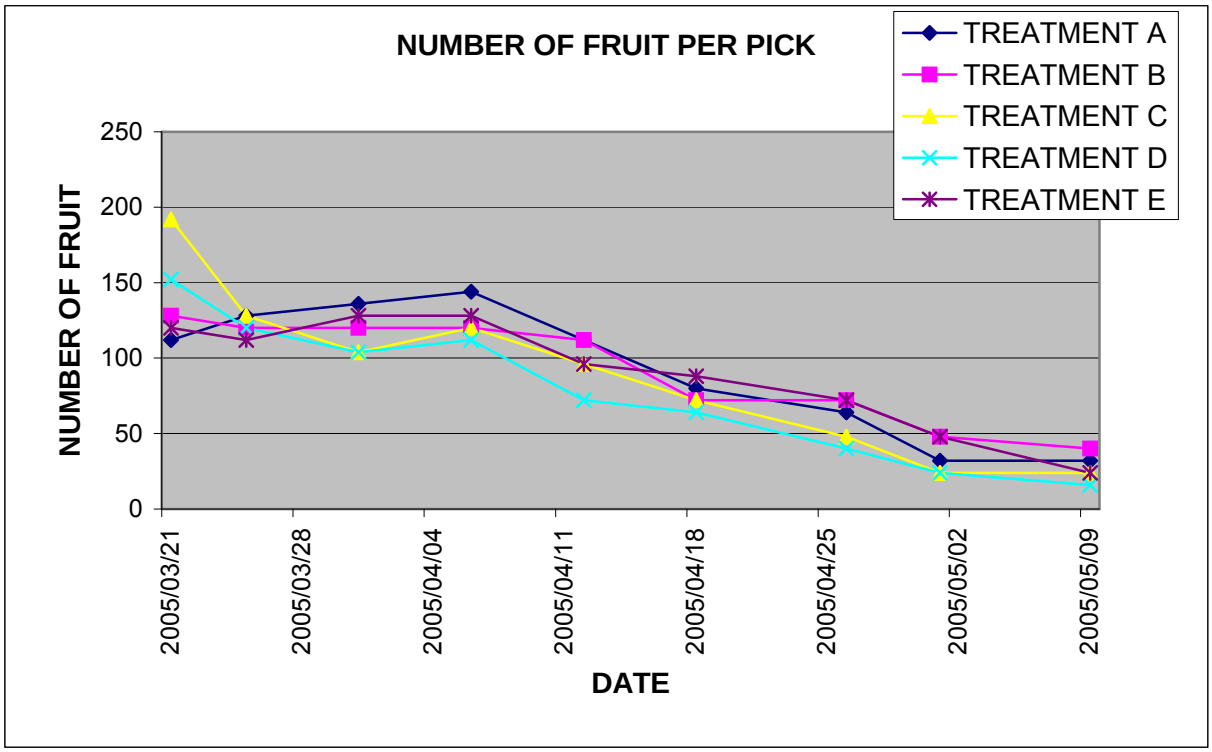


Figure 5.4. Number of fruit harvested at each pick for each of the treatments in trial 2.

In Figure 5.5, Treatment A had for the most part the lowest average fruit mass but produced the most fruit as can be seen in Figure 5.6. Treatment C gained well in average fruit mass notably towards the end of the production cycle. Treatment B had a good gain in average fruit mass around the 18 April 2005 but unfortunately did not keep it up thereafter but this gain ensured that it had the second highest yield as seen in Figure 5.6.

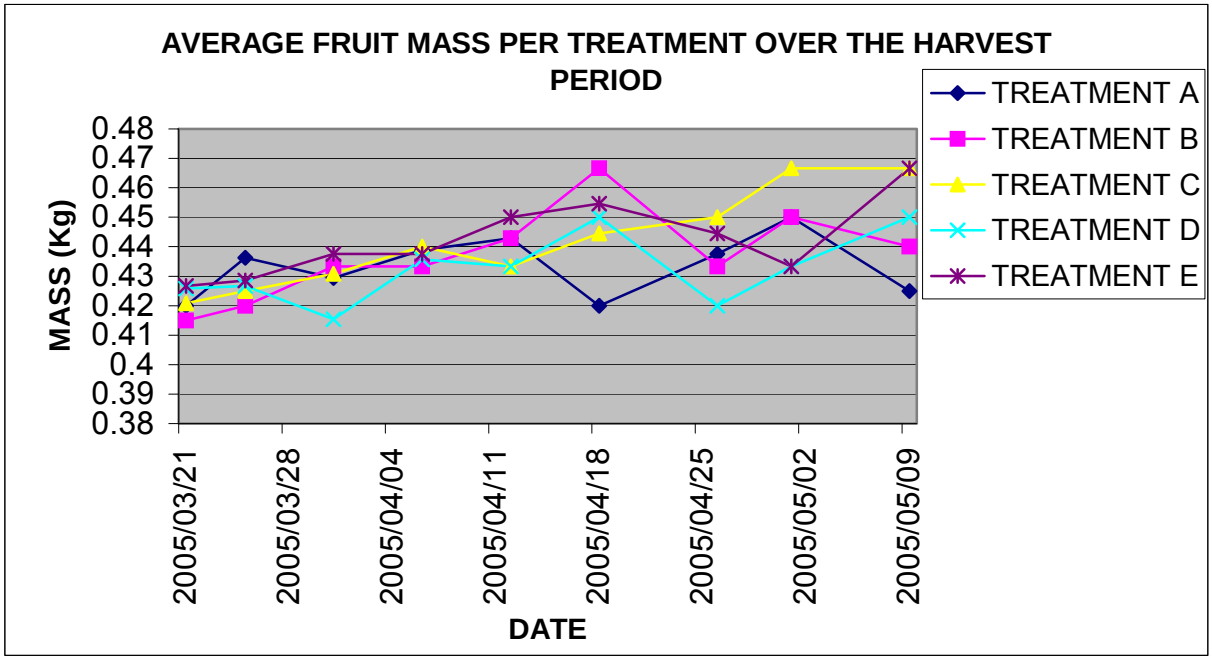


Figure 5.5. Average fruit mass per treatment for each pick over the period for trial 2.

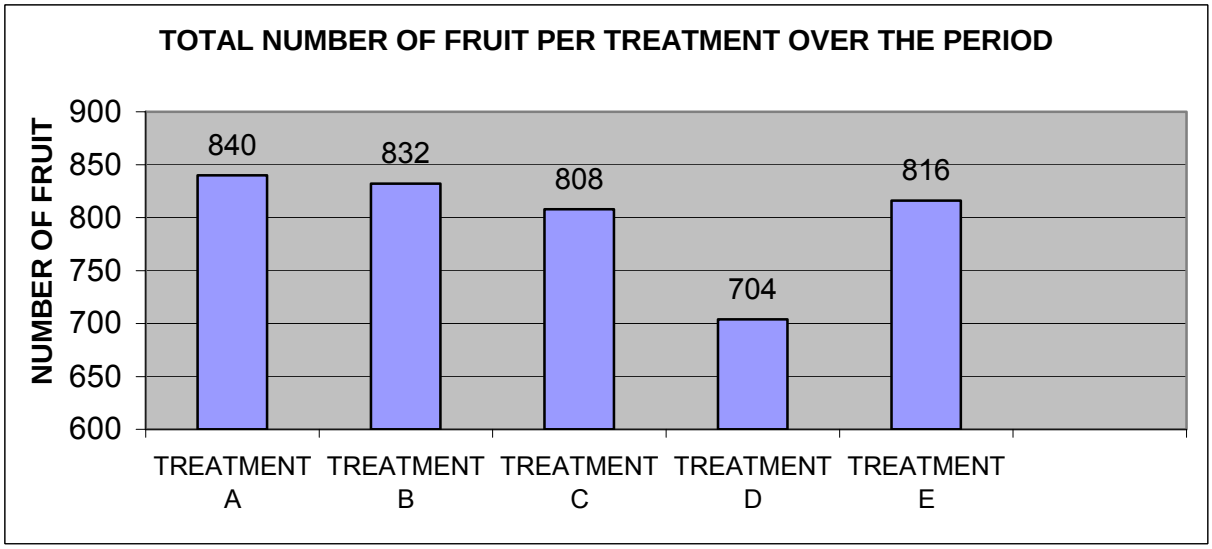


Figure 5.6. Total number of fruit per treatment over the harvest period for trial 2.

Fruit mass for each of the treatments at every pick date can be seen in Figure 5.7. Treatment A had the best overall yield (kg yield) as shown in Figure 5.8, superior to that of the control (Treatment E). Treatments C and D had a yield mass (kg) slightly lower than that of the control (Figure 5.8.).

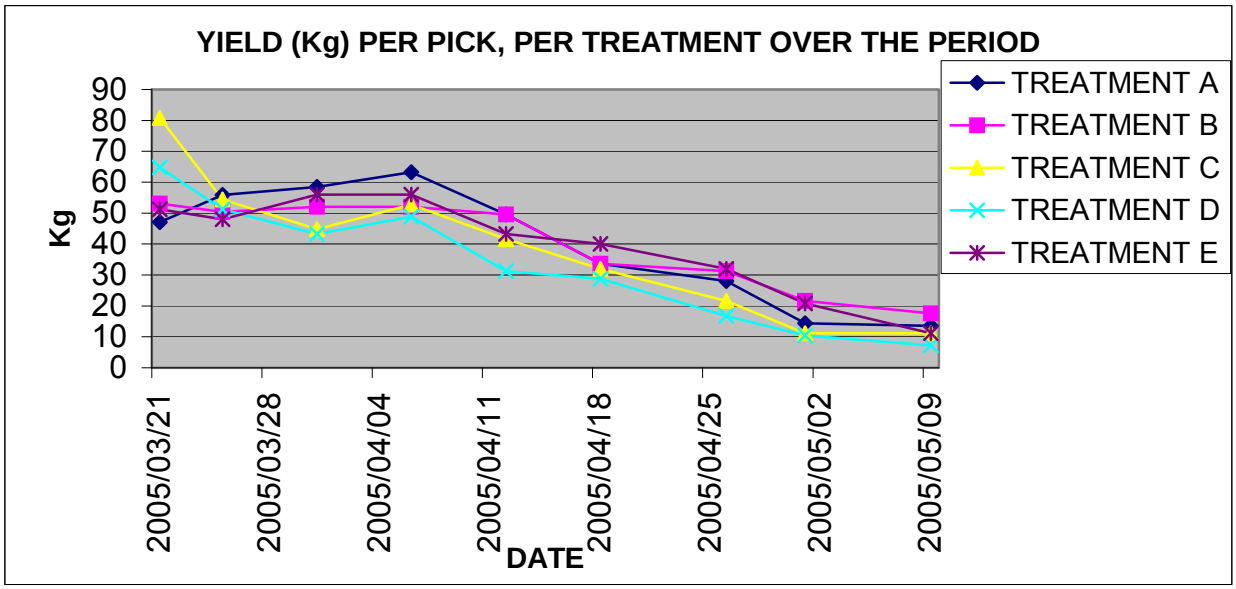


Figure 5.7. Yield mass per pick, per treatment over the entire harvest period.

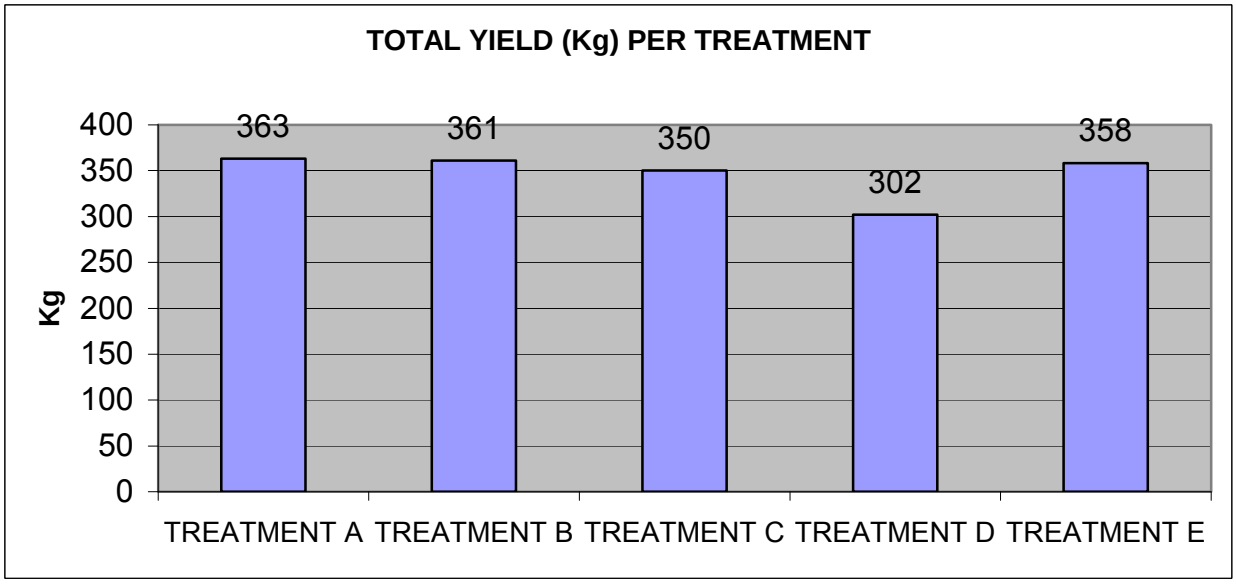


Figure 5.8. Total yield mass per treatment over the harvest period for trial 2.

Treatment D had the lowest average fruit mass and yield (kg), calculated over the entire harvest period (Figure 5.9.) and the control (Treatment E) had the highest average fruit mass, albeit by a narrow margin.

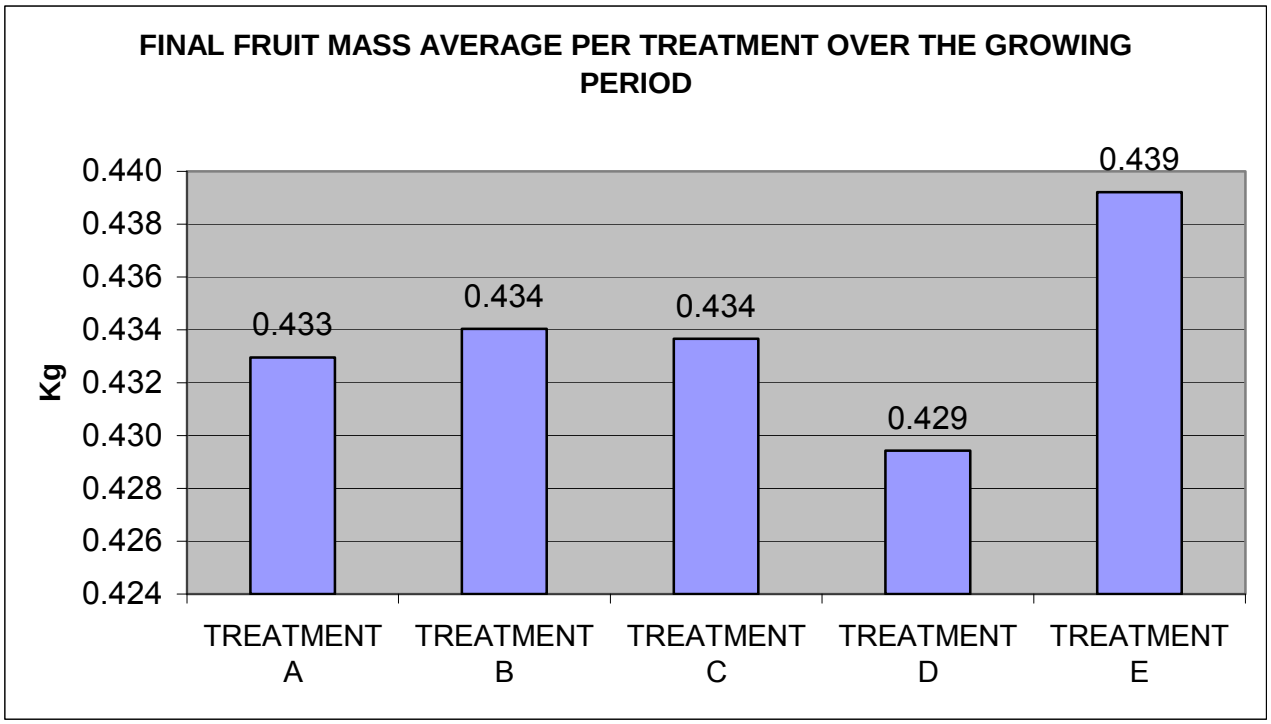


Figure 5.9. Final average fruit mass per treatment over the entire growing period.

In Figure 5.10, Treatment D had the highest degree of PM infection and also had the lowest yield mass (kg) as seen in Figure 5.8 which makes sense as (Hansen, 2000) states that both the yield and fruit size may be reduced with severe PM infections. Treatments A and B had the lowest PM infection and had the highest yield (kg) which again substantiates this claim as seen in Figure 5.8.

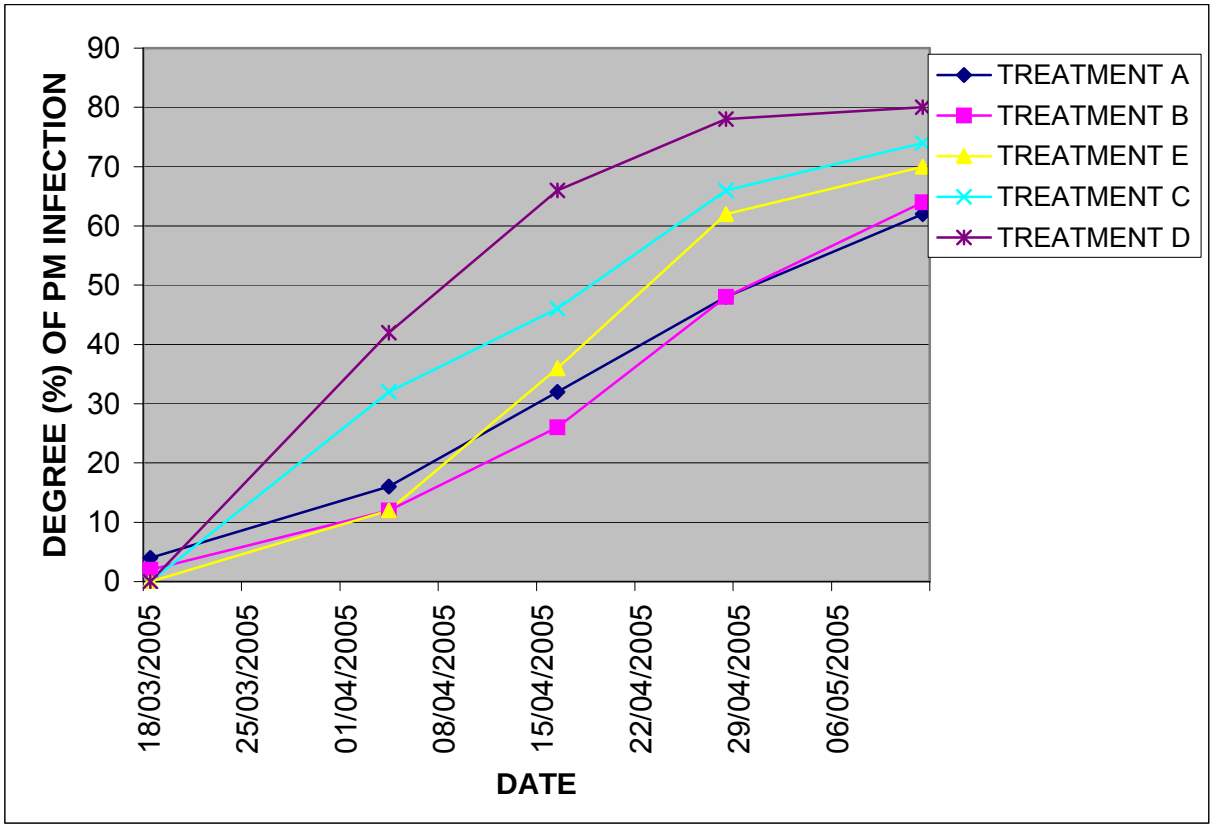


Figure 5.10. Degree (%) of PM infection per treatment over the period.

5.3 TRIAL 2: DISCUSSION

Treatment D had the highest degree of PM infection (Figure 5.10), thus having the most PM covered leaf area, which correlates with the low yield (Figure 5.8.). Treatments A and B had less PM infection than the control (Treatment E). The control had the greatest average fruit mass, but did not have the highest yield when compared to treatments A and B. Treatment A once again produced the highest number of fruit during the early stages, similar to trial 1 and also proved to be the most viable in terms of number of fruit (Figure 5.6) and total yield (Figure 5.8) making it the most viable option. Treatment A again produced the highest number of fruit, similar to trial 1. Although these infection rates seem high, the PM was localised generally on the lowest leaves, which were used in the rating of the PM infection. The rating system model, as per the original Coyier (1986) model, does not include the leaves higher up the plant that were generally free of powdery mildew.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

6.1 CONCLUSIONS DEDUCED FROM LITERATURE REVIEW

Past failures with BCAs are often a result of the incorrect use and timing of BCAs, and thus the criteria in section 1.2.5 were devised to ensure better application practices and ensure greater efficacy. A thorough knowledge of the modes of action (chapter 1.3.4) of BCAs gave a greater depth to the happenings at cellular level, which allowed one to understand which BCAs might be best suited to a specific application in bio-control.

Fungi-dominated mediums/substrates are shown to have 100% calcium retention (Ingham, 2003) but they require a more acidic environment (Table 1.3) and had a higher humidity requirement compared to bacteria used in bio-control. Consequently, fungi used in biological control programmes are highly suited to the root zone area, which generally met the above-mentioned criteria. Plant nutrients are most readily available to the plants in a similar pH range to fungi used as BCAs. Fungi are also shown to spread in the rhizosphere by means of hyphal growth, making them even better suited for substrate application. Fungi used in foliar applications needs to be done when humidity is high.

Sait (2003) stated that bacteria used in BCA programmes always dominated a liquid medium such as that used in true hydroponics. Certain bacteria used in BCA programmes were shown to be endophytic, and others were concentrated in the rhizosphere. As bacteria are less moisture dependent (Quarles, 2004) than fungal BCAs, they are highly suited to foliar application. Both fungi and bacteria have their own unique nutrient or food sources; therefore, it is possible to manipulate/stimulate fungi or bacteria BCAs and possibly keep a good balance between the two. Combinations of BCAs are often multi-modal and give enhanced efficacy in most cases. As found by Raupach & Kloepper, (1998), a mixture of introduced BCAs would mimic the natural situation closer and could broaden the spectrum of biological activity. An axenic culture would not be considered the norm in nature. Backman & Dorman (2003) stated that a combination of treatments in cucumbers increases yields above those of individual treatments. Raupach & Kloepper (1998) claimed that co-inoculants (chapter 1.3.3) were best, and furthermore, a combination of inputs was

advocated. However, such a combination requires a holistic approach and change in crop management to implement it.

Silicon (Section 1.2.4.) is also shown to be highly effective in controlling PM and appeared to be fully compatible with BCAs. Cultural practices (chapter 1.2.2) should also be considered standard practice and integrated with biological control programmes and silicon.

Sawdust is used as standard practice in South Africa as it is cost effective and readily available. This sawdust has a high C/N ratio and poor buffering capacity against pH change. Sawdust often compacts over time, leading to water logging which results in low oxygen levels in the root zone and impaired nutrition levels. Basics, such as medium amelioration, should be considered mandatory to further improve nutrient management. The above-mentioned PM control measures, when used with an integrated approach, are key to success in a biological control programme.

The pre-trial was originally explained in sections 2.2.1; 2.2.2 and in Table 2.3, and the results of the trial conducted were expounded in chapter 3.2. The shortcomings of the pre-trial led to the inclusion of silicon and application of a holistic approach in trials 1 and 2. The change in approach and the trials are illustrated and specified in Figure 2.4 and Table 2.3. This holistic approach included all beneficial inputs but with emphasis on using no synthetic chemicals, unlike IPM where chemicals are part of the disease or pest management programs in most cases. This approach is illustrated in Figure 6.1.

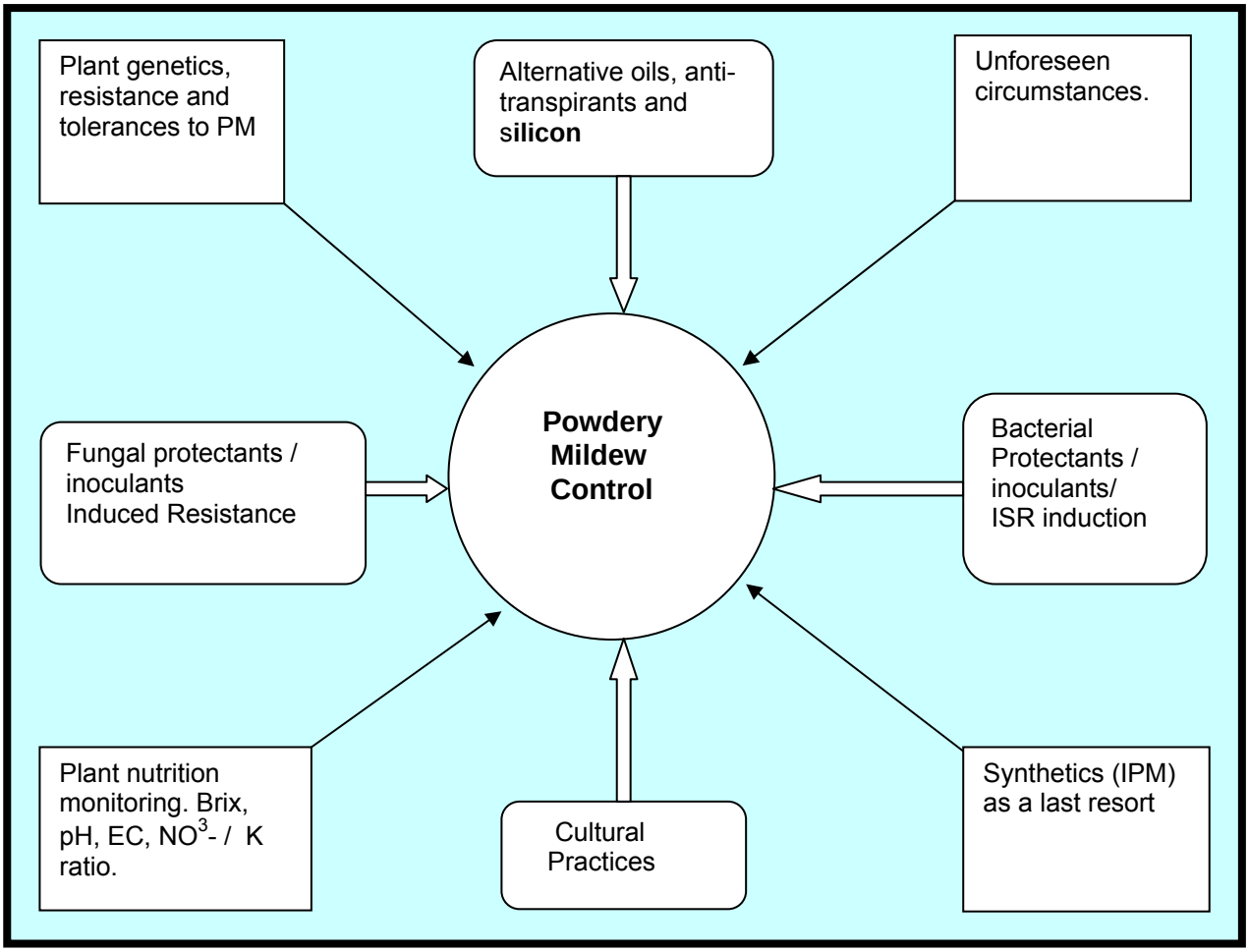


Figure 6.1. Representation of holistic approach to PM control.

6.2 STATISTICAL ANALYSIS- DATA AND INTERPRETATION

Trials 1 and 2 were combined as the second trial served as a replication of the first.

For each variable the following analysis was done. To test the hypothesis of equal medians for independent samples, the Kruskal-Wallis Anova by ranks test was used (the normality assumption was not satisfied, and therefore, a non-parametric test was used).

Three variables were considered:

1. Number of fruit harvested per treatment.

Results for the number of fruit harvested per treatment were as follows: Since p= 0.7964 $>$ 0.05, the hypothesis of equal medians was not rejected. There were no significant differences on a 5% level of significance in the median number of fruit harvested for the five treatments (four treatments and one control).

2. Average fruit mass.

Since p= 0.7430 $>$ 0.05, the hypothesis of equal medians was not rejected. There were no significant differences on a 5% level of significance in the median number of fruit harvested for the five treatments (four treatments and one control).

3. Yield, total mass of yield (kg yield)

Since p= 0.8693 $>$ 0.05, the hypothesis of equal medians was not rejected. There were no significant differences on a 5% level of significance in the median number of fruit harvested for the five treatments (four treatments and one control).

To determine which treatment resulted in the slowest progression of powdery mildew, the following analysis was done:

Trend analysis, using regression, was used to determine which treatment resulted in the slowest progression of powdery mildew based on the visual analysis of the plants.

Treatment A: b=0.5
Treatment B: b=0.3
Treatment C: b=0.6
Treatment D: b=0.7
Treatment E: b=0.5

(b represents the slope of the line of best fit for the regression model). The smaller b is, the more gradual the increase. The smallest b-value was achieved with treatment

B, and it seemed that B overall resulted in the most gradual increase in powdery mildew infection compared to the other three treatments and the control.

6.3 DISCUSSION AND CONCLUSIONS FROM TRIALS 1 AND 2

Treatment A (*Streptomyces griseovirdis & Streptomyces aureofaciens)* produced the most fruit (Figures 4.6 and 5.6) in trial 1 and 2. In South Africa cucumbers are sold per fruit so this could be the most feasible option. All the BCAs performed better in production, number and mass of fruit than the control (Treatment E) in trial 1. From this data it seems as though BCAs on this specific cucumber variant provide economically better alternatives than the specific synthetic used in the control. The data also indicates that BCAs can control PM infection similar to the synthetic used, for most of the time. The only BCA that could not control PM infection for a similar period as the control was Treatment A. However, Treatment A produced the best results with overall with the highest production, highest yield and largest number of fruit and could be an economically viable option to cucumber growers. The bacterial BCA used in Treatment A seemed to improve plant production and seemed to provide more consistent yield results compared to the fungal BCAs. It is important to note that the above is based on the physical data that has not been statistically analysed in chapter 6.2.

Treatment C *(Ampelomyces quisqualis)* controlled PM best in trial 1 and Treatment B *(Trichoderma harzianum* Rifai & *Trichoderma harzianum* Uppington) controlled PM best in trial 2, both of these being fungal based BCAs. It seems that fungal BCAs also have a place, especially in controlling the actual PM. The researcher would however recommend that more bacterial-based BCAs be used in future as they are said to be more climatically adaptable and also make economic sense, as they are often growth or yield enhancing (PGPR).

With some of the inconsistent results obtained in the trials, it became apparent that even inside a greenhouse the climatic conditions could not be controlled fully and consequently, a broader diversity of BCAs was required that would perform better and more consistently under such varied climatic conditions. It must be borne in mind that a grower would consider the financial return to be more important than actual PM

control. The BCAs did not always behave consistently as expected; therefore, co-inoculants would have a wider range of climatic adaptability than any singular BCA and a combination of both fungal and bacterial based BCAs are recommended by the researcher. Results showed BCA treatments combined with silicon, cucumber varieties with genetic tolerance to PM and cultural practices were no better than the control statistically, but rather they performed equally, thus being equal to the control (synthetic fungicide) and having benefits to the environment, workers and to the consumer.

The discussion on silicon and its modes of actions and its role in reducing PM opened the prospect of future inclusion to nutrient programmes. Silicon was the major contributor to the PM control in trials 1 and 2, but its efficacy was further enhanced by the BCAs, as could be seen in the variation in yield and the percentage of leaf area covered by PM. The intention of this trial was to investigate BCAs in controlling PM and not silicon on its own as a means of controlling PM, although a large part of the PM control was possibly due to silicon. As silicon has nutritional benefits to the plant as well as forming a physical barrier against PM, the shortfall of this study was that it did not have silicon on its own as the focus of the research is directed at BCAs and due to trial space restraints.

The yield-enhancing properties of certain bacterial based BCAs were an additional advantage, making these BCAs even more attractive to growers. These BCAs statistically provided similar PM control, as noted in the visual assessment ratings to the control (chlorothalonil) when such was used in combination with silicon, PM-tolerant varieties (genetics) and good cultural practices. This above combination provides a method of controlling PM and reduces risk to the consumer and the environment, thereby meeting the criteria desired in sustainable agriculture as stated by Cheah and Cox (1995).

Based on the pre-trial, it could be said that BCAs used alone could not control PM on a sustainable basis nor provide consistent results, thus a holistic approach was adopted. Therefore, the researcher could state that, based on the trials, biological control agents (BCAs) combined with silicon, genetic tolerance to PM and cultural practices are a sustainable alternative to synthetic fungicides in reducing powdery

mildew in tunnel cucumbers, as they were equal in terms of efficacy compared to the control. Trials 1 and 2 met the criteria stated for a sustainable means of controlling PM.

The increasing concerns for public health, the environment as well as the expanding competition in the agricultural market has motivated growers to seek disease control strategies that use reduced amounts of synthetic fungicides. For these reasons, a need exists for new and effective means of disease control that pose less risk to human health and the environment (Cheah and Cox, 1995:1).

6.4 RECOMMENDATION FOR FUTURE RESEARCH AND ACTIONS

Hydroponic mixes for cucumbers and possibly for other crops should contain a silicon source as a standard as this would provide part of the disease management and would be a sustainable means of control. Future studies should also do silicon applications as a treatment on its own as this trial could not accommodate another treatment and the topic is aimed at investigating BCAs. In addition to the silicon, a substitute or additive would be needed to ameliorate the poor qualities of sawdust that is used as a growing medium in South Africa.

Co-inoculants would be a more reliable and climatically adaptable and yield more consistent control results. Co-compatibility of BCAs is needed which would allow growers to formulate co-inoculants. Further studies need to be conducted to quantify the growth-enhancing properties of BCAs and to establish if fungal BCAs also enhance plant growth and yield, and also to what extent.

Further research should be done to establish the affect of these treatments on various cucumber varieties and then done on a much larger scale to demonstrate the full potential to commercial growers.

Tables 6.1 and 6.2 give an overview of results obtained in trials 1 and 2, considering the small sample size (80 plants per treatment) used for each treatment in the trials, treatment A, in trial 1, produced 75 more fruit than treatment E (control). This result would be worth an extra R150 to a grower based on R2-00 per cucumber. Although

statistically there are no differences, the commercial economic and environmental differences make BCAs justifiable and a sustainable option, as can be seen by the simplified feasibility tables below.

The crop value based per hectare i.e. 6250 plants, 1.60 plants per m² hypothetically R2-00 per fruit is relevant to the South African scenario as cucumbers are sold per fruit. The value based on yield (mass Kg) per hectare for 6250 plants and hypothetically using R5-00 per kg makes sense for growers in Spain. The table below does not allow for the cost of the synthetic fungicide used as a control or the cost of the BCAs used in the trials as some of the BCAs are still in the experimental phase and do not have a commercial cost yet.

Table 6.1. Overview of results of treatments in trial 1

Trail 1	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Number of fruit produced	**534**	471	479	470	459
Yield (Kg)	**228,61**	200,58	204,59	207,09	189,65
Average fruit mass	0,428	0,425	0,427	**0,440**	0,413
Value based on R2-00 per fruit for 80 plants	**R1068**	R942	R958	R940	R918
Crop value based on 1 hectare=6250 plants (1.60/m²) and R2-00/ fruit. (Relevant to South Africa)	**R83 437**	R73 593	R74 843	R73 437-50	R71 718
Value based on yield-mass (Kg) per hectare / 6250 plants and R5 / Kg (Relevant to Spain)	R89 278	R78 193	R79 895	R80 781	R74 049

Table 6.2. Overview of results of treatments in trial 2

Trial 2	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Number of fruit produced	**840**	832	808	704	816
Yield (Kg)	**363,68**	361,12	350,40	302,32	358,40
Average fruit mass	0,432	0,434	0,433	0,429	**0,439**
Value based on R2-00 per fruit for 80 plants	**R1680**	R1664	R1616	R1408	R1632
Crop value based on 1 hectare=6250 plants (1.60/m²) And R2-00/ fruit. (Relevant to South Africa)	**R131 250**	R130 000	R126 250	R110 000	R127 500
Value based on yield-mass (Kg) per hectare / 6250 plants and R5 / Kg (Relevant to Spain)	**R141 750**	R141 050	R136 665	R117 975	R139 931

APPENDIX

Table 7.1. Average fruit mass per treatment over the harvest period and total combined average per treatment during the pre-trial

PICK NO.	TREATMENT A	TREATMENT B	TREATMENT C	TREATMENT D	TREATMENT E
1	0.656493506	0.519175778	0.514791667	0.522613636	0.652222222
2	0.485555556	0.387804878	0.4263596	0.425675676	0.45756705
3	0.420631579	0.541544118	0.416695157	0.431738587	0.453125
4	0.393884712	0.430208333	0.409440559	0.381904013	0.417845118
5	0.405938494	0.490162602	0.446078431	0.425740741	0.412720588
6	0.413333333	0.432212476	0.403942308	0.426071429	0.424285714
7	0.40142513	0.397548426	0.412773109	0.438484848	0.395394737
8	0.415079365	0.433116883	0.435087719	0.413939394	0.396351351
9	0.451493506	0.48227027	0.478923077	0.496363636	0.453333333
10	0.419166667	0.458119658	0.469021739	0.530833333	0.448366477
11	0.424910394	0.432545732	0.453684211	0.448838798	0.437068966
12	0.466946779	0.46026087	0.511458333	0.516761364	0.539712919
13	0.528289474	0.594444444	0.606725146	0.527927928	0.554166667
14	0.427214377	0.437995338	0.450814607	0.456108597	0.469930876
15	0.44030303	0.453645833	0.470935484	0.463181818	0.442934609
16	0.431305903	0.469615385	0.462678571	0.48452381	0.436609337
17	0.451344477	0.473701299	0.473418803	0.464192037	0.464685315
18	0.461625076	0.486558442	0.483963816	0.467911255	0.455991627
19	0.433655706	0.46011611	0.446449893	0.447643443	0.446547619
20	0.461306043	0.474324324	0.464986559	0.45906298	0.445174769
21	0.459047619	0.470443964	0.447860792	0.467660099	0.455515873
22	0.580677913	0.472792937	0.427591837	0.444202899	0.48744824
23	0.4975	0.4575	0.775	0.505555556	0.7
24	0.507142857	0.535064935	0.45952381	0.53047619	0.49217033
25	0.426276276	0.424444444	0.425769231	0.412749706	0.460833333
26	0.473125	0.405357143	0.607236842	0.400292398	0.466034483
27	0.391989087	0.405	0.436375661	0.443518519	0.390360046
28	0.36260454	0.373529412	0.386540541	0.388333333	0.363257576
29	0.39724359	0.418611111	0.451215278	0.470629371	0.428409091
30	0.478571429	0.459983051	0.466681338	0.450749064	0.434225875
31	0.425739958	0.422604736	0.45666215	0.444465409	0.44143213
32	0.469127086	0.48287422	0.522020202	0.509957776	0.519693487
33	0.568300654	0.504607843	0.510714286	0.526121795	0.549102564
34	0.488333333	0.465277778	0.48125	0.517156863	0.487571429
35	0.496153846	0.496595528	0.478346793	0.527948718	0.497521858
Ave. Mass	**0.45747818**	**0.46028738**	**0.473457644**	**0.464838143**	**0.467931732**

Table 7.2. Number of fruit per pick for each of the treatments in the pre-trial.

Pick Number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
TREATMENT A		9	17	32	46	44	29	50	39	29	41	34	19	18	49	46	48	38	42
TREATMENT B		35	44	13	28	28	33	58	27	31	31	45	24	12	42	40	28	38	29
TREATMENT C		39	44	46	35	34	33	43	34	26	30	46	20	14	55	41	38	36	35
TREATMENT D		26	37	40	52	51	24	37	43	21	21	49	19	26	54	47	29	48	55
TREATMENT E		10	24	20	30	37	28	57	34	26	43	44	15	16	30	56	35	43	44
Combined yield		118	165	150	191	193	146	245	176	132	165	216	97	86	228	229	177	201	204
Date		22/5/ 2004	25/5/ 2004	27/5/ 2004	28/5/ 2004	30/5/ 2004	31/5/ 2004	02/6/ 2004	04/6/ 2004	07/6/ 2004	09/6/ 2004	11/6/ 2004	13/6/ 2004	15/6/ 2004	17/6/ 2004	20/6/ 2004	22/6/ 2004	25/6/ 2004	28/6/ 2004

Table 7.2. Continued

Pick number		**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	Total
TREATMENT A		75	92	79	82	18	15	28	13	48	29	40	49	44	40	18	21	21	1331
TREATMENT B		59	93	80	73	22	15	24	23	39	29	38	55	53	45	24	37	37	1324
TREATMENT C		69	86	85	50	7	26	37	12	38	31	30	84	68	39	28	40	40	1412
TREATMENT D		59	94	47	55	12	16	30	19	27	22	24	93	57	39	25	24	24	1336
TREATMENT E		81	119	71	54	7	21	21	20	52	37	39	64	64	37	21	27	27	1347
Combined yield		342	484	361	312	65	91	139	86	202	147	170	344	285	199	115	148	148	6749
Date		1/7/ 2004	5/7/ 2004	8/7/ 2004	12/7/ 2004	15/7/ 2004	19/7/ 2004	21/7/ 2004	22/7/ 2004	26/7/ 2004	29/7/ 2004	2/8/ 2004	9/8/ 2004	12/8 2004	16/8/ 2004	19/8/ 2004	23/8/ 2004	1/9/ 2004	1/9/ 2004

Table 7.3. Number of fruit, fruit mass and average fruit mass at each harvest for each treatment over the harvest period and combined totals for each treatment during trial 1

		15-11-2004	21-11-2004	24-11-2004	28-11-2004	03-12-2004	08-12-2004	13-12-2004	21-12-2004	Totals
TREATMENT A	NO. OF FRUIT	52	93	89	79	69	68	54	30	534
	FRUIT MASS	22.2	39.47	38.1	34.8	30.32	28.8	22.4	12.52	228.61
	AVE. FRUIT MASS	0.426923	0.424409	0.42809	0.440506	0.43942	0.4235294	0.414815	0.417333	0.428109
TREATMENT B	NO. OF FRUIT	51	56	66	58	68	72	73	27	471
	FRUIT MASS	21.75	23.15	27.72	24.19	29.9	30.8	31.58	11.49	200.58
	AVE. FRUIT MASS	0.426471	0.413393	0.42	0.417069	0.439706	0.4277778	0.432603	0.425556	0.42586
TREATMENT C	NO. OF FRUIT	54	49	69	71	63	61	62	50	479
	FRUIT MASS	23.4	20.7	29.49	30.7	27.7	25	26.2	21.4	204.59
	AVE. FRUIT MASS	0.433333	0.422449	0.427391	0.432394	0.439683	0.4098361	0.422581	0.428	0.427119
TREATMENT D	NO. OF FRUIT	47	54	63	85	67	66	54	34	470
	FRUIT MASS	19.6	32.6	26.25	35.3	28.1	25.69	25.27	14.28	207.09
	AVE. FRUIT MASS	0.417021	0.603704	0.416667	0.415294	0.419403	0.3892424	0.467963	0.42	0.440617
TREATMENT E	NO. OF FRUIT	40	49	64	76	59	66	64	41	459
	FRUIT MASS	16.4	20.28	25.99	30.5	25	27.3	27.06	17.12	189.65
	AVE. FRUIT MASS	0.41	0.413878	0.406094	0.401316	0.423729	0.4136364	0.422813	0.417561	0.413181

Table 7.4. Number of fruit, fruit mass, average fruit mass per pick and combined totals of each, per treatment over the harvest period in trial 2

		21/03/2005	25/03/2005	31/03/2005	06/04/2005	12/04/2005	18/04/2005	26/04/2005	01/05/2005	09/05/2005	**TOTALS**
TREATMENT A	NO. OF FRUIT	112	128	136	144	112	80	64	32	32	**840**
	FRUIT MASS	47.04	55.84	58.4	63.2	49.6	33.6	28	14.4	13.6	**363.68**
	AVE. FRUIT MASS	0.42	0.43625	0.4294118	0.4388889	0.4428571	0.42	0.4375	0.45	0.425	**0.432952**
TREATMENT B	NO. OF FRUIT	128	120	120	120	112	72	72	48	40	**832**
	FRUIT MASS	53.12	50.4	52	52	49.6	33.6	31.2	21.6	17.6	**361.12**
	AVE. FRUIT MASS	0.415	0.42	0.4333333	0.4333333	0.4428571	0.4666667	0.4333333	0.45	0.44	**0.434038**
TREATMENT C	NO. OF FRUIT	192	128	104	120	96	72	48	24	24	**808**
	FRUIT MASS	80.8	54.4	44.8	52.8	41.6	32	21.6	11.2	11.2	**350.4**
	AVE. FRUIT MASS	0.4208333	0.425	0.4307692	0.44	0.4333333	0.4444444	0.45	0.4666667	0.4666667	**0.433663**
TREATMENT D	NO. OF FRUIT	152	120	104	112	72	64	40	24	16	**704**
	FRUIT MASS	64.72	51.2	43.2	48.8	31.2	28.8	16.8	10.4	7.2	**302.32**
	AVE. FRUIT MASS	0.4257895	0.4266667	0.4153846	0.4357143	0.4333333	0.45	0.42	0.4333333	0.45	**0.429432**
TREATMENT E	NO. OF FRUIT	120	112	128	128	96	88	72	48	24	**816**
	FRUIT MASS	51.2	48	56	56	43.2	40	32	20.8	11.2	**358.4**
	AVE. FRUIT MASS	0.4266667	0.4285714	0.4375	0.4375	0.45	0.4545455	0.4444444	0.4333333	0.4666667	**0.439216**

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