



**MANAGEMENT OF FUSARIUM WILT DISEASES USING NON-PATHOGENIC
FUSARIUM OXYSPORUM AND SILICON**

By

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OVERVIEW

In the genus *Fusarium* are many important plant pathogens. The diversity of hosts the genus attacks, the number of pathogenic taxa and the range of habitats in which they cause disease are the greatest in plant pathology. One important species complex within the genus *Fusarium* is *Fusarium oxysporum* Schlecht. This species complex consists more than 80 pathogenic *forma specialis* and is particularly difficult to control. The fungi can survive in soil for decades as specialized spores, known as chlamydospores. Interestingly, however, this species complex also contains beneficial non-pathogenic forms that can be exploited to manage *Fusarium* wilt diseases.

In this study, the ability of non-pathogenic *F. oxysporum* strains, *Trichoderma harzianum* Rifai Eco-T[®], soluble silicon, and their combination was evaluated on two important crops, banana (*Musa* sp. L.) and beans (*Phaseolus vulgaris* L.), for their potential to suppress pathogenic strains of *F. oxysporum*. The ability of these crops to take up and accumulate silicon in their organs, and its effect on low temperature stress was also investigated.

Several endophytic fungi, mainly *Fusarium* spp. and bacteria, were isolated from healthy mature banana plants. After preliminary and secondary *in vivo* screening tests against *F. oxysporum* f.sp. *phaseoli* on beans (*Phaseolus vulgaris* L.) cv. Outeniqua, two non-pathogenic *F. oxysporum* strains were selected for further testing. These two non-pathogenic *F. oxysporum* strains were found to colonize banana (*Musa* sp.) cv. Cavendish Williams and bean plants, and to suppress *Fusarium* wilt of these crops. In order to improve the efficacy of these biocontrol fungi, soluble silicon was introduced.

The relationship between plant mineral nutrition and plant diseases have been reported by several authors. Plants take up silicon equivalent to some macronutrients, although it is not widely recognized as an essential element. In this study, we established that roots, the target plant organ for soilborne plant pathogens, accumulated the greatest quantity of silicon of any plant organs when fertilized with high concentrations of silicon. On the other hand, the corm and stem accumulated the least silicon. Such observations contradict the concept of passive uptake of silicon via the transpiration stream in these plants as the only uptake mechanism.

The prophylactic properties of silicon have been documented for many crops against a variety of diseases. *In vitro* bioassay tests of silicon against these wilt pathogens showed that silicon can be toxic to *Fusarium* wilt fungi at high concentrations ($>7840 \text{ mg } \ell^{-1}$), resulting in complete inhibition of hyphal growth, spore germination and sporulation. However, low concentrations of silicon ($<490 \text{ mg } \ell^{-1}$) encouraged hyphal growth. Silicon fertilization of banana and beans significantly reduced disease severity of these crops by reducing the impact of the *Fusarium* wilt pathogens on these crops. However, it could not prevent infection of plants from the wilt pathogens on its own. Synergistic responses were obtained from combined applications of silicon and non-pathogenic *F. oxysporum* strains against *Fusarium* wilt of banana. Combinations of silicon with the non-pathogenic *F. oxysporum* strains significantly suppressed disease severity of *Fusarium* wilt of banana, caused by *F. oxysporum* f.sp. *cubense* (E.F. Smith) Snyder & Hansen, better than applications of either control measure on their own.

Banana production in the subtropical regions frequently suffer from chilling injury, and from extreme variations between night and day temperatures. Such stress predisposes banana plants to *Fusarium* wilt disease. Silicon, on the other hand, is emerging as important mineral in the physiology of many plants, ameliorating a variety of biotic and abiotic stress factors. We established that silicon fertilization of banana plants significantly reduced chilling injury of banana plants. Membrane permeability, lipid peroxidation (MDA level) and proline levels were higher in silicon-untreated plants than the treated ones, all of which demonstrated the stress alleviating effect of silicon. Low temperatures damage the cell membrane of susceptible plants and cause desiccation or dehydration of these cells. Levels of sucrose and raffinose, recognized as cryoprotectants, were significantly higher in silicon-amended banana plants than unamended plants.

DECLARATION

I Eyob G. Kidane declare that

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Prof. Mark D. Laing

ABSTRACT

In the genus *Fusarium* are many important plant pathogens. The diversity of hosts the genus attacks, the number of pathogenic taxa and the range of habitats in which they cause disease are the greatest in plant pathology. One important species complex within the genus *Fusarium* is *Fusarium oxysporum* Schlecht. This species complex consists more than 80 pathogenic *forma specialis* and is particularly difficult to control. The fungi can survive in soil for decades as specialized spores, known as chlamydospores. Interestingly, however, this species complex also contains beneficial non-pathogenic forms that can be exploited to manage *Fusarium* wilt diseases.

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Prof. Mark D. Laing

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FOREWORD

Management of Fusarial wilt diseases using non-pathogenic *Fusarium oxysporum* have been investigated on a variety of crops. One of the significant developments in biological control of *Fusarium* wilt diseases is the discovery of suppressive soils in which non-pathogenic *F. oxysporum*, among other microbial activity, prevents the pathogens from establishing and/or causing damage in susceptible crops (Alabouvette et al., 1979; Baker and Cook, 1974; Kloepper et al., 1980; Scher and Baker, 1982). The consensus among researchers, in this area, it is unlikely that a single control strategy will successfully manage Fusarium wilt diseases. An integrated approach is the best way forward. Mineral nutrition in plants is another component of integrated control strategy. The use of silicon in the management of several plant diseases and insects, and abiotic stress factors has been proven (Epstein, 1999).

To our knowledge there is no published research on the use of non-pathogenic *F. oxysporum* and soluble silicon to manage Fusarium wilt diseases of banana and beans, and low temperature stress, that aggravates such diseases further. The overall aim of this study was to investigate the efficacy of using endophytic non-pathogenic *F. oxysporum* strains and soluble silicon, and their combination to manage Fusarium wilt diseases of banana and beans. The research pursued the following objectives:

- a. To review the literature on the banana and bean production in relation to Fusarium wilt diseases of these crops, and the basis and approaches of controlling these diseases
- b. To isolate of fungal and bacterial endophytes from healthy banana plants, and screening them against *F. oxysporum f.sp. phaseoli*, and their method of application and dosage
- c. To determine the silicon uptake and distribution in banana and bean plants and the relationship between the level of silicon fertilization and control of Fusarium wilt diseases of banana and beans
- d. To evaluate the toxicity of silicon to Fusarium wilt fungi *in vitro* and in pot trials
- e. To determine the interaction between selected non-pathogenic *F. oxysporum* strains and soluble silicon in the control of Fusarium wilt of beans and bananas

- f. To Determine the ability of silicon to alleviate cold damage on banana plants, the effect of chilling injury on Fusarium wilt, and to determine the different physiological responses of silicon-treated and untreated plants exposed to low temperature

In this thesis referencing followed the format of Crop Science using Endnote.

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CHAPTER ONE

Literature Review

Application of endophytic microorganisms and silicon for the control of Fusarium wilt of banana and beans

Fusarium species are among the most ubiquitous fungi in terrestrial ecosystems (Ploetz, 2006b), well adapted to different habitats and are believed to be the oldest fungal colonizers on earth (Snyder, 1981). They occur in such diverse environments as the arctic and deserts (Nelson, 1981). A number of species within this genus are widely distributed soilborne species that cause disease on a wide variety of economically important crops (Beckman, 1987; Moss and Smith, 1984). Many members of this genus are significant plant pathogens, and based on the diversity of hosts they attack, the number of pathogenic taxa and the range of habitats in which they cause disease is the greatest in plant pathology (Booth, 1971; Gerlach and Nirenberg, 1982; Leslie and Summerell, 2006; Nelson et al., 1983; Ploetz, 2006b). Recent reports indicate that *Fusarium* is a significantly emerging human pathogen in immunocompromised patients (Roilides et al., 2007; Walsh and Groll, 1999). The human pathogenic *Fusarium* can cause serious morbidity and mortality (Boutati and Anaissie, 1997; Roilides et al., 2007), and may mimic aspergillosis (Boutati and Anaissie, 1997).

One important species complex within the genus *Fusarium* is *Fusarium oxysporum* Schlecht. Principally, this group consists of common soil fungi that survive as harmless colonizers of root rhizospheres or weak invaders of the root cortex of many plants. However, in addition to such harmless populations or perhaps as integral part of them, there are more than 80 known *formae speciales* that show specific pathogenicity to particular crops, causing vascular wilt, and root and crown rot. On the other hand, non-pathogenic forms of *F. oxysporum* are known to reduce the impact of vascular wilt pathogens. For example, there are soils particularly suppressive to *Fusarium* wilt diseases

in which the non-pathogenic *F. oxysporum* plays a crucial role (Alabouvette and Couteaudier, 1992; Smith and Snyder, 1971; Tamietti et al., 1993).

1.1 Plant pathogenic *Fusarium oxysporum*

This “species”, as defined by morphology, is actually a diverse and complex collection of fungi (Kistler, 1997). These fungi share a common morphology (Snyder and Hansen, 1940). However, they can be separated into genetically distinct groupings. Collectively, pathogenic forms of this species complex have an impressive host range and show specificity for a common host tissue, the xylem. However, individual strains show selective pathogenicity to specific plants or groups of related plants, causing characteristic wilt symptoms and are designated as *formae speciales* (f.sp.). The pathogenic strains within this complex that cause vascular wilt diseases on bananas and beans are known as *F. oxysporum* Schlecht. f.sp. *cubense* (Smith) Snyder & Hans. and *F. oxysporum* Schlecht. f.sp. *phaseoli* Kendrick & Snyder, respectively. A brief review of these two important crops, banana and beans, which were used in this study is given below to acquaint the reader with the history and significance of these crops in relation to Fusarium wilt diseases, with an emphasis on the South African situation.

1.2 Banana production with respect to Fusarium wilt

1.2.1 Banana Production

Banana is a perennial monocotyledon that belongs to the family Musaceae. The family contains two genera, *Musa* and *Ensete*. Bananas originated in South East Asia and in the Indian subcontinent several thousand years ago and are now grown in virtually all areas located between 30°N and 30°S latitudes (Bentley et al., 1998; Simmonds and Shepherd, 1955). All edible banana cultivars except for the Fe'i group are essentially derived from the two wild, seeded species, *Musa acuminata* Colla that provides the A genome and *M. balbisiana* Colla that provides the B genome (Simmonds and Shepherd, 1955). The nature of banana genotypes is determined by their genetic makeup and can be divided into diploid, triploid and tetraploid (Nelson et al., 2006; Viljoen, 2002).

Edible bananas and plantains are perennial crops that grow quickly and can be harvested all year round. Export dessert bananas are the main fruit in the international fruit trade and the most popular fruit in the world. It ranks first in terms of volume of production and ranks second to citrus in terms of value. It is also a fruit of importance socially, environmentally, economically and politically (Anania, 2006; Josling and Taylor, 2003). In the year 2000 some 90 million hectares of land was cultivated to bananas. From 1998 to 2000 average world banana production was roughly 92 million tons per annum. This was raised to 99 million tons in 2001 (Arias et al., 2003). According to FAO (2005) the world banana exports in the year 2004 was 15.9 million tons. The total value of the international banana trade ranges between US\$4.5 and 5 billion per year (Arias et al., 2003). In many regions of Africa and other developing countries, banana is an important source of food. Of the entire global production, less than 15% leaves the countries in which it is produced, and 85% is consumed locally. Banana production is a major source of income and is an essential source of food for more than 70 million people in Africa (Rutherford and Viljoen, 2003). Africa produces over 30 million tons of bananas every year, which is mostly consumed locally, representing around 34% of world production (Lescot, 2000).

1.2.2 Banana production in South Africa

There is no recorded history of the banana production in South Africa in the literature. However, it is believed that it was introduced to KwaZulu-Natal (KZN) with the labourers from India, who worked on sugarcane farms between the mid-to-late 1800s (Ploetz et al., 1990; Viljoen, 2002). It is, however, also possible that it might have been introduced from neighboring African countries (Viljoen, 2002). Commercial planting first started in Natal and much later in the Transvaal (today the provinces of Limpopo and Mpumalanga) around the early 1950s. At present there are six major production areas in the country: Levubu, Letaba, Sabie, Burgershall, Onderberg (all in Limpopo and Mpumalanga), and KwaZulu-Natal (Ploetz et al., 1990; Viljoen, 2002).

According to Viljoen (2002) South Africa was not a banana exporter until 2001. All banana fruit produced was consumed locally. However, due to increases in banana fruit volumes produced, South Africa made its first shipment for export in June 2001. Although almost all of the fruit is sold locally, it is one of the largest agricultural industries in the country, with an annual income of approximately R600 million (Viljoen, 2003). The

banana industry creates employment for thousands of South Africans and provides a cheap and nutritious food for millions. The banana industry is well developed in South Africa, using a high yielding cultivar under optimized horticultural conditions. Large-scale commercial cultivation comprises almost 95% of all bananas produced in the country. A single cultivar triploid AAA of the Cavendish subgroup dominates banana production in South Africa (Viljoen, 2002).

1.2.3 Fusarium wilt of banana

The soilborne fungus, *F. oxysporum* f.sp. *cubense*, responsible for Fusarium wilt of banana was first recognized in 1874 in Australia by Dr. Joseph Bancroft (Pegg et al., 1996). This disease was next reported in banana plantations grown for export in Central America in 1890 (Ashby, 1913). Although the first report of the disease was given by Bancroft in 1876, the pathogen was first isolated from host tissue sent from Cuba by Smith (1910) who named it *Fusarium cubense* Smith. Ashby (1913) reported the first detailed description of the disease and the pathogen, while Brandes (1919) demonstrated pathogenicity conclusively. In 1940, Snyder and Hansen proposed the name *F. oxysporum* f.sp. *cubense* (Snyder and Hansen, 1940). By the 1950s, the disease had reached such epidemic proportions that it was considered one of the most destructive plant diseases in recorded history (Pegg et al., 1996). The commercial banana industry was mainly based on the cultivar Gros Michel in those times. From 1890 to the mid 1950s, it destroyed some 40,000ha of banana in Central and South America, threatening the demise of the banana industry (Pegg et al., 1996). Fusarium wilt is still regarded as one of the most significant threats to banana production worldwide (O'Donnell et al., 1998).

Since there are no recorded reports of the early history of Fusarium wilt in South Africa, much of what is known today is gathered from various reports and conversations with the older growers in the Natal (now KwaZulu-Natal) and those who were involved in the start of the former Transvaal industry (Ploetz et al., 1990; Viljoen, 2002). In 1940 Wager (1941) observed about 100 wilted Cavendish plants in a localized area 25 km southwest of Durban, which is the first verified case of Fusarium wilt in the country and according to Ploetz (1990), it may have also been the first report of Race 4 here. He described typical symptoms of the disease and isolated *F. oxysporum* from infected plants. The next outbreaks in KZN occurred in 1957 in Pinetown and shortly thereafter at Anerly on the

South Coast. New outbreaks have been regularly reported since 1966 from the province. From there it spread to Kiepersol in Mpumalanga with movement of infected plant materials (Ploetz et al., 1990). Deacon (1984) reported that the disease had been present in the Kiepersol area since at least 1970. In September and November 2000, infected banana plants were observed in a commercial Cavendish plantation in Tzaneen and Mpumalanga province, respectively (Grimbeek et al., 2001b) reflecting the spread of the disease to previously unaffected areas.

Fusarium wilt has already caused losses of up to 30% of fields in two of the six banana growing areas of South Africa (Viljoen, 2002), forcing growers to change to alternative crops. It is now threatening to bring banana production to an end on farms in these areas (Viljoen, 2002). It was assumed that the four previously unaffected production areas could be kept free of the disease because they are geographically separated and each area is large enough to supply its own planting material. Movement of plant material is strictly controlled by legislation within the country, and because growers understand the importance of permit system, they police the movement of planting materials (Ploetz et al., 1990; Viljoen, 2002). However, despite all the quarantine measures, the disease has been observed recently on two of the previously disease-free production areas, Tzaneen and Komatipoort (Grimbeek et al., 2001b).

1.2.4 Symptoms and disease development

Inoculum of *F. oxysporum* f.sp. *cubense* in the soil infects banana plants by penetrating the roots. It invades the xylem vessels, and if it is not blocked by vascular occluding responses of the host, then it advances into and colonizes the corm, eventually blocking the vascular system (Moore et al., 1995; Stover, 1962). This results in the typical and conspicuous external symptom, yellowing of the leaf margins of older leaves, which may initially be confused with potassium deficiency, especially under dry or cold conditions. Yellowing then advances to the youngest leaves until the dead leaves hang down to form a “skirt” around the pseudostem (Moore et al., 1995). Internally, at initial stages of disease development the vascular tissues of the roots and corm become yellowed later progressing to form a continuous yellow, red or brown discoloration of the vascular strands in the pseudostem and sometimes the bunch stalk. Longitudinal splits of the pseudostem are also common (Davis, 2005; Moore et al., 1995). No disease symptoms have been observed in

fruit (Moore et al., 1995). Disease symptoms are most severe in South Africa during a hot summer following a cold winter (Viljoen, 2002) because of cold stress.

The fungus grows out of the xylem vessels into the surrounding tissues of the dying plant, and over-winters in the soil by forming many chlamyospores when the plant decays. *F. oxysporum* f.sp. *cubense* can survive in the soil for up to 30 years as chlamyospores in infected debris or in the roots of alternative hosts. The fungus is also able to survive in the roots of symptomless alternative hosts such as close relatives of the banana and several species of weeds and grasses under field conditions (Armstrong and Armstrong, 1948; Armstrong et al., 1942; Hendrix and Nielsen, 1958; Katan, 1971; Moore et al., 1995; Smith and Snyder, 1975). The pathogen is most commonly spread by the movement of infected planting materials and soils attached to it (Pegg et al., 1996). It can also be disseminated via farm machinery and implements, and surface run-off water or irrigation reservoirs contaminated with spores of *F. oxysporum* f.sp. *cubense* (Moore et al., 1995).

1.3 Fusarium wilt and its importance in bean production

The center of origin for beans (*Phaseolus* spp.) is believed to be in the Central and Southern America (Bernal and Graham, 2001). According to du Plessis et al. (2002), in South Africa there are three species from the genus *Phaseolus* that are agronomically important. These are *P. vulgaris* L., *P. acutifolius* A. Gray and *P. coccineus* L. In relation to its high protein content and dietary benefits, the bean crop is currently considered as one of the most important field crops in South Africa harvested for dry seeds. In this study *P. vulgaris* was used throughout the experiments. There are many different types and colours within this species. The most important ones are, however: small white, red speckled or sugar beans, carioca and green beans, with percentages of local production being 10-20, 65-75 and 3-5, respectively (du Plessis et al., 2002). The instability in bean production in South Africa in the past has been partially blamed on diseases and pests. Fusarium yellows/wilt, caused by *F. oxysporum* f.sp. *phaseoli*, is one of the most important bean pathogens reported in South Africa (du Plessis et al., 2002).

Fusarium wilt of beans is an important disease of common beans (*P. vulgaris*) particularly in areas where high temperatures and drought prevail (Buruchara and Camacho, 2000).

The fungus may also infect *P. coccineus* (scarlet runner bean) and some lupin varieties (Armstrong and Armstrong, 1963). But it is not pathogenic to other types of beans such as lima beans (*P. limensis* Macfad. var. *limenanus* Bailey), cowpea (*Vigna sinensis* (L.) Savi ex Hassk.) or soybeans (*Soja max* (L.) Piper) (Brayford, 1997).

Fusarium yellows/wilt of beans was first reported in 1928 from the Sacramento Valley in California, USA (Harter, 1929). In the 1930s the disease re-appeared in the same localities, but disappeared when the affected fields were planted with other crops. However, 10yr later the disease re-emerged. This led to pathogenicity trials and the fungus was described as a distinct *forma specialis* (Kendrick and Snyder, 1942). The disease was subsequently reported from other *P. vulgaris* producing areas and from *P. coccineus* in UK (Brayford, 1997). The pathogen is widely distributed in areas where *P. vulgaris* is grown, including Brazil, Czech Republic, China, Colombia, Costa Rica, Egypt, Greece, Italy, Japan, Kenya, Mexico, Peru, Poland, Rwanda, Slovakia, The Netherlands, UK, USA and former Yugoslavia (Brayford, 1997). Severe outbreaks of *Fusarium* wilt of beans have been reported in Africa, Europe, Latin America and the United States (Abawi and Pastor-Corrales, 1990; Allen, 1995; Buruchara and Camacho, 2000; Kraft et al., 1981; Mutitu et al., 1988; Rusuku et al., 1997; Schwartz et al., 1989; Silbernagel and Mills, 1990). In some places such as Brazil, losses of up to 80% have been reported (Dhingra et al., 2006). In Rwanda annual yield losses because of *Fusarium* wilt of beans were estimated as 14,690 tonnes (Trutmann and Graf, 1993).

Based on differential responses of *F. oxysporum* f.sp. *phaseoli* strains to cultivars of *P. vulgaris*, seven races have been identified (Alves-Santos et al., 2002; Nascimento et al., 1995; Ribeiro and Hagedorn, 1979a; Ribeiro and Hagedorn, 1979b; Salgado and Schwartz, 1993; Woo et al., 1996). But it cannot be ruled out that there could be other races elsewhere in the world, e.g., in China (Brayford, 1997). Apparently, there is a relationship between races and geographic origin, Race 1 being from Europe and North America, Race 2 from Brazil, Race 3 from Colombia, Race 4 from USA (Colorado), Race 5 from Greece, although this needs to be confirmed by analysis of more strains (Woo et al., 1996). Recently, Races 6 and 7 have also been identified from Spain (Alves-Santos et al., 2002).

Like other *formae specialis* of *F. oxysporum* it does not require wound to infects the roots or hypocotyls from soil or seed-borne inoculum; yet, wounding by nematodes such as

Meloidogyne incognita (Kofoid & White) Chitwood or *M. javanica* (Treub) Chitwood (Brayford, 1997; France and Abawi, 1994) or insects such as fungus gnats larvae (*Bradysia* spp.) makes infection easier. Brayford (1997) described a lifeless yellow-green color on the lower or primary leaves as an indication of initial symptoms, which then progresses quickly upwards to the younger leaves. Infected young plants appear stunted. At advanced stages of the disease, leaves develop chlorosis, becoming progressively more conspicuous as they become bright yellow. The leaf margins may curl inwards and the leaflets droop. The yellowing is uniform, but in some cases unilateral yellowing affects one side of the plant first. Eventually the whole plant wilts and collapses. Infection of young plants causes stunting. Internally, the vascular tissue develops a dark reddish-brown discoloration extending from the roots up the stem to the petioles and into the pods. After the plant is dead, the fungus sporulates, forming slimy masses of microconidia and macroconidia at the host surface and forming chlamydospores in colonized tissues.

Like other *Fusarium* wilt fungi, dissemination occurs through a variety of ways. Man (Kommedahl et al., 1970), irrigation or flood-water, animals and wind (Kommedahl et al., 1970; Nelson, 1981) move infested soil and plant parts from one area to another. Ooka (1975) in Nelson (1981) found randomly isolated fungal colonies from soil from North Dakota which settled on snow deposited during a blizzard in January 1975 in St. Paul, Minnesota, of which 11% were *F. oxysporum*. The fungus is also seedborne by conidial contamination (Kendrick, 1934).

1.4 Disease management of fusarial wilt diseases

Early farmers prayed or offered sacrifice to the gods to cure their crops of diseases, and to forgive their sins because diseases of humans, their domestic animals and their crops were viewed as the wrath of the gods for their sins. With man's growing understanding of the environment, the real causes of the various plagues on our crops were identified and methods to combat them have been evolving for generations. Since the early 19th century, when man discovered the true causes of plant diseases, our understanding of the interaction between pathogens, plants and the environment has enabled us to develop a wide range of disease management measures. Based on this accumulated knowledge, a set of basic principles, mainly known as "traditional principles", were drawn to manage plant disease,

first by Whetzel (1929) and that was modified by other researchers. These traditional principles include the following (Arneson, 2006):

- (i) **Avoidance**, whereby disease is prevented either by choosing a time of the year or site where there is no pathogen inoculum or where the environment is not conducive for disease to develop
- (ii) **Exclusion**, whereby the introduction of pathogen inoculum is prevented
- (iii) **Eradication**, to eliminate, destroy, or inactivate pathogen inoculum
- (iv) **Protection**, to prevent infection by means of a toxicant or some other barrier to infection
- (v) **Resistance**, the use of cultivars that are resistant or tolerant to infection by the pathogen
- (vi) **Therapy**, to cure plants that are already infected by the pathogen

This set of principles has helped farmers over the years and there are a number of success stories where plant pathogens have been successfully managed. However, soilborne plant pathogens in general, and Fusarial wilts in particular, have proved to be very difficult to manage (Jaroszuk-Scisel et al., 2008; Ploetz, 2006a; Ploetz, 2006b; Tamietti and Valentino, 2006).

1.4.1 Avoidance

The ubiquitous nature of *Fusarium* spp. has been interestingly explained by Leslie and Summerell (2006) as, “if it is green, there is some *Fusarium* that can grow on it, or with it”. Most of the banana or bean production areas in South Africa are infested with *Fusarium* wilt inoculum. With the exception of the Mediterranean, Melanesia, Somalia, and some islands in the South Pacific (Anonymous, 1977; Ploetz and Pegg, 2000; Stover, 1962), all other banana growing areas of the world, including the world’s major banana producing countries in Central and South America, are affected by *F. oxysporum* f.sp. *cubense*. The pathogen existed long before the start of commercial production of bananas. Epidemics of Panama disease are more common where there is commercial monoculture of the crop. This is because pathogenic microorganisms increase during monoculture of a crop. Yields are reduced, regardless of the fertility program in place (Cook, 1986). For many years farmers have been clearing forests and bushes for agriculture when the existing agricultural land is no longer productive, either because of pests and diseases, or soil

infertility. Such actions have resulted in desertification and the shrinking of natural forests and extinction of wild animals. In some parts of the world, however, moving to virgin land is nearly impossible, either because there is no land left suitable for agriculture, or because the country's law protect the remaining forest or wildlife. On the other hand, due to the perennial nature of the banana plant and the biology of *Fusarium* wilt fungi, it is not possible to avoid the pathogen by using seasonal planting. They produce chlamydozoospores that help them survive in the soil for decades. Unlike most plant pathogens that thrive during summer, winter apparently also favours the subtropical Race 4, which attacks the Cavendish clones in the subtropics (Buddenhagen, 1990).

1.4.2 Exclusion

One of the basic principles in plant pathology is the disease triangle, where for disease to occur a suitable environment, a pathogenic organism and a susceptible host must be present. Based on this principle, disease can not develop, provided that plants and pathogens are separated. Thus, to prevent the introduction and spread of plant pathogens into new areas, quarantine laws are put in place by governments in order to regulate the conditions under which certain crops may be grown and distributed between production areas or countries (Agrios, 1997). Of the various control methods, regulatory control measures aimed at excluding a pathogen from the host is the only option available in the control of many fusarial wilt diseases in areas where they are not yet introduced. Viljoen (2002) noted that, in South Africa four out of the six banana production areas were then free of *Fusarium* wilt of banana. This was mainly because of policing of movement of plant material to those areas and awareness of farmers to the biology, epidemiology and management of the disease, in addition to the geographical isolation of the production areas and the use of tissue culture plantlets instead of suckers for replanting. However, such measures could not keep these areas permanently free from the introduction of the pathogen. Grimbeek et al. (2001a) reported the first occurrence of the disease in two of these areas, namely Tzaneen and Komatipoort.

1.4.3 Eradication

When crop production is at risk because of a heavy inoculum load of a given pathogen in the soil, the use of selective fungicides (Erwin, 1981), steam pasteurisation or solarization

of the soil (Beckman, 1987; Katan, 1980) may be used to reduce the inoculum load. Steam pasteurisation or sterilization of soil in glasshouse culture of intensively grown crops is a normal practice to destroy inoculum prior to planting. This prevents *Fusarium* wilt diseases as well as the root diseases caused by other soilborne pathogens (Erwin, 1981). Baker & Roistacher *in* Erwin (1981) claimed that soil could be sterilized by maintaining a temperature of 80°C for 30min with steam. Steam-air mixtures have also been used in some situations (Baker *in* Erwin, 1981).

However, in the field, steam is an expensive method of pasteurisation of soil. Moreover, due to condensation of water, the depth of penetration of soil by steam is limited (Erwin, 1981). Similarly, soil solarization is another safe, non-chemical method of controlling soilborne diseases. Its use, however, is limited to regions where the climate is suitable and the soil is free of a crop for about one month before mulching (Katan, 1980) and it is likely to be too expensive on widespread basis in the field.

The use of chemicals to control plant disease dates back to the late 1600s, in those days farmers treated their wheat seeds with brine (sodium chloride solution) to control bunt (Agrios, 1997). Fungicides have provided great advantages to agricultural producers for many years (Gamlie and Stapleton, 1995), but are limited by safety considerations, the need for complicated equipment, highly trained personnel, high cost, pesticide residues, phytotoxicity, and reinfestation of soil resulting from drastic reductions in antagonistic microbes (Katan, 1980). Although about 2.5 million tons of pesticides are used annually, one-third of all crop production is still lost (Campbell, 1989; Pimentel, 1995).

As described by Beckman (1987), use of fungicides is economically justified only under conditions in which the soil mass to be treated is limited or the crop value and increased yields warrant the considerable expense. In field situations the cost can be prohibitive. To maintain a low inoculum load by a continuous supply of synthetic fungicides, systemic chemicals or biocides alone is not practical to control soilborne diseases. The cost of treatment that results from the high degree of adsorption in soils and the need for a sustained dosage level has prevented their use except under special circumstances. Erwin (1981) noted that chemical control with systemic benzimidazole chemicals, and general biocides such as methyl bromide, chloropicrin, mixtures of these chemicals, and vapam

was limited to intensively grown crops because of the great expense of the chemicals, inefficiency of uptake by roots, and the tarping of the land with polyethylene sheeting.

Pesticides may influence populations of organisms affecting interactions among species within ecosystems, or they may destabilize these systems (USDA, 1980). One such non-target effect of pesticides is suppression of mycorrhizal fungi (Johnson and Zak, 1977; Kleinschmidt and Gerdemann, 1972). Fumigation with methyl bromide:chloropicrin (45:55) for control of *Verticillium* wilt in cotton has this effect (Erwin, 1981). The best-documented examples of nontarget effects are the zones bordering agroecosystems. For example, in U.S. agroecosystems more than 500,000 tons of pesticides are applied annually to control insect pests, plant pathogens, and weeds (Pimentel et al., 1991; USDA, 1980). However, as little as less than 0.1% of this may actually hit the target organisms (Pimentel, 1995; PSAC, 1965). Instead, most reaches non-target sectors of agroecosystems and/or is spread to surrounding ecosystems as chemical pollutants (Pimentel and Edwards, 1982). Chemical fumigants may cause negative effects on the environment or human beings (Yates et al., 2003).

Although the large costs (e.g. approx. \$1000 ha⁻¹ in export banana) of broadcast fumigant chemical treatments (Ploetz, 2001) have resulted in their limited use on broad-acre crops, health hazards and environmental problems, including the contamination of ground water have been widely reported. As a result, a number of products have been withdrawn from the market (Thomason, 1987). Some of the early pesticides were potent toxins (e.g., mercury and organochlorine insecticides). They persisted in the environment, accumulated in predators at the top of food chains and were shown to have long-term effects on non-target organisms. The use of such chemicals has been discouraged or prohibited in most countries (Campbell, 1989).

Modern pesticides have to pass stringent tests for safety and a lack of any environmental hazard. The fact remains that they are toxins and occasional examples of misuse or unexpected side effects do occur. It is estimated that there are about 3000 hospitalisations and 200 fatalities per year in the USA alone occur due to pesticides, apart from the other medical problems that are unrecognised or not considered serious enough to warrant medical attention (Pimentel et al., 1983). Though normal precautions are employed in the use of fungicides with the knowledge currently available, additional information, however,

sometimes comes to light that many cast suspicion on the safety of the continued use of some even well-established materials. For example, benomyl and related compounds prevent completion of mitosis (Seiler, 1973; Styles, 1973); and it has been reported that some of the carbamates and related compounds have deleterious cytogenetic effects on a variety of organisms (Seiler, 1973; Styles, 1973; Styles and Robert, 1974).

In 2000, the Antarctic ozone hole reached approximately 28m sq. km in size. Present trends indicate that the size is unlikely to start to decline (Anonymous, 2007). Under the Montreal Protocol of 1991, methyl bromide was defined as a chemical that contributes to depletion of the Earth's ozone layer. The definition was based on scientific data. Accordingly, the manufacture and importation of methyl bromide will be completely phased out in 2005 and 2015 in developed countries and developing countries, respectively (Vick, 2002). (Buschena et al., 1995) indicated that with the loss of methyl bromide as a soil fumigant, reduction of seedling growth and vigour, as well as increased seedling mortality is anticipated. Despite the comprehensive research worldwide, the Methyl Bromide Technical Option Committee still reports that no alternative has been found (Banks, 2002).

Fungicides are discovered by an essentially random process of testing as many chemicals as possible for their effect on fungi. A large agrochemical company may test tens of thousands of chemicals each year with the hope that one in 5000 might lead to a useful product. However, it has recently become clear that it is now more difficult to find new compounds; perhaps only 1 in 100,000 tested now becomes a new product. This is partly because new compounds have to pass more stringent tests than previously required, but there is also the suspicion that perhaps most of the effective chemicals have already been found (Campbell, 1989; Delp, 1977; Lewis, 1977). After discovery of a new disease-control agent, it may take 5 to 10yr to develop the necessary data on efficacy, residue, and toxicology to register the compound for sale (Delp, 1977). Moreover, according to estimates from Bayer CropScience, the cost of discovering a new fungicide and bringing it to market in 1990, 2000 and 2006 was around US \$30, US\$184 and US\$250 million, respectively (Chet, 1990; Russell, 2006).

Another negative side of fungicides is the issue of development of resistance. Fungi develop resistance to fungicides, therefore, new chemicals are constantly needed, but they

are becoming more difficult to find, and therefore, more expensive (Campbell, 1989; Delp, 1977; Lewis, 1977). Benomyl resistance in cucumber powdery mildew was reported within one year after introduction of this fungicide (Schroeder and Provvidenti, 1969). Bollen & Scholten (1971) showed that *Botrytis cinerea* Pers. isolated from diseased plant tolerated much higher concentrations of benomyl than the wild type fungus. Even at 1000ppm of benomyl there was not a complete inhibition of growth, while the wild type fungus was eliminated at 0.5ppm of benomyl in the medium. Paper disc bioassay tests by Magie and Wilfret (1974.) reported that isolations of *F. oxysporum* f.sp. *gladioli* (Massey) Snyder & Hansen from benomyl-treated corms tolerated 50 to 200 times more benomyl than those from non-tolerant corms. Bioassay data indicated that the growth of nontolerant strains was slightly restricted by concentrations of benomyl on the paper disc as low as 2-5mg⁻¹. Nakanishi & Oku (1969) also reported *F. oxysporum* f.sp. *niveum* (Smith) Snyder & Hansen and f.sp. *lycopersici* (Sacc.) Snyder & Hansen tolerant to quintozene (pentachloronitrobenzene).

1.4.4 Resistance

Where the pathogen is present, the use of resistant cultivars has been the primary choice of researchers and growers. However, the rise of new strains of pathogens may overwhelm the resistance of those crops over time. After the disastrous end of the banana export industry based on Gros Michel by *F. oxysporum* f.sp. *cubense* Race 1 (Simmonds, 1966), the export trade was rescued by the introduction of Cavendish clones, which has been resistant to Fusarium wilt of banana for more than 30 years (Buddenhagen, 1990). The regions where the Cavendish clones are planted such as the Caribbean and South America, where most of the international banana crop is produced, and in tropical Africa, is infested with Fusarium wilt. Occasional rare incidence of diseased plants in the last 30 years probably resulted from stress instead of changes in the pathogen, and no major disease epidemics were observed (Buddenhagen, 1990). In contrast, Cavendish clones grown in tropical Philippines, at the edge of the tropics in southern Taiwan, and the three widely separated subtropical regions, Australia, South Africa and the Canary islands, have been attacked by Fusarium wilt Race 4. Bananas grown in South Africa are mainly commercial (95%) and entirely based on the cultivar Cavendish. The rise of new virulent strains of the pathogen that attack the once-resistant clone, the absence of resistant cultivars with desirable quality to replace the existing Cavendish clone and the sterile and triploid nature

of the plant that make breeding a very difficult task (Morpurgo et al., 1994), are all issues that particularly trouble banana growers, where virulent strains are present, banana production is at risk.

1.4.5 Protection

Protection from infection from inocula in the soil can be achieved through the use of chemical fungicides or beneficial microorganisms. Since there are no effective fungicides against *Fusarium* wilt diseases on the market, this review will focus on the use of beneficial microorganisms to manage these diseases.

1.5 Biological control of *Fusarium* wilt diseases

There is an increasing interest in the biological control of pests and diseases as a result of the increase in public concern over the impact that chemicals have on the environment, their ineffectiveness against soilborne pathogens such as *Fusarium* wilts, and the need to find alternatives to the use of chemicals to control plant diseases (Whipps, 2001). Intensive studies have been made in the search for biocontrol agents to control diseases caused by plant pathogenic members of the genus *Fusarium*. However, in order to achieve successful and reproducible biological control of soilborne plant pathogens, it is essential to have knowledge of the ecological interactions that take place in the soil and root (Deacon, 1994; Whipps, 1997), and inside the plant. An interesting finding was the discovery of natural suppressiveness of soils to *Fusarium* wilts in the nineteenth century by Atkinson in 1892 (Weller et al., 2002). This was later observed at other sites around the world (Alabouvette, 1986; Dominguez et al., 2001; Hopkins et al., 1987; Peng et al., 1999; Scher and Baker, 1980; Smith and Snyder, 1971; Sneh et al., 1987; Toussoun, 1975). Much knowledge has been drawn from research on such soils. Modern methods for analyzing microbial community structures have been particularly valuable to help define the key organisms or groups of organisms responsible for such natural suppression (Abbasi et al., 1999; Buyer et al., 1999; Duineveld et al., 1998; Gamo and Shoji, 1999; Mazzola, 1999; Natsch et al., 1998; Postma et al., 2000; Shiomi et al., 1999; Smit et al., 1999; Tiedje et al., 1999). More biocontrol agents are reaching the market place, in part because such knowledge is behind them (Whipps and Davies, 2000; Whipps and Lumsden, 2001).

1.5.1 Basis for biological control of Fusarium wilt diseases

Understanding of the biology and epidemiology of Fusarium wilt pathogens, and the crop and its production system are important factors in determining the most appropriate approach for developing an effective biological control system. The selection of such an approach also depends on interaction between the Fusarium wilt pathogen, the control agent population and the host. These key interactions determine the mechanism for biological control, the population dynamics of the pathogen, the physiology of host:parasite interaction and the structure of biological community associated with the disease (Marois, 1990). Marois (1990) further suggested that the interaction between the host, the control agent and the environment is another crucial factor that determines success or failure of a biological control agent. This is because interaction between populations of beneficial organisms and pathogens do not occur separately; and understanding of the complex pathosystem is important, however, difficult this is to achieve.

1.5.2 Approaches for biological control

Cook and Baker (1983) identified three general approaches to biological control of plant pathogens: (1) control of pathogen inoculum, (2) protection of plant surfaces from infection, and (3) cross protection or induced resistance. The most appropriate approach is dependant upon the specific pathosystem being investigated. Each approach may be examined in relation to Fusarium wilt of beans and banana.

1.5.2.1 Control of pathogen inoculum

This includes the destruction of pathogen propagules through the use of antagonists, prevention of inoculum formation, weakening or displacement of the pathogen in infested residue (the food base) by competitors or antagonists, and reduction of vigour or virulence of the pathogen (Cook and Baker, 1983). In this case, competitive antagonists with high levels of parasitic ability and antibiosis activity are required. *Trichoderma* spp. are biocontrol organisms known to possess most of such characteristics. However, it is not possible for the rhizosphere biocontrol agents to protect all root infection sites and the endophytic/systemic nature of the wilt pathogens takes beyond reach for such antagonists, once infection has taken place. In addition, Marois (1990) suggested that biological control of inoculum of Fusarium wilts of perennial crops such as banana would be difficult in most

areas of production due to the perennial nature of the crop. He further reasons that because of the great diversity of biotic and abiotic conditions, and low energy inputs that exists in the perennial crops/forests, there is high level of competition. From ecological studies there is positive relationship between diversity and competition. Therefore, the introduction of an antagonist to an already high competition environment would not be effective. In greenhouse crops and annual field crops, however, there are several examples of successful biological control of diseases caused by *F. oxysporum* through increased competition (Dhingra et al., 2006; Locke et al., 1985; Marois and Locke, 1985; Marois et al., 1981; Rowe and Farley, 1978; Serra-Wittling et al., 1996).

1.5.2.2 Protection from infection

This approach to biological control involves the establishment of antagonistic microorganisms in or around the infection court so as to provide biological protection of the host against the pathogen (Cook and Baker, 1983). In soils where the pathogen is already established, protecting sites of potential infection is a better approach than trying to alter the entire soil microbial community through manipulation of the soil pH or the addition of organic matter (Marois, 1990). He suggested that such an approach is more appropriate when the pathogen does not yet occur in the soil. This approach also may involve some form of direct inhibition (limitation of prepenetration growth) of the pathogen by antagonists. The host provides the habitat and nutrients required for the control agent to be established at the infection site (Carter, 1971; Carter, 1983; Cook and Baker, 1983; Kawamoto and Lorbeer, 1976; Magie, 1980; Ordentlich et al., 1991; Sivan and Chet, 1986). This procedure is used in Taiwan, where plantlets are treated with specific antagonists before they are taken to the field (Marois, 1990). Biological control using resident antagonists, suppressive soils, and control using organic amendments fall into this category (Cook and Baker, 1983).

1.5.2.3 Cross protection/Induced resistance

According to Kuc (1982), all plants possess the genetic potential to express disease resistance. However, the key to resistance is the expression of this potential. For instance, *F. oxysporum* f.sp. *cubense* may infect both resistant and susceptible host cultivars (Baayen and Elgersma, 1985; Beckman et al., 1962) but its advance depends on how fast the host reacts to such invasion (Beckman, 1990). Resistant cultivars reacted faster than

susceptible cultivars, allowing them to contain the movement of secondary conidia upwards in the xylem vessels, stopping systemic colonization and disease. Therefore, susceptible cultivars could be “immunized” by stimulating the expression of resistance before the plants actually encounter the pathogen. This is the concept of cross protection or induction of resistance. This is the establishment of non-pathogenic or only mildly pathogenic microorganisms or agents within the plant or infected area in order to stimulate greater resistance of the plant to the pathogen (Cook and Baker, 1983).

Although most of the work on biological control of *Fusarium* wilt has been done using direct interaction of the control agent with the pathogen outside the host, research on cross protection or induced resistance to reduce host susceptibility to pathogen seems promising (Kuc, 1982). Marois (1990) suggested that induced resistance or cross protection may be the most promising approach for biological control of *Fusarium* wilt of banana. The use of non-pathogenic fusaria to induce resistance in plants against *Fusarium* wilt has been widely studied (Fravel et al., 2003; Larkin and Fravel, 1999; Larkin et al., 1996). Prior inoculation with a non-pathogenic *F. oxysporum* induced local and systemic resistance against wilt in watermelon (Larkin et al., 1996), cucumber (Mandeel and Baker, 1991), tomato (Fuchs et al., 1997), and sweet potatoes (Makino et al., 1996). Plant growth promoting rhizobacteria (PGPR) have also been investigated in order to induce systemic resistance against various pathogens, including *F. oxysporum* (Liu et al., 1995; Ramamoorthy et al., 2001; van Peer et al., 1991; Wei et al., 1996).

1.5.3 Non-pathogenic *Fusarium* species as biocontrol agents

Many attempts have been made to control *Fusarium* spp. by biological means. One of the significant phenomena in biological control of *Fusarium* wilt disease is the existence of suppressive soils in which microbial activity prevents the pathogen from establishing and/or causing damage in susceptible crops (Alabouvette et al., 1979; Baker and Cook, 1974; Kloepper et al., 1980; Scher and Baker, 1982). Such soils are naturally suppressive to *Fusarium* wilt. Non-pathogenic strains of *F. oxysporum* play a role in their indigenous microbiota (Alabouvette and Couteaudier, 1992; Smith and Snyder, 1971; Tamietti et al., 1993). The interaction between pathogenic and non-pathogenic *Fusarium* strains in those soils in part results in the control of the disease (Fravel et al., 2003; Larkin et al., 1993; Larkin et al., 1996; Paulitz et al., 1987; Schneider, 1984).

Smith and Snyder (1971) studied the importance of biological factors in soils, naturally suppressive to *Fusarium*, especially to the saprophytic microorganisms such as nonpathogenic *Fusarium* spp. Soil suppressiveness was eliminated when soil was treated with moist heat, methyl bromide and gamma irradiation (Alabouvette, 1986; Scher and Baker, 1980) and was transferable. This suggests that microbial activity is the reason for their disease suppressive properties. Large populations of non-pathogenic *Fusarium* species have been reported in soils naturally suppressive to *Fusarium* wilt (Toussoun, 1975). Louvet et al. (1976) also reported that soils from Châteaurenard, France, supported large populations of *F. oxysporum* and *F. solani*. Using a simple form of Koch's Postulates, the role of nonpathogenic populations of *Fusarium* spp. in the suppression of *Fusarium* wilts in these soils was confirmed when the soil was heat treated to >55°C, resulting in the loss of suppressiveness. Suppressiveness was then restored when the soil was amended by artificial introduction of strains of nonpathogenic *F. oxysporum* or *F. solani* to the heat-treated soil (Rouxel et al., 1979). Many other authors from different parts of the world have also reported the role of non-pathogenic *Fusarium* species in *Fusarium*-suppressive soils (Larkin et al., 1993; Larkin et al., 1996; Paulitz et al., 1987; Schneider, 1984).

Nonpathogenic *Fusarium* spp. are known to protect plants from *Fusarium* wilt pathogens through their ability to compete with pathogenic fusaria for nutrients such as organic carbon and iron, and/or for infection sites in the rhizoplane (Alabouvette and Couteaudier, 1992; Lemanceau and Alabouvette, 1991; Lemanceau et al., 1993). The induction of resistance to *Fusarium* wilt, as in the case of cucumber (Mandeel and Baker, 1991) and chickpea (Hervas et al., 1995), or formae speciales of *F. oxysporum*, such as f.sp. *melonis* on cucumber (Gessler and Kuc, 1982) and f.sp. *dianthi* on tomato (Kroon et al., 1991) using nonpathogenic strains of *F. oxysporum* have also been reported. More recently, nonpathogenic *F. oxysporum* strain Fo47 was shown to induce resistance to *Fusarium* wilt in tomato (Fuchs et al., 1997).

Strains of non-pathogenic *Fusarium* species differ considerably in their efficacy to suppress *Fusarium* wilt (Alabouvette et al., 1993; Larkin and Fravel, 1998; Larkin and Fravel, 1999) because such screening of several isolates to obtain efficient ones is crucial. Furthermore, according to Larkin and Fravel (1998; 1999) strains of non-pathogenic *Fusarium* species not only differ in their efficacy but also in their mode of action(s) and dosage required to suppress disease. For instance, *F. oxysporum* Strain CS-20 required as

little as 100 chlamydospores per gram of soil to significantly suppress disease, while Strain Fo47 required as much as 10^4 - 10^5 chlamydospores per gram of soil to effectively suppress disease.

1.6 Silicon in crop production

Silicon (Si) is the second most abundant element (31%) on the surface of the earth next to oxygen (49%), and SiO_2 comprises 50-70% of the soil mass (Ma and Yamaji, 2006). As a result, various amount of silicon is found in plant tissues. It is present in plants in amounts equivalent to those of certain macronutrients such as calcium, magnesium and phosphorus (Epstein, 1999), although its physiological role in higher plants is controversial (Clarkson and Hanson, 1980; Epstein, 1994; Jones and Handreck, 1967; Simpson and Volcani, 1981; Werner and Roth, 1983). Its role in plant growth and development was unmarked until the beginning of the 20th century (Epstein, 1999; Ma and Takahashi, 2002; Richmond and Sussman, 2003). However, repeated cropping and constant application of chemical fertilizers such as nitrogen, phosphorus and potassium have depleted the levels of plant-available silicon in the soil to a point where supplemental silicon fertilization is needed to maximize production. Furthermore, some soils naturally contain little plant-available silicon (Ma and Yamaji, 2006). Soils that are low in silicon are typically highly weathered, leached, acidic and low in base saturation (Foy, 1992). Plants that grow on highly organic Histosols that contain little mineral matter may obtain small amount of silicon. Similarly, soils that are mainly composed of quartz sand (SiO_2) such as sandy Entisols may contain very little plant-available silicon. Many agricultural lands in Africa, Asia, Latin America, the southeastern USA (Datnoff et al., 1997) and Australia (Goldie, 1998) have such conditions.

There are many reports in relation to the role of silicon in higher plants. Silicon has been reported to benefit plants (e.g. rice) in a number of ways: (i) increasing canopy photosynthesis by keeping leaves erect, (ii) increasing resistance to fungi, bacteria, and insects, (iii) reducing the toxicity of heavy metals, (iv) reducing cuticular transpiration, and (v) improving water use efficiency (Epstein 1999; Savant, Snyder, and Datnoff 1997; Yoshida, Ohnishi, and Kitagishi 1962).

1.7 Silicon in plant disease control

There are several reports on the role of silicon in protecting plants from a number of biotic stresses. Japanese researchers were the first to report that silicon can be used to effectively control plant diseases, especially on rice (*Oryza sativa* L.) (Kozaka, 1965). They showed that application of silicon from various sources considerably reduced the incidence and severity of rice blast, caused by *Magnaportha grisea* (Hebert) Yaegashi and Udagawa, and brown spot, caused by *Cochliobolus miyaheanus* (Ito and Kuribayashi in Ito) Drechs. Ex Dastur (Ohata et al., 1972; Okuda and Takahashi, 1964; Takahashi, 1967; Volk et al., 1958). The incubation period of *M. grisea* was lengthened by silicon accumulation, whereas lesion length, rate of lesion expansion, and disease leaf area dramatically decreased (Seebold et al., 2001). Other rice disease such as leaf scald, caused by *Gerlachia oryzae* (Hashioka and Yokogi) Gams, sheath blight, caused by *Rhizoctonia solani* (Frank) Donk, and stem rot, caused by *Magnaporthe sahinii* (Cattaneo) Krause and Webster (Savant et al., 1997) were also effectively managed by application of silicon fertilizers. Silicon has been reported to reduce the incidence of powdery mildew in cucumber, barley and wheat; ring spot on sugarcane; rust on cowpea; leaf spot on Bermuda grass; and gray leaf spot on St. Augustine grass and perennial ryegrass (Datnoff, 2007). Belanger (1995) reported that foliar and soilborne diseases of cucumber and other cucurbits can also be suppressed by applying silicon. It has been suggested that silicon enhances plant resistance to disease in two main ways: creation of a physical barrier and induced systemic resistance (Ma and Yamaji, 2006). Alleviation of abiotic stresses is also equally important especially in relation plant diseases such Fusarium wilt of banana, because abiotic stresses such drought, heat and cold result in increased disease levels.

1.7.1 Physical barrier

In plants silicon is mostly deposited beneath the cuticle to form a cuticle-silicon double layer (Fauteux et al., 2005). Increasing levels of silicon have been detected in the outer regions of epidermal cell walls of rice leaves (Kim et al., 2002). The silicon is initially absorbed in the form of silicic acid [$\text{Si}(\text{OH})_4$], then accumulates and polymerizes into silica gel in host cell walls (Heath and Stumpf, 1986; Heath et al., 1992; Yoshida et al., 1962b). More than 90% of the silicon within rice leaves forms a silica gel layer (Yoshida et al., 1962a). It is an immobile compound that accumulates with tissue age (Takahashi,

1995). This layer is believed to mechanically prevent insect attack, and fungal penetration of the plant tissue by disrupting the infection process (Hayasaka et al., 2008; Kim et al., 2002; Yoshida et al., 1962a). Fungal hyphae and parasite haustoria successfully infecting plant cells must breach the cell wall, and they do so by mechanical and/or chemical means. Therefore, mechanically reinforced cell walls are likely to be harder to penetrate. Blaich & Grundhöfer (1998) reported that silicon accumulated at sites of attempted pathogen penetration. In contrast, Fauteux et al. (2005) suggests that such accumulation probably reflects the higher transpiration rates at infection sites, rather than active transport of silicon as part of a plant defense response. Another study by Samuel et al. (1991) disputed the role of silicon as physical barrier to pathogen penetration. When silicon application to cucumber plants was interrupted, the prophylactic effects of silicon against powdery mildew were lost, despite accumulation of silicon in the plant tissue. On the other hand, Hayasaka et al.(2008) suggested that silicon confers physical resistance to fungal appresorial penetration and physiological resistances against infection after penetration. They reported that appresorial penetration of the blast fungus on rice was significantly reduced relative to the proportion of silica level on the leaves. Appresoria that penetrated also failed to sporulate.

Fusarium wilt fungi survive in the soil in a quiescent state for many years by forming resistant spores called chlamydospores. Like many other pathogenic soilborne fungi, in order for these fungi to infect plant roots, their propagules must be stimulated by molecules present in seed and root exudates. Without the release of such stimulatory molecules, in most cases, root infections hardly take place (Nelson, 1990). Silicon can reduce amino acid and starch formation, which promote fungal pathogen growth (Takahashi, 1995). Thus, silicon may reduce or delay spore germination and fungal growth indirectly.

1.7.2 Systemic acquired resistance

Application of silicon has been reported to enhance a cascade of biochemical defense mechanisms in infected plants (Belanger et al., 1995; Epstein, 1999). Cherif et al. (1994) and Cherif et al. (1992) reported that addition of 1.7mM of silicon to nutrient solution enhanced resistance expression to *Pythium* spp. attack and the appearance of precursors of fungitoxic aglycones, possibly acting like phytoalexins. They also identified other potentially protective metabolic processes and substances inhibitory to *Pythium* spp.

Application of silicon enhanced resistance of cucumber plants to powdery mildew by increasing the antifungal activity of infected leaves. One of the low molecular weight metabolites that had antifungal activity in the leaves was identified as flavonol aglycone rhamnetin (3,5,3',4'-tetrahydroxy-7-*O*-methoxyflavone) (Fawe et al., 1998). The defense mechanisms mobilized by silicon include the accumulation of lignin, and phenolic compounds, as well as chitinases and peroxidases (Belanger et al., 1995). Based on such information, silicon-induced systemic resistance is related to the “systemic acquired resistance”, which is characterized by the accumulation of salicylic acid pathogenesis-related (PR) proteins (Belanger et al., 1995). This kind of response is typically elicited by pathogens; however, “activators” such as silicon can enhance or accelerate the response (Belanger et al., 1995; Schneider and Ullrich, 1994).

1.7.3 Alleviation of abiotic stress

Vigorously growing plants are more resistant to disease than weak and stressed plants. Silicon is known to alleviate some abiotic factors that cause stress to plants, such as: (i) heavy metal toxicity, (ii) drought stress, (iii) salinity, and (iv) heat and cold.

1.7.3.1 Heavy metal toxicity

Williams and Vlamis (1957) were the first to discover the importance of silicon in alleviating heavy metal toxicity. They grew barley, *Hordium vulgare* cv. Atlas 46, in a conventional Hoagland culture solution with 9.1 μM manganese. The leaves developed dark brown necrotic spots, which were eliminated by the addition of silicon at 137 μM (10mg ℓ^{-1}). Silicon caused even distribution of manganese in the leaves rather than being concentrated in discrete necrotic spots. Horst and Marschner (1978) also reported that manganese distribution on bean leaf blade was inhomogeneous, characterized by spot-like accumulations, even at optimal manganese levels (10^{-4} mM). This was rectified by addition of silicon to the nutrient solution. Addition of silicon increased the ability of bean plants to tolerate concentrations of manganese 10 times higher than the lowest level of manganese needed to cause toxicity effects.

The discovery of the ability of silicon to ameliorate manganese toxicity led to the findings that it could reduce the toxicity of other metals as well. Another highly toxic metallic ion at high concentration in acidic soils is aluminum. It is considered the most detrimental soil

mineral because of its presence in all soils and its high solubility under acidic conditions, especially in heavily weathered tropical soils (Epstein, 1999). Its importance in forest decline due to anthropogenic soil acidification is of a serious concern (Godbold et al., 1988). Li et al. (1989) found that cotton is highly sensitive to 200 μ M Al in solution. Addition of silicon to the solution attenuated the effect of aluminum. Barceló et al. (1993) found that teosinte, a wild form of *Zea mays* L., exposed to toxic levels of aluminum (60 μ M or 120 μ M) was less inhibited when treated with silicon as low as 4 μ M in a culture solution.

There are several mechanisms proposed by which silicon alleviates aluminum and manganese toxicity. One of the mechanisms is a direct interaction between silicon and the metals, reducing the activity of toxic metallic ions in the medium (Hiradate et al., 1998). The formation of aluminum-silicon complexes may be responsible for the protection of maize from aluminum toxicity (Ma et al., 1997). Another mechanism is the solubility of silicon in the medium. Silicon level in the soil solution shows little change over the entire physiological pH, which is in the range between 0.1-0.6mM (Epstein, 1994). However, polymerization and precipitation becomes a problem in culture solutions containing high concentrations of silicon. External factors may also be responsible in mitigating heavy metal toxicity. For example, aluminum toxicity to soybean was diminished due to precipitation of subcolloidal, inert hydroxyaluminosilicate species in solution (Baylis et al., 1994). Iron and manganese toxicity to plants was also eased by addition of silicon, mainly due to increased internal tolerance level of the plants to excess amounts of these metals, in addition to its role in reducing their absorption (Jones and Handreck, 1967; Lian, 1976; Okuda and Takahashi, 1964).

1.7.3.2 Drought stress

Soilborne plant pathogens that can grow at relatively low osmotic potentials (e.g., -10 to -15bars) and that stop growing only when the water potential is between -90 and -100 bars or less (e.g. *Fusarium* spp. (Cook in Nelson et al., 1981) cause the most disease under dry soil conditions and in plants with low osmotic potentials (Cook and Papendick, 1972; Cook and Duniway, 1987; Harris et al., 1981). According to Cook and Papendick (1972), *Fusarium* wilt fungi grow maximally at osmotic potentials between -10 and -20 bars. Although they cause the most disease where crops are irrigated or soils are wet on a regular

basis, they cease growth only when the osmotic potential is below about -120 bars. Their ability to grow at such low osmotic potentials enables them to outcompete other competitors and cause more damage on an already water stressed plant.

With some exceptions, transpiration favors silicon translocation in plants, where it often accumulates in the transpiration termini such as at leaf edges, in trichomes, specialized bulliform cells, and other sites of intense transpiration (Sangster and Hodson, 1992). On the other hand, although the findings are contradictory and inconclusive, there are reports on the effects of silicon on water relations (Matoh et al., 1986; Savant et al., 1997). Its effect on water relation is related to its ability to increase cell wall rigidity through the formation of opal once deposited (Epstein, 1994; Rafi and Epstein, 1997; Raven, 1983), the osmotic effect of silicic acid (H_4SiO_4), its tendency to form opal, and its intimate association with the cell wall matrix (Perry et al., 1987). One way or another, reduced water stress means increased plant vigour. Vigorous plants are less likely to be easily killed by pathogens such as Fusarium wilt of banana, especially in the subtropics where the disease causes more damage on stressed plants.

1.7.3.3 Salinity stress

Many coastal areas have highly saline soils that restrict the kind of plants that can thrive in those soils. Plants such as mangrove have the ability to even grow in seawater because of their special adaptation to such soil-water conditions. Salinity can also be a problem in agricultural areas far away from the coast. Stress from soil salinity can be mitigated by addition of silicon. Matoh et al. (1986) planted rice plants in hydroponic nutrient solution with addition of either NaCl, seawater, or polyethylene glycol in order to produce a salinity and osmotic pressure level equivalent to 20% seawater. Na^+ uptake and its translocation to the shoot were discouraged by the addition of 0.89mM silicon. Dry matter production also increased for the salinity stressed, but silicon treated plants, compared with the controls. Similar findings were also reported from work on barley (Liang et al., 1996) and wheat (Ahmad et al., 1992) where plants were grown in salinized solutions. Silicon amendments to the solution repressed Na^+ transport and improved plant growth over plants grown in salinized solutions but not amended with silicon.

In South Africa, farming is becoming increasingly difficult and many farmers are losing the battle. Pests and diseases are major factors to blame, in addition to poor economic fundamentals. Banana farmers are particularly desperate, with several of them already out of business because of Fusarium wilt. There is an urgency in the need to solve this critical problem to avoid the repeat of the disastrous end of the Gros Michel-based banana industry. At the ProMusa symposium held in White River, South Africa, 10-14 September 2007, the seriousness of the problem and the critical need for a solution was repeatedly articulated. Although the bean farmers are not as desperate as the banana growers, the bean yellows fungus is seriously affecting productivity. The increase in demand for organic green beans and the relatively higher prices farmers obtain for organic food are also additional factors that drive the need for alternative management options.

1.9 Reference

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CHAPTER TWO

Screening of fungal endophytes against Fusarium wilt of beans caused by *Fusarium oxysporum* f.sp. *phaseoli*

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Abstract

Several endophytic fungi and bacteria were isolated from old banana plants in banana plantations from both the South and North Coast of KwaZulu-Natal. The isolates were initially screened for their ability to reduce disease severity of Fusarium wilt/yellows of beans, caused by *Fusarium oxysporum* Schlecht. f.sp. *phaseoli*. Only a few fungal isolates reduced disease severity. Selected isolates were re-screened, and the two best isolates, identified as *F. oxysporum* Strains N7 and Strain N16, were selected for further experiments. These two isolates effectively reduced disease severity to below 25%. The pathogen treated control had disease severity levels of more than 75%. No significant difference in the efficacy as a result of different dosage or methods of application (seed treatment, dusting, drenching) of these isolates was observed. This may be because of the time advantage given to the isolates to establish before inoculating the pathogen. They readily colonized the vascular tissue of beans, maize and banana, a most important and valuable feature.

2.1 Introduction

Fusarium wilts, caused by a group of soilborne fungi known as *Fusarium oxysporum* Schlecht., are economically important soilborne diseases affecting many crops worldwide (Weller et al., 2002). The most effective chemical control of Fusarium wilt has been the widely used fumigant, methyl bromide. However, it has been banned for the hazards to the ozone layer and to food health (Anonymous, 2007; Pelley, 2002). The development of resistant cultivars is an attractive strategy against Fusarium wilts; however, the rise of new virulent strains renders resistant cultivars susceptible and often does not give sustainable control. Moreover, some crops, for example bananas, are very difficult to breed due to their sterile and triploid nature (Morpurgo et al., 1994). The use of beneficial microorganisms gives an alternative approach to the protection of plants against Fusarium wilts (Alabouvette et al., 1993).

Many attempts have been made to control *Fusarium* spp. by biological means. One of the significant phenomena in biological control of *Fusarium* wilt disease is the existence of suppressive soils in which microbial activity prevents the pathogen from establishing and/or causing damage in susceptible crops (Alabouvette et al., 1979; Baker and Cook, 1974; Kloepper et al., 1980; Scher and Baker, 1982). Such soils are naturally suppressive to Fusarium wilt, and non-pathogenic *F. oxysporum* play a key role in the antagonistic microbiota (Alabouvette and Couteaudier, 1992; Rouxel et al., 1979; Smith and Snyder, 1971; Tamietti et al., 1993). The interaction between pathogenic and non-pathogenic *Fusarium* strains in those soils result in natural suppression of the disease (Larkin et al., 1993; Larkin et al., 1996; Magie, 1980; Paulitz et al., 1987; Schneider, 1984).

More attention has been given to the importance of biological factors in soils naturally suppressive to *Fusarium*, such as the work of Smith and Snyder (1971), especially to the saprophytic soil microorganisms including nonpathogenic *Fusarium* spp. In these soils, suppressiveness was eliminated when soil was treated with moist heat, methyl bromide or gamma irradiation (Alabouvette, 1986; Scher and Baker, 1980), and was transferable. This suggested that microbial activity was the reason for the disease suppressive properties of these soils. Large populations of non-pathogenic *Fusarium* species were reported in soils naturally suppressive to Fusarium wilt by Toussoun (1975) and it was also reported by Louvet et al. (1976) that soils from Chateaubriand, France, supported high populations of

F. oxysporum and *F. solani* Snyder & Hansen. Using a simple form of Koch's postulates, the role of nonpathogenic populations of *Fusarium* spp. in the suppression of Fusarium wilts in these soils was confirmed when the soil was heat treated to >55°C, resulting in the loss of suppressiveness. Suppressiveness was restored when the soil was amended by artificial introduction of strains of nonpathogenic *F. oxysporum* or *F. solani* to the heat treated soil (Rouxel et al., 1979). Many other authors from different parts of the world have also reported on the role of non-pathogenic *Fusarium* species in Fusarium-suppressive soils (Larkin et al., 1993; Larkin et al., 1996; Paulitz et al., 1987; Schneider, 1984).

Nonpathogenic *Fusarium* spp. are known to protect plants from Fusarium wilt pathogens through their ability to compete with pathogenic fusaria for nutrients such as organic carbon and iron, and/or for infection sites at the rhizoplane (Alabouvette and Couteaudier, 1992; Lemanceau and Alabouvette, 1991; Lemanceau et al., 1993; Olivain and Alabouvette, 1997). The induction of resistance to Fusarium wilt have also been reported, as in the case of cucumber (Mandeel and Baker, 1991) and chickpea (Hervas et al., 1995), or formae speciales of *F. oxysporum*, such as f.sp. *melonis* Snyder & Hansen in cucumber (Gessler and Kuc, 1982) and f.sp. *dianthi* (Prill. and Del.) Snyder and Hansen in tomato (Kroon et al., 1991) using nonpathogenic strains of *F. oxysporum*. Recently, nonpathogenic *F. oxysporum* Strain Fo47 was shown to induce resistance to Fusarium wilt in tomato (Fuchs et al., 1997).

Strains of non-pathogenic *Fusarium* species differ considerably in their efficacy to suppress Fusarium wilt (Alabouvette et al., 1993; Larkin and Fravel, 1998; Larkin and Fravel, 1999). As such, screening of many isolates in order to obtain efficient ones is crucial. Furthermore, according to Larkin and Fravel (1998; 1999), strains of non-pathogenic *Fusarium* species not only differ in their efficacy but also in their mode of action(s) and the dosage required to suppress disease. For instance, *F. oxysporum* Strain CS-20 required as little as 100 chlamydospores per gram of soil to significantly suppress disease while Strain Fo47 required as many as 10^4 - 10^5 chlamydospores g⁻¹ of soil to effectively suppress disease.

In this study several nonpathogenic strains of *Fusarium* species isolated from healthy banana plants were screened against *F. oxysporum* f.sp. *phaseoli* Kendrick & Snyder on green beans under greenhouse conditions to find the best isolates. Because nonpathogenic

Fusarium spp. are not known for their antibiotic or mycoparasitic effect (Fravel et al., 2003), no *in vitro* screening on Petri dishes was conducted.

2.2 Materials and methods

2.2.1 Source of plant material

Bean seeds of a susceptible cultivar, Outeniqua, provided by Pro-Seed[®] cc¹ was used throughout the experiments.

2.2.2 Isolation of endophytes

Fourty three fungal and 21 bacterial endophytes were isolated from the xylem area of healthy banana roots and corms, obtained from banana farms on the North Coast and South Coast area of KwaZulu-Natal. The roots and corms were surface sterilized with 3.5% sodium hypochlorite for five minutes and subsequently rinsed three times with sterile distilled water. Roots and corms were cut into pieces and blended in a sterile Waring Commercial blender. To isolate fungal endophytes the juice was then plated onto Potato Dextrose Agar (PDA) (Merck) supplemented with chloramphenicol and streptomycin sulphate to inhibit bacterial growth, and Nash and Snyder Medium (Nash and Snyder, 1962), and incubated at 25-27°C. Bacterial endophytes were isolated by plating the juice onto Tryptone Soy Agar (TSA) (Merck) and incubated at 30°C. Isolated colonies of the fungi and bacteria were subcultured onto Potato Carrot Agar (PCA) and Nutrient Agar (NA) (Merck), and stored in 50% and 15% glycerol, respectively, at -80°C. PCA was prepared as follows: A grade potatoes, 20 g, carrots, 20 g, agar, 15 g and 1 ℓ of tap water. Potatoes and carrots were grated and cooked for 30min. The juice was then filtered through cheesecloth without straining. The agar was added to the filtrate and autoclaved at 121°C for 15 min.

2.2.3 Isolation of the pathogen

Fusarium oxysporum f.sp. *phaseoli* was isolated from diseased bean plants from Ukulinga Farm, Pietermaritzburg, South Africa. The plants were surface sterilized using 3.5%

¹ Pro-Seed cc, P.O.Box 101477, Scottsville 3209, Pietermaritzburg, South Africa

sodium hypochlorite for 5 min and subsequently rinsed three times with sterile distilled water. Small disks of the stem cut in cross section were placed on Nash and Snyder Medium and incubated at 27°C for 5 d. *Fusarium oxysporum* f.sp. *phaseoli* colonies were then subcultured on to PCA and stored in 50% glycerol at -80°C.

2.2.4 Production of inoculum of fungal endophytes and the pathogen

Inoculum of the fungi was produced by growing them on barley grains. Fifty grams of barley grains were soaked overnight in 250 ml Erlenmeyer flask containing a 100 ml of water. The excess water was drained off and the flasks were sterilized consecutively for two days at 121°C for 20 min. Each flask was then inoculated with three disks of a fungus grown on PDA for 3 d at 27°C in the dark. After two weeks at 27°C the grains were air-dried, milled using a coffee grinder and stored at 4°C.

2.2.5 Production of inoculum of endophytic bacteria

Endophytic bacteria were grown in 250 ml Erlenmeyer flasks each containing a 100 ml nutrient broth (Merck) for 4 d at 30°C in a water bath (Gesellschaft für Labortechnik (GFC), mbH, D-30938, Burgwedel, Germany) shaker at 150 oscillations min⁻¹. Flasks were inoculated with bacteria previously grown in TSA for 48 h. After 4 d bacteria were harvested by centrifugation in a Beckman J2-HS Centrifuge at 9000 rpm for 15 min. The broth was decanted and the bacterial pellets were resuspended in sterile distilled water. The bacterial cells were then counted using a plate dilution technique in a TSA and adjusted to a concentration of 10⁷ c.f.u. ml⁻¹ of water.

2.2.6 Preliminary screening of fungal endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

The fungal and bacterial isolates were tested against *F. oxysporum* f.sp. *phaseoli* in a greenhouse with temperatures ranging from 26-28°C. Beans were planted in 150 mm pots with composted pine bark as a sole growing medium. Soils were drenched with a 50 ml inoculum of 10⁷ c.f.u. ml⁻¹ of fungal and bacterial endophytes after planting. After germination, plants were drenched with 50 ml inoculum of 10⁷ c.f.u. ml⁻¹ of *F. oxysporum* f.sp. *phaseoli*. Irrigation, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio, was supplied three times a day for 3 min. The experiment was

arranged in a randomized complete block design replicated three times. Two months later plants were rated for disease severity, and dry weight was taken after the biomass was dried in an oven for 72 h at 70°C.

2.2.7 Secondary screening of endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

Nine selected, non-pathogenic *Fusarium* isolates were tested against *F. oxysporum* f.sp. *phaseoli* in a greenhouse with temperatures ranging from 26-28°C. Beans were planted in 150 mm pots with composted pine bark as a sole growing medium. Soils were drenched with 50 ml inoculum of 10^7 c.f.u. ml⁻¹ after planting. After germination plants were drenched with 50 ml inoculum of 10^7 c.f.u. ml⁻¹ of *F. oxysporum* f.sp. *phaseoli*. Irrigation, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio, was supplied three times a day for 3 min. The experiment was arranged in a randomized complete block design replicated six times. Two months later plants were rated for disease severity, and dry weight was taken after the biomass was dried in an oven for 72 h at 70°C.

2.2.8 Method of application of non-pathogenic *Fusarium oxysporum*

The selected non-pathogenic *F. oxysporum* Strain N7 and Strain N16 were applied in three different ways: (1) seed treatment - seeds were soaked in a suspension of 5×10^9 c.f.u. ml⁻¹ of non-pathogenic *F. oxysporum* isolates supplemented with 2% carboxymethyl cellulose (CMC) as a sticker and immediately dried in a Laminar Flow overnight; (2) drenching – each pot was drenched with 50 ml suspension of the non-pathogenic *F. oxysporum* isolates, with a concentration of 10^7 c.f.u. ml⁻¹ on planting; (3) dusting – seeds were dusted with 0.1 g of non-pathogenic *F. oxysporum* isolates with a concentration of 10^8 c.f.u. g⁻¹ on planting. After germination plants were inoculated with 50 ml inoculum of 10^7 c.f.u. ml⁻¹ of *F. oxysporum* f.sp. *phaseoli*. Irrigation, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio, was supplied three times a day for 3 min. The experiment was arranged in a factorial 2×3 factorial in a randomized complete block design replicated six times. Two months later plants were rated for disease severity, their fresh green pods were harvested and weighed immediately, and dry weight was taken after the biomass was dried in an oven for 72 h at 70°C.

2.2.9 Concentration of non-pathogenic *Fusarium oxysporum*

The selected non-pathogenic *F. oxysporum*, Strain N7 and Strain N16, were applied by drenching to the pots on planting with three different concentrations: 10^6 , 10^7 or 10^8 c.f.u. ml⁻¹. After germination, the plants were inoculated with a 50 ml spore suspension of *F. oxysporum* f.sp. *phaseoli* with a concentration of 10^7 c.f.u. ml⁻¹. Irrigation, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio, was supplied three times a day for 3 min. The experiment was arranged in a 2×3 factorial in randomized complete block design replicated six times. Two months later plants were rated for disease severity, their fresh green pods were harvested and weighed immediately, and dry weight was taken after the biomass was dried in an oven for 72 h at 70°C.

2.2.10 Disease Rating

Disease severity was recorded based on visual assessment of symptoms arising from infection of by *F. oxysporum* f.sp. *phaseoli* according to a five-point key (Carver et al., 1996):

- 0 = healthy plants
- 1 = initial signs of wilting (yellowing)
- 2 = up to 25% of the leaves with symptoms
- 3 = up to 50% of the leaves with symptoms
- 4 = up to 75% of the leaves with symptoms
- 5 = plants dead

The disease severity index was calculated as follows (Mak et al., 2004; Zhang et al., 1996):

$$\text{Disease severity index (DSI)} = \frac{\sum (\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum (\text{Number of treated seedlings})}$$

2.2.11 Statistical Analysis

Experiments were repeated twice, unless otherwise stated. Data was analyzed using GenStat[®] Executable release 9 Statistical Analysis Software. Analysis of variance (ANOVA) was used to analyse the biocontrol experiments to determine treatment effects. Significant difference between treatments was determined using Fisher's protected least

significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

2.3 Results

2.3.1 Preliminary screening of fungal endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

Forty three endophytic fungal isolates, applied by drenching conidial and mycelial suspensions to beans seeds, were tested for their potential to reduce disease severity of Fusarium wilt of beans, caused by *F. oxysporum* f.sp. *phaseoli*. Of all the isolates tested, only 16% of the endophytes very significantly ($P < 0.001$) reduced disease severity to less than 2.5; and 11.6% reduced disease severity very significantly ($P < 0.001$) to less than 2 on the scale. On the contrary, 18% of the isolates increased disease severity as compared to the *F. oxysporum* f.sp. *phaseoli* only inoculated control, though not significantly. Of those isolates which reduced disease severity very significantly ($P < 0.001$) to less than 2.5, 71% were isolated from banana fields on the North Coast area of KwaZulu-Natal, South Africa. The North Coast area is virtually free from Fusarium wilt of banana so far. Results on preliminary screening of fungal endophytes are presented in Table 3.1 and Figure 3.1-3.2.

Only three of the 43 isolates or 6.9% of the isolates caused very significantly ($P < 0.001$) higher dry matter. The rest of the isolates caused no significant increase in dry matter (Table 3.1; Figure 3.2).

Table 3.1. Preliminary screening of fungal endophytes against *Fusarium oxysporum* f.sp. *phaseoli*, causal agent for *Fusarium* wilt of beans

Fungal endophytes	DSI		Dry weight	
N1	5.00	j	1.73	abcdef
N2	5.00	j	1.91	abcdef
N3	2.75	bcdef	2.92	defghi
N4	3.75	efghij	2.38	abcdefg
N5	3.00	cdefg	3.15	fghi
N6	2.50	abcde	1.97	abcdef
N7	1.75	abc	3.91	hi
N8	4.00	fghij	2.48	abcdefgh
N11	4.75	ij	1.29	abc
N12	4.25	ghij	1.59	abcde
N13	4.25	ghij	2.18	abcdefg
N15	4.00	fghij	1.63	abcdef
N16	1.25	a	4.34	i
N17	4.25	ghij	1.71	abcdef
N18	1.91	abc	1.41	abcd
N19	4.75	ij	2.31	abcdefg
N20	4.50	hij	1.33	abc
N21	2.00	abcd	2.31	abcdefg
N22	5.00	j	1.89	abcdef
N23	4.75	ij	2.68	cdefgh
N24	5.00	j	1.74	abcdef
N25	4.00	fghij	2.67	cdefgh
N26	4.50	hij	2.25	abcdefg
N27	5.00	j	2.13	abcdef
N28	3.75	efghij	1.86	abcdef
N29	4.75	ij	1.13	ab
S1	4.25	ghij	1.64	abcdef
S2	3.25	defgh	2.21	abcdefg
S3	5.00	j	1.63	abcdef
S4	5.00	j	1.06	a
S5	4.25	ghij	2.17	abcdefg
S6	3.75	efghij	1.45	abcd
S7	3.50	efgh	3.66	ghi
S8	5.00	j	2.21	abcdefg
S9	2.75	bcdef	2.45	abcdefgh
S10	4.25	ghij	2.49	abcdefgh
S11	3.25	defgh	3.08	efghi
S12	4.25	ghij	1.86	abcdef
S13	3.03	cdefg	1.61	abcde
S14	3.50	efghi	2.09	abcdef
S15	4.50	hij	2.55	abcdefgh
S16	1.50	ab	2.65	bcdefgh
S17	1.50	ab	1.56	abcde
<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	4.00	fghij	1.82	abcdef
F pr.	<0.001		0.014	
l.s.d.	1.2804		1.524	
s.e.d.	0.6470		0.770	
cv%	24.1		50.4	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

- N = Isolates from the North Coast; S = Isolates from the South Coast

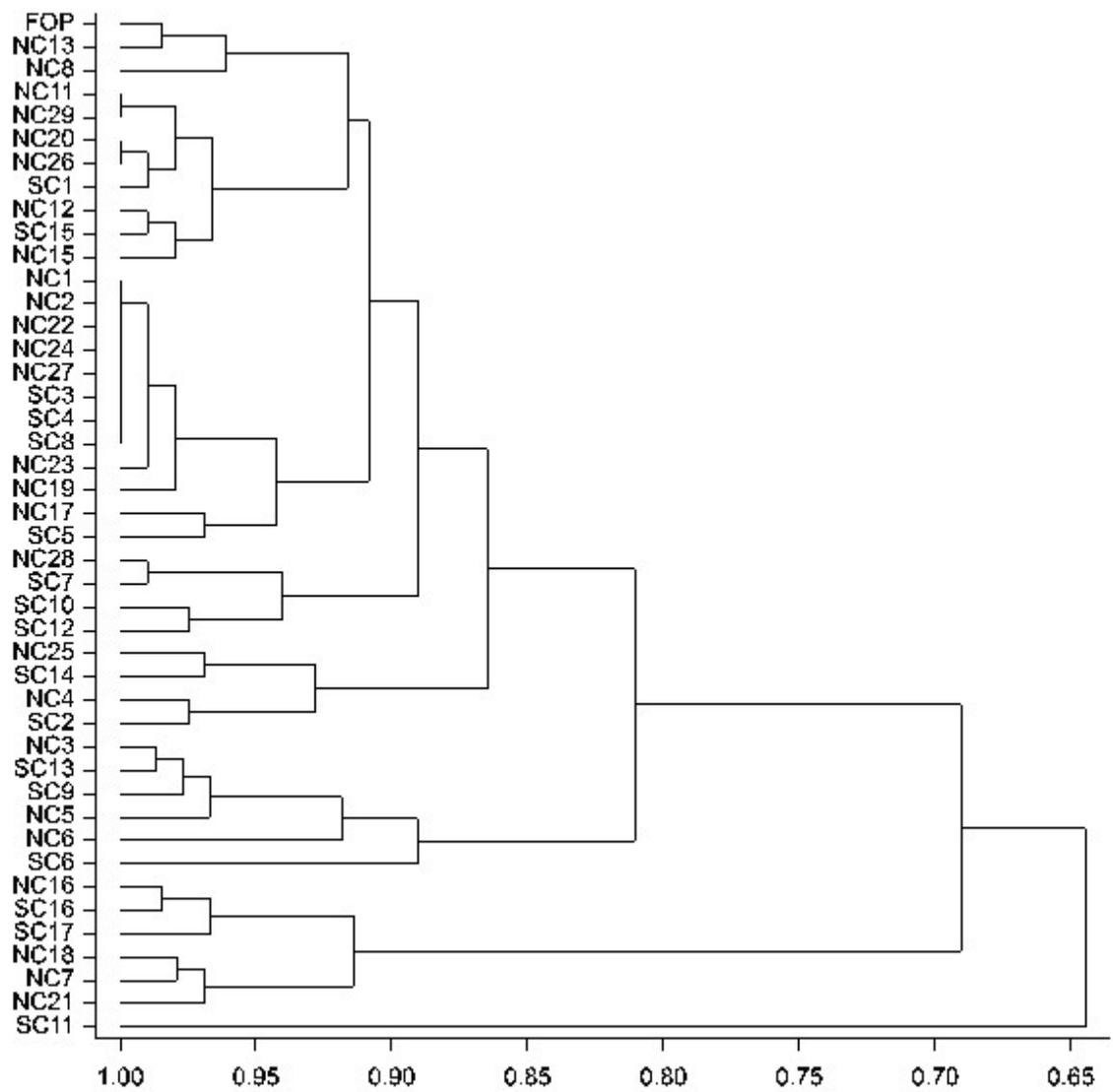


Figure 3.1 Dendrogram of fungal endophytes groupings based on disease severity index of bean plants treated with the endophytes

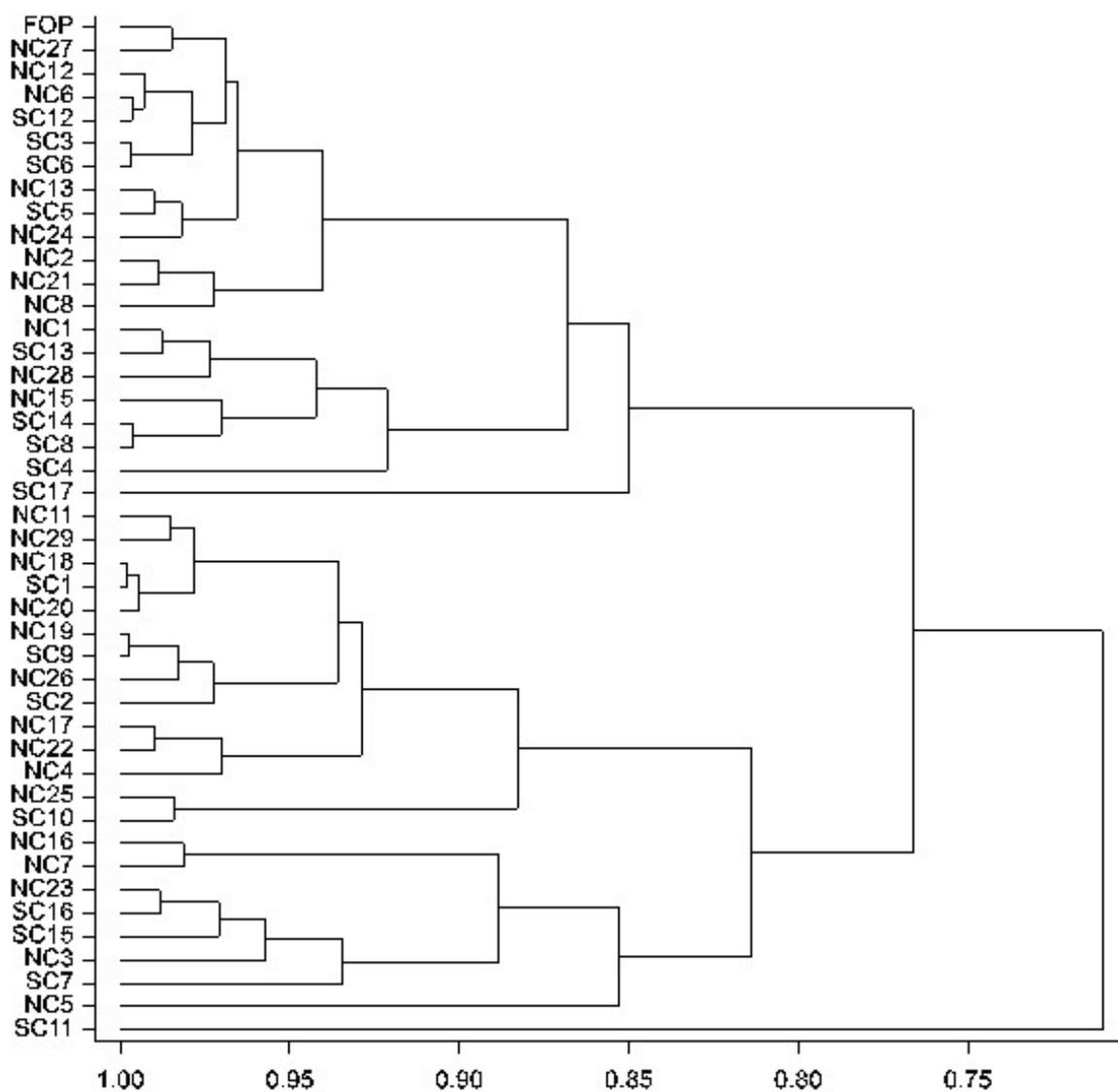


Figure 3.2 Dendrogram of fungal endophytes groupings based on dry biomass of bean plants treated with the endophytes

2.3.2 Preliminary screening of bacterial endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

None of the bacterial endophytes tested against *F. oxysporum* f.sp. *phaseoli* on beans decreased disease severity to below ‘3’ on the scale (Table 3.2). Although two isolates caused a significant reduction in disease severity as compared to the *F. oxysporum* f.sp. *phaseoli* inoculated control (P<0.001), the disease severity observed was still high, up to 50% (Table 3.2).

Table 3.2 Preliminary screening of bacterial endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

Bacterial endophytes	DSI		Dry weight (g)	
SB1	4.50	ab	0.33	d
SB10	5.00	b	0.12	ab
SB11	5.00	b	0.21	abcd
SB12	3.50	ab	0.15	abc
SB13	4.00	ab	0.20	abcd
SB14	4.75	ab	0.09	a
SB15	4.00	ab	0.18	abcd
SB16	5.00	b	0.18	abcd
SB17	4.00	ab	0.11	ab
SB18	3.00	a	0.24	abcd
SB19	4.50	ab	0.10	ab
SB2	3.25	ab	0.27	bcd
SB20	5.00	b	0.25	abcd
SB21	5.00	b	0.26	abcd
SB3	4.00	ab	0.21	abcd
SB4	3.25	ab	0.21	abcd
SB5	4.25	ab	0.34	d
SB6	5.00	b	0.18	abcd
SB7	4.75	ab	0.17	abcd
SB8	3.00	a	0.31	cd
SB9	3.75	ab	0.31	cd
Control (no pathogen)	0.25	a	0.75	e
<i>F. oxysporum</i> f.sp. <i>phaseoli</i> only	5.00	b	0.21	abcd
F pr.	<0.001		<0.001	
s.e.d.	0.888		0.088	
l.s.d.	1.773		0.175	
cv%	30.8		53.2	

- Means with the same letter in the same column are not significantly different at P = 0.05

2.3.3 Secondary screening of fungal endophytes against *Fusarium oxysporum* f.sp. *phaseoli*

Secondary screening was carried out using nine fungal endophytes, five isolated from the North Coast and four from the South Coast area, selected from preliminary screening trials. Although isolates were stored by freezing them at -80C protected by 50% glycerol, most lost their ability to reduce disease severity to the level obtained during the preliminary screening trials. All isolates except *Fusarium* sp. Strain S9 significantly reduced disease severity, but high levels of disease still developed, compared to the *F. oxysporum* f.sp. *phaseoli* only inoculated control. However, *Fusarium oxysporum* Strain N7 Accession # 08296 and Strain N16 Accession # 08297 significantly ($P < 0.001$) reduced disease severity to 2 and 1.5 on the scale, respectively (Table 3.3, Figure 3.3). There was no significant ($P < 0.001$) difference in disease severity reduction between these two isolates.

All isolates significantly ($P = 0.011$) increased fresh green pod weight compared to the *F. oxysporum* f.sp. *phaseoli* only inoculated control. *Fusarium oxysporum* Strain N7 and Strain N16 caused significantly higher fresh green pod weight compared to the other isolates tested ($P = 0.011$). These isolates increased fresh green pod weight more than six fold of the pathogen only inoculated control. Similarly, the two best isolates caused a significant ($P = 0.003$) increase in dry weight compared to the pathogen only treated control and the other isolates except Strains N6 and S17. Results for secondary screening of fungal endophytes are presented in Table 3.3 and Figure 3.3.

Table 3.3. Secondary screening of selected endophytic *Fusarium* isolates against *Fusarium oxysporum* f.sp. *phaseoli*

Fungal endophytes	DSI		Pod weight		Dry weight	
N3	3.25	cde	159	c	30.4	ab
N5	3.00	cd	204	c	33.5	b
N6	2.50	bc	194	c	35.9	bc
N7	2.00	ab	343	d	54.5	c
N16	1.50	a	295	d	54.2	c
S9	4.00	ef	102	b	25.3	ab
S13	3.50	de	153	bc	34.7	b
S16	3.50	de	153	bc	23.8	ab
S17	3.00	cd	181	c	38.7	bc
<i>F. oxysporum</i> f.sp. <i>phaseoli</i> only	4.75	f	49	a	13.8	a
F pr.	<0.001		0.011		0.003	
l.s.d.	0.8561		52.8		18.64	
s.e.d.	0.4172		68.4		9.07	
cv%	18.7		52.8		37.2	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

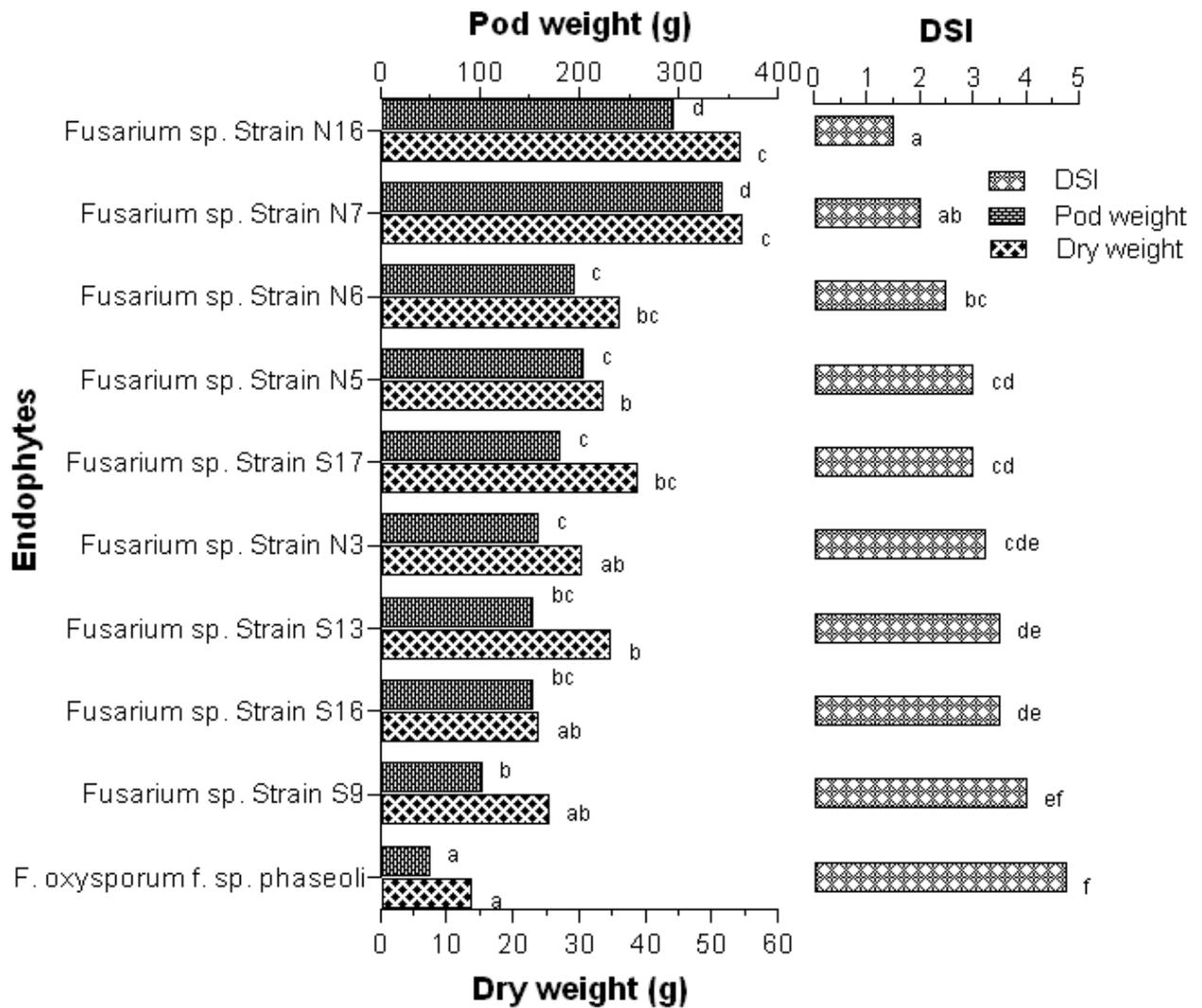


Figure 3.3 Secondary screening of selected endophytic *Fusarium* isolates against *Fusarium oxysporum* f.sp. *phaseoli*, pooled mean of three trials

2.3.4 Effect of method of application of selected endophytic non-pathogenic *Fusarium oxysporum* on the control of *Fusarium oxysporum* f.sp. *phaseoli*

The two selected isolates, *F. oxysporum* Strain N7 and Strain N16, were tested for their response to three different methods of applications: seed treatment, drenching with spore suspensions after planting and dusting propagules on top of the seeds during planting. Although there was a significant ($P=0.027$) difference in reducing disease severity between the two isolates, there was no significant difference between the three different methods of applications used, either on disease severity ($P=0.308$), fresh green pod weight ($P=0.531$) or dry weight ($P=0.597$) (Table 3.4). There was also no interaction between the endophytes and their method of application for all three parameters measured (Table 3.4).

Table 3.4 The effect of method of application of selected endophytic non-pathogenic *Fusarium oxysporum* on the control of *Fusarium oxysporum* f.sp. *phaseoli*

Source	Level	DSI	Pod weight (g)	Dry weight (g)
Main effect				
Endophytes		$p=0.027$	$p=0.284$	$p=0.549$
	Non-pathogenic <i>Fo</i> * N16	1.306a	192a	59.3a
	Non-pathogenic <i>Fo</i> * N7	1.694b	221a	57.2a
Application method		$p=0.308$	$p=0.531$	$p=0.597$
	Seed treatment	1.33a	191a	57.2a
	Dusting	1.542a	227a	56.7a
	Drenching	1.625a	201a	60.8a
Endophytes × Application method		$p=0.133$	$p=0.474$	$p=0.292$

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

- **Fo* = *Fusarium oxysporum*

2.3.5 Effect of dosage of selected endophytic non-pathogenic *Fusarium oxysporum* on the control of *Fusarium oxysporum* f.sp. *phaseoli*

The two selected isolates showed significant ($P=0.015$) response to dosage in reducing disease severity where application at a rate of 10^7 c.f.u. ml⁻¹ reduced disease severity to 0.71 (Table 3.5). There was a significant ($P=0.015$) difference between applications at a rate of 10^7 c.f.u. ml⁻¹ and 10^6 c.f.u. ml⁻¹. However, there was no significant difference between applications at a rate of 10^7 c.f.u. ml⁻¹ and 10^8 c.f.u. ml⁻¹, although disease severity at 10^8 c.f.u. ml⁻¹ was higher than at 10^7 c.f.u. ml⁻¹, and between 10^6 c.f.u. ml⁻¹ and 10^8 c.f.u. ml⁻¹ (Table 3.5). Pod weight ($P=0.834$ and $P=0.546$) and dry weight ($P=0.669$ and $P=0.647$) did not show significant differences across the isolates or their dosage, respectively (Table 3.5). No significant change was observed on either disease severity ($P=0.189$), fresh green pod weight ($P=0.259$) or dry weight ($P=0.259$) as a result of interactions between isolates and dosage (Table 3.5).

Table 3.5. The effect of inoculum dosage of selected endophytic non- pathogenic *Fusarium oxysporum* on the control of *Fusarium oxysporum* f.sp. *phaseoli*

Source	Level	DSI	Pod weight (g)	Dry weight (g)
Main effect				
Endophytes		$p=0.109$ NS	$p=0.834$ NS	$p=0.669$ NS
Non-pathogenic <i>Fo</i> N16		1.14a	276a	72.64a
Non-pathogenic <i>Fo</i> N7		1.64a	267a	69.10a
Dosage		$p=0.015$	$p=0.546$ NS	$p=0.647$ NS
	10^6	2.04b	239a	66.01a
	10^7	0.71a	276a	71.19a
	10^8	1.42ab	300a	75.41a
Endophytes × Dosage		$p=0.189$ NS	$p=0.259$ NS	$p=0.774$ NS

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

- NS = Non-significant at $P \leq 0.05$

2.4 Discussion

Isolation and screening for potential biocontrol organisms are crucial steps in biocontrol research to obtain efficient antagonists for the biocontrol of plant diseases. There is a need to develop simple, inexpensive and time-efficient procedure with repeatable and reliable results. For instance, an *in vitro* screening procedure which provides rapid and *in situ* repeatable results is an important step in screening efficient antagonists for biocontrol of plant diseases (Anith et al., 2003). However, such procedures are not readily available.

In this study biocontrol organisms were isolated from inside plant tissue that is normally a niche for a *Fusarium* wilt pathogen as well. Endophytes were chosen because rhizosphere biocontrol agents cannot reach the pathogen once it is inside the vascular tissue of the plant where it causes the most damage. Two banana production areas were selected for isolation of the beneficial microorganisms. The North Coast of KwaZulu-Natal is virtually free of Panama disease, either because the pathogen has not been introduced, or because it is being suppressed for some reason. The other site selected was the South Coast of KwaZulu-Natal where Panama disease has caused widespread damage to the banana plantation there. Healthy uninfected plants were identified in plantations and samples of plant tissue, root and corm were taken and isolations were made.

From our field observations in the South Coast and pathogenicity tests done in the greenhouses (data not shown), the banana plants take several months to show symptoms of disease after infection with *F. oxysporum* f.sp. *cubense*. Therefore, screening of the isolated biocontrol agents using banana plants was considered too time consuming for a three year PhD study. As a result, screening of the selected biocontrol isolates were carried out on an important crop, green bean, against a pathogen, *F. oxysporum* f.sp. *phaseoli*, which is in the same species complex as Panama wilt fungus, *F. oxysporum* f.sp. *cubense*. This disease is important for the bean growers in South Africa and other parts of Africa. Although the biocontrol fungi and bacteria were isolated from banana plants, they readily colonized the vascular tissues of bean and maize plants as well. The ability to colonize tissues of plants other than the one it was isolated from is an important characteristic required in the commercial development of an endophytic biocontrol organism.

Competitive endophytes are not known to use antibiosis or mycoparasitism (Fravel et al., 2003). Therefore, *in vitro* dual culture tests on Petri dishes were not conducted. Instead the biocontrol organisms were tested against the pathogen using target plants in greenhouse pot experiments. Of all the isolates tested, only a few reduced disease severity to below 25%. The bacterial isolates only managed to reduce disease severity to below 50%. Most of them did not reduce disease severity at all. Such ineffectiveness of most of the isolates may be because of a change in environment, and because they are selective in the kind of plants they can live in. Some fungal endophytes were observed to increase disease severity more than the wilt-pathogen treated control. Peters and Grau (2002) observed such a phenomenon when non-pathogenic *F. solani* was co-inoculated with *Aphanomyces euteiches* Drechsler onto pea seedlings. However, the two non-pathogenic fungal isolates selected after secondary screening, *F. oxysporum* Strain N7 and Strain N16, were consistently effective against *F. oxysporum* f.sp. *phaseoli*, reducing disease severity to less than 25%. Although the number of effective isolates obtained was small, the ability of these isolates to live outside their normal niche is an added advantage, enabling the microorganisms to be effectively used on other crops against Fusarium wilt diseases. These strains effectively colonized the vascular tissues of banana, beans and maize (data not shown). The well researched non-pathogenic *F. oxysporum* Strain Fo47, first isolated from a soil suppressive to Fusarium wilt at Châteaurenard, France, has been reported to effectively colonize and reduced Fusarium wilt and Pythium diseases in carnation

(Lemanceau et al., 1992), cucumber (Benhamou et al., 2002), eucalyptus (Salerno et al., 2000), and tomatoes (Olivain et al., 2006).

The two non-pathogenic *F. oxysporum* strains, N7 and N16, did not respond to differences in the method of applications used, and were not very sensitive to dosage of application. This may be attributed to their ability to induce resistance, and to the time advantage they were given to establish themselves in the vascular tissue of the plant before the pathogen was inoculated. Farmers' of banana growers and other horticultural crops planted via TC plantlets can easily exploit the time advantage, unlike green bean growers. The TC plantlets can be inoculated with the endophytes before they are transplanted into the field to allow the endophytes to establish themselves in the plant vascular system.

2.5 References

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CHAPTER THREE

Silicon uptake and distribution, and its effect on Fusarium wilt of banana and bean plants

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Abstract

Bean (*Phaseolus vulgaris* L.) and banana (*Musa* sp. L.) plants were grown under greenhouse conditions. They were submitted to a wide range of silicon fertilization levels, 0-2000 mg ℓ^{-1} , to quantify their silicon uptake and distribution, as well as to determine the effect of silicon on disease severity of Fusarium wilt of these crops. Silicon uptake differed with different levels of silicon supply and its distribution in the different plant parts varied widely. Initially bean plants accumulated the highest amounts of silicon in the leaf followed by the root. However, with high levels of silicon fertilization, silicon deposition in the root exceeded that of the leaf. The stem accumulated the lowest levels and the levels did not change with increased silicon supply. Similarly, in banana plants, silicon distribution at low silicon fertilization levels was: leaf > root > petiole \approx midrib > pseudostem > corm. Silicon levels in the root increased with increased silicon fertilizer levels and exceeded the silicon levels in the leaf. In both banana and bean plants, leaf silicon levels saturated quickly and remained steady, regardless of the increase in soil silicon level. Silicon applications significantly reduced disease severity of Fusarium wilt of both banana and beans.

3.1 Introduction

Plants take up and accumulate silicon in amounts equivalent to those of certain macronutrients such as calcium, magnesium and phosphorus (Epstein, 1999). Plants take up silicon mostly in the form of uncharged silicic acid [$\text{Si}(\text{OH})_4$] molecules when the pH of the soil solution is below nine (Ma and Takahashi, 2002). Different plant species have different abilities to take up and accumulate silicon, ranging from 0.1% to 10.0% silicon (dry weight) (Epstein, 1999; Ma and Takahashi, 2002; Richmond and Sussman, 2003). Angiosperms that belong to the commelinoid monocots of the orders Poales and Arecales accumulate considerably more silicon in their shoots than do species from the other monocot clades; and within the order Poales, species from the Graminae and Cyperaceae families accumulate high levels of silicon (Ma and Takahashi, 2002). On the other hand, most other plants, particularly dicots, are unable to accumulate such high levels of silicon in their shoots. Such difference is believed to be due to the difference in the ability of their roots to take up silicon (Ma and Takahashi, 2002).

Different plant parts accumulate different levels of silicon (Henriet et al., 2006). It is assumed that such differences are associated with silicon uptake mechanisms used in plants. It has been widely accepted that silicon is transported via the transpiration stream and all sink tissues along the xylem path passively unload silicon (Jones and Handreck, 1967). Such an assumption would indicate that large amounts of silicon should be deposited in plant organs containing numerous stomatal openings, such as leaf lamina. However, plant roots accumulate substantial amounts of silicon (Henriet et al., 2006) although they are not furnished with stomata or active transpiration organs.

Silicon is especially important in reducing the impact of soilborne pests and diseases and buffering against abiotic stresses. It reportedly benefits plants in a number of ways. Some of these benefits so far known for some crops (e.g. rice) include: (i) increasing canopy photosynthesis by keeping leaves erect, (ii) increasing resistance to fungi, bacteria, and insects, (iii) reducing the toxicity of heavy metals, (iv) reducing cuticular transpiration, and (v) improving water use efficiency (Epstein, 1999; Rodrigues et al., 2001; Yoshida et al., 1962). Although silicon is not widely accepted as an essential element, its beneficial effects on growth, development, yield and disease resistance have been observed in a wide variety of plants (Ma, 2004) such as rice, sugar cane, most other cereals and several

dicotyledons (Belanger et al., 1995; Jones and Handreck, 1967; Savant et al., 1997). In the 1930s and 1940s Japanese researchers first showed that silicon can effectively control plant diseases, especially in rice (Kozaka, 1965; Suzuki, 1935). They showed that application of various sources of silicon to silicon-deficient paddy soils considerably reduced the incidence and severity of rice blast disease, caused by *Magnaportha grisea* (Hebert) Yaegashi and Udagawa, and brown spot, caused by *Cochliobolus miyaheanus* (Ito and Kuribayashi in Ito) Drechs. Ex Dastur (Ma and Takahashi, 2002; Okuda and Takahashi, 1965; Volk et al., 1958). Other rice diseases suppressed by silicon fertilization are leaf scald, caused by *Gerlachia oryzae* (Hashioka and Yokogi) Gams, sheath blight, caused by *Rhizoctonia solani* (Frank) Donk, and stem rot, caused by *Magnaporthe sahinii* (Cattaneo) Krause and Webster (Savant et al., 1997). Belanger et al. (1995) reported that foliar and soilborne diseases of cucumber and other cucurbits can be suppressed by applying silicon.

To our knowledge, there are few, if any, studies conducted on the effect of silicon on Fusarium wilt diseases in general and Fusarium wilts of banana and beans, in particular. In this study, these two agriculturally important crops, banana, a monocot, and beans, a dicot, were used. These two crops are seriously affected by vascular diseases caused by fungi in the *Fusarium oxysporum* Schlecht. species complex. The Fusarium wilt pathogen can survive for several decades in soil. Once a farm is infested there is no commercially viable way to eliminate the pathogen from the soil (Smith et al., 2005). The banana industry is particularly under threat from this fungus known as *F. oxysporum* f.sp. *cubense* (Smith) Snyder & Hansen. Fusarium wilt of beans, caused by *F. oxysporum* f.sp. *phaseoli* Kendrick & Snyder, is also an important disease, affecting bean production worldwide. The objectives of this study were to determine the level and the distribution of silicon in the different plant parts and to determine any correlation between silicon uptake and disease reduction.

3.2 Materials and methods

3.2.1 Silicon

Silicon was applied in the form of liquid potassium silicate Product K2550 containing 20.5-20.9% SiO₂. It was provided by PQ Corporation (Pty) Ltd².

3.2.2 Source of plant materials

Bean seeds (*Phaseolus vulgaris* L.) of the cultivar Outeniqua were provided by ProSeed cc (Pty) Ltd³. Tissue culture banana plantlets of the cultivar Cavendish Williams were provided by DuRoi Laboratory⁴ at 50 mm in length.

3.2.3 Isolation of pathogens

Fusarium oxysporum f.sp. *cubense* was isolated from a diseased banana plant from the South Coast of KwaZulu-Natal while *F. oxysporum* f.sp. *phaseoli* was isolated from diseased bean plants from Ukulinga Farm, Pietermaritzburg, South Africa. The plants were surface sterilized using commercial bleach containing 3.5% sodium hypochlorite for 5 min and were subsequently rinsed three times with sterile distilled water. Small disks of the corm/stem cut in cross-section were placed on Nash and Snyder Medium (Nash and Snyder, 1962) and incubated at 27°C for 5 d. Colonies were then subcultured on to Potato Carrot Agar (PCA). PCA was prepared as follows: A grade potatoes, 20 g, carrots, 20 g, agar, 15 g and 1 l of tap water. Potatoes and carrots were grated and cooked for 30min. The juice was then filtered through cheesecloth without straining. The agar was added to the filtrate and autoclaved at 121°C for 15 min. Mycelial and conidial suspensions of the fungi grown on PCA were freeze-stored in Eppendorf tubes at -80°C, supplemented with 50% glycerol, until further use.

3.2.4 Production of inoculum of the pathogens

Inoculum was produced by growing the fungi on barley grains. Fifty grams of barley grains were soaked overnight in 250 ml Erlenmeyer flasks containing a 100 ml of water.

² PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

³ Pro-Seed cc, P.O.Box 101477, Scottsville 3209, Pietermaritzburg, South Africa

⁴ Du Roi Laboratory, P.O.Box 1147, Letsitele 0885, South Africa

The excess water was drained off and the flasks were sterilized consecutively for two days at 121°C for 20 min. Each flask was then inoculated with three disks of the pathogens grown on Potato Dextrose Agar (PDA) (Merck®) for 3 d at 27°C in the dark. After 2wk incubation at 27°C, the grains were air-dried, milled using a coffee grinder, sieved and stored at 4°C until further use.

3.2.5 Silicon uptake under greenhouse condition

Bean plants were grown in 150 mm pots containing composted pine bark and treated with 200 ml of potassium silicate with a concentration of silicon ranging from 150-500 mg ℓ^{-1} weekly. Banana plants were grown in 250 mm pots, with pine bark as a growing medium, and were treated weekly with 1 ℓ of potassium silicate with concentrations of silicon ranging from 200-4000 mg ℓ^{-1} . The experiment was arranged in a randomized complete block design replicated four times. Each plot consisted of four pots/plants. The experiment was conducted under greenhouse condition with temperatures varying between 26-28°C. Banana and bean plants were watered via drip irrigation three times a day for 5 and 3 min, respectively, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio. After pod formation the roots, stems and leaves of bean plants were separately placed in paper bags and oven dried at 70°C for 72 h. For the banana experiment, the roots, corm, pseudostem, petiole, midrib and leaf lamina were chopped into pieces and separately placed in paper bags, and were oven dried at 70°C for 5 d. Five grams of the milled and oven-dried plant material was ashed at 650°C for 12 h in a furnace. The ash was placed in a nickel crucible and digested by adding 5 ml of 15% NaOH and evaporated on a hot plate at low heat. The digested ash was dissolved using 10 ml of ultra pure water and measured for silicon using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES), Varian 720-ES.

3.2.6 Determination of soluble silicon in banana plants

One gram of fresh banana leaves (young, middle and old) or roots, obtained from banana plants treated with 1 ℓ of potassium silicate containing 0 or 1000 mg ℓ^{-1} , weekly, were frozen in liquid nitrogen and ground using mortar and pestle. The powder was then ultrasonicated using an Ultrasonic cell disrupter (Virsonic 100) for 1min in 10ml ultra pure water. The homogenate was left shaken overnight, centrifuged twice at 20,000 g for 10 min

and filtered using a 0.8/0.2 μm filter. The amount of soluble silicon was then determined using ICP-OES.

3.2.7 Silicon concentration trial against Fusarium wilt diseases of banana and beans under greenhouse conditions

Bean plants were grown in 150 mm pots containing composted pine bark and treated weekly with 200 ml of potassium silicate with a concentration of silicon ranging from 150-500 mg l^{-1} . Banana plants, on the other hand, were grown in a composted pine bark in 250 mm pots, treated weekly with 1 l of potassium silicate with concentrations of silicon ranging from 200-2000 mg l^{-1} . Silicon application commenced at planting. Bean plants were inoculated with 50 ml of 10^7 c.f.u. ml^{-1} of a *F. oxysporum* f.sp. *phaseoli* inoculum 1 wk post germination while banana plants were treated with 150 ml of 10^7 c.f.u. ml^{-1} of a *F. oxysporum* f.sp. *cubense* inoculums 3 wk later. The experiments were arranged in a randomized complete block design replicated four times and each plot consisted four pots/plants. The experiment was conducted under greenhouse condition with temperature varying between 26-28°C. Banana and bean plants were watered via drip irrigation three times a day for 5 and 3min, respectively, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio. Two months later, after pod formation, bean plants were rated for disease severity, fresh green pods were weighed immediately after harvest and dry weight was taken after total biomass was oven dried for 72 h at 70°C. Banana plants, on the other hand, were rated for disease severity 6 mo later.

3.2.7 Disease rating

Evaluation of disease severity of bean plants was based on a visual assessment of symptoms arising from infection of by *F. oxysporum* f.sp. *phaseoli* was according to a five-point key (Carver et al., 1996):

- 0 = healthy plants
- 1 = initial signs of wilting (yellowing)
- 2 = up to 25% of the leaves with symptoms
- 3 = up to 50% of the leaves with symptoms
- 4 = up to 75% of the leaves with symptoms
- 5 = plants dead

Disease severity ratings of banana plants was based on a visual assessment of the internal symptoms caused by *F. oxysporum* f.sp. *cubense*, according to the INIBAB's Technical Guideline 6 (Carlier et al., 2002):

- 1= Corm completely clean, no vascular discoloration
- 2 = Isolated points of discoloration in vascular tissue
- 3 = Discoloration of up to one-third of vascular tissue
- 4= Discoloration of between one-third and two-thirds of vascular tissue
- 5 = Discoloration of greater than two-thirds of vascular tissue
- 6 = Total discoloration of vascular tissue

The overall disease severity index (DSI) was calculated as follows (Mak et al., 2004; Zhang et al., 1996):

$$DSI = \frac{\sum(\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum(\text{Number of treated seedlings})}$$

3.2.8 Statistical analysis

Experiments were repeated twice. Data was analyzed using GenStat[®] Executable release 9 Statistical Analysis Software. A One-way analysis of variance (ANOVA) was used to analyse results to determine treatment effects. Differences between treatments was distinguished using Fisher's protected least significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

3.3 Results

3.3.1 Silicon uptake and distribution in bean plants

Results on silicon uptake and its distribution in bean plants are presented in Table 3.1 and Figure 3.1. Significantly higher level of silicon accumulated in the silicon-treated plants than silicon-untreated ones. Silicon distributed unevenly over the different parts of bean plants. Silicon accumulation was higher in leaves than any other part of the plants, when low concentrations of silicon were applied. Bean plants treated with $150 \text{ mg } \ell^{-1}$ of silicon resulted in 5.20 , 2.19 and $18.04 \text{ g kg}^{-1} \text{ DM}$ (where, $\text{g kg}^{-1} \text{ DM}$ = gram of silicon per kg of plant dry matter) silicon accumulated in the roots, stems and leaves, respectively. With an increase in soil silicon levels, silicon levels in the root increased and exceeded that in the leaf. Silicon in the leaf did not significantly increase with the increases in silicon applications beyond $150 \text{ mg } \ell^{-1}$ ($P < 0.001$).

Although application of silicon with concentrations greater than $300 \text{ mg } \ell^{-1}$ did not result in significantly higher accumulation of silicon in the roots of bean plants ($P < 0.001$), a 12% increase in silicon level was observed in plants fertilized with $500 \text{ mg } \ell^{-1}$ silicon, as compared to those treated with $300 \text{ mg } \ell^{-1}$. No significant difference in silicon accumulation in the root was also observed between untreated plants and plants treated with $150 \text{ mg } \ell^{-1}$ ($P < 0.001$). However, quantitatively, plants treated with $150 \text{ mg } \ell^{-1}$ silicon accumulated 44% more silicon than the untreated control. The untreated control accumulated up to $3.55 \text{ g kg}^{-1} \text{ DM}$.

Silicon accumulated five fold more in the root or leaf than in the stem. No significant increase in the accumulation of silicon in the stem resulted from application of silicon at levels higher than $150 \text{ mg } \ell^{-1}$ ($P = 0.002$).

Table 3.1 Silicon uptake and its distribution in bean plants treated with different concentrations of silicon under greenhouse condition

Silicon applied (mg ℓ^{-1})	Silicon concentration (g kg^{-1} DM)					
	Root		Stem		Leaf	
0	3.5(0.7)	a	0.6(0.2)	a	2.0	a
150	5.2(0.8)	a	2.2(0.5)	b	18.0	b
200	8.3(1.0)	b	2.8(0.6)	b	18.3	b
250	15.9(1.2)	bc	2.9(0.6)	b	17.1	b
300	19.8(1.3)	cd	2.4(0.5)	b	16.4	b
350	21.4(1.3)	cd	3.4(0.6)	b	18.2	b
400	22.5(1.3)	cd	3.2(0.6)	b	19.5	b
450	21.2(1.3)	cd	3.4(0.6)	b	19.6	b
500	22.3(1.4)	d	2.4(0.5)	b	17.6	b
F pr. (Silicon conc.)	(<0.001)		(0.001)		<0.001	
l.s.d.	(0.09)		(0.06)		2.83	
s.e.d.	(0.19)		(0.12)		5.99	
cv%	(9.6)		(12.9)		21.2	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$
 - Values in parenthesis represent means transformed using log transformation.

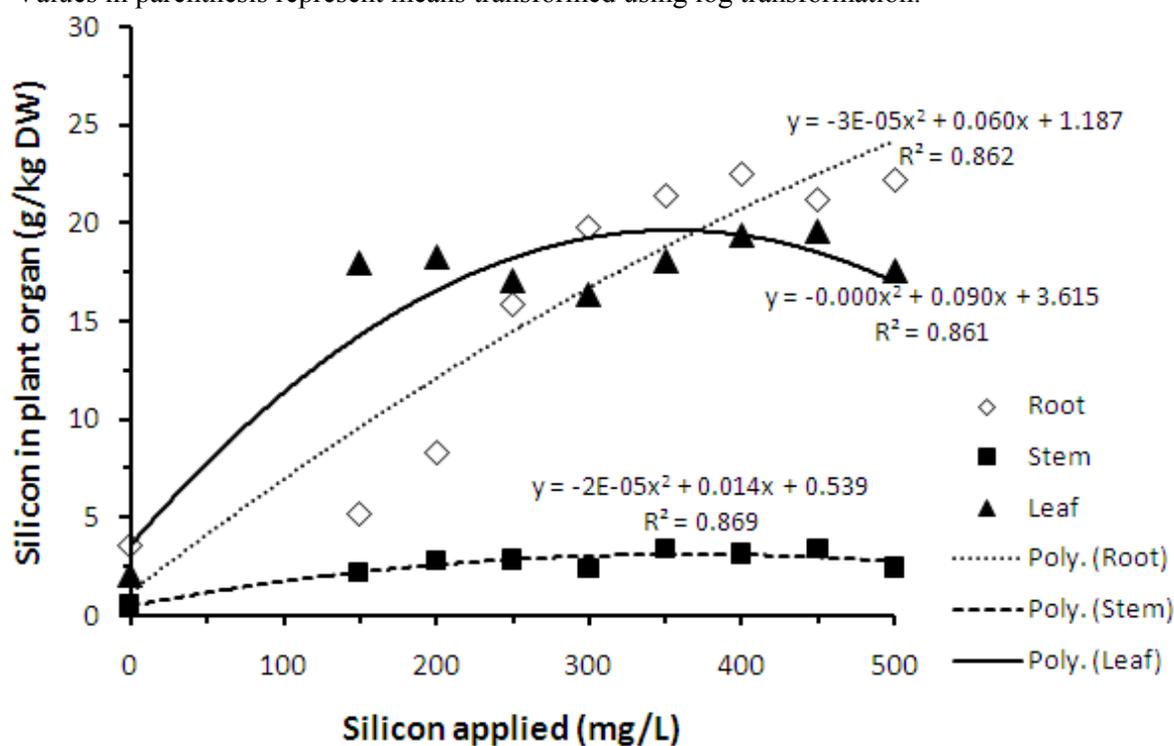


Figure 3.1 Silicon uptake and distribution bean plants fertilized with different concentrations of silicon under greenhouse conditions

3.3.2 Silicon uptake and distribution in banana plants

Application of silicon resulted in significantly higher accumulation of silicon in the plant organs analysed ($P < 0.001$). Generally, silicon concentration in banana plants increased in sequence from root > leaf > petiole > midrib > pseudostem > corm (Table 3.2, 3.3; Figure 3.2, 3.3). However, this was not the case at all the concentrations of silicon applied to the banana plants. At low soil silicon levels, plant silicon level increased in sequence from leaf > root > petiole \approx midrib > pseudostem > corm (Table 3.2, 3.3; Figure 3.2, 3.3).

Banana plants treated with silicon concentrations from 200-500 mg ℓ^{-1} and 3500-4000 mg ℓ^{-1} did not show significant differences in silicon accumulation in their roots. Plants treated with 3500-4000 mg ℓ^{-1} were much smaller in size than the rest. The highest level of root silicon accumulation was observed when plants were treated with 2000-2500 mg ℓ^{-1} of silicon, i.e., 18.15 g kg^{-1} DM compared to 0.81 and 8.54 g kg^{-1} DM in untreated plants and plants treated with 200 mg ℓ^{-1} of silicon, respectively. Silicon levels in banana leaf laminas remained more or less steady at all concentrations of silicon applied, with the silicon untreated control accumulating 2.03 g kg^{-1} DM while plants treated with 200 mg ℓ^{-1} of silicon accumulated 13.28 g kg^{-1} DM.

The amount of silicon accumulated in the corm was the lowest for all banana organs. Silicon accumulation in the corm did not increase with increases in the concentration of silicon applied to the banana plants. Similarly, silicon accumulation in the pseudo-stem, petiole and midrib did not respond to changes in silicon concentrations applied to soil.

EDX analysis and silicon mapping on banana roots revealed that most of the silicon accumulated in the roots was located in the epidermis (Figure 3.4a-e, 3.5). However, all epidermal cells did not contain the same level of silicon. Swollen epidermal cells had higher levels of silicon than the surrounding cells. On the other hand, the xylem and stele area accumulated lower levels of silicon. The epidermis in the root of the untreated control plants did not have any peak areas for silicon.

Table 3.2 Silicon uptake and its distribution in banana plants treated with different concentrations of silicon under greenhouse conditions measured with ICP-OES

Silicon applied (mg ℓ^{-1} wk $^{-1}$)	Silicon concentration (g kg $^{-1}$ DM)			
	Root		Leaf lamina	
0	0.81	a	2.03	a
200	8.54	bc	13.28	bc
250	8.60	bc	12.18	b
300	8.64	bc	13.67	bc
350	8.41	bc	14.65	bcd
400	9.99	c	15.02	cd
500	8.43	bc	13.70	bc
750	9.00	c	14.18	bcd
1000	13.44	d	13.66	bc
1500	13.91	d	14.43	bcd
2000	17.71	e	14.60	bcd
2500	18.15	e	15.73	cd
3000	13.90	d	15.25	cd
3500	6.46	b	14.06	bcd
4000	8.00	bc	16.49	d
F pr. (Silicon conc.)	<0.001		<0.001	
s.e.d.	1.258		1.357	
l.s.d.	2.533		2.734	
cv%	17.3		14.2	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

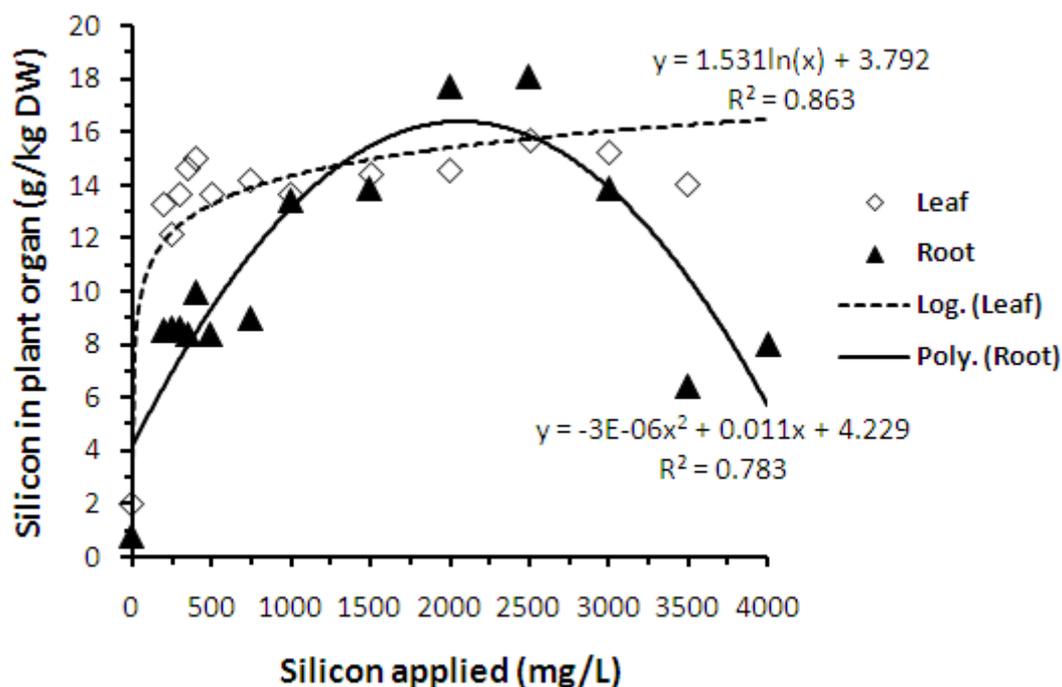


Figure 3.2 Silicon uptake and distribution in banana plants fertilized with different concentrations of silicon under greenhouse conditions

Table 3.3 Silicon uptake and its distribution in banana plants treated with different concentrations of silicon under greenhouse conditions

Silicon applied (mg ℓ^{-1} wk $^{-1}$)	Silicon concentration (g kg $^{-1}$ DM)							
	Corm		Pseudo-stem		Petiole		Midrib	
0	0.46	a	0.53	a	0.61	a	0.68	a
1000	2.00	cd	2.80	b	4.05	b	3.79	b
1500	2.13	d	3.47	d	4.29	b	4.49	c
2000	1.69	b	3.65	de	5.45	d	5.48	e
2500	1.89	bc	3.79	e	4.92	c	5.36	e
3000	1.70	b	3.14	c	6.55	e	4.64	c
3500	2.58	e	3.76	e	6.67	e	5.64	e
4000	2.62	e	4.98	f	7.69	f	4.97	d
F pr. (Silicon conc.)	<0.001		<0.001		<0.001		<0.001	
s.e.d.	0.103		0.091		0.253		0.137	
l.s.d.	0.213		0.188		0.523		0.283	
cv%	7.8		3.9		7.1		4.4	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

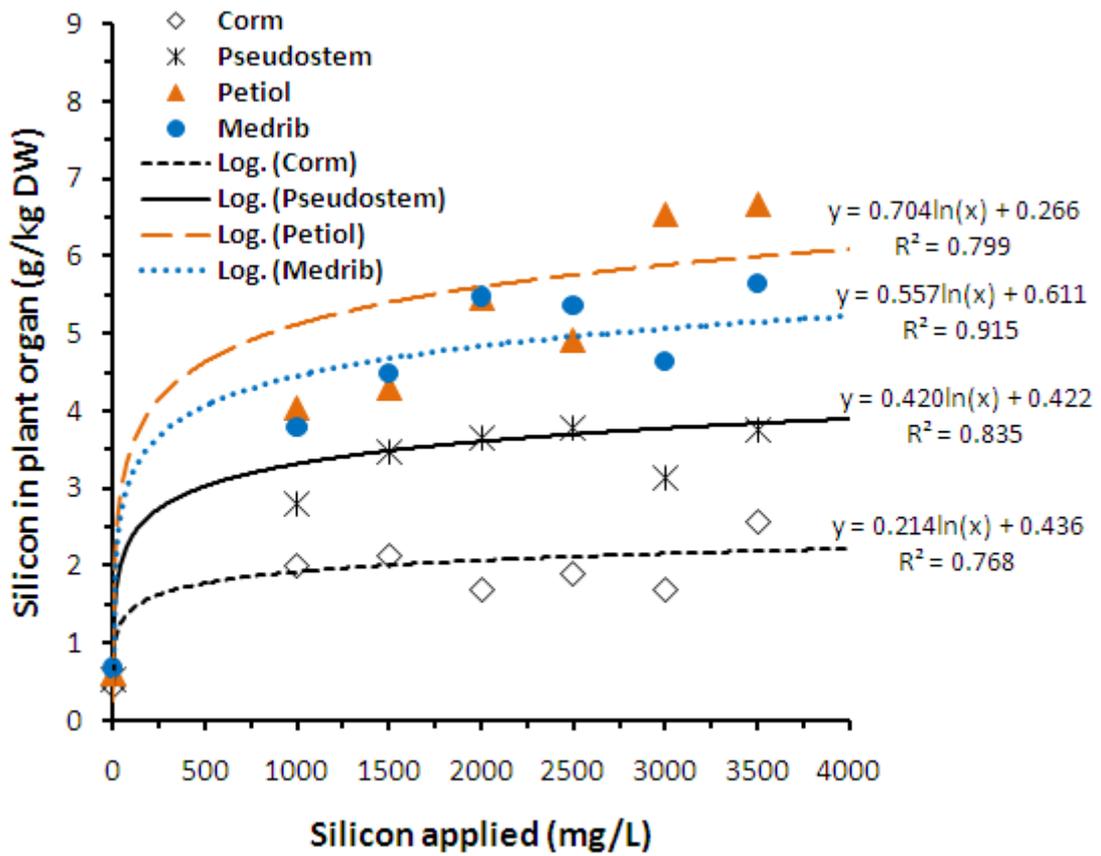


Figure 3.3 Silicon uptake and distribution in banana plants fertilized with different concentrations of silicon under greenhouse conditions

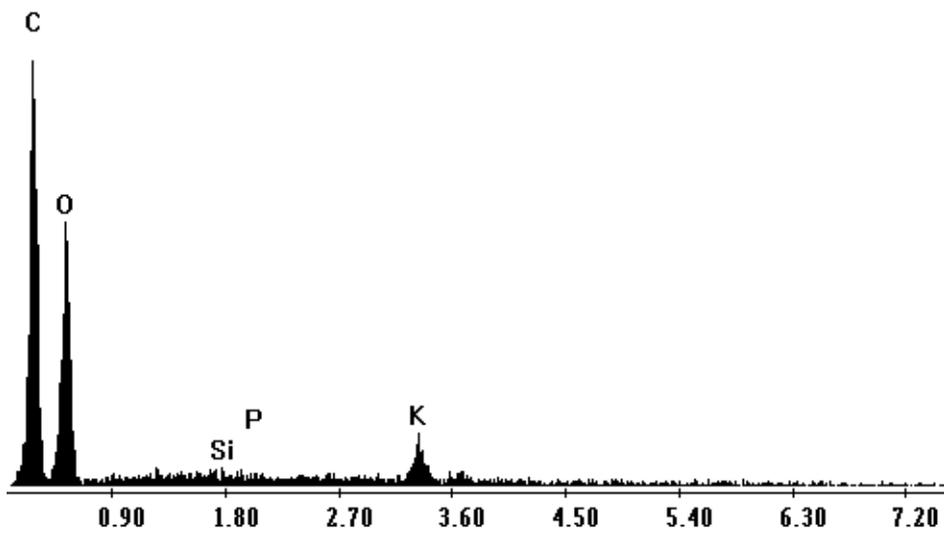


Figure 3.4 a Silicon level of root epidermis of silicon-untreated banana plant measured using EDX

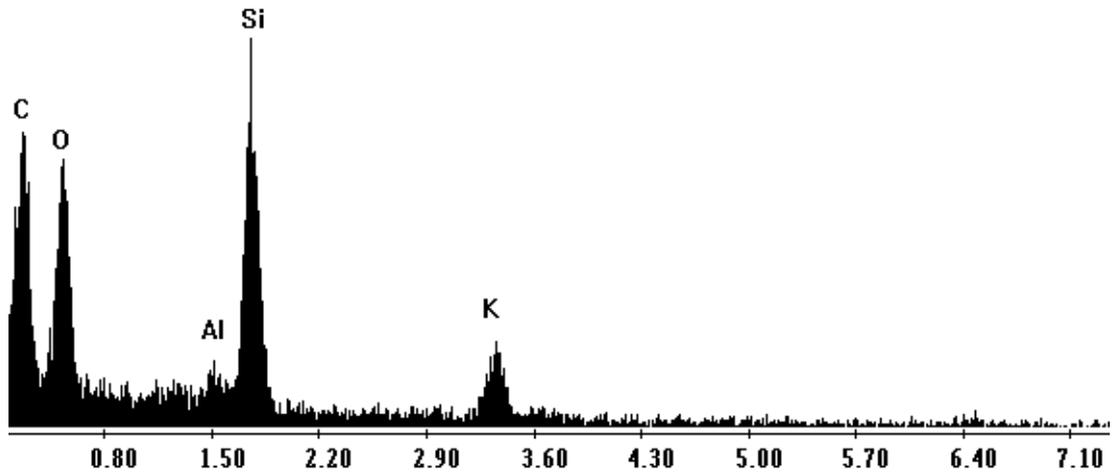


Figure 3.4 b Silicon level of root epidermis measured using EDX on banana plant treated with $1500 \text{ mg } \ell^{-1}$ silicon weekly

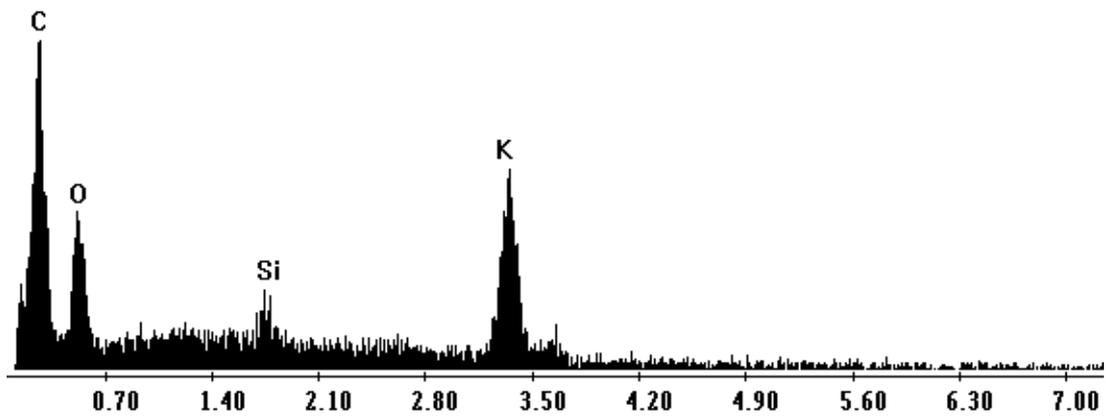


Figure 3.4 c Silicon level of the xylem of banana root measured using EDX on a plant treated with $1500 \text{ mg } \ell^{-1}$ silicon weekly

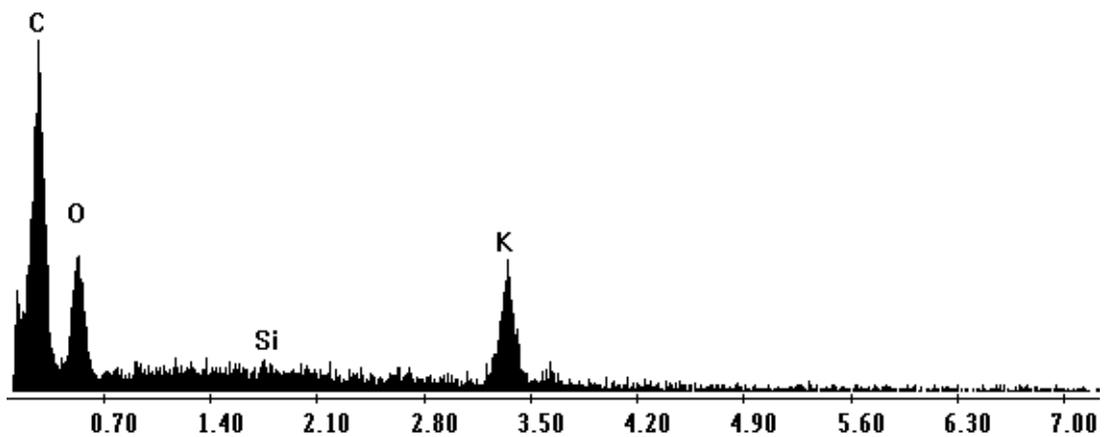


Figure 3.4 d Silicon level of the stele of banana root measured using EDX on a plant treated with $1500 \text{ mg } \ell^{-1}$ silicon weekly

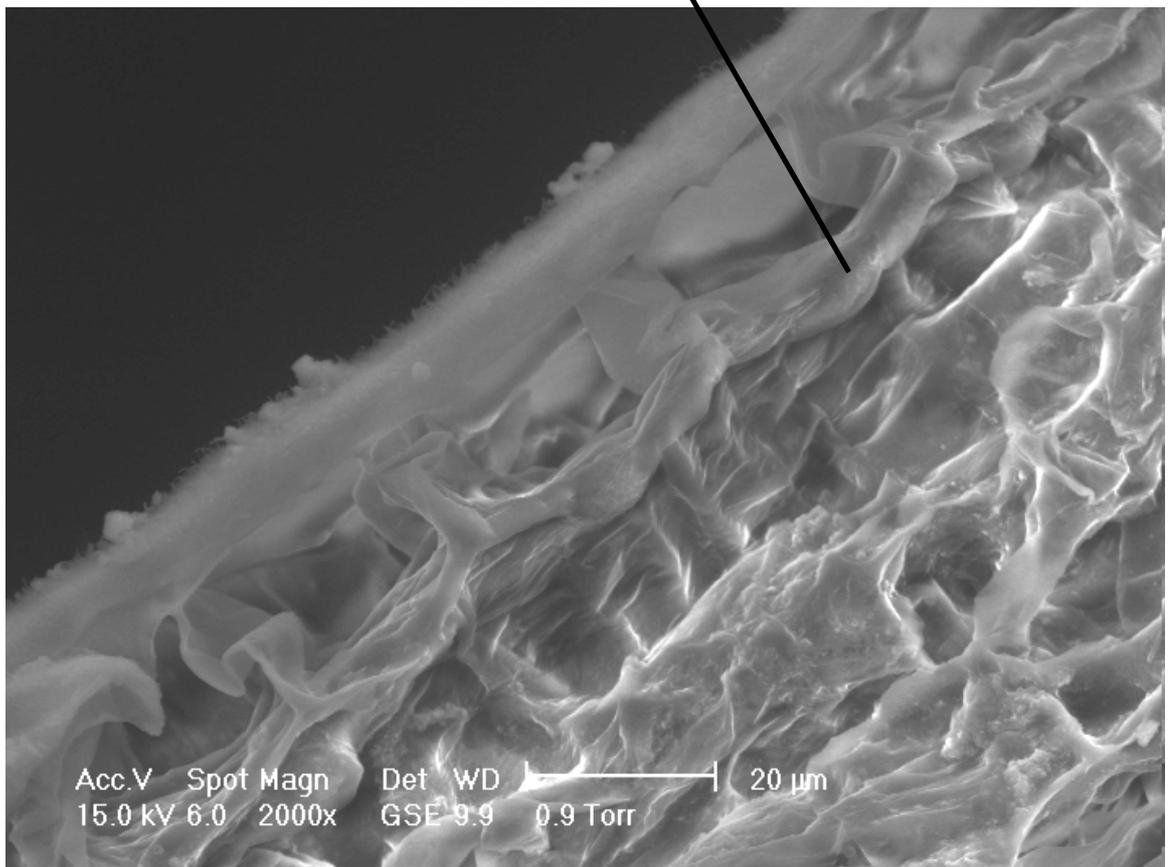
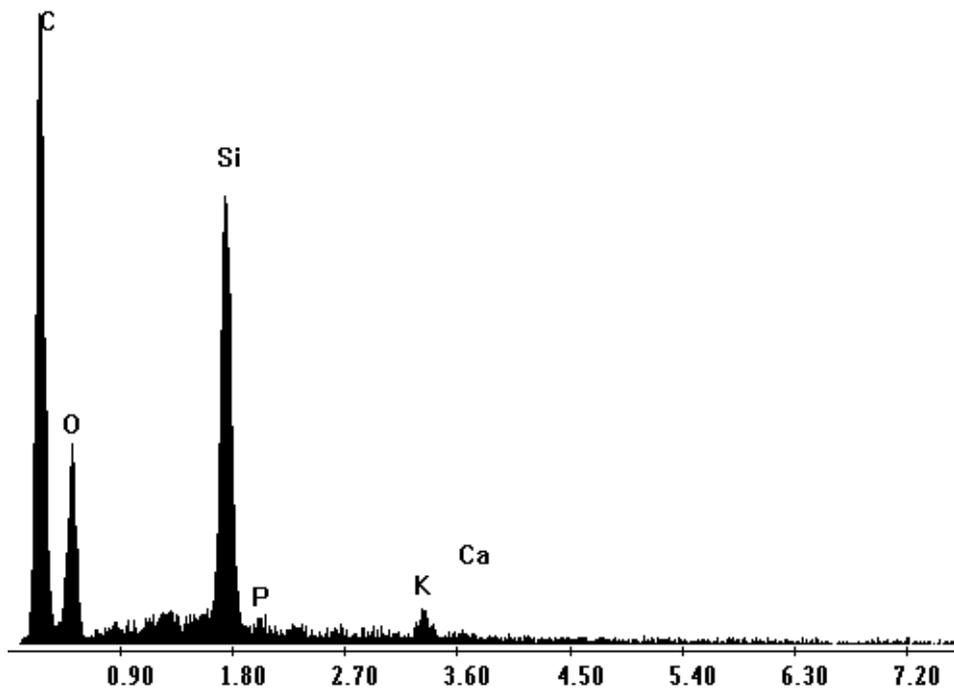


Figure 3.4 e Silicon level of swollen cells in the root epidermis of banana plants treated with 1500 mg ℓ^{-1} silicon weekly, measured using EDX

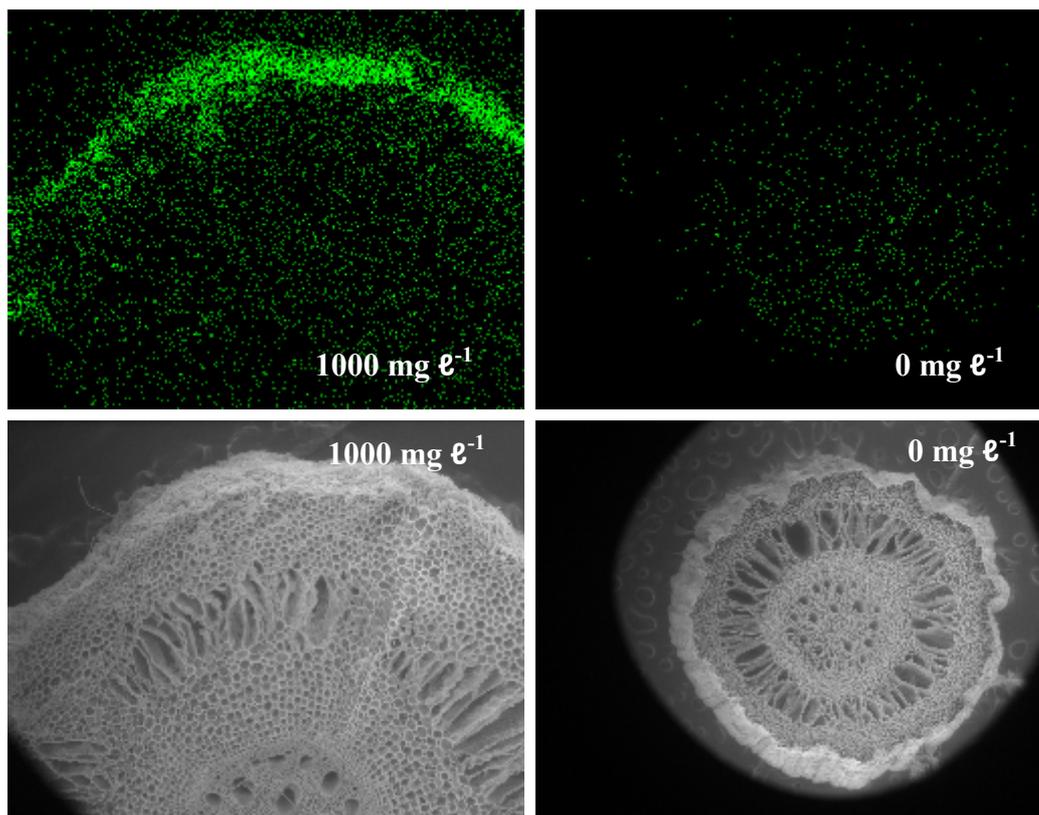


Figure 3.5 Mapping of silicon distribution using Environmental Scanning Electron Microscopy in the cross-section of the roots of banana plants treated with (A) 1000 mg ℓ^{-1} silicon and (B) 0 mg ℓ^{-1} silicon

3.3.3 Soluble silicon in banana plants

The level of soluble silicon was consistently higher in banana roots than in the leaves and in the silicon-treated than in the control banana plants (Table 3.4; Figure 3.6). The roots of silicon-treated and untreated control banana plants had significantly higher level of soluble silicon than the leaves of these plants.

Table 3.4 Soluble silicon extracted from silicon treated and untreated banana plants

Silicon applied to soil (mg l ⁻¹)	Soluble silicon (g Kg ⁻¹ DW)							
	Young leaf		Middle leaf		Old leaf		Root	
0	0.2	a	0.3	a	0.3	a	0.9	d
1000	0.5	b	0.8	cd	0.7	c	1.7	e
Plant part × Si conc	<0.001							
s.e.d.	0.07							
l.s.d.	0.14							
cv%	12.0							

- Means with the same letter are not significantly different at $P \leq 0.05$

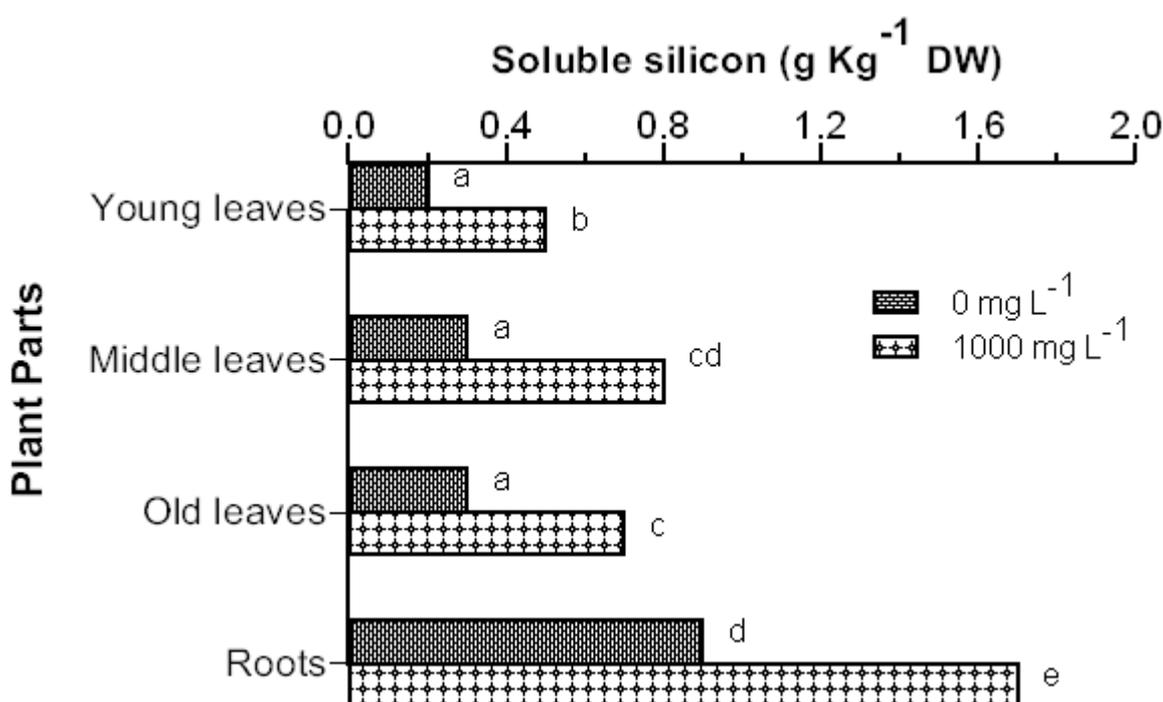


Figure 3.6 Soluble silicon extracted from silicon treated and untreated banana plants

3.3.3 The effect of silicon applications on Fusarium wilt of beans

Application of different concentrations of silicon to bean plants inoculated with *F. oxysporum* f.sp. *phaseoli* significantly reduced Fusarium wilt disease severity ($P=0.002$) (Table 3.5; Figure 3.7a, b). Disease severity levels of the pathogen inoculated control plants were rated at more than 4 (up to 75%) while plants treated with silicon had disease severity ratings ranging from 2 (up to 25%) to 3 (up to 50%) (Table 3.5; Figure 3.7a, b).

However, there was no significant difference in disease severity, green pod weight or biomass dry weight as a result of the different silicon application dosages, although disease severity ratings decreased with increased in silicon concentrations. In addition, there was no correlation between silicon accumulated in the roots of bean plants, and disease severity ($R^2=0.331$), fresh pod weight ($R^2=0.0.174$) and dry weight ($R^2=0.0.139$) (Figure 3.8, 3.9).

Table 3.5 Effect of different concentrations of silicon applied to bean plants against *Fusarium oxysporum* f.sp. *phaseoli*

Silicon conc. applied (mg t^{-1} wk$^{-1}$)	DSI*		Pod weight (g)		Dry weight (g)	
0	4.31	c	26.77	a	31.60	a
150	2.56	b	68.45	a	32.47	a
200	3.03	bc	61.49	a	35.57	a
250	2.92	bc	67.36	a	30.87	a
300	2.74	b	70.43	a	36.73	a
350	3.08	bc	41.98	a	29.19	a
400	2.94	bc	58.91	a	34.83	a
450	2.00	b	74.36	a	37.86	a
500	2.17	b	75.92	a	39.50	a
Untreated control	0.00	a	206.86	b	60.72	b
F pr.	0.002		0.004		0.121	
s.e.d.	0.682		32.839		9.201	
l.s.d.	1.439		68.992		19.413	
cv%	32.50		53.400		30.500	

*DSI – Disease severity index

-Means with the same letter in the same column are not significantly different at $P \leq 0.05$

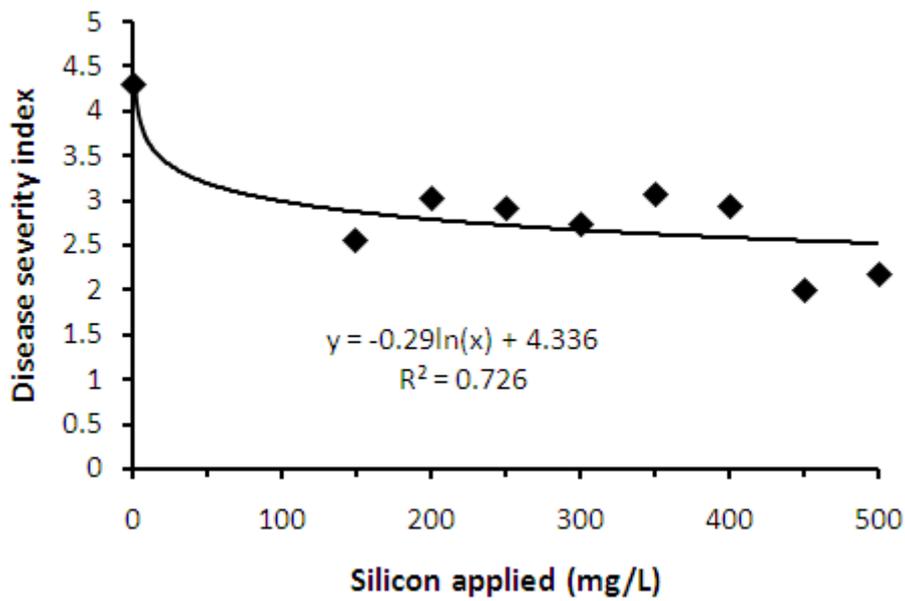


Figure 3.7a Effect of different concentrations of silicon on disease severity of bean plants inoculated with *Fusarium oxysporum* f.sp. *phaseoli*

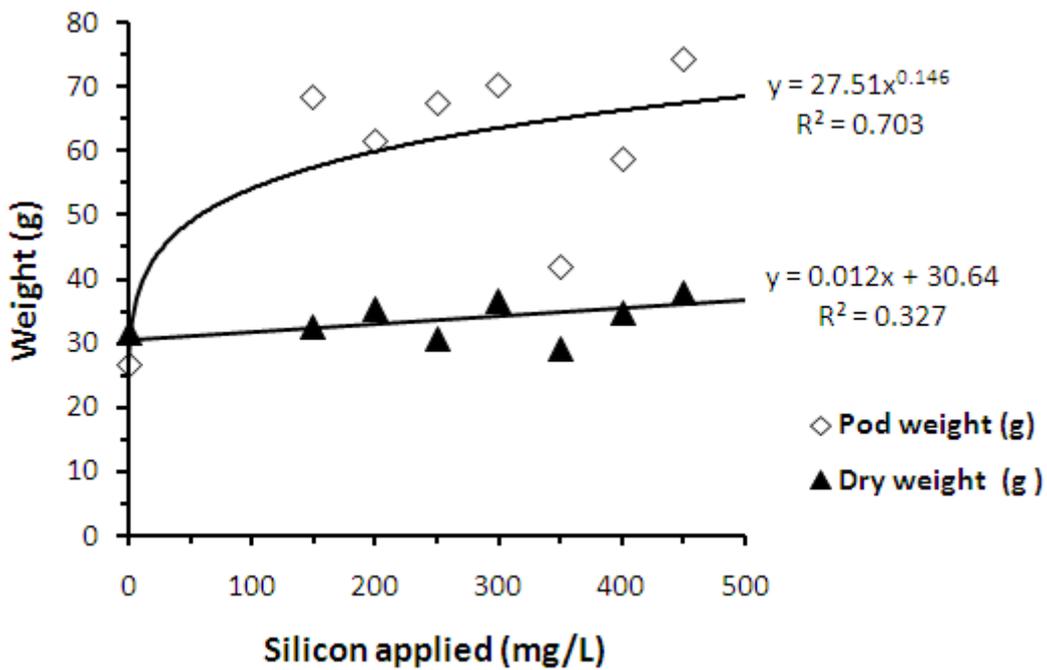


Figure 3.7b Effect of different concentrations of silicon on fresh pod weight of bean plants inoculated with *Fusarium oxysporum* f.sp. *phaseoli*

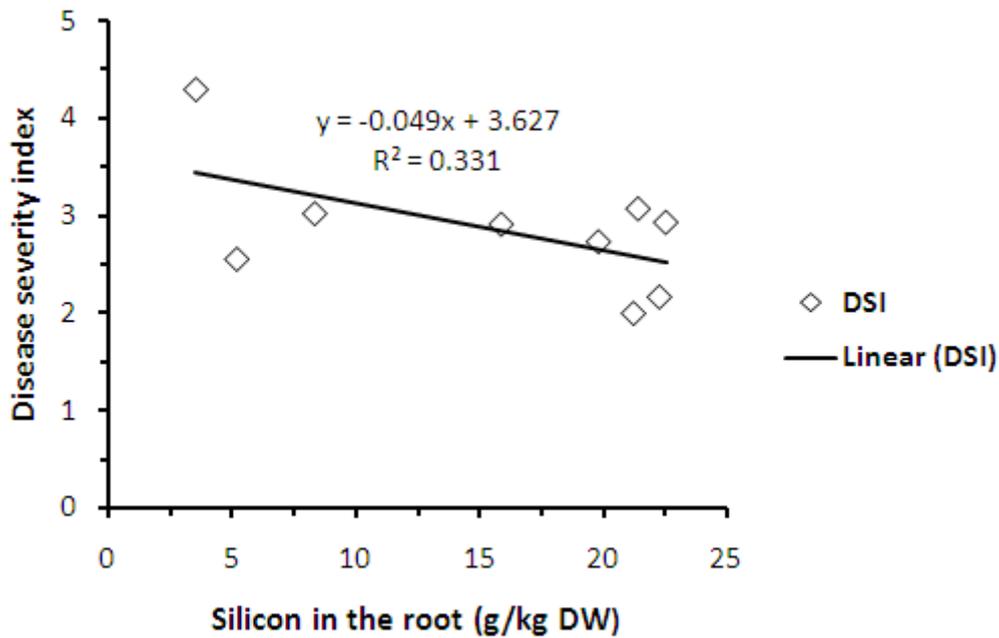


Figure 3.8 Relationship between silicon accumulated in the roots of bean plants and disease severity caused by *Fusarium oxysporum* f.sp. *phaseoli*

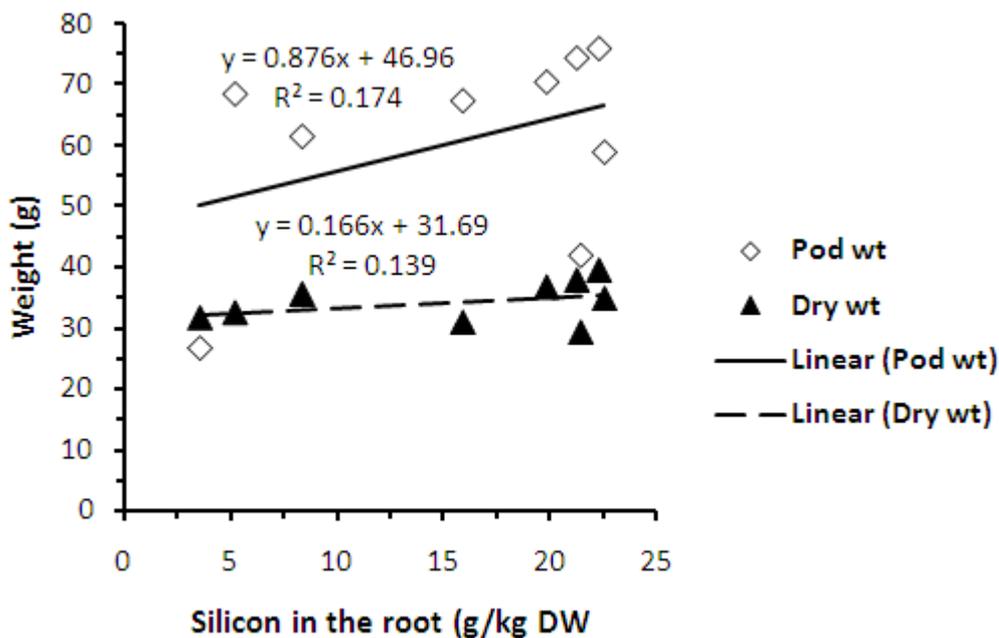


Figure 3.9 Relationship between silicon accumulated in the roots of bean plants, and fresh pod weight and dry weight of bean plants inoculated with *Fusarium oxysporum* f.sp. *phaseoli*

3.3.4 The effect of silicon application dose on *Fusarium oxysporum* f.sp. *cubense*

Application of silicon significantly reduced foliar wilting/yellowing ($P=0.003$) and corm discoloration ($P<0.001$) of banana plants inoculated with *F. oxysporum* f.sp. *cubense*. There was a negatively linear ($R^2=0.801$) and logarithmic relationship ($R^2=0.775$) between the levels of silicon applied, and foliar wilting and corm discoloration of banana plants, respectively (Figure 3.10). Plants treated with 200-400 mg ℓ^{-1} wk $^{-1}$ of silicon did not have significantly different levels of foliar wilting/yellowing compared to the pathogen treated control. Conversely, silicon concentrations higher than 400 mg ℓ^{-1} significantly reduced foliar wilting/yellowing of banana plants. Corm discoloration was significantly reduced by all silicon concentrations applied to banana plants, with the application of silicon at 2000 mg ℓ^{-1} resulting in corm that were almost as free of symptoms as the control (Table 3.6; Figure 3.10, 3.12). There was a strong negative linear relationship between silicon accumulated in the roots of banana plants, and corm discoloration ($R^2=0.851$) and foliar wilting/yellowing ($R^2=0.860$) (Figure 3.11).

Table 3.6 Effect of different concentrations of silicon applied to banana plants against *Fusarium oxysporum* f.sp. *cubense*

Silicon applied (mg ℓ^{-1} wk $^{-1}$)	Foliar severity index		Corm discoloration index	
0	3.64	c	5.67	c
200	2.78	bc	2.89	b
250	2.67	bc	3.56	b
300	2.89	bc	3.56	b
350	2.67	bc	3.22	b
400	2.78	bc	3.33	b
450	2.22	b	2.89	b
500	2.39	b	3.56	b
750	2.56	b	3.22	b
1000	1.89	b	2.89	b
1500	2.11	b	2.74	b
2000	0.81	a	1.21	a
F pr.	0.003		<0.001	
s.e.d.	0.487		0.599	
l.s.d.	1.009		1.242	
cv%	24.3		22.7	

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$

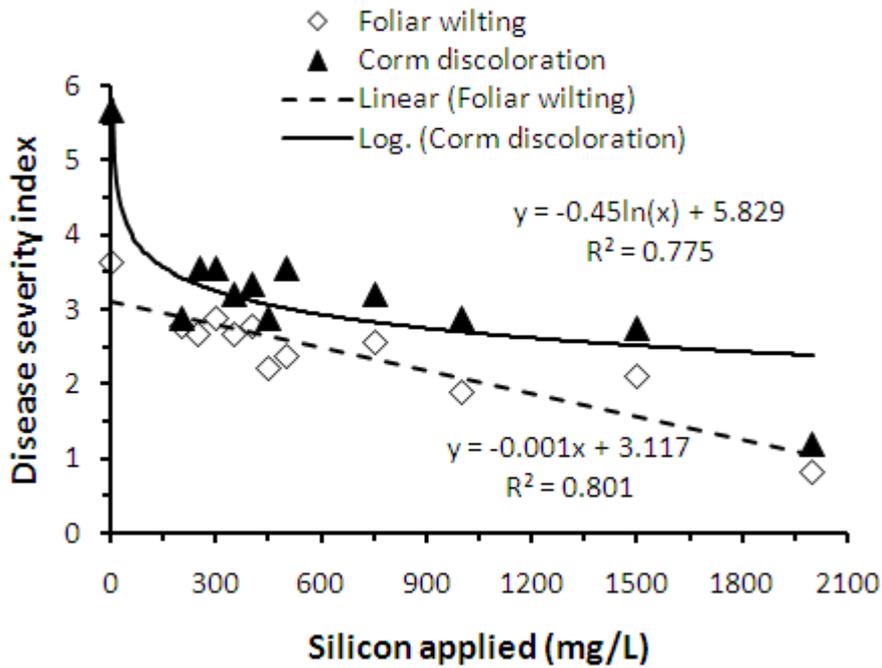


Figure 3.10 Effect of different concentrations of silicon applied to banana plants against *Fusarium oxysporum* f.sp. *cubense*

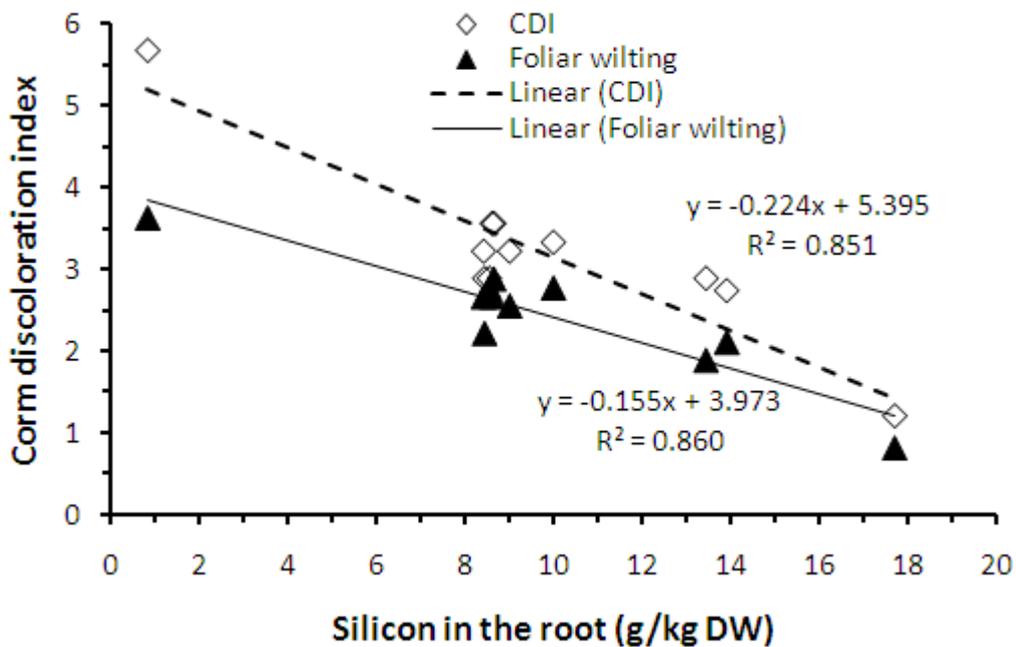


Figure 3.11 Relationship between silicon accumulated in the roots of banana plant and disease severity of caused by *Fusarium oxysporum* f.sp. *cubense*

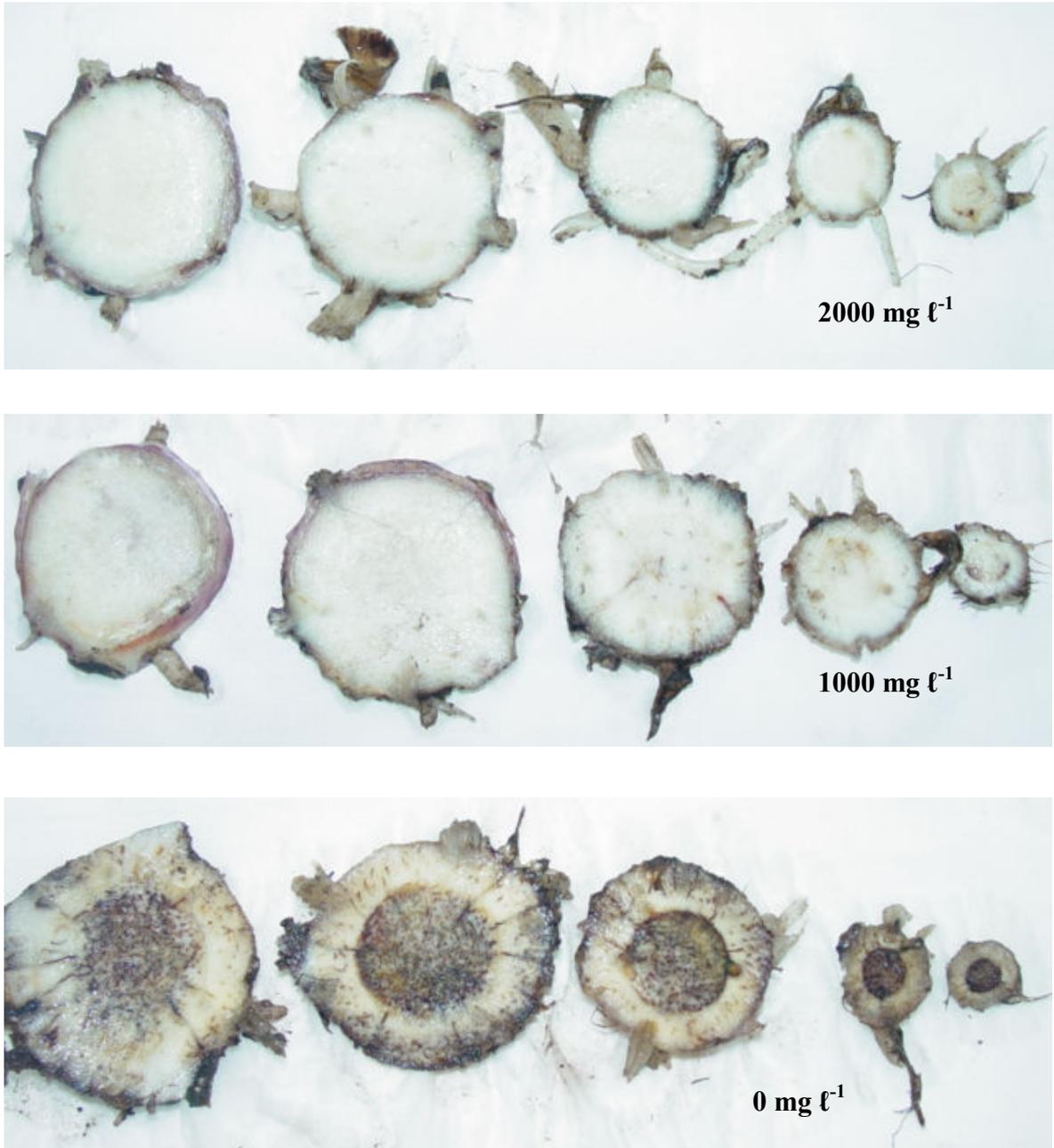


Figure 3.12 Progress of corm discoloration, caused by *Fusarium oxysporum* f.sp. *cubense*, in banana plants treated with different concentrations of silicon

3.4 Discussion

Previously it was assumed that silicon was translocated to the different parts of plants via passive transport along with the water column towards the transpiration termini, where it is deposited as biogenic opal (Jones and Handreck, 1967; Raven, 1983; Raven, 2001). Jones and Handreck (1967) suggested that, “all sink tissues along the xylem path unload silicon passively; the distribution of silicon among shoot organs should closely follow the cumulated transpiration of these organs, which is a function of their transpiration rate and age”.

The results of this study do not agree with the quantitative predictions of Henriot et al. (2006), based on the assumption made by Jones and Handreck (1967), where the gradient of silicon concentration in banana plant should be in the order: root < pseudostem < petiole \approx midrib < lamina, whatever the silicon concentration in the nutrient solution was. However, our quantitative data placed the order as: corm < pseudostem < midrib \approx petiole < root < leaf lamina. This order changed when soil silicon application levels increased to 1000 mg ℓ^{-1} and above. At these levels, the amount of silicon in the root exceeded that of the leaf lamina. Silicon levels in the leaf lamina remained more or less steady, regardless of the soil silicon content above 200 mg ℓ^{-1} . On the other hand, the corm was the least silicon-accumulating plant organ. The petiole, midrib and pseudostem take part in transpiration. Roots, on the other hand, are not one of the transpiring plant organs yet they accumulate a substantial amount of silicon, even more than that in the leaf at high soil silicon levels. This was also observed in bean plants, where the roots accumulated more than six times more silicon than the stem. Soluble silicon in the roots was higher than in the leaves whether banana plants were treated with silicon or not.

Such observations contradict the concept of passive uptake of silicon in these plants as the only uptake mechanism. The existence of some active absorption mechanism seems clearly evident. A silicon transporter gene (*Lsi1*) has been identified in rice that is involved in active uptake of silicon from the soil (Ma et al., 2002; Ma et al., 2004; Ma et al., 2006). Such mechanisms may also exist in banana and beans.

The prophylactic properties of silicon have been documented for many crops against a variety of diseases, mainly fungal (Belanger et al., 1995; Epstein, 1999; Ma, 2004). In this

study, silicon reduced the disease severity level of Fusarium wilt of banana and beans. Silicon accumulation in the root of banana plants relative to silicon fertilization levels was negatively correlated with corm discoloration ($R^2=0.775$) and foliar wilting ($R^2=0.801$) on banana plants. Similarly, there was a negatively linear relationship between silicon levels in the roots of banana plants, and corm discoloration and foliar wilting of the plants caused by *F. oxysporum* f.sp. *cubense*. However, silicon accumulation in the roots of bean plants along the different levels of silicon fertilization weakly correlated ($R^2=0.331$) with disease severity of bean plants. This could be due to the reduced ability of bean plants to respond to increase in soil silicon levels.

Disease suppression may have resulted from the high level of silicon deposition observed in the epidermal tissue, believed to play a role in delaying infection (Hayasaka et al., 2008; Kim et al., 2002; Yoshida et al., 1962), and the soluble silicon, believed to enhance plant defense responses (Belanger et al., 1995; Cherif et al., 1994; Cherif et al., 1992; Epstein, 1999; Hayasaka et al., 2008). In general, disease severity reduced with the increase in silicon fertilization. Application of silicon, however, did not prevent infection of plants by the wilt pathogens. Silicon suppressed disease severity significantly. Application of silicon reduced disease severity of bean plants by more than 50%. Similarly, foliar wilting/yellowing and corm discoloration of banana plants was substantially reduced through application of silicon. The corms of banana plants treated with 2000 mg ℓ^{-1} of silicon were almost as clear of symptoms as the uninoculated control plants (Figure 3.12). However, these plants were smaller in size. High levels of potassium silicate increase soil pH. At high soil pH, uptake of nutrients such as nitrogen become limited, resulting in reduced plant growth and development. However, pH cannot be a problem in areas where soils are acidic, as it is the case in most African soils (Anonymous, 2004).

The weight of green pods of bean plants treated with silicon was higher than silicon-untreated pathogen-inoculated control plants. However, dry weight did not increase because of silicon applications. Fusarium wilt diseases cause serious damage on stressed plants. Although the Fusarium wilt pathogens infect plants at any stage, marked symptoms of the disease appear more clearly during the reproductive stage of plants, depending on the soil inoculum levels. Plant's demand for carbohydrates and mineral nutrients is at a peak during flowering and grain-fill, and root growth declines at this stage (Albers, 1993; Gerik et al., 1998; Hamlin and Mills, 2001). A combination of such factors may increase

the stress level in the plant, which leads to pronounced expression of wilt symptoms. During the reproductive stage, vegetative growth of plants declines considerably because the plant priorities shift from feeding the source (leaves and roots) to feeding the sink (seed). When resources are limited, the plant nutrients are supplied to the highest priority structures first (Albers, 1993). As a result, no significant difference was expected in dry weights. However, green pod weight from silicon treated plants was almost three times higher than from the pathogen treated control.

3.5 References

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CHAPTER FOUR

Sensitivity of *Fusarium oxysporum* strains to silicon

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Abstract

The direct toxicity effects of soluble silicon on *Fusarium* wilt pathogens was addressed. Conclusive evidence is presented that soluble silicon inhibits hyphal growth, sporulation and germination of *F. oxysporum* spores. It also suppressed disease severity and delayed symptom expression in banana and bean plants. In PDA, silicon concentrations as high as 490 mg ℓ^{-1} of silicon stimulated *F. oxysporum* strains, which grew vegetatively faster. However, with an increase in silicon concentration in the media complete inhibition of mycelia growth occurred at concentrations higher than 7840 mg ℓ^{-1} . At these concentrations, the fungi could not grow, sporulate and their conidia did not germinate. In greenhouse pot experiments, application of soluble silicon at 8820 mg ℓ^{-1} significantly reduced disease severity and delayed symptom expression. However, plants did not escape infection.

4.1 Introduction

Living organisms have a tolerance to exposure to certain elements or compounds beyond which it becomes toxic. Chemicals have been used since the 1800s in agriculture such as copper based fungicides. There has been no major change in their use and mode of action where the fungicides are applied to the site of infection in amounts sufficient enough to be toxic to bacterial or fungal infections (Schroth et al., 1993). Copper is an essential element for plant and fungal metabolism as micronutrient but is toxic at higher concentrations (Graham et al., 1986). Its deficiency reduces biological function and could potentially lead

to death (Van Zwieten et al., 2004). The addition of iron to copper based fungicides improves their efficacy (Schroth et al., 1993). Manganese based fungicides such as mancozeb are also widely used fungicides in agriculture.

Silicon is the second most abundant element on earth next to oxygen. Silicon dioxide comprises 50-70% of the soil mass (Ma and Yamaji, 2006). However, plant-available silicon concentrations of most soils are in the range 0.5–0.65 mM (Jones and Handreck, 1967) and this low level prompts the need for silicon fertilization. Many plants absorb silicon in amount equivalent to macronutrients such as calcium, magnesium and phosphorus (Epstein, 1999a), although its physiological essentiality in higher plants is controversial (Clarkson and Hanson, 1980; Epstein, 1994; Werner and Roth, 1983). Interestingly, excess accumulation of silicon in plants causes no damage, making it the only element with such a unique and important property (Alves-Santos et al., 2002).

Silicon is believed to improve plant health by reducing biotic and abiotic stresses on plants (Epstein, 1999b; Rodrigues et al., 2001; Yoshida et al., 1962). Some of the benefits that plants obtain from silicon include (i) increasing canopy photosynthesis by keeping leaves erect, (ii) increasing resistance to fungi, bacteria, and insects, (iii) reducing the toxicity of heavy metals, (iv) reducing cuticular transpiration, and (v) improving water use efficiency (Epstein, 1999b; Rodrigues et al., 2001; Yoshida et al., 1962). With the work of Japanese researchers in the 1930s and 1940s it became clear that silicon could suppress plant diseases, especially in rice (Kozaka, 1965; Suzuki, 1935). Application of various sources of silicon to silicon deficient paddy soils considerably reduced the incidence and severity of rice blast disease, caused by *Magnaportha grisea* (T. T. Hebert) Yaegashi and Udagawa, and brown spot, caused by *Cochliobolus miyaheanus* (Ito and Kuribayashi in Ito) Drechs. Ex Dastur (Volk et al., 1958)(Volk et al., 1958; Okuda and Takahashi, 1964; Takahashi, 1967; Ohata et al., 1972). Other rice diseases suppressed by silicon fertilization are leaf scald, caused by *Gerlachia oryzae* (Hashioka and Yokogi) W. Gams, sheath blight, caused by *Rhizoctonia solani* (A. B. Frank) Donk, and stem rot, caused by *Magnaporthe sahinii* (Cattaneo) R. Krause and R.K. Webster (Savant *et al.*, 1997). Belanger et al. (1995) reported that foliar and soilborne diseases of cucumber and other cucurbits can also be suppressed by applying silicon.

There are several reports on the mechanisms by which silicon achieve its prophylactic properties in plants. Some studies suggested that the formation of a silicated epidermal cell layer (Yoshida, 1975; Ou, 1985; Takahashi, 1995) prevents physical penetration by fungi and makes the plant cell walls less susceptible to enzymatic degradation by fungal pathogens (Datnoff et al., 1997). The redistribution of silicon around the infection peg and its preferential accumulation at the point of infection further supports such claim as a physical barrier preventing hyphal growth and haustoria formation (Heath and Stumpf, 1986; Carver *et al.*, 1987; Samuels *et al.*, 1991). However, the importance of such mechanisms has been downplayed, with recent research findings that silicon catalyses the production of plant biochemical defense responses. Rapid deposition of phenolics or lignin at infection sites are known response of plants to attack by plant pathogens, and the presence of soluble silicon may facilitate this response (Datnoff et al., 1997). It has recently been proposed that soluble silicon acts as a modulator of host resistance to pathogens (Ma and Yamaji, 2006). Several studies on monocots (rice and wheat) and dicots (cucumber) show that when these plants were treated with silicon, they produced phenolics and phytoalexins in response to fungal infection such as those causing rice blast and powdery mildew (Belanger et al., 2003; Fawe et al., 1998; Rémus-Borel et al., 2005; Rodrigues et al., 2004). Kaiser et al. (2005) reported that soluble silicon inhibited mycelial growth of pathogenic fungi from avocado plants. However, the direct effect of silicon on plant pathogenic and non-pathogenic *F. oxysporum* has not been reported so far. Application of high levels of copper or manganese based chemical compounds results in fungal toxicity. Therefore, application of silicon at high concentrations may have a similar effect. In this study, therefore, the direct or fungistatic effect of silicon on *F. oxysporum* f.sp. *cubense* and f.sp. *phaseoli*, the causal agents of Fusarium wilt of banana and beans, respectively, was investigated.

4.2 Materials and Methods

4.2.1 Silicon

Silicon was applied in the form of soluble potassium silicate K2550 from PQ Silicas (Pty) Ltd⁵, containing 20.5-20.9% SiO₂.

⁵ PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

4.2.2 Source of Plant Materials

Bean seeds of the cv. Outeniqua, provided by ProSeed cc (Pty) Ltd⁶, and 50 mm sized tissue culture banana plantlets of the cv. Cavendish Williams, provided by DuRoi Laboratories⁷, were used throughout the experiments.

4.2.3 Pathogens

Fusarium oxysporum f.sp. *cubense* was isolated from a diseased banana plant from the South Coast of KwaZulu-Natal while *F. oxysporum* f.sp. *phaseoli* was isolated from diseased bean plants from Ukulinga Farm, Pietermaritzburg, South Africa. The plants were surface sterilized using commercial bleach containing 3.5% sodium hypochlorite for 5 min and were subsequently rinsed three times with sterile distilled water. Small disks of the corm/stem cut in cross-section were placed on Nash and Snyder Medium and incubated at 27°C for 5 d. Colonies were then subcultured onto Potato Carrot Agar (PCA). PCA was prepared as follows: A grade potatoes, 20 g, carrots, 20 g, agar, 15 g and 1 l of tap water. Potatoes and carrots were grated and cooked for 30 min. The juice was then filtered through cheesecloth without straining. The agar was added to the filtrate and autoclaved at 121°C for 15 min. The fungi were grown on PCA, and their mycelial and conidial suspensions were freeze-stored in Eppendorf tubes at -80°C, protected with 50% glycerol.

4.2.4 Non-pathogenic *Fusarium oxysporum*

The non-pathogenic *F. oxysporum* endophytes were isolated from the xylem area of healthy banana roots, from banana plants from the North Coast area of KwaZulu-Natal. The roots and corms were surface sterilized using commercial bleach containing 3.5% sodium hypochlorite for 5 min and subsequently rinsed three times using sterile distilled water. Roots were cut into pieces and blended in a sterile Waring Commercial blender. To isolate fungal endophytes, the juice was then plated onto Nash and Snyder Medium and incubated at 27°C for 5 d. Isolated colonies were subcultured onto PCA. The mycelia and conidial suspensions of the fungi, grown on PCA, were freeze-stored in Eppendorf tubes at -80°C supplemented with 50% glycerol. These isolates were selected for their biocontrol potential against the pathogenic *F. oxysporum* f.sp. *phaseoli* and *cubense* on beans and bananas, respectively.

⁶ Pro-Seed cc, P.O.Box 101477, Scottsville 3209, Pietermaritzburg, South Africa

⁷ Du Roi Laboratory, P.O.Box 1147, Letsitele 0885, South Africa

4.2.5 Fungal inoculum production

Inoculum was produced by growing the fungi on barley grains. Fifty grams of barley grains were soaked overnight in 250 ml Erlenmeyer flasks containing 100 ml of water. The excess water was drained off and the flasks were autoclaved consecutively for 2 d at 121°C for 20 min. Each flask was then inoculated with three disks of the pathogens grown on Potato Dextrose Agar (PDA) (Merck®) for 3 d at 27°C in the dark. After two weeks at 27°C the grains were air-dried, milled using a coffee grinder, sieved and stored at 4°C.

4.2.6 Hyphal growth of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended agar

Both the pathogenic and non-pathogenic *F. oxysporum* were subcultured on PDA by scraping off pieces of the frozen fungal suspension. After 3 d at 27°C in the dark approximately 4×4 mm² mycelial plugs taken from the margins of *F. oxysporum* f.sp. *phaseoli*, *F. oxysporum* f.sp. *cubense*, non-pathogenic *F. oxysporum* N7 and non-pathogenic *F. oxysporum* N16 were subcultured onto PDA supplemented with 0-9800 mg ℓ⁻¹ silicon. The high pH (11.32) of the commercial formulation of potassium silicate increased the pH of PDA supplemented with potassium silicate, whereas the untreated PDA had a pH of 5.3 (Table 4.2; Figure 4.5). Therefore, to determine the effect of pH on the growth of these fungi, potassium hydroxide was added to PDA to raise the pH up to 11.5 (Table 4.2; Figure 4.5).

4.2.7 Sporulation of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended agar

Both the pathogenic and non-pathogenic *F. oxysporum* were subcultured on PDA by scraping off pieces of the frozen fungal suspension. After 3 d at 27°C in the dark three 4×4 mm² mycelial plugs from the margins of the pathogenic and non-pathogenic *F. oxysporum* were subcultured in 250 ml Erlenmeyer flasks containing 50 ml Yeast Extract Peptone Glucose Medium, prepared by mixing 5 g yeast extract, 5 g peptone, 10 g glucose and 1 ℓ distilled water. The flasks were amended with 0-9800 mg ℓ⁻¹ silicon. The flasks were then incubated for 5 d in a rotary shaker at 27°C at 120 oscillations min⁻¹. The broth was then filtered through two layers of cheesecloth and conidia were counted using a Neubauer Improved Double Haemocytometer.

4.2.8 Conidial germination of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended agar

Both the pathogenic and non-pathogenic strains of *F. oxysporum* were subcultured onto banana leaf agar by scraping off pieces of the frozen fungal suspension, which was stored at -80°C. All culture plates were incubated in a controlled cabinet with an alternating temperature of 25°C day and 20°C night and a photoperiod of 12 h (Nelson et al., 1983) for 14 d to stimulate formation of conidia especially, macroconidia. Conidia were harvested by flooding the plates with 10 ml sterile distilled water and gently scrapping the plates with a glass hockey stick. The conidial and mycelial suspensions were then filtered through cheesecloth to remove the mycelia. Water agar, amended with 0, 490, 4900 and 8820 mg ℓ^{-1} silicon, was plated with 0.1 ml of conidial suspensions and smeared as evenly as possible using a hockey stick. The plates were then incubated for 12 h at 25°C. To arrest the further growth of the fungi, 0.5 ml of lactaphenol cotton blue was then added to each plate. The number of germinated *F. oxysporum* microconidia and macroconidia were counted using a compound microscope with the 40× magnifying lens.

4.2.9 Greenhouse experiments on the control of *Fusarium oxysporum* f.sp. *phaseoli* and *Fusarium oxysporum* f.sp. *cubense* using soluble silicon

Plants were grown in a 150 mm pot containing composted pine bark as the sole growing medium and were treated with 100 ml silicon with a concentration of 0-8820 mg ℓ^{-1} at the following frequencies: (i) only once; (ii) twice with 1 wk intervals between applications; (iii) three times with 1 wk intervals between applications; (iv) every 2 wk; and (v) every 3 wk. There were two controls; one was inoculated with a pathogen but not treated with soluble silicon and the other was treated with neither of them. Banana plants were exposed to artificial cold stress after 1 mo for 3 wk at 10°C in a cold room with a 12 h photoperiod, and were subsequently kept for another 3wk back to the greenhouse, where the temperature range was 26-28°C. Two months later bean plants were rated for disease severity, their fresh green pods were harvested and weighed immediately, and dry weights were taken, after the biomass has been dried in the oven for 72 h at 70°C. Bananas plants were rated for disease severity according to the INIBAB's Technical Guideline 6 (Carlier et al., 2002). Plants were watered via drip irrigation three times a day for 3 min, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio.

4.2.10 Disease Rating

Fusarium disease severity ratings of bean plants were recorded based on a visual assessment of symptoms arising from infection by *F. oxysporum* f.sp. *phaseoli*, according to a five-point key (Carver *et al.*, 1996):

- 0 = healthy plants
- 1 = initial signs of wilting (yellowing)
- 2 = up to 25% of the leaves with symptoms
- 3 = up to 50% of the leaves with symptoms
- 4 = up to 75% of the leaves with symptoms
- 5 = plants dead

Fusarium disease severity ratings on banana plants were recorded based on a visual assessment of the internal symptoms caused by *F. oxysporum* f.sp. *cubense*, according to the INIBAB's Technical Guideline 6 (Carlier *et al.*, 2002):

- 1 = Corm completely clean, no vascular discoloration
- 2 = Isolated points of discoloration in vascular tissue
- 3 = Discoloration of up to one-third of vascular tissue
- 4 = Discoloration of between one-third and two-thirds of vascular tissue
- 5 = Discoloration of greater than two-thirds of vascular tissue
- 6 = Total discoloration of vascular tissue

The overall disease severity index (DSI) was calculated as follows (Mak *et al.*, 2004; Zhang *et al.*, 1996):

$$DSI = \frac{\sum(\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum(\text{Number of treated seedlings})}$$

4.2.11 Statistical Analysis

Experiments were repeated twice. Data was analyzed using GenStat® Executable release 9 Statistical Analysis Software. Analysis of variance (ANOVA) was used to determine treatment effects. Differences between treatments were distinguished using Fisher's protected least significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

4.3 Results

4.3.1 Hyphal growth of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended agar

Hyphal growth of the pathogenic and non-pathogenic *Fusarium oxysporum* strains increased at low concentrations of silicon after 4d of incubation. However, as the concentration of silicon in the media increased hyphal growth decreased (Table 4.1; Figure 4.1). Hyphal growth of *F. oxysporum* f.sp. *cubense* and non-pathogenic *F. oxysporum* N7 was significantly higher on PDA amended with 490-1960 mg ℓ^{-1} of silicon than the unamended control. There was no significant increase in hyphal growth of *F. oxysporum* f.sp. *phaseoli* on PDA amended with 490-1960 mg ℓ^{-1} of silicon compared to the unamended control. On the other hand, hyphal growth of non-pathogenic *F. oxysporum* N16 was significantly higher on PDA amended with 490-980 mg ℓ^{-1} of silicon compared to the unamended control at 4 d, but not after 7 d. Silicon at levels higher than 3920 mg ℓ^{-1} significantly inhibited hyphal growth of all four *Fusarium* strains tested. At 7840-9800 mg ℓ^{-1} growth was negligible. At this concentration there was no significant difference in hyphal growth of *F. oxysporum* N16 between 4d and 7d. Similarly, hyphal growth of *F. oxysporum* f.sp. *phaseoli* did not significantly increase at silicon levels higher than 8820 mg ℓ^{-1} . However, at all other concentrations there were significant increase in hyphal growth from 4d to 7d incubation period.

Higher pH did not inhibit the growth of the four *F. oxysporum* strains (Table 4.2, 4.3; Figure 4.3, 4.4). Instead, hyphal growth was stimulated by an increase in the pH of the PDA media. However, as the pH increased beyond 10.0 growth declined but it was still not significantly lower than the control. The effect of pH on hyphal growth of the fungi changed with length of incubation period, except for *F. oxysporum* Strain N16.

Table 4.1 Hyphal growth of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended PDA media

Silicon (mg l ⁻¹)	Hyphal growth (mm)							
	<i>F. oxysporum</i> f.sp. <i>cubense</i>		<i>F. oxysporum</i> f.sp. <i>phaseoli</i>		<i>F. oxysporum</i> Strain N16		<i>F. oxysporum</i> Strain N7	
	4d	7d	4d	7d	4d	7d	4d	7d
0	19.8 h	34.0 l	24.8 i	40.2 m	25.0 j	41.2 p	24.0 k	38.4 p
490	28.0 k	40.0 p	27.6 jk	40.6 m	30.0 l	40.8 p	29.8 n	40.2 q
980	25.4 j	40.0 p	24.6 i	40.0 m	29.6 l	40.4 p	27.8 n	40.0 q
1960	22.2 i	37.9 n	23.4 hi	1.0 m	19.4 h	38.8 n	25.2 l	39.8 q
2940	19.0 h	36.4 m	22.0 h	38.2 l	15.0 g	34.6 m	22.2 j	39.6 q
3920	5.4 cd	20.0 h	10.0 f	29.0 k	8.2 f	27.2 k	9.0 g	26.4 m
4900	6.4 d	15.6 g	7.8 e	26.5 j	5.2 e	23.6 i	6.2 e	21.8 j
5880	3.8 b	12.0 f	3.4 cd	15.6 g	4.0 d	15.8 g	4.6 d	16.6 i
6860	0.8 a	9.4 e	1.0 ab	4.6 d	1.8 b	2.8 c	2.4 c	15.2 h
7840	0.2 a	4.6 bc	0.2 a	2.2 bc	0.0 a	0.1 a	0.6 a	7.4 f
8820	0.2 a	3.6 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.0 b
9800	0.0 a	5.2 cd	0.8 ab	0.9 ab	0.0 a	0.0 a	0.0 a	3.8 d
Time×Si	<0.001		<0.001		<0.001		<0.001	
l.s.d.	1.306		1.587		0.866		1.103	
s.e.d.	0.658		0.798		0.435		0.554	
cv%	6.4		5.7		4.3		3.4	

Means with the same letter in the same strain are not significantly different at P≤0.05.

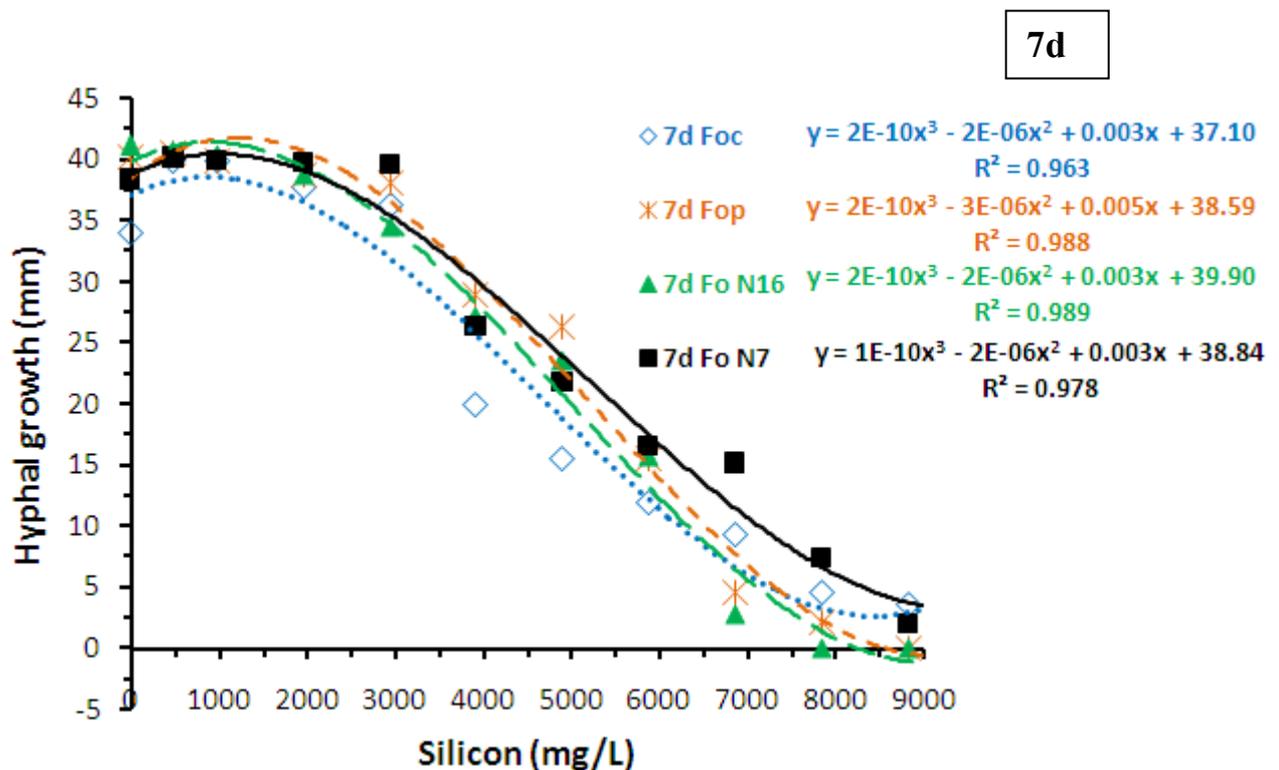
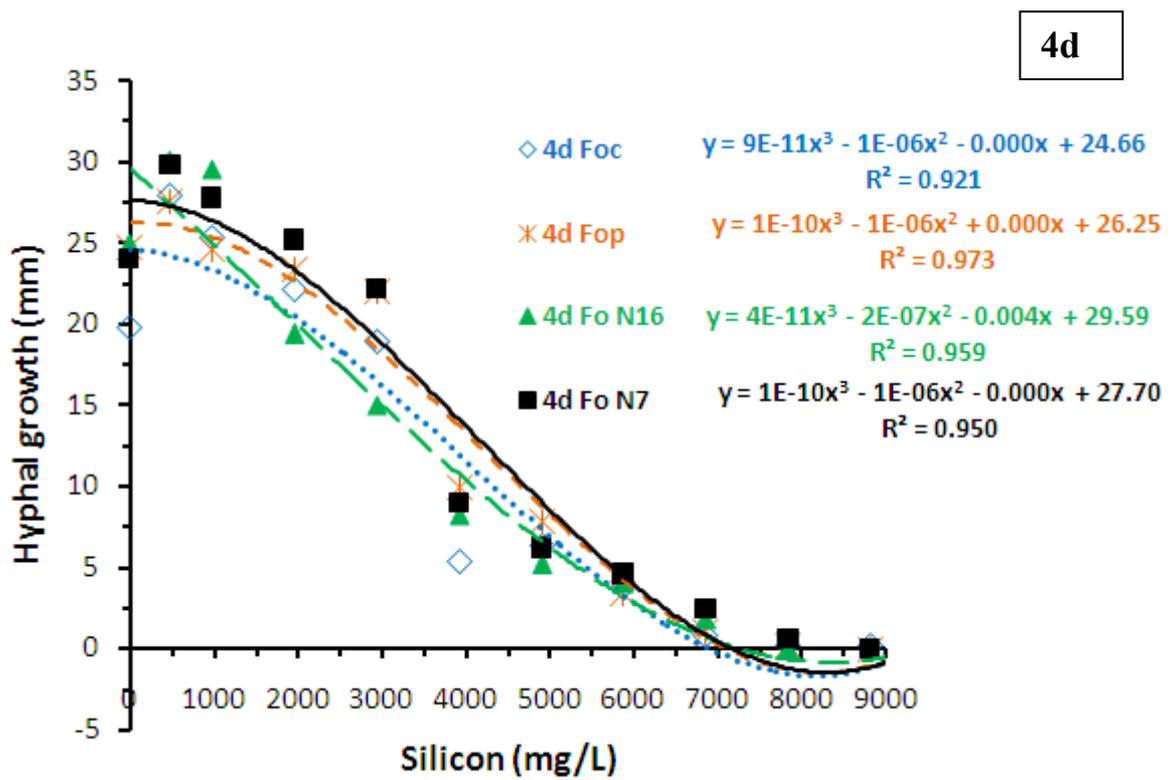


Figure 4.1 Hyphal growth of pathogenic and non-pathogenic *Fusarium oxysporum* in potassium silicate amended PDA media after 4d and 7d incubation period

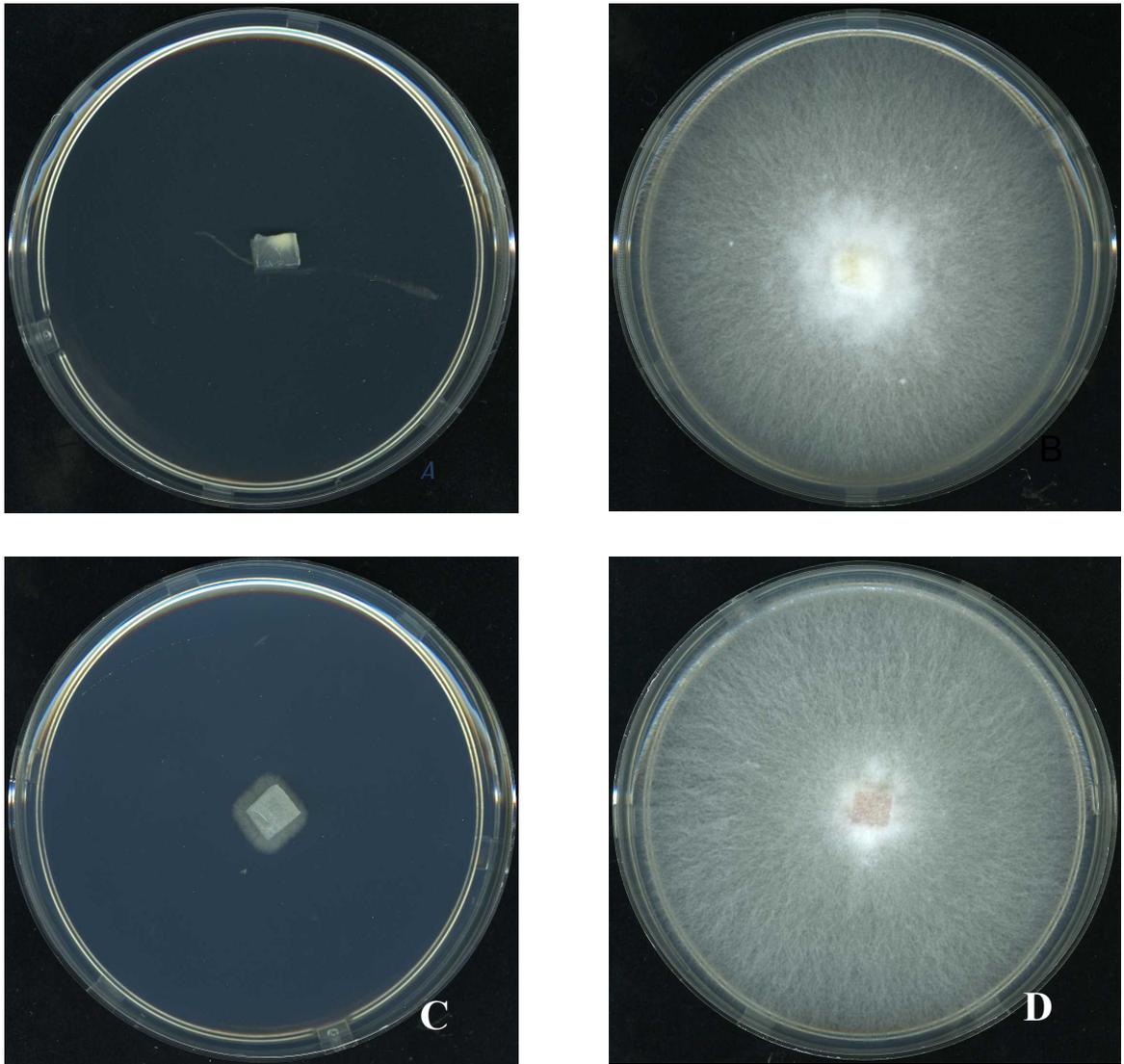


Figure 4.2 Hyphal growths of *F. oxysporum* f.sp. *phaseoli* (A, B) and non-pathogenic *F. oxysporum* Strain N7 (C, D) on PDA amended with 0 mg l^{-1} (B, D) and 8820 mg l^{-1} (A,C) of silicon

Table 4.2 Hyphal growths of pathogenic and non-pathogenic *Fusarium oxysporum* strains on PDA, where the pH was raised by the addition of potassium hydroxide

pH	Hyphal growth (mm)							
	<i>F. oxysporum</i> f.sp. <i>cubense</i>		<i>F. oxysporum</i> f.sp. <i>phaseoli</i>		<i>F. oxysporum</i> Strain N16		<i>F. oxysporum</i> Strain N7	
	4d	7d	4d	7d	4d	7d	4d	7d
5.3*	26.4 a	37.8 c	25.0 a	35.6 d	27.6 ab	44.0 c	25.6 a	34.8 c
7.0	26.4 a	37.4 c	26.4 b	38.8 e	28.2 b	47.4 e	28.6 b	44.2 de
8.0	26.8 a	43.6 d	28.8 c	45.4 h	28.6 b	47.2 e	28.4 b	45.4 e
9.0	28.6 b	50.2 f	26.6 b	41.0 fg	28.2 b	46.4 de	29.0 b	44.6 de
10.0	28.0 ab	48.5 ef	25.8 ab	41.4 g	27.6 ab	45.4 cd	27.4 ab	43.8 de
11.5	27.4 ab	47.6 e	25.0 a	40.0 ef	26.6 a	44.6 c	25.6 a	42.6 d
Time.pH	<0.001		<0.001		0.137NS		0.002	
s.e.d.	0.862		0.686		0.742		1.155	
l.s.d.	1.736		1.384		1.492		2.324	
cv%	4.2		3.8		3.0		5.7	
					Time <0.001†		pH <0.001†	

* Control, where no potassium hydroxide was added

† Main effects were significant at $P \leq 0.05$.

Means with the same letter in the same strain are not significantly different at $P \leq 0.05$.

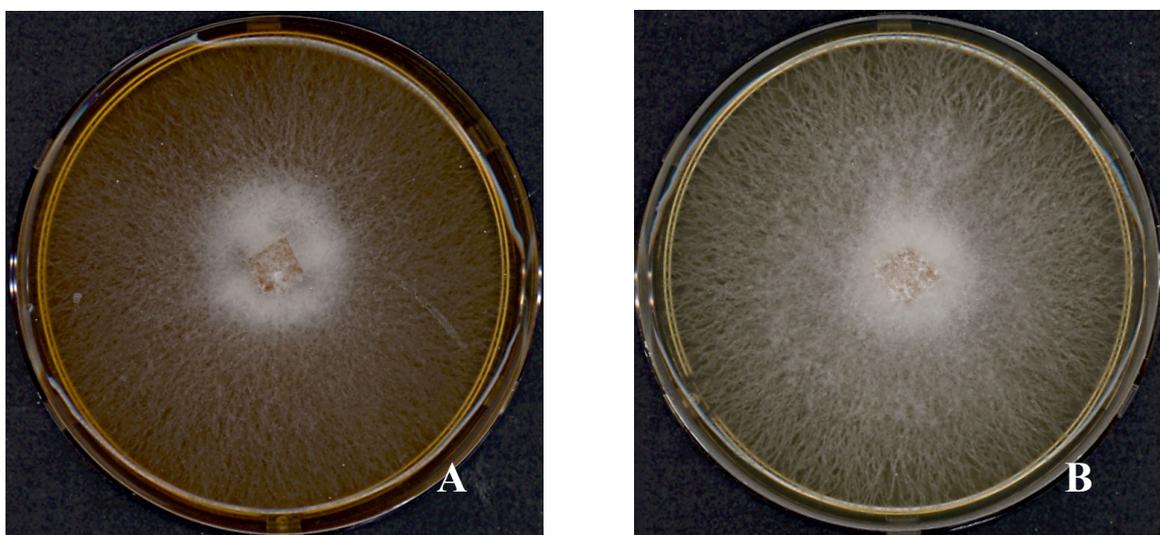


Figure 4.3 Hyphal growths of *F. oxysporum* f.sp. *phaseoli* on PDA where pH was adjusted to 11.5 (A) and 9.0 (B) using potassium hydroxide

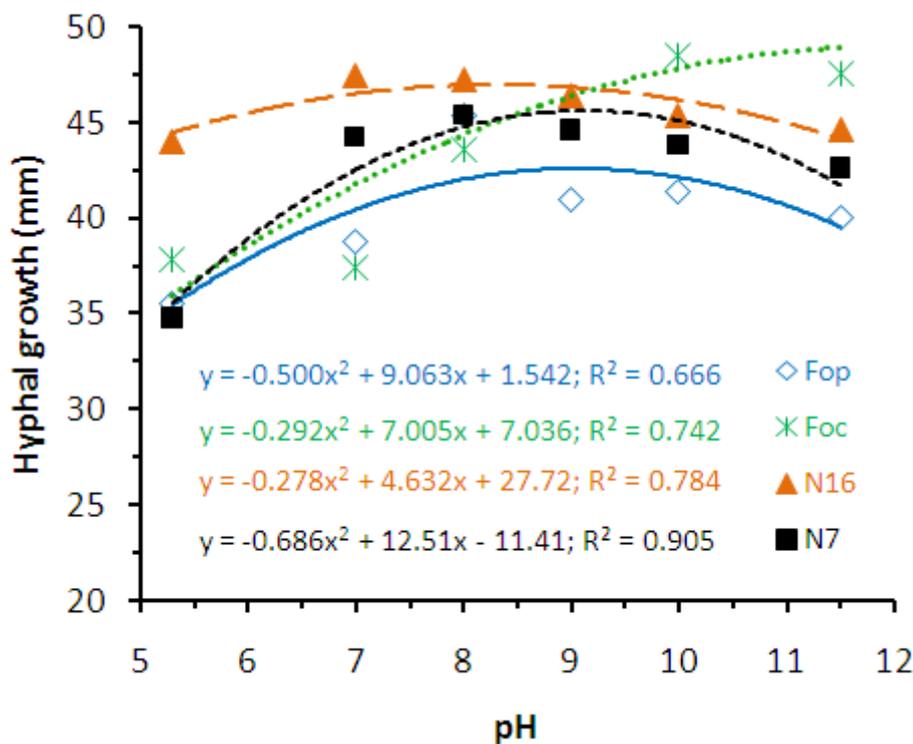
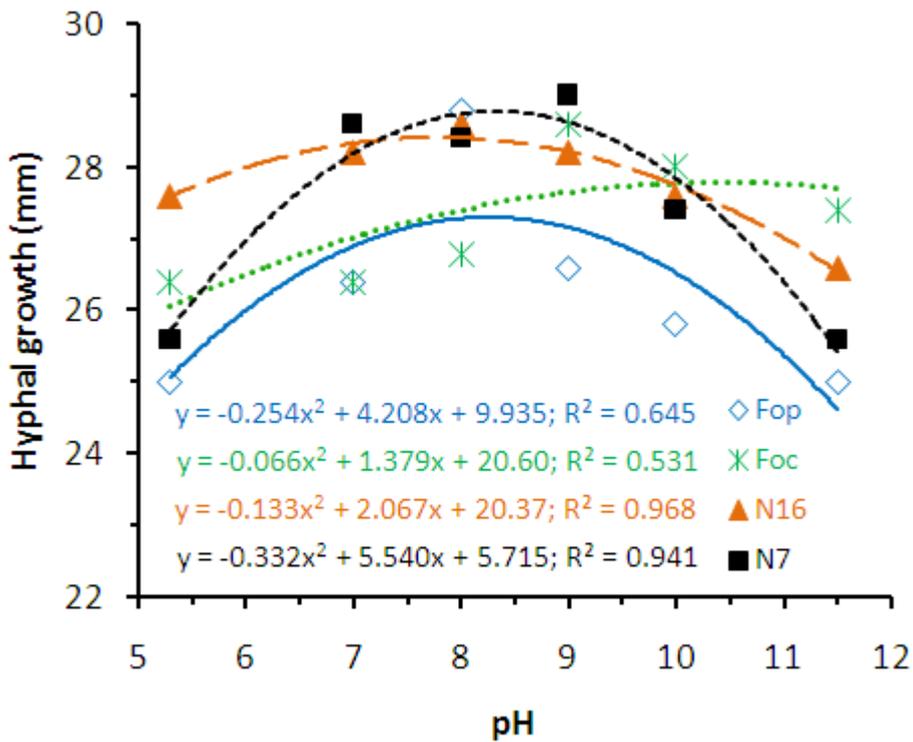


Figure 4.4 Hyphal growths of *F. oxysporum* f.sp. *cube*nse (Foc), *F. oxysporum* f.sp. *phaseoli* (Fop), non-pathogenic *F. oxysporum* Strain N7 (Fo N7) and Strain N16 (Fo N16) on PDA, in which potassium hydroxide was added to raise medium's pH

Table 4.3 The pH of PDA media amended with different concentrations of silicon

Silicon (mg/L PDA)	pH of media
0	5.35
490	8.90
980	9.58
1960	10.13
2940	10.40
3920	10.56
4900	10.68
5880	10.76
6860	10.82
7840	10.87
8820	10.91

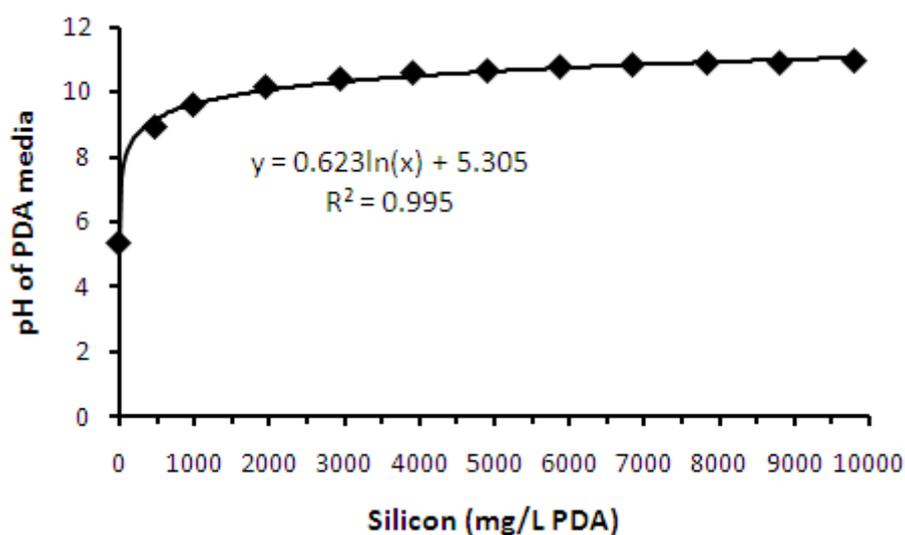


Figure 4.5 The pH of PDA media amended with different concentrations of silicon

4.3.2 Sporulation of pathogenic and non-pathogenic *Fusarium oxysporum* on soluble silicon amended agar

Sporulation of *F. oxysporum* strains was significantly ($P < 0.001$) higher when no silicon was added to the medium (Table 4.4; Figure 4.6). Addition of soluble silicon significantly reduced sporulation. Spore formation was reduced with an increase in the levels of silicon in the media for *F. oxysporum* Strain N16. The other strains, however, produced significantly higher conidia at $980 \text{ mg } \ell^{-1}$ silicon than at $490 \text{ mg } \ell^{-1}$ and decreased

thereafter (Table 4.4; Figure 4.6). Sporulation of the *Fusarium* strains was negligible at concentrations more than 2940 mg ℓ^{-1} (Table 4.4; Figure 4.6). No major difference in sporulation due to exposure to silicon was observed between the pathogenic and non-pathogenic *F. oxysporum* strains. Values with zero (Table 4.4) do not necessarily indicate that no single spore was produced at all but none were observed under the microscope.

Table 4.4 Sporulation by pathogenic and non-pathogenic strains of *Fusarium oxysporum* grown on Yeast Extract Peptone Glucose Medium amended with different concentrations of potassium silicate

Silicon (mg ℓ^{-1})	Sporulation ($\times 10^6$ conidia ml^{-1})			
	<i>F. oxysporum</i> f.sp. <i>cubense</i>	<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	<i>F. oxysporum</i> Strain N16	<i>F. oxysporum</i> Strain N7
0	18.80 e	5.25 e	6.40 e	29.23 e
490	7.38 c	0.68 b	4.75 d	5.40 c
980	10.40 d	2.47 d	3.57 c	6.90 d
1960	7.62 c	2.53 d	0.62 b	1.27 b
2940	2.90 b	1.44 c	0.21 ab	0.42 a
3920	1.15 a	0.07 a	0.0 a	0.0 a
4900	0.0 a	0.0 a	0.0 a	0.0 a
5880	0.0 a	0.0 a	0.0 a	0.0 a
6860	0.0 a	0.0 a	0.0 a	0.0 a
7840	0.0 a	0.0 a	0.0 a	0.0 a
8820	0.0 a	0.0 a	0.0 a	0.0 a
9800	0.0 a	0.0 a	0.0 a	0.0 a
F pr.	<0.001	<0.001	<0.001	<0.001
s.e.d.	0.721	0.162	0.231	0.246
l.s.d.	1.572	0.352	0.502	0.535
cv%	17.9	15.6	17.8	6.8

Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

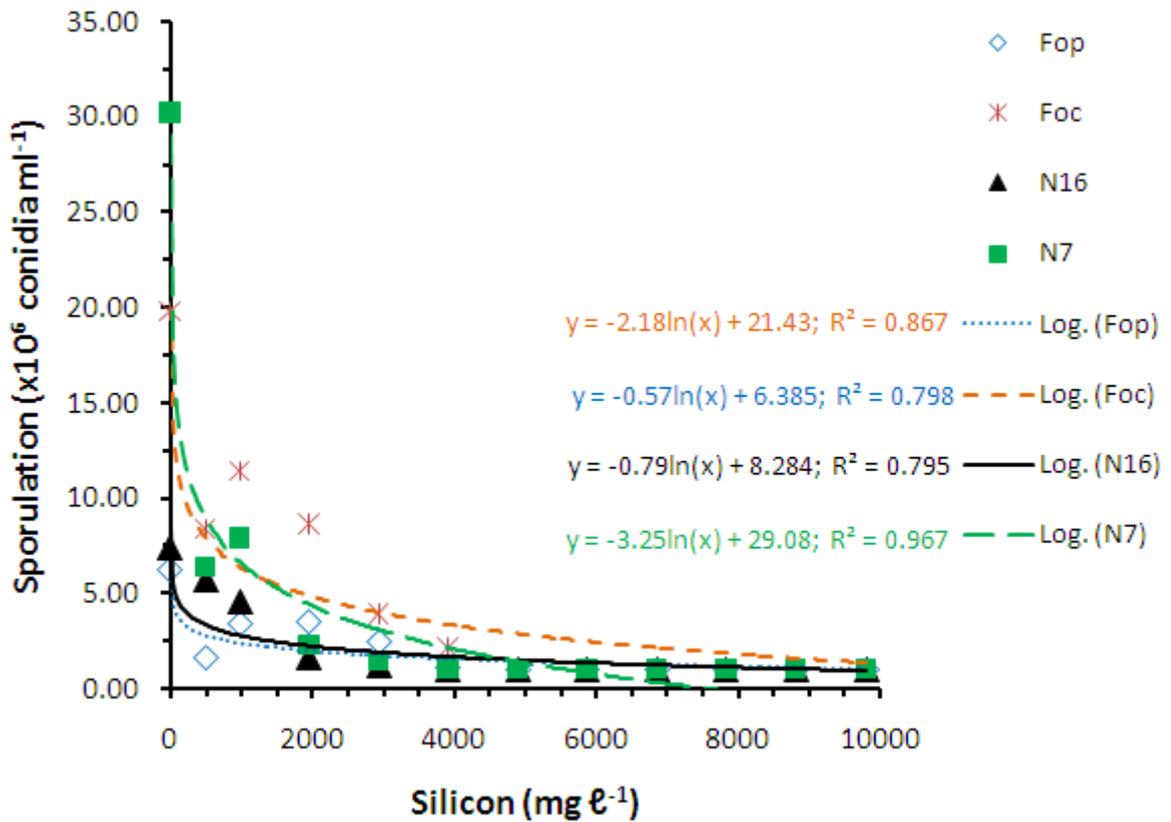


Figure 4.6 Sporulation of *F. oxysporum* f.sp. *cubeense* (Foc), *F. oxysporum* f.sp. *phaseoli* (Fop), non-pathogenic *Fusarium oxysporum* Strain N7 (Fo N7) and Strain N16 (Fo N16) grown in Yeast Extract Peptone Glucose Medium amended with different concentrations of potassium silicate

4.3.3 Germination of pathogenic and non-pathogenic *Fusarium oxysporum* spores on soluble silicon amended agar

Spore germination was significantly reduced as the concentration of soluble silicon in the media increased (Table 4.5; Figure 4.8). There was no significant difference in conidial germination between the untreated control and media treated with 490 mg l⁻¹ of silicon. At 4900 mg l⁻¹ of silicon spore germination was significantly inhibited. Those that germinated had very small germtubes compared to either the untreated control or media treated with 490 mg l⁻¹ (Figure 4.7). Spores did not germinate on media with 8820 mg l⁻¹ of silicon. Both the pathogenic and non-pathogenic *F. oxysporum* strains showed similar responses when exposed to different levels of soluble silicon.

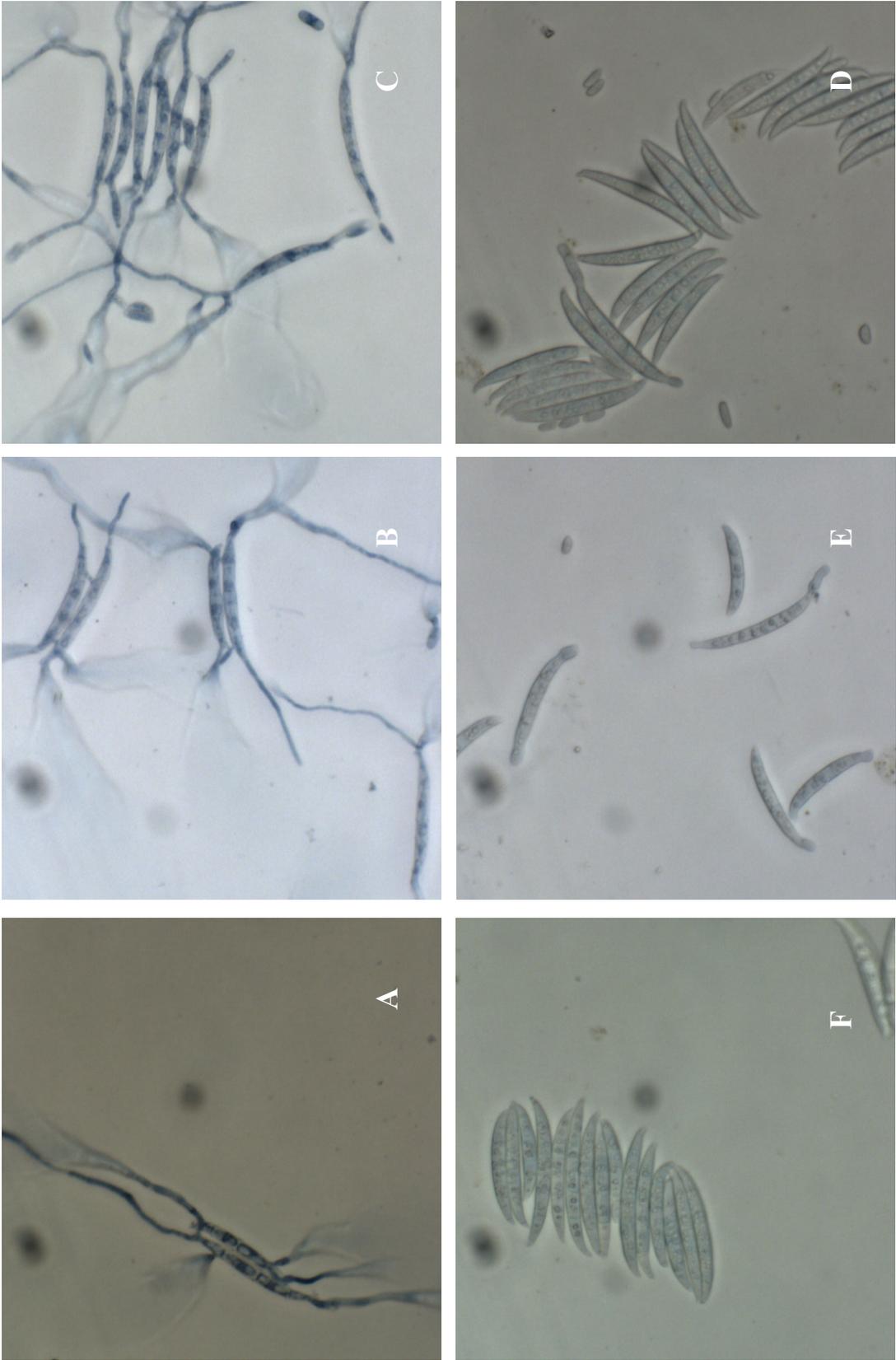


Figure 4.7 Conidial germination and germ tube length of *F. oxysporum* f.sp. *phaseoli* (A, B, D, F) and f.sp. *cubense* (C, E) on water agar amended with 0mg l⁻¹ (A), 490 mg l⁻¹ (B, C), 4900 mg l⁻¹ (D, E), and 8820 mg l⁻¹ (F) soluble silicon

Table 4.5 Germination of conidia of pathogenic and non-pathogenic strains of *Fusarium oxysporum* grown on water agar amended with different concentrations of potassium silicate

Silicon (ml l ⁻¹)	Conidial germination (%)											
	<i>F. oxysporum</i> f.sp. <i>cubense</i>			<i>F. oxysporum</i> f.sp. <i>phaseoli</i>			<i>F. oxysporum</i> Strain N16			<i>F. oxysporum</i> Strain N7		
	Micro- conidia	Macro- conidia		Micro- conidia	Macro- conidia		Micro- conidia	Macro- conidia		Micro- conidia	Macro- conidia	
0	94.59c	58.0(49.7)c		100(89.74)c	93.4(75.55)d		100(89.74)c	88.79(70.86)c		100(89.74)c	97.38(82.49)c	
490	98.30c	56.2(49.0)c		96.7(89.74)c	84.6(67.60)c		100(89.74)c	96.55(80.78)d		100(89.74)c	94.33(76.27)c	
4900	31.67b	23.7(28.6)b		25.5(29.91)b	30.1(33.16)b		50.9(45.46)b	34.61(35.99)b		49.58(44.76)b	7.56(15.63)b	
8820	0.0a	0.0(0.3)a		0.0(0.26)a	0.0(0.26)a		0.0(0.26)a	0.0(0.26)a		0.0(0.26)a	0.0(0.26)a	
F pr.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	
s.e.d.	2.663	4.37		2.090	2.734		2.526	2.422		0.936	2.216	
l.s.d.	5.556	9.22		4.391	5.703		5.286	5.052		1.952	4.655	
cv%	8.2	23.7		6.9	10.7		7.8	8.9		2.9	8.8	

Means with the same letter in the same column are not significantly different at P≤0.05.

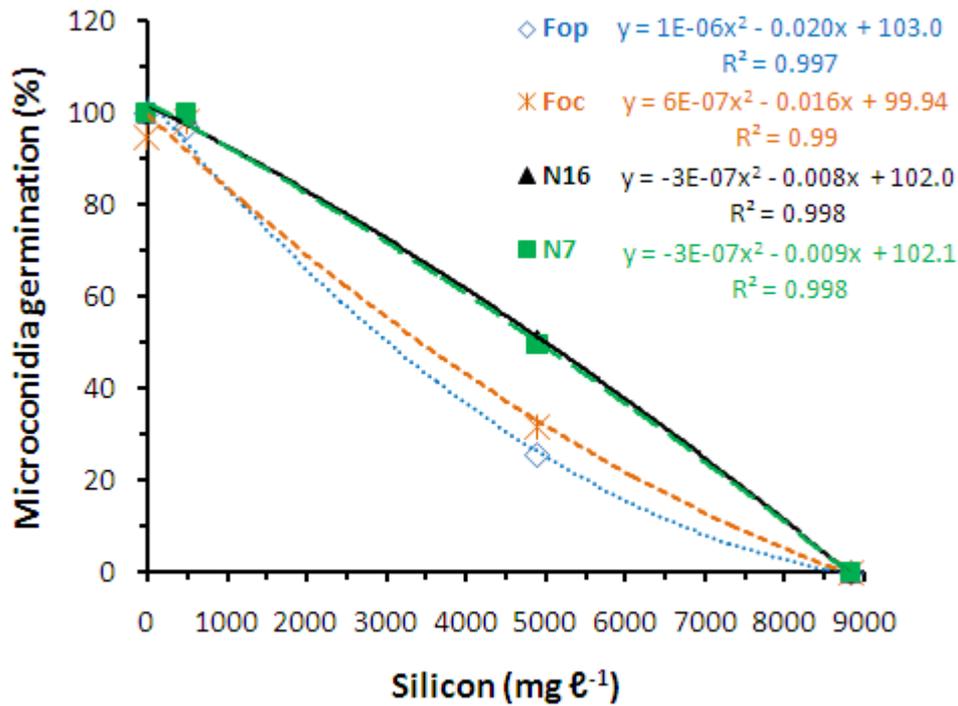


Figure 4.8a Microconidial germination of pathogenic and non-pathogenic strains of *Fusarium oxysporum* grown on water agar amended with different concentrations of potassium silicate

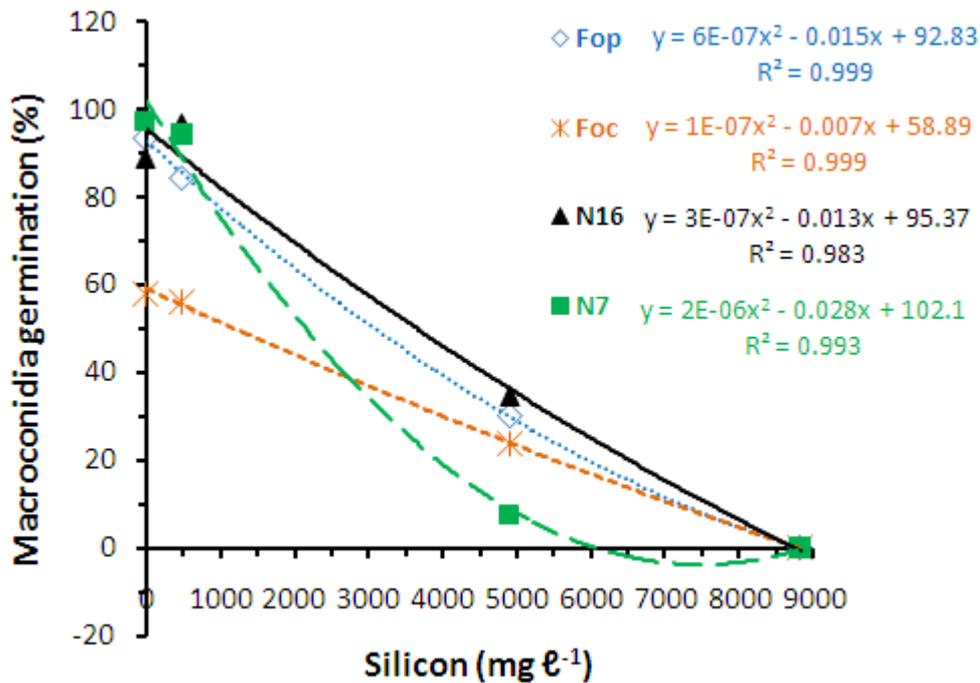


Figure 4.8b Macroconidial germination of pathogenic and non-pathogenic strains of *Fusarium oxysporum* grown on water agar amended with different concentrations of potassium silicate

4.3.4 Greenhouse pot experiments using soluble silicon against *Fusarium oxysporum* f.sp. *phaseoli* on beans applied with varying frequencies

Application of different concentrations of silicon significantly suppressed disease severity of bean plants infected with *F. oxysporum* f.sp. *phaseoli* (Table 4.6). However, there was no interaction ($P=0.418$) between the different concentrations of silicon and their frequency of application. Frequency of application of silicon had no effect on disease severity ($P=0.479$), green pod weight ($P=0.549$) and biomass dry weight ($P=0.359$). Silicon applied at concentrations of 4900 and 8820 mg ℓ^{-1} caused significantly lower disease levels compared to 0 or 490 mg ℓ^{-1} . Application at 4900 mg ℓ^{-1} caused significantly higher green pod weight ($P=0.042$) and biomass dry weight ($P=0.081$) of beans.

Table 4.6 Greenhouse pot experiment on the effect of different concentrations of potassium silicate applied at three different frequencies against *Fusarium oxysporum* f.sp. *phaseoli* on beans

Source	Level	DSI	Pod weight (g)	Dry weight (g)
Main effect				
Silicon (mg ℓ^{-1})		$P=<0.001$	$P= 0.042$	$P= 0.081$ NS
	0	3.31 c	226.43 a	44.06 a
	490	2.58 ab	366.61 ab	72.09 b
	4900	2.79 b	419.57 c	73.21 b
	8820	2.38 a	399.34 ab	62.60 ab
Frequency		$P=0.479$ NS	$P= 0.549$ NS	$P= 0.359$ NS
	Once	2.74 a	356.71 a	69.94 a
	Every 2wk	2.88 a	318.34 a	54.91 a
	Every 3wk	2.68 a	383.93 a	64.12 a
Silicon \times Frequency		$P=0.418$ NS	$P= 0.801$ NS	$P= 0.505$ NS

Means with the same letter in the same column are not significantly different at $P\leq 0.05$.

To improve the results obtained in the first experiment, a second experiment was designed with the idea that high concentrations of silicon in the first three weeks might reduce spore germination more than applications over an extended period. Application of 8820 mg ℓ^{-1} silicon at a 2 wk interval significantly ($P<0.001$) reduced disease severity on beans, and both 2 wk and 3 wk interval applications on banana compared to the pathogen treated control (Table 4.7, 4.8; Figure 4.9, 4.10). However, there was no significant difference between the other frequencies of applications. Application of silicon to bean plants at 8820 mg ℓ^{-1} at all frequencies of application did not cause significantly ($P<0.001$) higher green pod weight or biomass dry matter (Table 4.7; Figure 4.9).

Table 4.7 Greenhouse pot experiment on the effect of application frequencies of potassium silicate applied at a concentration of 8820 mg ℓ^{-1} against *Fusarium oxysporum* f.sp. *phaseoli* on beans

Frequency	DSI	Pod weight (g)	Dry weight (g)
Untreated control	0.12 a	989 b	146.94 b
Every 2wk	2.33 b	613 a	91.96 a
2wk	2.62 bc	622 a	90.39 a
Every 3wk	2.82 bc	640 a	99.97 a
3wk	3.04 bc	432 a	69.52 a
Once	3.12 c	495 a	83.84 a
<i>F. oxysporum</i> f.sp. <i>phaseoli</i> only	3.25 c	547 a	80.65 a
Frequency of application	<0.001	0.015	0.005
s.e.d.	0.344	121.5	14.821
l.s.d.	0.75	264.7	32.292
cv%	17.0	24.0	19.2

Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

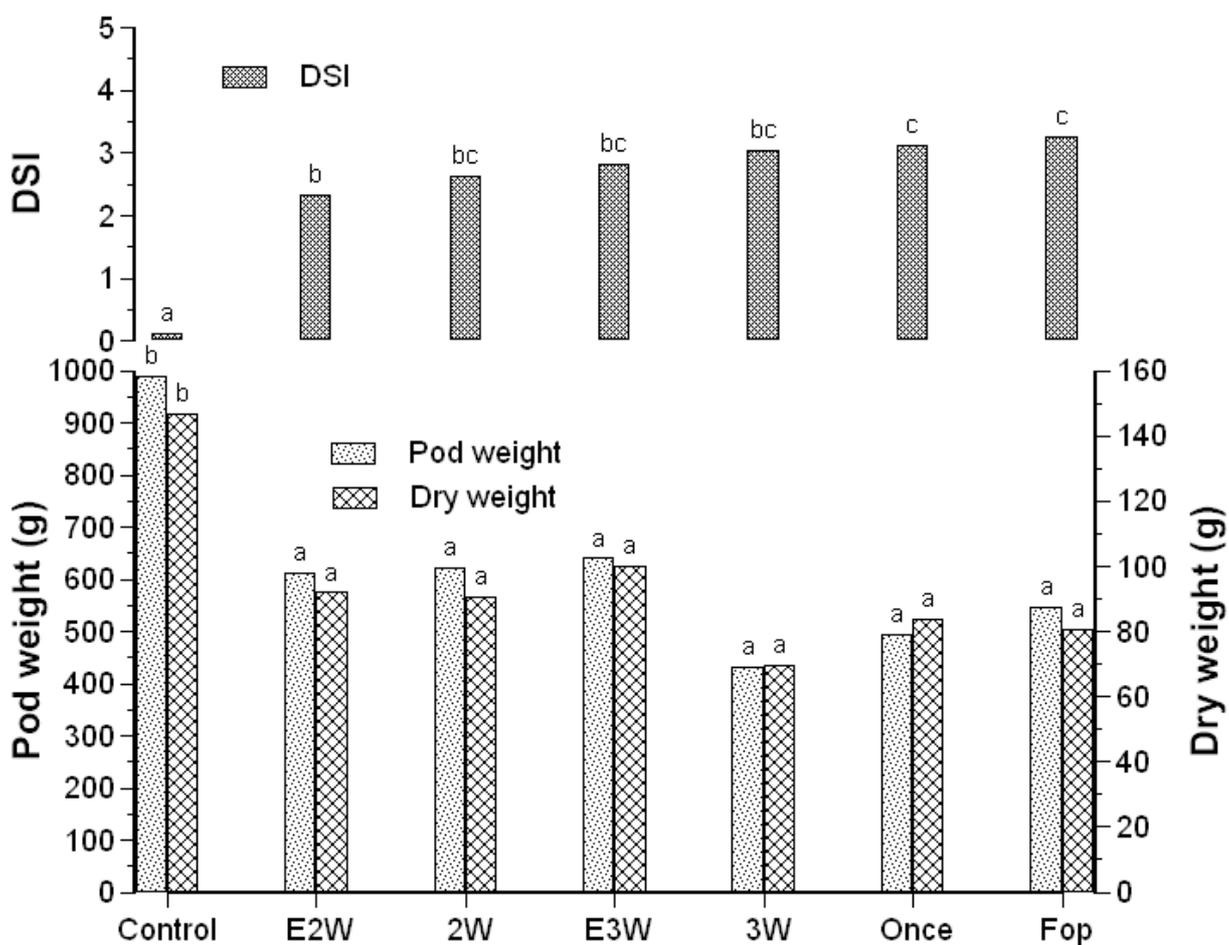


Figure 4.9 Greenhouse pot experiment on the effect of application frequencies of potassium silicate applied at a concentration of 8820 mg ℓ^{-1} against *Fusarium oxysporum* f.sp. *phaseoli* on beans

Table 4.8 Greenhouse pot experiment on the effect of application frequencies of potassium silicate applied at a concentration of 8820 mg ℓ^{-1} against *Fusarium oxysporum* f.sp. *cubense* on bananas

Frquency	Corm discoloration	Foliar wilting/yellowing
Untreated control	1.00 a	0.00 a
2wk	3.07 bc	2.00 b
Every 2wk	2.33 b	2.33 b
3wk	2.67 bc	2.57 bc
Every 3wk	2.20 b	2.67 bc
Once	2.73 bc	4.07 cd
<i>F. oxysporum</i> f.sp. <i>cubens</i> only	3.47 c	4.33 d
Frequency of application	0.005	0.002
s.e.d.	0.459	0.718
l.s.d.	1.001	1.599
cv%	22.5	34.2

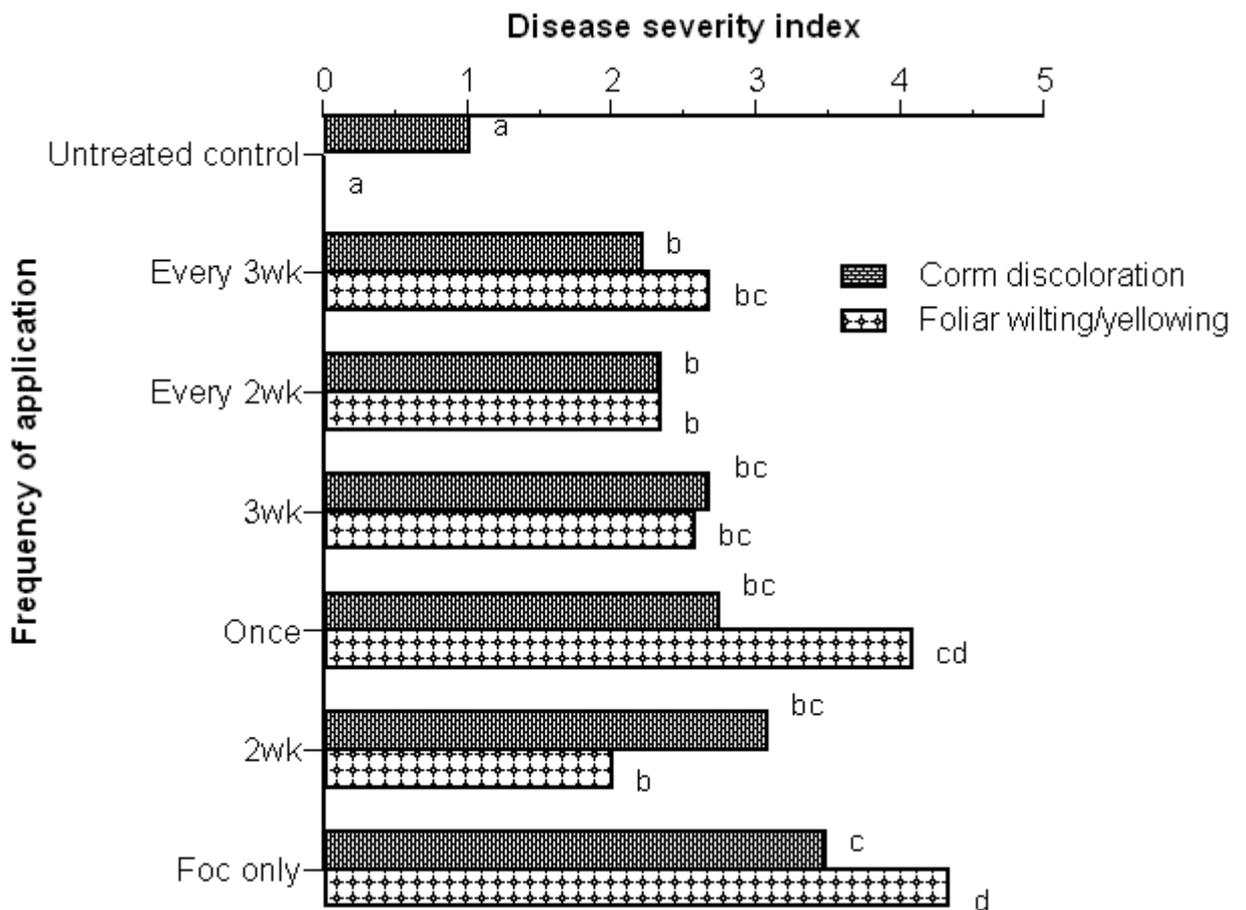


Figure 4.10 Greenhouse pot experiment on the effect of application frequencies of potassium silicate applied at a concentration of 8820 mg ℓ^{-1} against *Fusarium oxysporum* f.sp. *cubense* on bananas

4.4 Discussion

The anti-fungal activity of chemicals against pathogenic fungi is usually measured in the laboratory by exposing the fungi to the chemicals and measuring toxicity responses. These toxicity responses are usually inhibition of fungal growth, spore germination, or actual plant infection, in cases where the fungus cannot be cultured (Bhuiyan et al., 2000; Damicone, 2004; Iacomi-Vasilescu et al., 2004).

The results presented in this study demonstrated that high concentrations of soluble silicon inhibit fusarial hyphal growth, sporulation, spore germination and reduced disease severity. However, hyphal growth was higher at 490 mg ℓ^{-1} silicon than the untreated control. This actually indicates that vegetative growth of these fungi was stimulated by lower levels of silicon but at high concentrations it becomes toxic, much like elements such as Cu and Mn. Although pH is an important factor in the growth and development of many fungi, the increase in pH due to the addition of potassium silicate to PDA did not make any contribution to the inhibition of fungal growth. This was clearly shown by the growth of the Fusarial strains at pH as high as 11.5 equaling growth of the control with a pH of 5.3.

Sporulation of the fusarial strains was significantly inhibited at concentrations higher than 3920 mg ℓ^{-1} , when sporulation of the fungi was negligible. Fungi produce spores to spread and colonize new areas through a variety of mechanisms. They also produce spores to withstand sub-optimal conditions and survive. Successful colonization in Fusarium wilt pathogens occurs by means of successive passage of microconidia via the transpiration stream in the xylem vessels (Beckman, 1990). Inhibition of spore formation would then greatly reduce the multiplication and spread of the pathogens if plants can actually maintain such high levels of soluble silicon in their xylem vessels. Nevertheless, it is applicable in reducing the spread of postharvest pathogens (e.g. *Penicillium* spp. in citrus). Sporulation at 490 mg ℓ^{-1} of soluble silicon was lower than at 980 mg ℓ^{-1} . This could mainly be because at 490 mg ℓ^{-1} of silicon the fungi grew more vegetatively. Spore production in most fungi is low in media rich in carbohydrates. In these media the fungi grow vegetative faster but spore production was low.

Spore germination is very important in the life cycle of all fungi. It is a complex multi step process initiated when nutrients are provided (Joseph-Strauss et al., 2007). Conidial

germination of the fusarial strains was significantly inhibited. The conidia could not germinate or slowly germinated under conditions where there were high concentrations of silicon. Relatively early in the process of germination, conidia go through a process of un-coating in which they lose their unique spore wall and becomes more sensitive to different environmental stresses (Herman and Rine, 1997). This study did not investigate if the conidia, which did not germinate, were on the process of un-coating. However, it can be deduced from the conidia, which were slowly germinating in the presence of $4900 \text{ mg } \ell^{-1}$ of silicon, that they were on the process of germination, which makes them vulnerable if exposed to suboptimal conditions.

In pot experiments, application of silicon significantly reduced disease severity, slowing disease progress. The reduction in disease could not be entirely attributed to direct toxicity of silicon because silicon absorbed by the plants also plays a role in suppression of disease through a variety of other ways. The level of control obtained, however, was not as impressive as the *in vitro* plate experiments. This could mainly be because of the complexity of soil chemistry and the difficulty of maintaining high levels of soluble forms of silicon in the growing medium, which was under constant irrigation. Silicon may bind to heavy metals such as Al and thereby change into insoluble forms (Epstein, 1999a). It is a useful property of silicon in reducing heavy metal toxicity in plants. But its availability may be reduced in the soil solution. Silicon is a non-polluting, naturally occurring soil component that is safe and has multiple beneficial effects in agriculture, in addition to the fungistatic effect it has at high concentrations.

4.5 References

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CHAPTER FIVE

Effect of solo and combined application of non-pathogenic *Fusarium oxysporum*, *Trichoderma harzianum* Eco-T[®] and soluble silicon against *Fusarium* wilt of banana and beans

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Abstract

The importance of *Fusarium* wilt diseases of banana and beans in agriculture has been widely documented. The lack of viable control options and their significance to the livelihood of millions of people around the world makes them particularly important. In this study, the integration of biological and agronomic control methods was investigated under greenhouse conditions on banana and beans, and field conditions on banana plants. Integration of non-pathogenic *Fusarium oxysporum* Schlecht Strain N7 and Strain N16, and silicon were tested against *F. oxysporum* f.sp. *cubense* and *F. oxysporum* f.sp. *phaseoli* on banana and beans, respectively, in greenhouse pot experiments. The endophytes were also tested in combination with *Trichoderma harzianum* Rifai Eco-T[®] against *Fusarium* wilt of beans. Banana plants were cold stressed to induce disease expression quickly. The non-pathogenic *F. oxysporum* Strain N7 and Strain N16 were effective biocontrol agents against *Fusarium* wilt of banana and beans. Silicon fertilization of plants exposed to the non-pathogenic *F. oxysporum* strains improved the health of cold-stressed banana plants compared to those treated with endophytes only. However, silicon did not produce an equivalent effect on beans. Similarly, use of a combination of *T. harzianum* Eco-T[®] and the non-pathogenic *F. oxysporum* strains did not result in increased disease suppression relative to application of the endophytic strains only. In the field plants that were treated with combinations of non-pathogenic *F. oxysporum* Strain N16, *T. harzianum* Eco-T[®], silicon and mulch had significantly greater shoot height and pseudostem girth than solo

applications of the treatments. Mulching increased growth of feeder roots. The combined application of biocontrol organisms, silicon and mulching can provide an effective control option for banana growers dealing with *Fusarium* wilt in their plantations.

5.1 Introduction

Fusarium wilt of banana and beans, caused by *Fusarium oxysporum* (Schlecht) f.sp. *cubense* (Smith) Snyder & Hansen and f.sp. *phaseoli* Kendrick and Snyder, respectively, are important diseases of these crops in many parts of the world (Buruchara and Camacho, 2000; Ploetz, 2006). These *Fusaria* have the ability to establish themselves systemically in the xylem vessels of their hosts, causing yellowing and wilting symptoms (MacHardy & Beckman, 1981).

Severe outbreaks of *Fusarium* wilt of beans have been reported in Africa, Europe, Latin America and the United States (Abawi and Pastor-Corrales, 1990; Allen, 1995; Buruchara and Camacho, 2000; Kraft et al., 1981; Mutitu et al., 1988; Rusuku et al., 1997; Schwartz et al., 1989; Silbernagel and Mills, 1990). It is estimated that yield losses in beans may reach 80% in Brazil (Dhingra et al., 2006) and annual losses exceed 14,690 tonnes in Rwanda (Trutmann and Graf, 1993). Similarly, *Fusarium* wilt of banana, commonly known as Panama disease, is one of the most important diseases of banana plants worldwide. It has been threatening the banana industry for many years. Although the disease was first reported in 1874 in Australia (Simmonds, 1966), it is most famous for the epidemic it caused in Central and South America, where it destroyed 40,000 ha of Gros Michel banana plantation from 1890 to 1950 (Pegg et al., 1996). It is ranked as the most destructive disease of all times, along with wheat rust and potato blight (Carefoot and Sprott, 1969). The export banana industry was only saved by the introduction of Cavendish cultivar, which was resistant to *F. oxysporum* f.sp. *cubense* (O'Donnell et al., 1998). However, recent outbreaks of the disease on Cavendish clones in Australia, Canary Islands, South Africa (Ploetz, 1990) and Southern Asia (O'Donnell et al., 1998) is threatening the continued use of these clones for export trade and local production. The disease was first reported in South Africa in 1940 in KwaZulu-Natal. It subsequently spread to Kiepersol, where it caused 30% loss in banana fields between 1991 and 2000 (Viljoen, 2002); and it continues spread. Industry estimates were that banana production is

reduced by more than a third in South Africa because of Fusarium wilt. Because it is not profitable to grow banana on *F. oxysporum* f.sp. *cubense* infested fields, many growers are shifting to growing other crops such as macadamia. The value of Fusarium wilt-infested land also declines considerably.

Several studies have shown the potential of non-pathogenic *F. oxysporum* to reduce the impact of their pathogenic counterparts on several crops. The role played by non-pathogenic *F. oxysporum* on soils suppressive to Fusarium wilt diseases is a prime example (Alabouvette and Couteaudier, 1992; Smith and Snyder, 1971; Tamietti et al., 1993). Similarly, several reports indicate that *Trichoderma* species can effectively suppress fusarial wilt pathogens (Calvet et al., 1990; Sivan and Chet, 1986; Sivan et al., 1987). Another important component of this study was the use of soluble silicon. Silicon is believed to improve plant health by reducing biotic and abiotic stresses on different plants (Belanger et al., 1995; Epstein, 1999; Rodrigues et al., 2001; Yoshida et al., 1962).

During initial screening and subsequent trials in this study (Chapter 2), the selected non-pathogenic, endophytic *F. oxysporum* strains reduced disease severity of Fusarium wilt of beans significantly. However, pronounced discoloration of the vascular tissue was still visible. The control agents could only suppress expression of disease but could not protect plants from infection. In this study, the selected endophytic *F. oxysporum* organisms were tested against Fusarium wilt of banana and beans, in combination with soluble silicon or *Trichoderma harzianum* Eco-T[®], a commercial biocontrol product effective against several soilborne plant pathogens. However, when soluble silicon in the form of potassium silicate was applied at different rates to bean or banana plants challenged by Fusarium wilt pathogens, the level of control provided was not adequate (Chapter 3). Therefore, in this study, a combination of the non-pathogenic *F. oxysporum* strains and silicon was tested against *F. oxysporum* f.sp. *cubense* and f.sp. *phaseoli* on banana and beans, respectively. The efficacy of combinations of the non-pathogenic *F. oxysporum* strains and *T. harzianum* Eco-T[®] were also tested against *F. oxysporum* f.sp. *phaseoli* on beans.

5.2 Materials and methods

5.2.1 Source of Plant Material

Bean (*Phaseolus vulgaris* L.) seeds of the cultivar Outeniqua, provided by ProSeed cc (Pty) Ltd.⁸, Pietermaritzburg, South Africa, and 50 mm banana (*Musa* sp.) tissue cultured plantlets of the cultivar Cavendish, Williams, provided by DuRoi Laboratory⁹ were used throughout the experiments.

5.2.2 Biocontrol agents

Biocontrol microorganisms, non-pathogenic *F. oxysporum* Strains N7 Accession # 08296 and N16 Accession # 08297, and *T. harzianum* Eco-T^{®10}, were used throughout the experiments. Non-pathogenic *F. oxysporum* Strain N7 and Strain N16 were selected after the screening of many endophytes, isolated from healthy banana plants, against *F. oxysporum* f.sp. *phaseoli* (Chapter 2). *Trichoderma harzianum* Eco-T[®] is a registered, formulated biocontrol product, effective against several soilborne pathogens such as *Pythium*, *Rhizoctonia* and *Sclerotinia*, was provided by Plant Health Products (Pty) Ltd.

5.2.3 Soluble silicon

Soluble silicon was applied in the form of liquid potassium silicate, product K2550, containing 20.5-20.9% SiO₂, provided by PQ Silicas (Pty) Ltd¹¹.

5.2.4 Inoculum production of the *Fusarium oxysporum*

Inocula of the fungi were produced by growing them in barley grains. Fifty grams of barley grains were soaked overnight in 250 ml Erlenmeyer flask containing a 100 ml of water. Excess water was drained off and the flasks were autoclaved consecutively for two days at 121°C for 20 min. Each flask was then inoculated with three disks of a fungus grown on PDA for 3 d at 27°C in the dark. The fungi had been freeze stored in 50% glycerol at -80°C in Eppendorf tubes and were grown by scraping off a small piece onto PDA plates.

⁸ Pro-Seed cc, P.O.Box 101477, Scottsville 3209, Pietermaritzburg, South Africa

⁹ Du Roi Laboratory, P.O.Box 1147, Letsitele 0885, South Africa

¹⁰ Plant Health Products (Pty) Ltd, P.O.Box 207, Nottingham Road, KwaZulu-Natal, South Africa

¹¹ PQ Silicas, PO Box 14016, Wadeville 1422, South Africa

After 2wk at 27°C, the grains were air-dried, milled using a coffee grinder and stored at 4°C.

5.2.5 Combination of non-pathogenic *Fusarium oxysporum* and soluble silicon on beans

Bean plants were grown in 150 mm pots containing composted pine bark as a growing medium. Plants were treated with the non-pathogenic *F. oxysporum* strains with or without soluble silicon. The non-pathogenic *F. oxysporum* strains were applied as a drench at a rate of 100 ml per plant, containing mycelial and conidial suspensions at a concentration of 10^6 c.f.u ml⁻¹, at planting. Similarly, *F. oxysporum* f.sp. *phaseoli* was applied at the same dose but 1wk later, after bean seeds had germinated. Silicon was applied once a week at 400 mg ℓ⁻¹. The experiment was arranged in a randomized complete block design with each treatment replicated four times in a greenhouse with temperatures varying between 26-28°C. Plants were drip irrigated three times a day for 3 min supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio. Nine weeks later, bean plants were rated for disease severity, their fresh green pods were harvested and weighed immediately, and subsequently the whole plant dry weight was taken after the plants were oven-dried for 72 h at 70°C.

5.2.6 Combination of non-pathogenic *Fusarium oxysporum* and soluble silicon on bananas under greenhouse conditions

As with the bean trials, banana plants were treated with the non-pathogenic *F. oxysporum* strains and *T. harzianum* Eco-T[®], with or without soluble silicon, and were challenged by *F. oxysporum* f.sp. *cubense*. Banana plantlets were treated with the non-pathogenic strains of *F. oxysporum* and *T. harzianum* Eco-T[®] by dipping their roots in a conidial and mycelial suspension containing 10^7 c.f.u ml⁻¹ and planted immediately in a 75 mm pot containing composted pine bark as a growing medium. Silicon was applied weekly at a concentration of 1000 mg ℓ⁻¹. Three weeks later all plants except the untreated control were inoculated with 100 ml of 10^7 c.f.u ml⁻¹ of *F. oxysporum* f.sp. *cubense*. After 1mo post inoculation with the pathogen, the banana plants were stressed with cold to induce early disease expression by placing them in a cold room at 10°C for 3 wk. After 3 wk in the cold room, the banana plants were transplanted into 250 mm pots. One month later corm discoloration was rated according to the INIBAB's Technical Guideline 6 (Carlier et al., 2002).

5.2.7 Integration of biological and agronomic control methods on banana plants in the field to control *Fusarium* wilt

Banana plantlets were treated with the non-pathogenic *F. oxysporum* N16 by dipping their root in a spore suspension containing 10^7 c.f.u ml⁻¹ and immediately planted. Plants were kept in the greenhouse for 3 wk before being taken to the field. They were planted in a commercial banana plantation¹² with a history of *Fusarium* wilt, following the normal practices used by the farmer. *T. harzianum* Eco-T[®] was applied at a rate of 400 ml per tree at a concentration of 10^6 conidia ml⁻¹ at planting. Plants were also treated monthly with 4 l tree⁻¹ of 900 mg l⁻¹ of silicon. In addition, 250 mm of macadamia husks were placed at the bottom of banana plants to cover their drip zones as mulch. After 5 mo, agronomic measurements were taken according to the INIBAB's Technical Guideline 6 (Carrier et al., 2002). However, disease severity measurements were not taken as the experiment still running and it is too soon to destructively harvest the banana plants.

5.2.8 Combination of non-pathogenic *Fusarium oxysporum* and *Trichoderma harzianum* Eco-T[®] on beans

Beans were planted in a composed pine bark growing medium in 150 mm pots. The *T. harzianum* Eco-T[®] and non-pathogenic *F. oxysporum* Strain N7 or N16 were applied either a seed treatment or as a drench.

Seed treatment:

Bean seeds were coated with 2 g of 2×10^9 conidia g⁻¹ of *T. harzianum* Eco-T[®], or non-pathogenic *F. oxysporum* Strains N7 or N16, supplemented with 2% (w/v) carboxymethylcellulose (CMC) which served as an adhesive, then immediately dried by ventilation under a laminar flow.

Drenching:

After planting seeds coated with either *T. harzianum* Eco-T[®], or *F. oxysporum* Strain N7 or N16, pots were drenched with either 50 ml of 10^7 conidia ml⁻¹ *T. harzianum* Eco-T[®], or *F. oxysporum* Strains N7 or N16, depending on what the planted seed was coated with. If the seed planted was coated with *T. harzianum* Eco-T[®], then it was drenched with either non-pathogenic *F. oxysporum* Strain N7 or N16 and vice versa. After germination, plants were

¹² Tony Abbot, Clearwater farm, District Road 595, Port Edward

drenched with 100 ml inoculum of 10^6 c.f.u. ml^{-1} of *F. oxysporum* f.sp. *phaseoli*. Irrigation, supplemented with NPK soluble fertilizer [3:1:3(38)] and calcium nitrate (48) in a 1:1 ratio, was supplied three times a day for 5 min. The experiment was arranged in a randomized complete block design replicated three times. Nine weeks later plants were rated for disease severity, fresh green pods were weighed and dry weight was taken after the biomass was dried in the oven for 72 h at 70°C.

5.2.9 Disease Rating

Disease severity levels of beans were based on visual assessment of symptoms arising from infection of by *F. oxysporum* f.sp. *phaseoli* according to a five-point key (Carver et al., 1996):

- 0 = healthy plants
- 1 = initial signs of wilting (yellowing)
- 2 = up to 25% of the leaves with symptoms
- 3 = up to 50% of the leaves with symptoms
- 4 = up to 75% of the leaves with symptoms
- 5 = plants dead

Disease severity levels in the banana trials were recorded based on visual assessment of the internal symptoms caused by *F. oxysporum* f.sp. *cubense* according to the INIBAB's Technical Guideline 6 (Carlier et al., 2002):

- 1 = Corm completely clean, no vascular discoloration
- 2 = Isolated points of discoloration in vascular tissue
- 3 = Discoloration of up to one-third of vascular tissue
- 4 = Discoloration of between one-third and two-thirds of vascular tissue
- 5 = Discoloration of greater than two-thirds of vascular tissue
- 6 = Total discoloration of vascular tissue

The over all disease severity index was calculated as follows (Mak et al., 2004; Zhang et al., 1996):

$$\text{DSI} = \frac{\sum (\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum (\text{Number of treated seedlings})}$$

5.2.10 Statistical Analysis

Experiments were repeated twice. Data was analysed using GenStat® Executable Release 9 Statistical Analysis Software. Analysis of variance (ANOVA) was used to analyse the biocontrol experiments to determine whether the treatment effects were significant. This was made using Fisher's protected least significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

5.3 Result

5.2.1 Combination of non-pathogenic *Fusarium oxysporum* and soluble silicon on bananas under greenhouse conditions

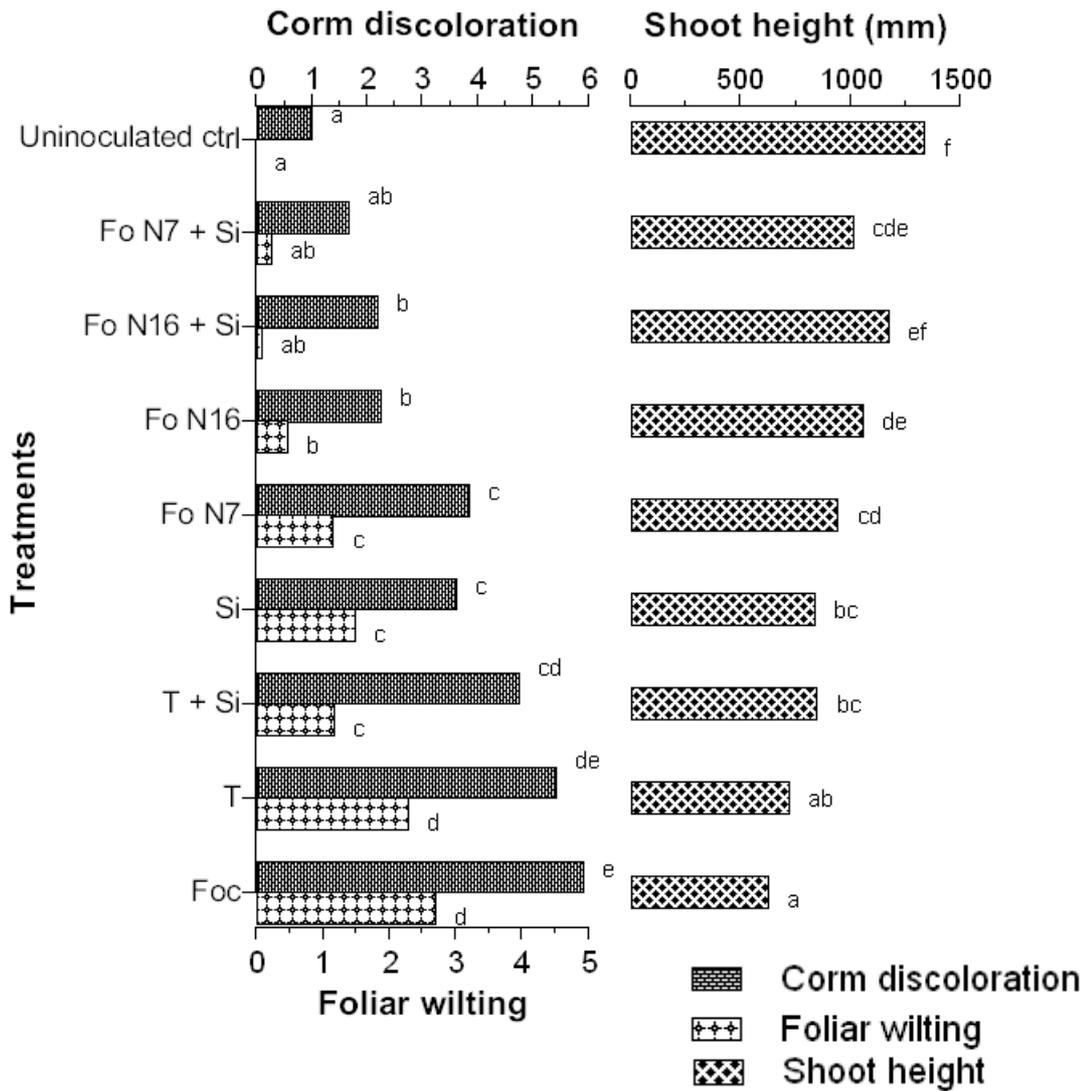
Disease severity (corm discoloration and shoot yellowing/wilting) was significantly ($P < 0.001$) reduced on banana plants treated with either the non-pathogenic *F. oxysporum* or their combination with silicon. Application of *T. harzianum* Eco-T® and silicon also produced significantly lower disease severity level compared to the pathogen-treated control ($P < 0.001$). Applications of soluble silicon to banana plants treated with the non-pathogenic *F. oxysporum* Strain N7 at planting reduced corm discoloration and shoot yellowing/wilting to a level not significantly different to the pathogen-untreated control ($P < 0.001$). Combined applications of non-pathogenic *F. oxysporum* Strain N16 and soluble silicon did not suppress disease severity significantly better compared to treatment with the non-pathogenic *F. oxysporum* Strain N16 alone, but plants were taller and shoot wilting/yellowing was reduced. On the contrary, addition of silicon to banana plants treated with non-pathogenic *F. oxysporum* Strain N7 significantly reduced disease severity ($P < 0.001$) and increased shoot height ($P < 0.001$), compared to a solo application of the non-pathogenic *F. oxysporum* Strain N7. Application of silicon reduced corm discoloration by more than 50% and shoot yellowing/wilting by 80%. Non-pathogenic *F. oxysporum* Strain N16 was superior to non-pathogenic *F. oxysporum* Strain N7 in suppressing disease and improving shoot height. Shoot height was significantly higher as a result of all treatments ($P < 0.001$), except *T. harzianum* Eco-T®, compared to the pathogen-treated control. Endophytes and their combination with silicon resulted in shoot height almost double that of the pathogen-treated control.

Table 5.1 Solo and combined application of biocontrol fungi and soluble silicon to control *Fusarium oxysporum* f.sp. *cubense* infecting banana plants

Treatments	Corm		
	discoloration	Shoot wilting	Shoot height (mm)
Uninoculated control	1.0 a	0.0 a	1342.3 f
N7 + Si	1.7 ab	0.3 ab	1015.0 cde
N16 + Si	2.2 b	0.1 ab	1175.3 ef
N16	2.3 b	0.6 b	1057.5 de
N7	3.9 c	1.4 c	946.8 cd
Si	3.6 c	1.8 c	840.3 bc
T + Si	4.8 cd	1.4 c	850.8 bc
T	5.4 de	2.8 d	725.0 ab
<i>F. oxysporum</i> f.sp. <i>cubense</i>	5.9 e	3.3 d	627.9 a
F pr.	<0.001	<0.001	<0.001
s.e.d.	0.46	0.25	95.84
l.s.d.	0.98	0.54	207.04
cv%	16.50	25.0	12.1

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

- Key: N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; T = *T. harzianum* Eco-T[®]; Si = Potassium silicate



Key: Foc = *Fusarium oxysporum* f.sp. *cabense*; Fo N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; Fo N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; T = *T. harzianum* Eco-T[®]; Si = Potassium silicate

Figure 5.1 Solo and combined applications of biocontrol fungi and soluble silicon to control *Fusarium oxysporum* f.sp. *cabense*

5.2.2 Integration of biological and agronomic control methods on banana plants in the field

In the field, banana plants that were treated with combinations of non-pathogenic *F. oxysporum*, *T. harzianum* Eco-T[®], silicon and mulch had significantly greater stem heights and greater pseudostem girths size than solo applications of the treatments (Table 5.2;

Figure 5.2). Mulching increased growth of feeder roots. However, there was no significant difference on the number of leaves (Table 5.2; Figure 5.2).

Table 5.2. Measurements of banana plants treated with biocontrol agents, silicon and/or mulch in a field infested with *Fusarium oxysporum* f.sp. *cubense*.

Treatment	Shoot height (cm)	Stem girth (cm)	Number of leaves
Fo N16	161.9 a	66.5 ab	13.6 a
Fo N16 + Si	144.8 a	64.9 a	12.8 a
Fo N16 + Si + T + Mulch	182.8 b	75.0 b	13.9 a
Untreated control	148.6 a	60.5 a	13.1 a
F pr.	0.017	0.024	0.345
s.e.d.	12.4	4.62	0.62
l.s.d.	25.04	9.33	1.25
cv%	21.3	19.0	12.7

-Values followed by the same letter within the same column are not significantly different at $P \leq 0.05$.

Key: Fo N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; T = *T. harzianum* Eco-T[®]; Si = Potassium silicate

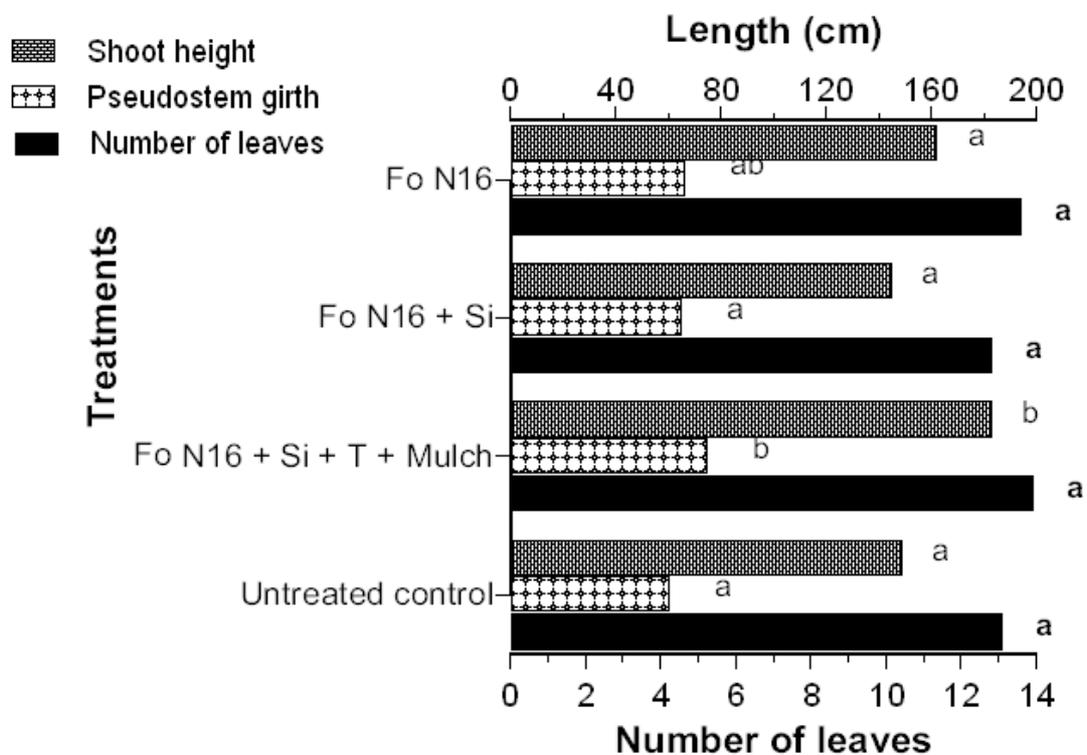


Figure 5.2 Measurements of banana plants treated with biocontrol agents, silicon and/or mulch in a field infested with *Fusarium oxysporum* f. sp. *cubense*

5.3.3 Combination of non-pathogenic *Fusarium oxysporum* and silicon on beans

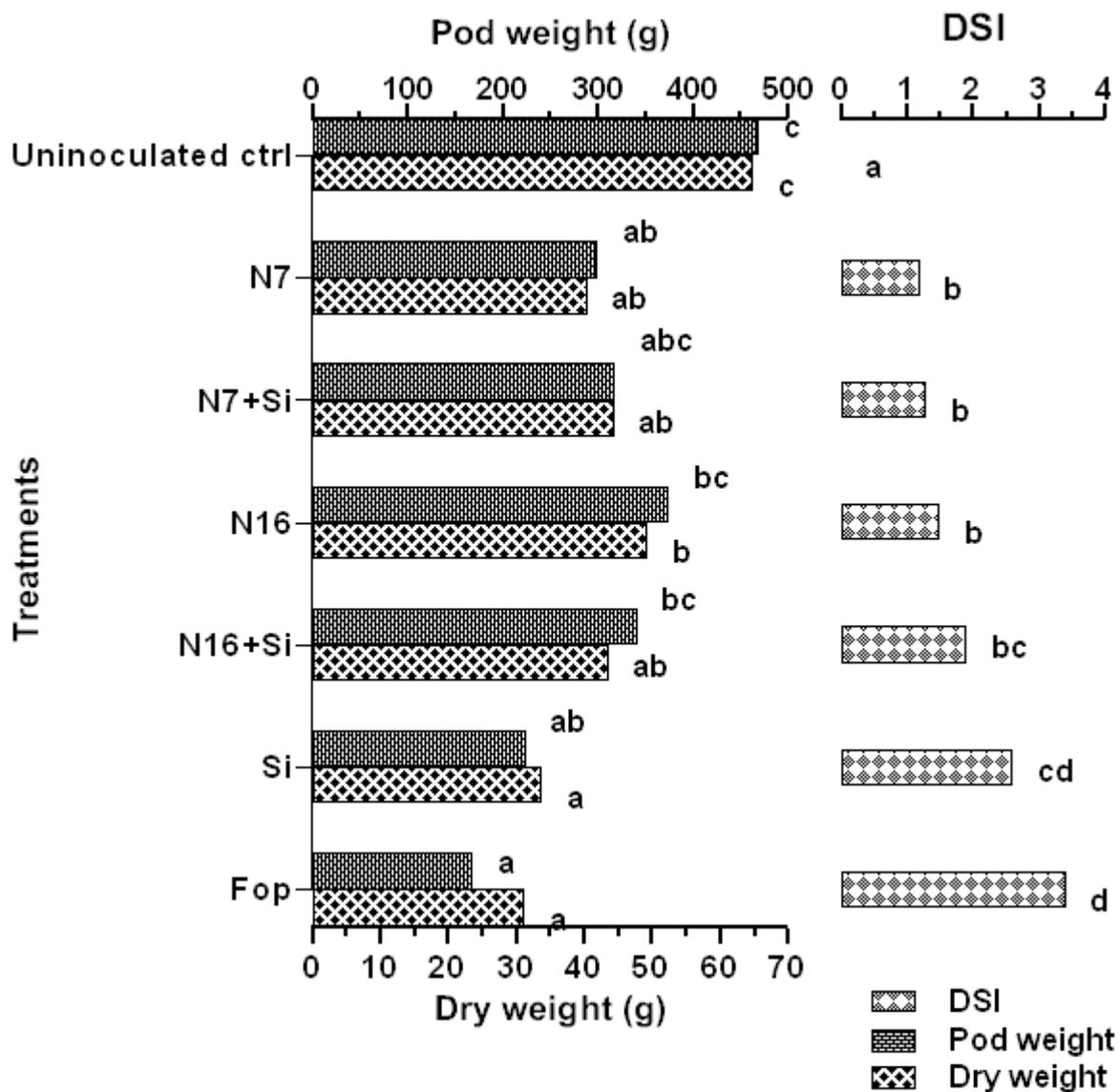
With the exception of application of silicon alone, solo and combined applications of non-pathogenic *F. oxysporum* strains and silicon significantly reduced disease severity compared to the pathogen-treated control ($P < 0.001$). Addition of silicon to bean plants treated with endophytic non-pathogenic *F. oxysporum* strains did not cause a significant reduction in disease severity compared to treatment with the non-pathogenic *F. oxysporum* strains alone ($P < 0.001$) (Table 5.3; Figure 5.3). In the same experiment conducted using hydroponics, a significantly higher disease severity occurred with the addition of silicon ($P < 0.001$) (Table 5.4; Figure 5.4). No significant differences in fresh green pod weights or dry weights of biomass was observed between the silicon amended and non-amended plants that were also treated with non-pathogenic *F. oxysporum* strains in drip-irrigated beans (Table 5.3; Figure 5.3). On the other hand, under hydroponic condition, there was a significantly lower fresh green pod weight ($P = 0.011$) and dry weight of biomass ($P = 0.002$) in plants amended with silicon (Table 5.4; Figure 5.4). A slight yellowing of leaves and diminished growth that were not typical symptoms of bean yellows disease were prominent on beans treated with silicon in the hydroponic situation.

Table 5.3 Solo and combined treatments of endophytic non-pathogenic *Fusarium oxysporum* and silicon to control *F. oxysporum* f.sp. *phaseoli* on beans in pots

Treatments	DSI	Pod weight (g)	Dry weight (g)
Control	0.0 a	469.6 c	64.9 c
N7	1.2 b	299.8 ab	40.5 ab
N7 + Si	1.3 b	318.7 abc	44.4 ab
N16	1.5 b	374.7 bc	49.4 b
N16 + Si	1.9 bc	341.5 bc	43.6 ab
Si	2.6 cd	224.2 ab	33.9 a
<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	3.4 d	169.2 a	31.3 a
F pr.	<0.001	0.019	0.004
s.e.d.	0.370	69.35	6.31
l.s.d.	0.810	151.10	13.76
cv%	26.900	27.10	17.6

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

Key: N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; Si = potassium silicate



Key: Fop = *Fusarium oxysporum* f.sp. *phaseoli*; N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; Si = potassium silicate

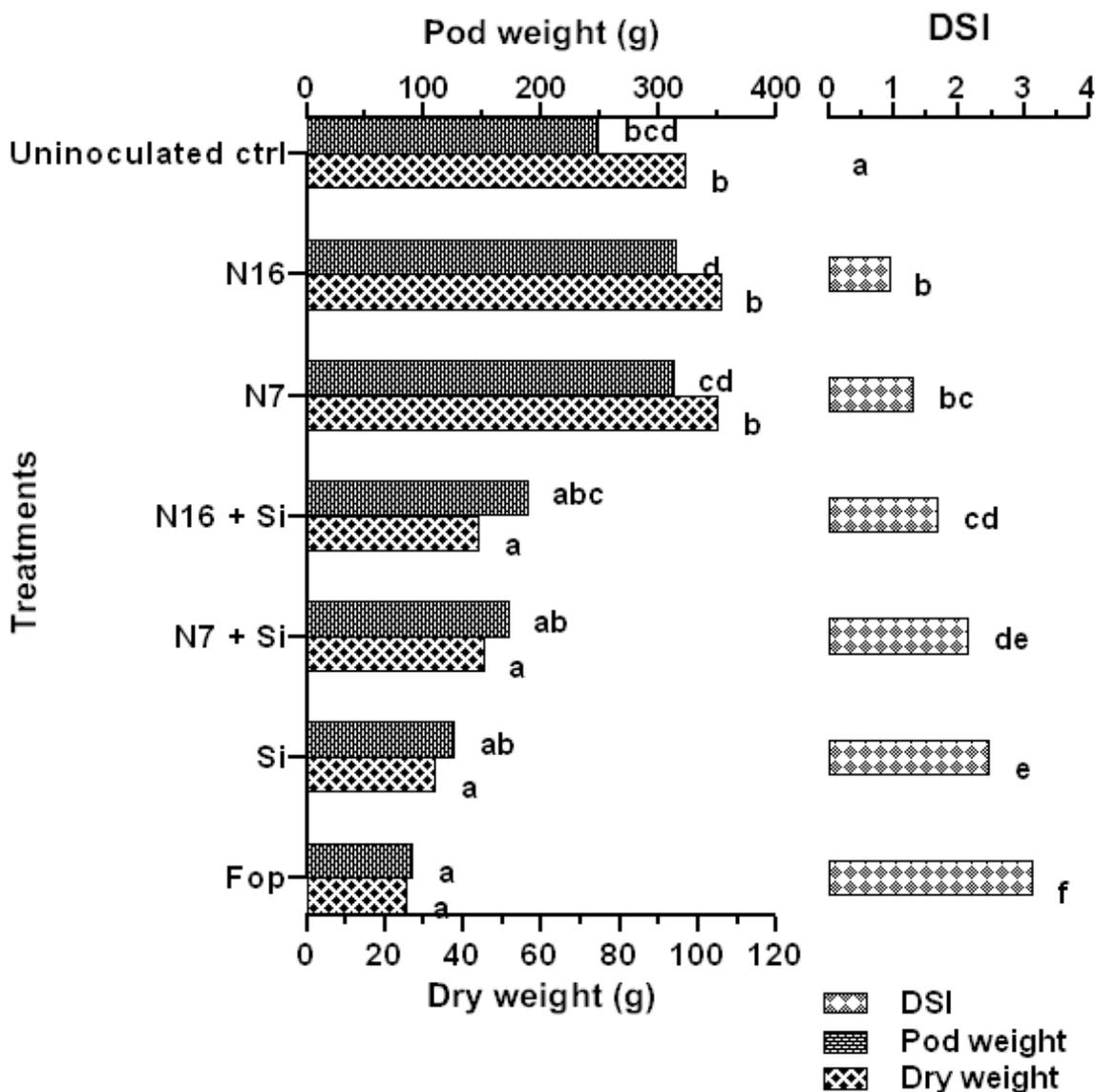
Figure 5.3 Solo and combined applications of endophytic non-pathogenic *Fusarium oxysporum* and soluble silicon to control *F. oxysporum* f.sp. *phaseoli* on beans in pots

Table 5.4 Solo and combined applications of endophytic non-pathogenic *Fusarium oxysporum* and soluble silicon to control *Fusarium oxysporum* f.sp. *phaseoli* on beans under hydroponic conditions

Treatments	DSI	Pod weight (g)	Dry weight (g)
Uninoculated control	0.00 a	249.7 bcd	97.3 b
N16	0.96 b	316.4 d	106.3 b
N7	1.33 bc	313.8 cd	105.6 b
N16 Si	1.71 cd	189.4 abc	44.4 a
N7 Si	2.17 de	173.3 ab	45.7 a
Si	2.49 e	127.5 ab	33.3 a
<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	3.15 f	91.3 a	26.0 A
F pr.	<0.001	0.011	0.002
s.e.d.	0.292	57.564	18.44
l.s.d.	0.636	125.421	40.18
cv%	21.2	33.8	34.5

- Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

- Key: N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; Si = potassium silicate



Key: Fop = *Fusarium oxysporum* f.sp. *phaseoli*; N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; Si = Silicon

Figure 5.4 Solo and combined application of endophytic non-pathogenic *Fusarium oxysporum* and soluble silicon to control *Fusarium oxysporum* f.sp. *phaseoli* under hydroponic conditions

5.3.4 Combination of *Trichoderma harzianum* Eco-T[®] with endophytic *Fusarium* isolates to control *Fusarium oxysporum* f.sp. *phaseoli* on beans

The non-pathogenic *F. oxysporum* strains and their combination with *T. harzianum* Eco-T[®] very significantly reduced disease severity compared to the pathogen-treated control

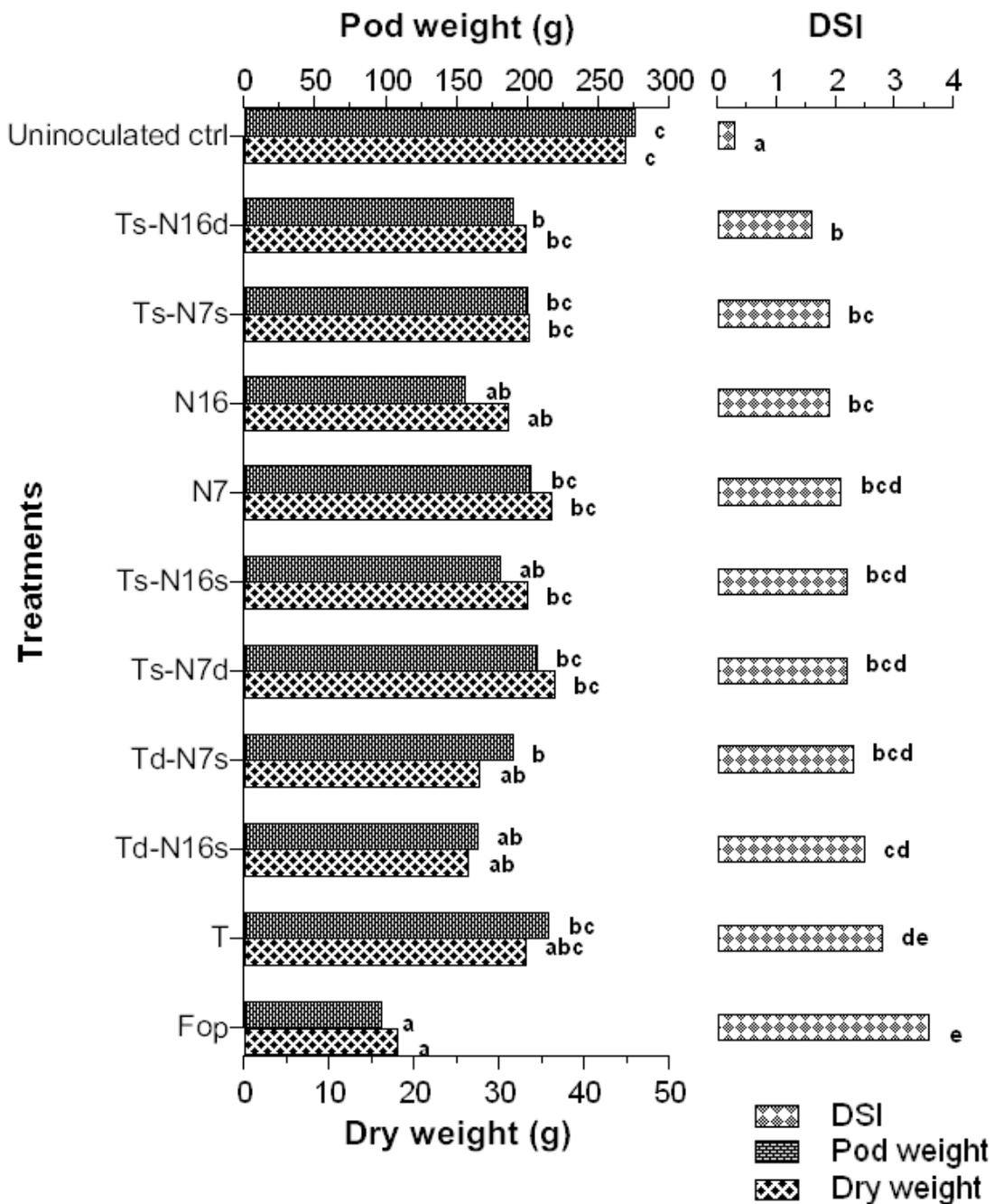
($P < 0.001$). However, there was no added effect in reducing disease severity due to the combination of the rhizosphere and endophytic control agents. A solo application of the endophytes was as good as their combined application. Although *T. harzianum* Eco-T[®] alone reduced disease severity, it was not significant compared to the pathogen-treated control ($P < 0.001$). On the other hand, *T. harzianum* Eco-T[®] did not seem to have deleterious effect on the non-pathogenic *F. oxysporum* strains. A few plants in the pathogen-untreated control showed symptoms of bean yellows, possibly due to inter-plot interference mediated by fungus gnats. Results are shown on Table 5.5 and Figure 5.5.

Table 5.5 Combination of *Trichoderma harzianum* Eco-T[®] with endophytic *Fusarium* isolates to control *Fusarium oxysporum* f.sp. *phaseoli* on beans

Treatments	DSI		Pod weight (g)		Dry weight (g)	
Uninoculated control	0.3	a	275.64	c	44.78	c
Ts+N16d	1.6	b	190.13	b	33.22	bc
Ts+N7s	1.9	bc	199.64	bc	33.47	bc
N16	1.9	bc	156.36	ab	31.04	ab
N7	2.1	bcd	202.78	bc	36.19	bc
Ts+N16s	2.2	bcd	180.36	ab	33.32	bc
Ts+N7d	2.2	bcd	206.84	bc	36.45	bc
Td+N7s	2.3	bcd	189.26	c	27.75	ab
Td+N16s	2.5	cd	165.14	ab	26.42	ab
Ts	2.8	de	215.10	c	33.15	bc
<i>F. oxysporum</i> f.sp. <i>phaseoli</i>	3.6	e	97.68	a	18.11	a
F pr.	<0.001		0.046		0.055	
s.e.d.	0.42		39.64		6.264	
l.s.d.	0.87		82.688		13.067	
cv%	23.90		25.700		23.800	

Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

Key: T = *Trichoderma harzianum* Eco-T[®]; N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; s = seed treatment; d = drenching



Key: T = *Trichoderma harzianum* Eco-T[®]; N7 = Non-pathogenic *Fusarium oxysporum* Strain N7; N16 = Non-pathogenic *Fusarium oxysporum* Strain N16; Fop = *Fusarium oxysporum* f.sp. *phaseoli*; s = seed treatment; d = drenching

Figure 5.5 Combination of *Trichoderma harzianum* Eco-T[®] with endophytic *Fusarium* isolates to control *Fusarium oxysporum* f.sp. *phaseoli* on beans

5.4 Discussion

The efficacy of biocontrol agents in reducing disease is strongly affected by environmental factors. Such factors affect the establishment, survival and activity of the control agents (Burpee, 1990; Burrage, 1971; Elad et al., 1994; Hallan and Juniper, 1971; Hannusch and Boland, 1996; Shtienberg and Elad, 1996), and may favor the pathogen and reduce plant vigor, making it vulnerable to pathogen attack. In the subtropics, where South Africa lies, banana plants infected with *F. oxysporum* f.sp. *cubense* wilt severely during a hot summer following a cold winter (Buddenhagen, 1990; Cook and Baker, 1983; Viljoen, 2002). To mimic the effect of a cold winter, banana plants were cold treated at 10°C for 1mo following inoculation with *F. oxysporum* f.sp. *cubense*. A significant level of disease was observed on the pathogen-treated control (Table 5.3; Figure 5.3). Such level of disease was not observed when plants were kept under summer temperatures in the greenhouse without cold treatment. The need for cold treatment was revealed when banana plants infected with *F. oxysporum* f.sp. *cubense* showed high level of wilting in summer 2007, following winter. Tropical plants such as banana exhibit a marked physiological dysfunction when exposed to low or nonfreezing temperatures below about 10°C to 12°C (Lyons, 1973).

The non-pathogenic *F. oxysporum* strains significantly reduced disease severity even when young banana plants went through a severe cold treatment. But the addition of silicon considerably improved plant health. A strong synergistic response was observed for non-pathogenic *F. oxysporum* Strain N7, wherein addition of silicon reduced corm discoloration and shoot yellowing/wilting by more than 50% and 80%, respectively, compared to application of the non-pathogenic *F. oxysporum* Strain N7 alone.

The endophytes were by far better control agents against Fusarium wilt of banana than the rhizosphere control organism, *T. harzianum* Eco-T[®]. One of the important limitations of rhizosphere biological control agents against vascular wilt diseases is their inability to reach the pathogen once it is in the xylem vessel. However, the non-pathogenic *F. oxysporum* Strains N7 and N16 were isolated from the vascular tissue of banana plants and can readily colonize the vascular tissue of other plants such as common bean and maize (data not shown) as well.

The non-pathogenic *F. oxysporum* strains significantly suppressed disease severity of bean plants infected with *F. oxysporum* f.sp. *phaseoli*. However, plants were not entirely protected from infection. The endophytes could not prevent infection, but suppressed its expression, limiting its effect on plant growth and development. However, addition of silicon to bean plants, also treated with the non-pathogenic *F. oxysporum* strains on planting, did not result in improved plant health. Surprisingly, bean plants grown under hydroponic conditions showed higher level of disease when supplemented with potassium silicate. Reduced plant growth and slight yellowing of the leaves was observed. This was probably because of the increase in pH, resulting in the unavailability of sufficient quantities of nitrogen and other elements essential to bean plants. Composted pine bark that was used as a growing medium has a low pH, and addition of silicon increases soil pH to near neutral. As a result, weekly application of silicon to the drip irrigated bean plants did not negatively affect plant growth and development. However, under hydroponic conditions, the solution has no buffering effect; and addition of weak acids such as acetic acid to lower the pH would have resulted in polymerization (gelling) of the potassium silicate, making it unavailable to plants. African soils are mainly acidic (Anonymous, 2004). Repeated use of ammonium based nitrogen fertilizers in acidic or poorly buffered soils reduces productivity due to increasing soil acidity unless measures are taken to increase soil pH (Bache and Heathcote, 1969; Djokoto and Stephens, 1961; Jones, 1976). Liming can enhance use of applied fertilizer (Yamoah et al., 1992) and substantially increase exchangeable Ca, pH, extractable P, and effective cation-exchange capacity (Lungu et al., 1993; Pieri, 1987; Yamoah et al., 1992). Similarly, potassium silicate provides effects similar to those obtained from liming and at the same time supply plants with soluble silicon.

Mixed bioinocula have been pioneered in the control of soilborne diseases (Sivasithamparam and Parker, 1978). Many researchers have reported the role of application of mixture of biocontrol agents to overcome the variability and inconsistent performance of biocontrol agents, particularly under field conditions, where they are highly influenced by abiotic and biotic conditions (Duffy et al., 1996; Fukui et al., 1999; Guetsky et al., 2001; Raupach and Kloepper, 1998).

Different species of *Trichoderma* have been reported to suppress fusarial wilt diseases in many crops (Calvet et al., 1990; Hervás et al., 1998; Prasad et al., 2002; Sivan and Chet,

1986; Sivan et al., 1987). Rhizosphere biocontrol organisms, e.g., *Trichoderma* spp., protect plants from infection by a variety of soilborne pathogens. However, once the plant is infected and pathogen is established inside the xylem vessels, then these rhizosphere organisms may have limited effect. In addition, the introduced rhizosphere organism cannot protect all potential infection sites, especially relative to the speed of plant root growth and movement, unless they can induce strong plant defense responses. There are reports that indicate that *Trichoderma* spp can also induce plant defense reactions such as the hypersensitivity response and phytoalexin production (Calderon et al., 1993; Chang et al., 1997).

In this study, the non-pathogenic *F. oxysporum* Strains N7 and N16 proved to be effective against their wilt-causing relatives, reducing disease severity. However, they could not prevent bean plants from infection, causing pronounced discoloration of the vascular tissue. Therefore, bearing in mind that *Trichoderma* spp. are effective rhizosphere control agents, a combined application of *T. harzianum* Eco-T[®], known to effectively control root rot and damping off pathogens, and non-pathogenic *F. oxysporum* Strains N7 and N16 was tested against *F. oxysporum* f.sp. *phaseoli*. Disappointingly, however, no major increase in disease suppression was observed. The lack of improvement in control level probably did not occur because of antagonism between the biocontrol agents because their combination did not result in more disease. However, the lack of improvement could be because there was no additive effect due to mixing of the control agents. In the field trial on banana, on the other hand, plants treated with the combination of the non-pathogenic endophyte, *T. harzianum* Eco-T[®], silicon and mulching had far better agronomic parameters than solo or dual application of the treatments. According to Guetsky et al. (2002), control efficacy achieved by biocontrol organisms exhibiting several distinct mechanisms of control will result in additive effects or synergism, but not antagonism. They further noted that if multiple mechanisms are involved, under a certain set of conditions, one mechanism may compensate for the other. For instance, mulching encourages growth of feeder roots and creates a conducive microenvironment, increasing microbial activity in the soil.

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CHAPTER SIX

Application of soluble silicon to ameliorate cold stress and its impact on Fusarium wilt of banana

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Abstract

Banana production in the subtropics frequently suffers from cold damage, because banana originated from a fully tropical region of the Earth. This abiotic stress also aggravates the effect of the biotic stress caused by the *Fusarium* wilt fungus. In this study, the effect of silicon in alleviating cold stress on banana and its impact on *Fusarium* wilt was investigated. Tissue culture banana plantlets were treated with 0, 1000 or 2000 mg ℓ^{-1} of silicon (in the form of potassium silicate) for six weeks. Half of the plants were treated with *Fusarium oxysporum* Schlecht. f.sp. *cubense* (E.F. Smith) Snyder & Hansen and were subjected to cold stress for 0-8 d at 4°C. Cold damage and corm discoloration due to *Fusarium* wilt were significantly reduced by the application of silicon.

6.1 Introduction

Environmental stresses such as cold, drought and salinity affect plants in many respects. Due to their widespread occurrence they cause the most economic losses in agriculture (Beck et al., 2007). These stress factors affect the water relations of plants, resulting in a variety of specific and unspecific reactions, damage and adaptation reactions (Beck et al., 2007). Plants of temperate origin are generally resistant to chilling. In contrary, plants of

tropical origin suffer chilling damage when exposed to non-freezing temperatures below 12°C (Lafuente et al., 1991).

Bananas are tropical plants which grow best under warm conditions. Most banana growing areas of the world are located between the Equator and latitudes 20°N and 20°S. The climate of these areas show relatively small temperature fluctuations from day to night and from summer to winter (Robinson, 1996). Because of the ability of banana to adapt to a relatively wide range of environments, warm subtropical countries such as China, Egypt, Israel, Morocco, South Wales, South Africa, South Queensland and Western Australia, Spain (the Canary Islands), Taiwan and southern parts of Brazil grow bananas (Gubbuk and Pekmezci, 2004). However, subtropical climates have wide temperature fluctuations between day and night, and low/high temperature extremes in winter and summer, respectively. Some typical physiological problems that banana plants develop after cold periods in subtropical climates are choke throat, November dump, winter leaf sunburn, under-peel discoloration, growth cessation (Robinson, 1996), and frost damage (Ganjun and Chunyu, 2008). The recent damage in Southern China is an example, where a ferocious weather condition, coupled with chilling rain and temperature destroyed banana plantations. The damage has been reported as a serious blow to the Chinese banana industry, which has been expected to compete with the Philippines's banana industry for both domestic and export trade (Ganjun and Chunyu, 2008). Chilling injury is important in the South African banana industry, frequently occurring during winter and spring when temperature amplitudes are at a maximum.

In addition to environmental/abiotic stresses, banana plants are affected by biotic stresses. One of the biotic stresses that has global importance is Fusarium wilt, caused by *Fusarium oxysporum* Schlecht. f.sp. *cubense* (E.F. Smith) Snyder & Hans. The Fusarium wilt strain in the subtropical climate of South Africa is relatively mild. However, the banana plants succumb to the disease when a cold winter is followed by a hot summer. The cold winter causes severe stress on the plants, which leads to expression of the Fusarium wilt symptoms. In this study, the ameliorating effect of silicon in banana plants to stresses resulting from biotic stress (Fusarium wilt) and an abiotic stress (low temperature) and their combination was assessed.

6.2 Materials and methods

6.2.1 Silicon

Silicon was applied in the form of liquid potassium silicate Product K2550 containing 20.5-20.9% SiO₂. It was provided by PQ Silica (Pty) Ltd¹³.

6.2.2 Source of plant material

Tissue cultured banana plantlets, 50 mm in size, of the cultivar Cavendish Williams that were provided by DuRoi Laboratory (Pty) Ltd¹⁴, were used for all experiments.

6.2.3 Isolation of the pathogen

Fusarium oxysporum f.sp. *cubense* was isolated from a diseased banana plant from the South Coast of KwaZulu-Natal. The plants were surface sterilized using commercial bleach containing 3.5% sodium hypochlorite for 5 min and were subsequently rinsed three times with sterile distilled water. Small disks of the corm/stem cut in cross-section were placed on Nash and Snyder Medium (Nash and Snyder, 1962) and incubated at 27°C for 5 d. Colonies were then subcultured on to Potato Carrot Agar (PCA). PCA was prepared as follows: A grade potatoes, 20 g, carrots, 20 g, agar, 15 g and 1 l of tap water. Potatoes and carrots were grated and cooked for 30 min. The juice was then filtered through cheesecloth without straining. The agar was added to the filtrate and autoclaved at 121°C for 15 min. Mycelial and conidial suspensions of the fungi grown on PCA were freeze-stored in Eppendorf tubes at -80°C, supplemented with 50% glycerol.

6.2.4 Production of inoculum of the pathogens

Inoculum was produced by growing the fungi on barley grains. Fifty grams of barley grains were soaked overnight in 250 ml Erlenmeyer flasks containing 100 ml of water. The excess water was drained off and the flasks were autoclaved consecutively for two days at 121°C for 20 min. The flasks were then inoculated with three disks of the pathogen grown

¹³ PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

¹⁴ Du Roi Laboratory, P.O.Box 1147, Letsitele 0885, South Africa

on Potato Dextrose Agar (PDA) (Merck®) for 3 d at 27°C in the dark. After two weeks at 27°C the grains were air-dried, milled using a coffee grinder, sieved and stored at 4°C.

6.2.5 Growth conditions and stress treatments

Tissue cultured banana plantlets, 50 mm in size, were planted in 150 mm pots containing pine bark as a growing medium. Plantlets were treated with 0, 1000 or 2000 mg ℓ^{-1} of silicon weekly for six weeks. Half of the plants were inoculated with *F. oxysporum* f.sp. *cubense* by dipping seedlings in a conidial and mycelial suspension of inoculum with a concentration of 10^7 c.f.u. ml^{-1} . One month post-inoculation, plants were subjected to temperatures of 0, 4 or 20°C for a period of 12 h, 1, 2, 4 or 8 d in a cold room with a 12 h photoperiod. Before subjecting plants to the target temperatures, they were acclimated to the cold treatment by dropping the temperature by 2°C every 2 h. After plants were cold treated, they were kept for 1 wk in a glasshouse with a temperature ranging from 26-28°C, and were then rated for cold damage. Banana plants inoculated with *F. oxysporum* f.sp. *cubense* were rated for corm discoloration.

6.2.10 Disease and cold damage rating

Corm discoloration was recorded based on visual assessment of the internal symptoms caused by *F. oxysporum* f.sp. *cubense* according to the INIBAB's Technical Guideline 6 (Carlier et al., 2002):

- 1= Corm completely clean, no vascular discoloration
- 2 = Isolated points of discoloration in vascular tissue
- 3 = Discoloration of up to one-third of vascular tissue
- 4= Discoloration of between one-third and two-thirds of vascular tissue
- 5 = Discoloration of greater than two-thirds of vascular tissue
- 6 = Total discoloration of vascular tissue

Cold damage on the foliage due to exposure to low temperatures was also recorded according to a five-point key where:

- 0 = healthy plants
- 1 = initial signs of cold damage/browning
- 2 = up to 25% of the leaves brown
- 3 = up to 50% of the leaves brown

4 = up to 75% of the leaves brown

5 = plants completely brown

The over all disease severity index (DSI) was calculated as follows (Mak et al., 2004; Zhang et al., 1996):

$$DSI = \frac{\sum(\text{Number on scale} \times \text{Number of seedlings in that scale})}{\sum(\text{Number of treated seedlings})}$$

6.2.8 Statistical analysis

Experiments were repeated twice. Data was analyzed using GenStat® Executable release 11 Statistical Analysis Software. Analysis of variance (ANOVA) was used to determine treatment effects. Differences between treatments were distinguished using Fisher's protected least significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

6.3 Results

6.3.1 Chilling injury

Chilling tolerance of banana seedlings treated with different levels of silicon was examined by visually rating the damage after exposing seedlings to freezing and near freezing temperatures. Application of silicon substantially reduced chilling damage of banana plants at 4°C. At 0°C, however, irreversible damage occurred to both silicon treated and untreated plants. Unlike at 4°C, the cold room temperature had hysteresis and a setting of 0°C oscillated between -2 and 0°C. This might have led to the death of all plants exposed to this temperature regime, irrespective of the length of time plants spent in the cold room.

No damage was visible on plants kept at room temperature. Silicon-untreated plants, on the other hand, showed extensive cold damage proportional to the period of exposure to low temperatures. After a few hours at 4°C, the leaves of silicon-untreated banana plants started folding downwards, while the leaves of the silicon-treated plants remained erect

and open, even 4 d later. At 4°C the level of cold damage on plants treated with silicon was not significantly different from those plants kept at room temperature (Table 6.1-6.2; Figure 6.1-6.2). The damage on plants treated with 1000 mg ℓ^{-1} of silicon at 4°C was significantly lower than untreated plants, regardless of the time that plants were exposed to this temperature (Table 6.1-6.2; Figure 6.1-6.2). After 8 d at 4°C, silicon-untreated plants showed irreversible damage. However, in spite of the extensive injury on plants treated with 1000 mg ℓ^{-1} of silicon, the first leaves of these plants were not killed, which was important in the recovery of these plants after they were placed under optimal temperatures (Figure 6.3A,B). Plants treated with 1000 mg ℓ^{-1} of silicon and kept at 4°C for 8 d showed equivalent levels of damage to silicon-untreated plants 4 d later, doubling their period of survival at such chilling temperatures.

On plants treated with 1000 mg ℓ^{-1} of silicon, chilling damage was reduced by 42% on plants inoculated with *F. oxysprum* f.sp. *cubense* and by 60% on uninoculated ones, irrespective of the period of time the banana plants were exposed to 4°C (Table 6.1; Figure 6.1). Cold damage on banana plants inoculated with *F. oxysporum* f.sp. *cubense* was worse than the uninoculated plants. There was a significant interaction effect Fusarium×Silicon×Temperature ($P<0.001$) and Silicon×Temperature×Time ($P<0.001$) on the cold damage of banana plants. However, Fusarium×Silicon×Temperature×Time ($P<0.067$) had no significant interaction effect on cold damage. Similarly, their interaction did not significantly affected disease severity ($P=0.793$). This may be mainly due to the relatively short period of time that the banana plants were exposed to the chilling temperatures. Therefore, the interaction between time of exposure to chilling temperatures and Fusarium wilt on cold damage and disease severity was not significant. Under field condition, where plants have to endure cold winter conditions for months, then the relationship between time of exposure and Fusarium wilt on cold damage and diseases severity might be established.

Table 6.1 The level of chilling tolerance of banana seedlings treated with silicon in the presence of Fusarium wilt

Temp. (°C)	Silicon (mg l ⁻¹)	Cold damage index	
		Foc uninoculated	Foc inoculated
0	0	5.00 d	5.00 d
	1000	5.00 d	5.00 d
	2000	5.00 d	5.00 d
4	0	2.50 b	3.25 c
	1000	1.00 a	1.88 a
	2000	2.44 b	1.94 a
20	0	0.00 a	0.00 a
	1000	0.00 a	0.00 a
	2000	0.00 a	0.00 a
Fusarium×Silicon×Temperature		<0.001	
s.e.d.		0.0992	
l.s.d.		0.1955	
cv%		11.70	

Means with the same letter are not significantly different at P≤0.05.

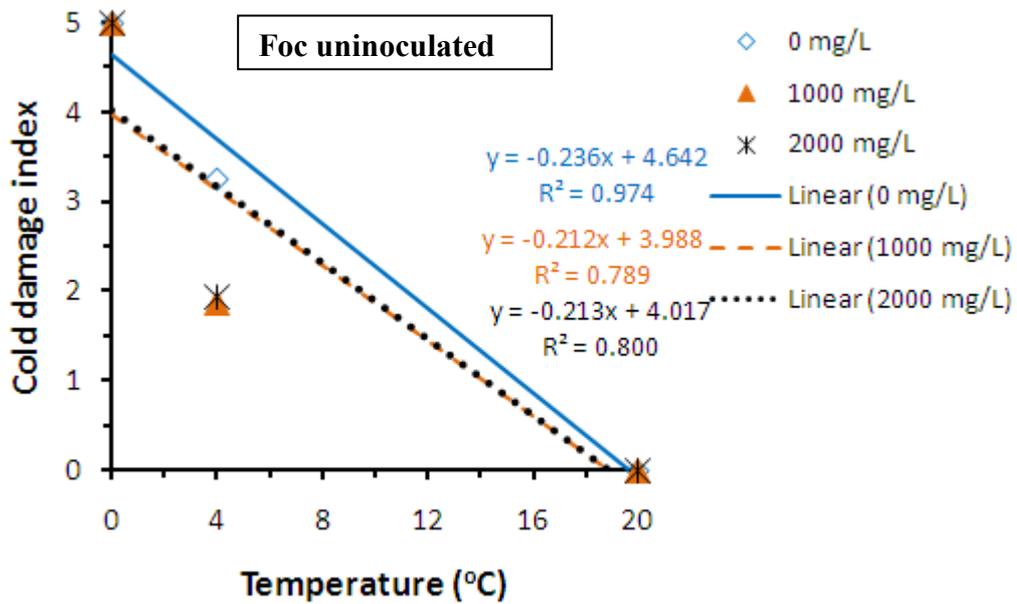
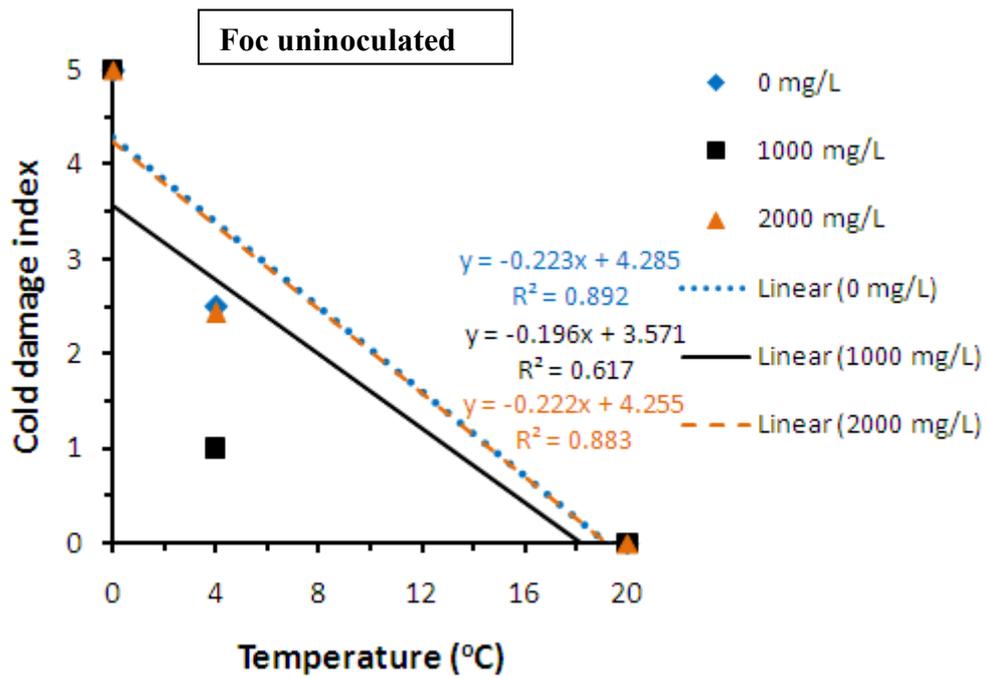


Figure 6.1 The level of chilling tolerance of banana seedlings treated with silicon in the presence and absence *Fusarium oxysporum* f.sp. *cubense*

Table 6.2 The level of chilling tolerance of banana seedlings treated with silicon subjected to different temperature for varying lengths of time

Temp. (°C)	Silicon(mg l ⁻¹)	Time (d)	Cold damage index							
			1	2	4	8	1	2	4	8
0	0		5.00	g	5.00	g	5.00	g	5.00	g
	1000		5.00	g	5.00	g	5.00	g	5.00	g
	2000		5.00	g	5.00	g	5.00	g	5.00	g
4	0		0.50	b	2.50	e	3.50	f	5.00	g
	1000		0.13	a	0.50	b	1.50	c	3.63	f
	2000		0.75	b	1.38	c	1.88	d	4.75	g
20	0		0.00	a	0.00	a	0.00	a	0.00	a
	1000		0.00	a	0.00	a	0.00	a	0.00	a
	2000		0.00	a	0.00	a	0.00	a	0.00	a
Silicon×Temperature×Time		<0.001								
s.e.d.		0.1145								
l.s.d.		0.2765								
cv%		11.70								

Means with the same letter are not significantly different at P≤0.05.

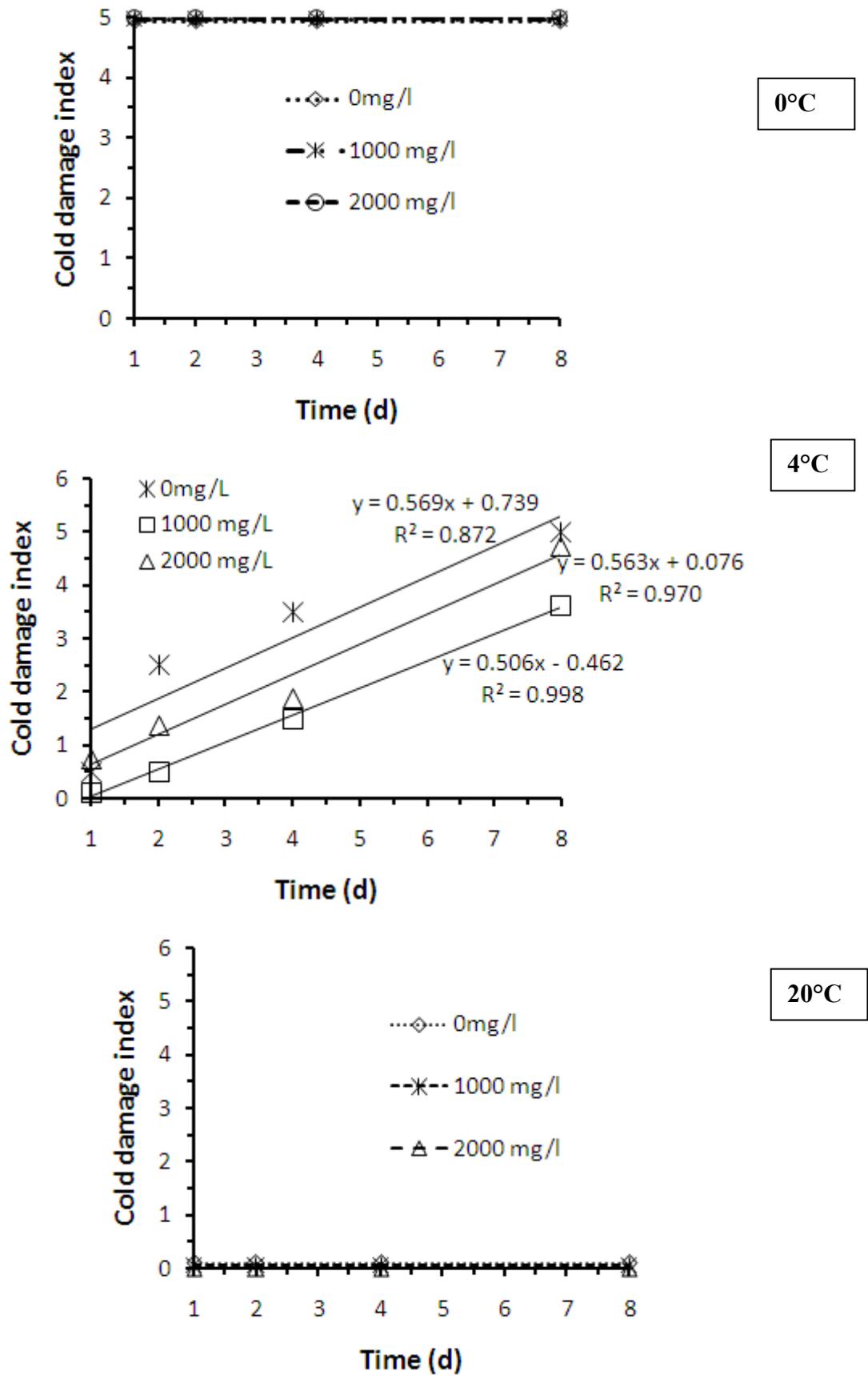


Figure 6.2 The level of chilling tolerance of banana seedlings treated with silicon

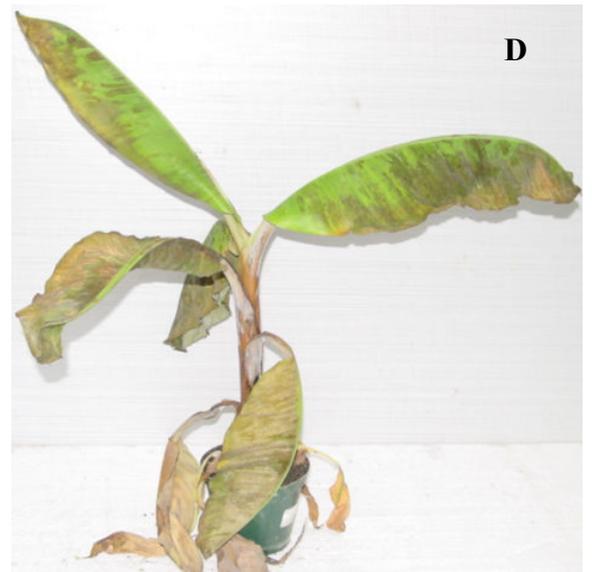


Figure 6.3 Chilling damage on the first leaf of silicon treated (A, C) and untreated (B, D) banana plants after 4 d (C, D) and 8 d (A, B) at 4°C

6.3.2 Disease severity

Corm discoloration of banana plants inoculated with *F. oxysporum* f.sp. *cubense* increased with decreases in temperature and increases in the length of exposure of the plants to chilling temperatures (Table 6.3-6.5.; Figure 6.4-6.6). Higher levels of corm discoloration was observed on plants kept at 4°C than those kept at room temperatures, with both silicon treated and untreated plants, except for plants treated with 1000 mg ℓ⁻¹. Cold damage was lowest on plants treated with 1000 mg ℓ⁻¹ (Section 6.3.1). Silicon treated plants showed significantly lower corm discoloration than the untreated plants, whether exposed to cold or not, and at all lengths of exposure to these temperatures. Corm discoloration of plants treated with 2000 mg ℓ⁻¹ was not significantly different from the pathogen-untreated control plants until 4 d at 4°C (Table 6.4; Figure 6.5). At 20°C, corm discoloration of banana plants treated with 2000 mg ℓ⁻¹ of silicon was not significantly different from pathogen-untreated plants and was almost 70% less than silicon untreated plants (Table 6.3; Figure 6.4). Corm discoloration was lowest on plants treated with 2000 mg ℓ⁻¹ regardless of the length of time subjected to 4°C (Table 6.4; Figure 6.5) and was >75% lower than silicon-untreated plants (Table 6.3; Figure 6.4). No corm discoloration was observed on plants that were not treated with *F. oxysporum* f.sp. *cubense*.

Table 6.3 Corm discoloration of Foc uninoculated and Foc inoculated banana plants treated with different levels of soluble silicon and exposed to different temperature regimes for a period of 1-8d

Temperature (°C)	Silicon (mg ℓ ⁻¹)	Corm discoloration index			
		Foc uninoculated		Foc inoculated	
4	0	1.00	a	5.44	e
	1000	1.00	a	3.58	c
	2000	1.00	a	1.59	b
20	0	1.00	a	4.58	d
	1000	1.00	a	3.66	c
	2000	1.00	a	1.20	a
Fusarium×Silicon×Temperature		0.015			
s.e.d.		0.159			
l.s.d.		0.314			
cv%		20.70			

Means with the same letter are not significantly different at P≤0.05.

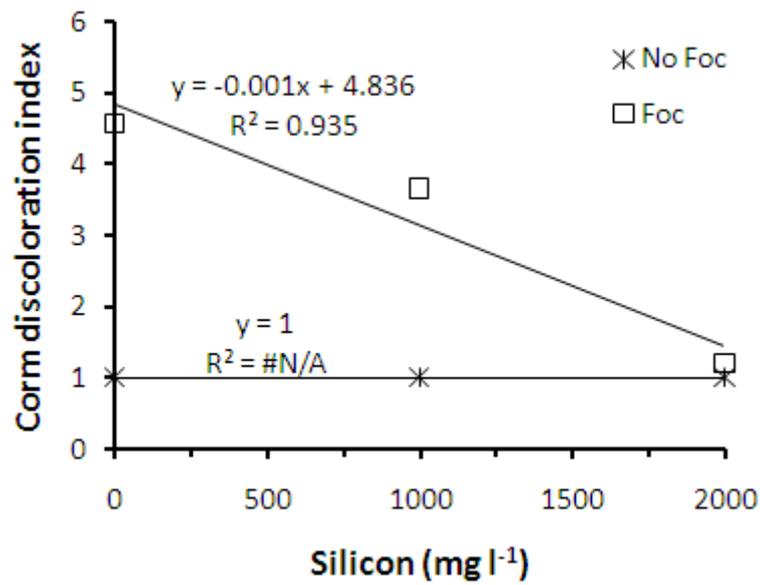
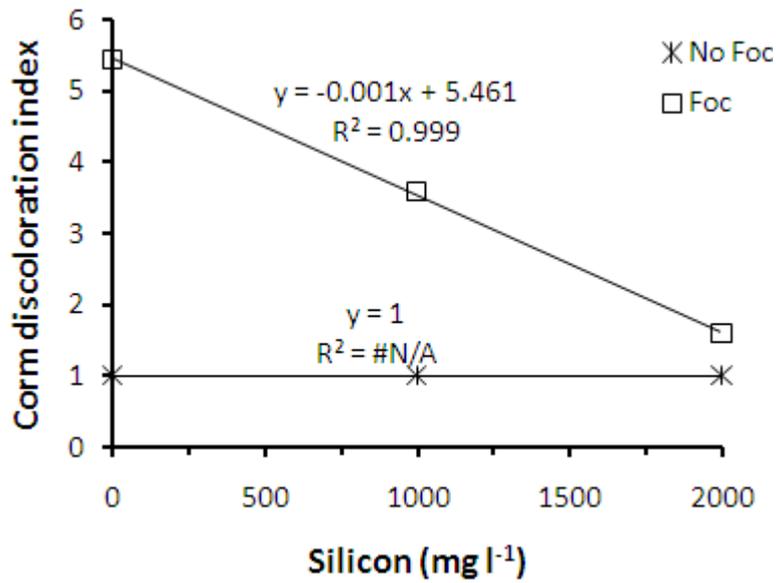


Figure 6.4 Corm discoloration of Foc uninoculated and Foc inoculated banana plants treated with different levels of soluble silicon and exposed to different temperature regimes for a period of 1-8d

Table 6.4 Corm discoloration of *Foc* uninoculated (A) and *Foc* inoculated (B) banana plants treated with different levels of soluble silicon and exposed to chilling temperatures for a period of 1-8d

<i>F. oxysporum</i> <i>f.sp. cubense</i>	Silicon (mg l ⁻¹)	Time (d)	Corm discoloration index							
			1		2		4		8	
Uninoculated	0		1.00	a	1.00	a	1.00	a	1.00	a
	1000		1.00	a	1.00	a	1.00	a	1.00	a
	2000		1.00	a	1.00	a	1.00	a	1.00	a
Inoculated	0		4.66	de	4.97	ef	5.16	f	5.25	f
	1000		2.78	c	2.88	c	4.38	d	4.44	d
	2000		1.25	ab	1.31	ab	1.41	ab	1.63	b
Fusarium×Silicon×Time		0.002								
s.e.d.		0.225								
l.s.d.		0.445								
cv%		20.70								

Means with the same letter are not significantly different at $P \leq 0.05$.

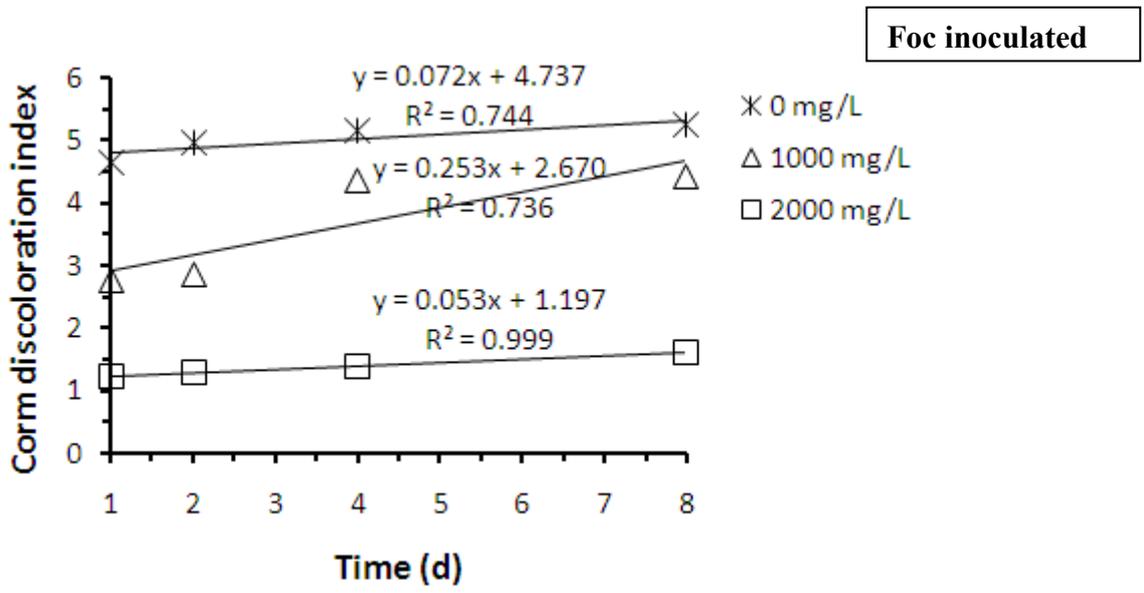
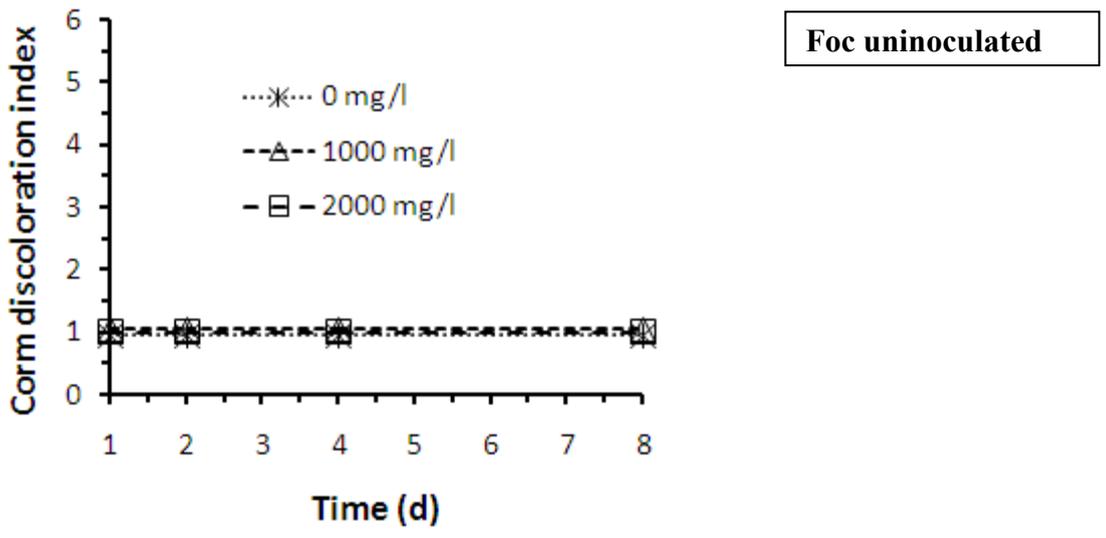


Figure 6.5 Corm discoloration of banana plants treated with different levels of soluble silicon and exposed to chilling temperatures for a period of 1-8d

Table 6.5 The effect of the interaction between the Fusarium wilt fungus, temperature and time of exposure to these temperatures on corm discoloration of banana plants

<i>F. oxysporum</i> <i>f.sp. cubense</i>	Temp. (°C)	Time (d)	Corm discoloration index							
			1	2	4	8				
Uninoculated	4		1.00	a	1.00	a	1.00	a	1.00	a
	20		1.00	a	1.00	a	1.00	a	1.00	a
Inoculated	4		2.90	b	3.19	bc	3.77	d	4.29	e
	20		2.90	b	2.92	b	3.52	cd	3.25	bc
Fusarium×Temperature×Time.		0.032								
s.e.d.		0.184								
l.s.d.		0.363								
cv%		20.70								

Means with the same letter are not significantly different at $P \leq 0.05$.

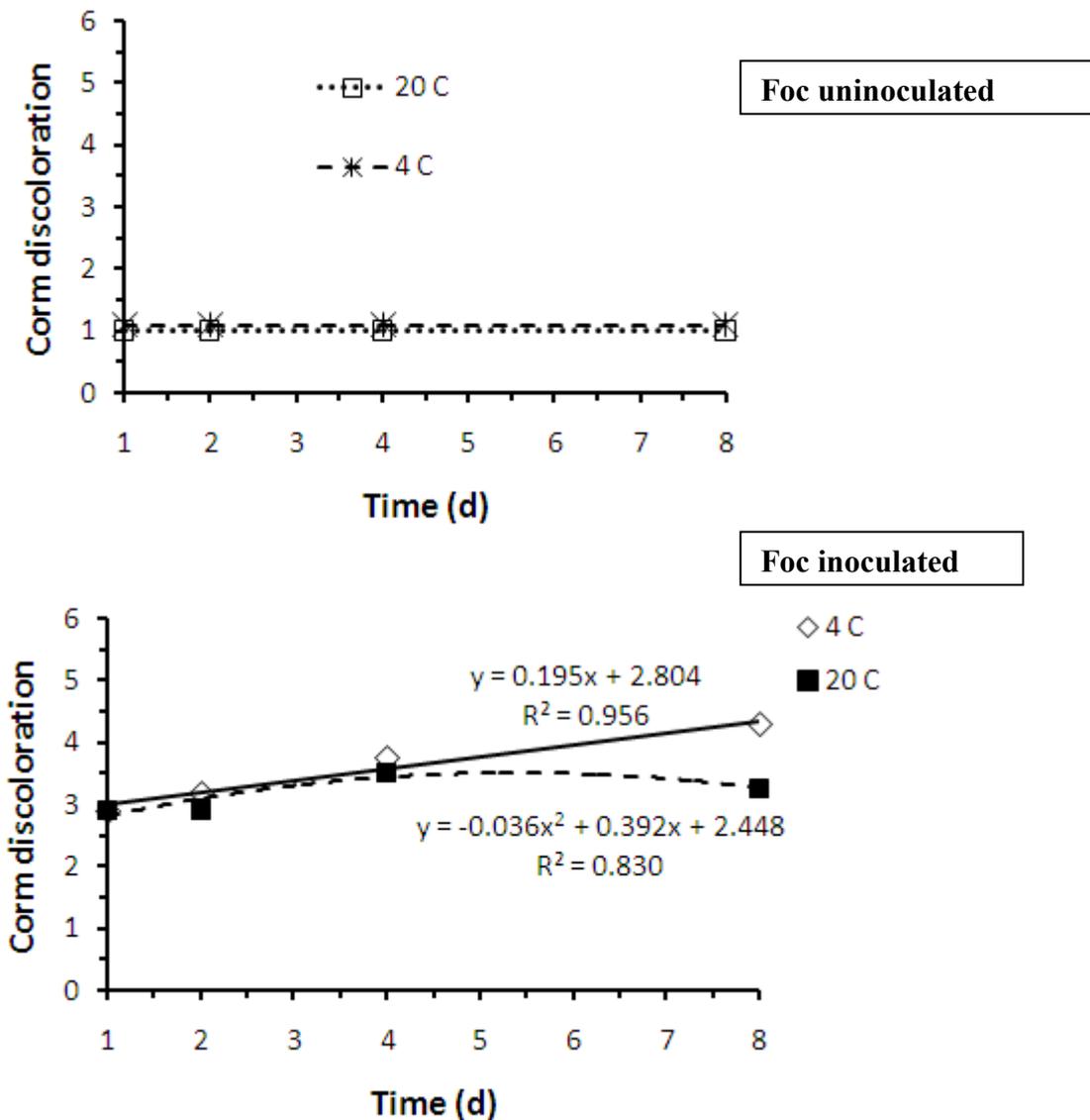


Figure 6.6 The level of corm discoloration of banana plants kept at 4 and 20°C for a period of 1-8d

6.4 Discussion

Subtropical banana plants must cope with a variety of abiotic stresses in the environment including extremes in temperature. In this region, the ability to withstand freezing or near freezing temperatures during winter and spring, and the high variation between day and night temperatures, is an essential trait. Many tropical plants, such as banana, are not able to acclimatize to cold temperatures; as a result suffer injury, and even irreversible damage, upon exposure to chilling temperatures between 0°C and 10°C (Tomashow and Browse, 1999).

In this study, the effect of soluble silicon in alleviating chilling damage on banana plants and its relation to Fusarium wilt severity was studied. Cold stress caused irreversible damage on unprotected banana plants. The effects of low temperature on the growth and development of banana, a tropical plant, is well documented (Ganjun and Chunyu, 2008; Tomashow and Browse, 1999). Chilling damage was significantly reduced in banana plants amended with silicon. Several authors have reported the ameliorating effect of silicon under different oxidative stresses, such as heavy metal toxicity in barley, cowpea, cucumber, maize and rice (Gunes et al., 2007; Guo et al., 2005; Iwasaki et al., 2002; Liang et al., 2005; Morikawa and Saigusa, 2002; Rogalla and Romheld, 2002; Treder and Cieslinski, 2005), salt stress in tomato (Al-aghabary et al., 2005; Romero-Aranda et al., 2006) and cucumber (Zhu et al., 2004), and drought stress in wheat (Gong et al., 2005) and sorghum (Hattori et al., 2005). However, there have been no reports on the effects of silicon on cold stress on banana plants.

As reported by Richmond and Sussman (2003) silicon irreversibly precipitates as $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ in the cell walls and cell lumens. This silicon reinforcement of cell walls protects the plants from abiotic stresses (Epstein, 1999). Although no measurement was taken, it was clearly evident that silicon treated banana leaves were tougher than silicon-untreated ones. The leaves of silicon-untreated banana plants folded down after 12 h in the cold room while the leaves of silicon-treated banana plants remained turgid and open, even 4 d later. However, this could also be due to management of turgor pressure.

The problem of Fusarium wilt of banana in the subtropics is exacerbated because of cold stress on banana plants. Cold stressed banana plants succumb to this disease easily than

vigorous or unstressed plants. The alleviation of cold stress on banana plants by supplying silicon as a fertilizer therefore has a double advantage. Besides its indirect effect on Fusarium wilt by decreasing stress, it also reduces disease directly, even under stress-free conditions. Application of silicon to tropical plants, growing in marginal sub-tropical environments to protect them from frost or cold damage, may have applications on other crops as well.

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CHAPTER SEVEN

Physiological effects of soluble silicon on bananas subjected to cold stress

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Abstract

The effect of silicon fertilization on the membrane permeability, lipid peroxidation (MDA), proline production and soluble sugars of cold stressed banana (*Musa* sp.) plants were investigated under cold room and greenhouse conditions. Tissue culture banana plantlets were treated with 0 or 1000 mg ℓ^{-1} of silicon in the form of potassium silicate for six weeks, and were subjected to cold stress for 0-8 d at 4°C. Silicon-treated plants showed significantly lower levels of lipid peroxidation and electrolyte leakage or membrane damage. Decreased production of proline was measured in silicon-treated plants. Application of silicon also resulted in significantly increased sucrose and raffinose levels in both the roots and leaves of banana plants. Reductions in lipid peroxidation, electrolyte leakage and proline production are signs of reduced stress levels. Sugars such as sucrose and raffinose are believed to be cryoprotectants, increasing plant tolerance to cold damage.

7.1 Introduction

In their natural environments, plants have evolved to cope with weather changes and make structural and physiological changes that help them cope to such changes. Winter seasons are generally characterized by low temperatures, which adversely affect plants from warm climates. Some chilling-sensitive plants can be hardened when exposed to intermediate temperatures (Moynihan et al., 1995), whereas a few chilling sensitive plants such as rice

(Sato and Saruyama, 2006) and banana (Tomashow and Browse, 1999) do not show hardening.

Low temperature stress induces various biochemical and physiological responses in plants (Bray et al., 2000). Some of these responses include: accumulation of various substances such as sugars, sugar alcohols, some amino acids (e.g. proline), quaternary ammonium compounds (e.g. betaine) and several other heterogeneous substances (Hinch et al., 2006; Yancey et al., 1982). These molecules are believed to play a role in osmotic adjustment in plants (Shinozaki and Yamaguchi-Shinozaki, 2006). Plants that cannot increase the proportion of unsaturated fatty acids in order to increase the fluidity of the biomembranes can be injured when exposed to low, non-freezing temperatures. The damage leads to membrane leakage, which may be worsened by the temperature-dependent inactivation of ion pumps or channels (Beck et al., 2007). During hardening, plants make a transition from the common starch-dominated carbohydrate metabolism to an oligosaccharide-dominated metabolism, using sucrose and its galactosides as cryoprotectants (Hansen et al., 1996). Low temperatures retard metabolic processes, and thus promote the formation of free radicals and reactive oxygen species by delayed dissipation of photosynthetic energy (Vogg et al., 1998). Unless the level of free radicals and reactive oxygen species are balanced through enhancement of the radical scavenging system, such as antioxidants, then cell damage or death may result. In this study, the effects of silicon on the physiological responses of cold-stressed banana plants was investigated.

7.2 Materials and Methods

7.2.1 Silicon

Silicon was applied in the form of liquid potassium silicate Product K2550 containing 20.5-20.9% SiO₂. It was provided by PQ Corporation (Pty) Ltd¹⁵.

7.2.2 Source of plant material

Tissue cultured banana plantlets, 50 mm in size, of the cultivar Cavendish Williams that were provided by DuRoi Laboratory (Pty) Ltd¹⁶, were used for all experiments.

¹⁵ PQ Corporation, PO Box 14016, Wadeville 1422, South Africa

7.2.3 Growth conditions and stress treatments

Tissue culture banana plantlets, 50 mm in size, were planted in 150 mm pot containing composted pine bark as a growing medium. Plantlets were treated with 0 or 1000 mg ℓ^{-1} of silicon weekly for 6wk. One month post-inoculation, plants were subjected to temperatures of 4°C or 20°C for a period of 12 h, 1, 2, 4 or 8 d in a cold room with a 12 h photoperiod. Before subjecting plants to the target temperatures, they were acclimated to the cold treatment by dropping the temperature by 2°C every 2 h.

7.2.6 Electrolyte leakage/membrane permeability

Fresh banana leaf discs were cut using a 10 mm diameter cork borer. Fifteen discs were placed in a 100 ml Erlenmeyer flask containing 40 ml ultra pure water, and were shaken in a water bath for 24 h at 22°C. The electrolyte leakage in the solution was measured using a Hanna Instruments HI991300 conductivity meter. Total conductivity was measured after the leaves were autoclaved at 121°C for 20 min and subsequently shaken for 24 h at 22°C. Results were expressed as percentage of total conductivity.

7.2.7 Proline analysis

Proline concentration was determined by means of a rapid colorimetric method, developed for plant tissue by Bates et al. (Bates et al., 1973). A 500 mg sample of freeze-dried banana leaves or roots was homogenized in a test tube containing 10 ml sulphosalicylic acid (3% w/v) for 1 min and filtrated through Whatman filter paper #2. Two milliliters of the filtrate were mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, and incubated at 100°C for 1 h. Acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M H_3PO_4 . After incubation at 100°C for 1 h, the reaction was stopped by cooling the test tube containing the mixture in an ice bath. Four milliliters of toluene were added to the mixture and vortexed vigorously for 15–20 s. The toluene phase was aspirated and warmed to room temperature. The absorbance was measured in a Du[®] Beckman Coulter spectrophotometer at 520 nm using pure toluene as a blank. A calibration curve was prepared using pure proline. Results were expressed as $\mu\text{g proline g}^{-1}$ dry matter (DM).

¹⁶ Du Roi Laboratory, P.O.Box 1147, Letsitele 0885, South Africa

7.2.8 Lipid peroxidation

Levels of lipid peroxidation were measured as the amount of malondialdehyde (MDA), reacted with thiobarbituric acid (TBA) (Sigma Chemical Co., St. Louis, USA) to form TBA–MDA complex. Crude extract was prepared as described by Heath and Packer (1968): a 500 mg sample of freeze-dried leaf or root of banana plants was homogenized in 10ml of 0.1% (w/v) trichloroacetic acid (TCA) (Merck, USA) using an ultrasonic cell disrupter (Virsonic-100) for 1 min. The homogenate was then centrifuged at 20,000 g for 10 min. One milliliter of supernatant from the crude extract was added to a test tube containing 4 ml of 0.5% TBA in 20% TCA. Samples were then mixed vigorously, heated at 95°C for 30 min, then the reaction was stopped in an ice bath. The mixture was centrifuged again at 20,000 g for 10 min. Absorbance of the supernatant was read at 532 nm by using a Du[®] Beckman Coulter spectrophotometer. The value for non-specific absorption at 600 nm was subtracted. Total MDA equivalents were calculated according to Heath and Packer (1968) as:

$$\text{Total MDA (nmol g}^{-1}\text{)} = \frac{\text{Extraction buffer (ml)} \times \text{Supernatant (ml)} \times \left[\frac{(\text{Abs}_{532} - \text{Abs}_{600})}{115} \right] \times 10^3}{\text{Amount of sample (g)}}$$

7.2.9 Soluble sugars

A sample of 0.1 g freeze-dried banana leaf or root material was homogenized using an ultrasonic cell disrupter in 10 ml ethanol 80% (v/v) for 1 min. Thereafter, the mixture was incubated in a water bath for 1 h at 80°C to extract the soluble sugars. The mixture was incubated at 4°C overnight. After centrifugation at 12000 g for 15 min at 4°C, the supernatant was filtered through glass wool and taken to dryness in a vacuum concentrator. Dried samples were resuspended in 2 ml ultra-pure water, filtered through a 0.45 µm nylon filter and analyzed using an isocratic HPLC system equipped with a refractive index detector on a Phenomenex[®] column (Rezex RCM-Monosaccharide) (Liu et al., 2002). The concentration of individual sugars was determined by comparison with sugar standards.

7.2.8 Statistical analysis

Experiments were repeated twice. Data was analyzed using GenStat[®] Executable release 11 Statistical Analysis Software. Analysis of variance (ANOVA) was used to analyse

results to determine treatment effects. Differences between treatments were distinguished using Fisher's protected least significant difference (LSD). Because significant ANOVA interactions override the main effects (Keppel and Wickens, 1973), main effects are not presented in the tables or discussed in the text.

7.3 Results

7.3.3 Electrolyte leakage/membrane permeability

Cold stress increased membrane permeability of excised banana leaves significantly ($P < 0.001$) (Table 6.6; Figure 6.7). As the length of exposure to chilling temperature increased, electrolyte leakage from the leaves increased consistently. Membrane deterioration due to cold injury on silicon treated plants was significantly less than the injuries suffered by the silicon-untreated control plants ($P < 0.001$). There was no significant difference in membrane permeability until after 2 d of cold exposure ($P < 0.001$). Application of $1000 \text{ mg } \ell^{-1}$ of silicon reduced electrolyte leakage of banana plants by $>75\%$ at 4 d. Electrolyte leakage of silicon-treated plants exposed to 4°C for 8 d was almost as much as the electrolyte leakage on silicon-untreated plants at 4 d.

Table 7.1 The effect of silicon application on the electrolyte leakage of banana leaf tissue after chilling stress (4°C)

Silicon (mg l ⁻¹)	Time (d)	Electrical conductivity (EC) (%)
0	0.5	4.3 (12.0) a
	1	4.4 (12.0) a
	2	5.1 (12.6) a
	4	21.8 (27.8) b
	8	50.0 (45.0) d
1000	0.5	4.1 (11.6) a
	1	5.4 (13.2) a
	2	3.4 (10.5) a
	4	5.2 (13.1) a
	8	28.2 (31.9) c
Silicon×Time		<0.001
s.e.d.		1.59
l.s.d.		3.19
cv%		14.5

Means with the same letter in the same column are not significantly different at P≤0.05.

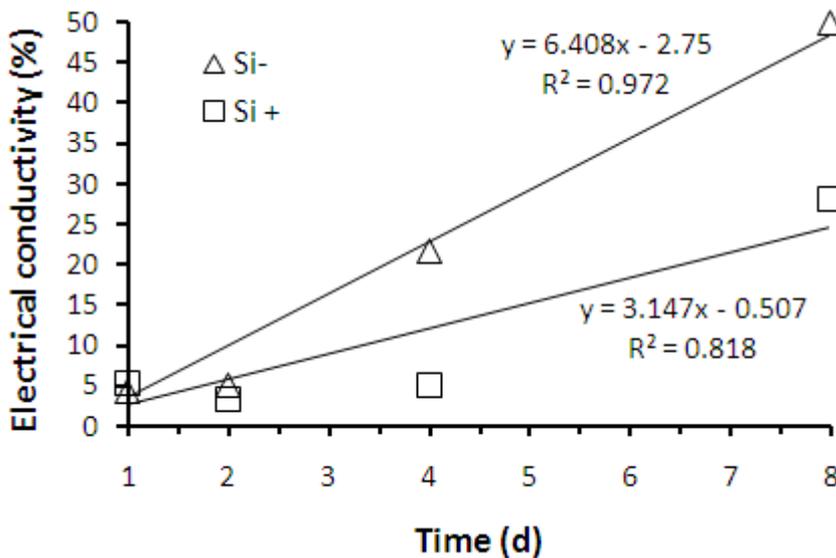


Figure 7.1 The effect of silicon application on the electrolyte leakage of banana leaf tissue after chilling stress (4°C)

7.3.4 Proline analysis

The banana roots accumulated less proline than the leaves, whether treated with silicon or not. Proline accumulation was significantly higher both in the leaves and roots of silicon-untreated plants than silicon-treated plants ($P < 0.001$), both before and after cold treatment (Table 6.7; Figure 6.8). Proline levels in the leaves of silicon-treated plants after 4 d at 4°C was less than silicon-untreated plants after 1 d at 4°C. After 2 d and 4 d at 4°C the levels of proline in the leaves of silicon-untreated plants were 55 and 61% higher, respectively, than in the leaves of silicon-treated plants subjected to 4°C for 4 d.

After cold treatment, the level of proline in the roots of silicon-treated banana plants was significantly lower than untreated ones ($P < 0.001$) (Table 6.7; Figure 6.8). Silicon-treated plants kept at 4°C for 8 d accumulated 22% less proline than silicon-untreated plants kept at 4°C for only 2 d. Similarly, the roots of silicon-untreated plants subjected to cold treatment for only 12 h accumulated almost twice as much proline as silicon-treated plants kept at 4°C for 2 d.

Table 7.2 Proline content of banana plant organs after cold treatment

Silicon (mg ℓ^{-1})	Time (d)	Proline ($\mu\text{g g}^{-1}$ DW)	
		Leaf	Root
0	0		171.80 i
	0.5	139.08 g	309.27 n
	1	250.08 m	321.92 o
	2	404.59 r	426.06 s
	4	646.01 y	440.60 t
	8	620.33 x	518.73 w
1000	0	132.31 f	60.92 a
	0.5	93.37 b	129.25 e
	1	142.82 h	122.53 c
	2	179.16 j	184.50 k
	4	246.48 l	351.18 q
	8	481.51 v	332.86 p
Plant organs \times Silicon \times Time		<0.001	
l.s.d.		0.617	
s.e.d.		0.310	

Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

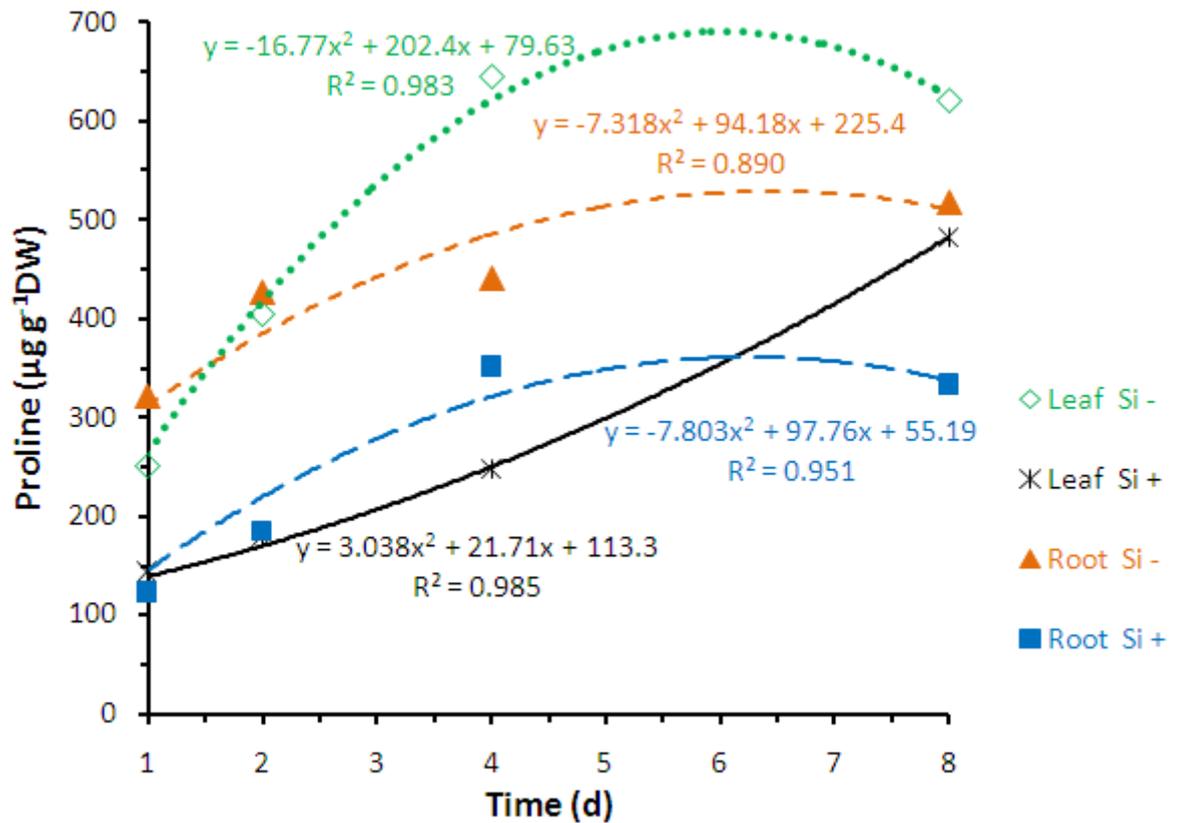


Figure 7.2 Proline content of banana plant organs after cold treatment

7.3.5 Lipid peroxidation

Application of silicon to banana plants significantly reduced lipid peroxidation or MDA level ($P < 0.001$) (Table 6.8; Figure 6.9). Similarly, there were significantly lower levels of MDA in the root than in the leaves of banana plants, whether treated with silicon and subjected to cold treatment or not ($P < 0.001$) (Table 6.8; Figure 6.9). In general, MDA level increased with the increase in the time plants were exposed to chilling temperature (Table 6.8; Figure 6.9). Before plants were treated with cold, application of silicon reduced the level of MDA in banana leaves by 29% as compared to silicon-untreated plants. The level of MDA produced in the leaves and roots of silicon-treated banana plants kept at 4°C for 8 d was less than the levels of MDA produced in silicon-untreated banana plants kept at 4°C for only 2 d.

Table 7.3 Lipid peroxidation of banana roots and leaves subjected to chilling temperature

Silicon (mg l ⁻¹)	Time (d)	MDA (nmol g ⁻¹ DW)	
		Leaf	Root
0	0	39.98 k	28.87 f
	0.5	43.36 l	26.57 e
	1	50.51 n	21.86 c
	2	66.28 q	32.96 h
	4	64.28 p	40.41 k
	8	62.28 o	37.20 j
1000	0	28.20 f	21.17 c
	0.5	44.07 l	23.70 d
	1	46.75 m	19.26 b
	2	35.19 i	17.96 a
	4	43.81 l	30.86 g
	8	51.09 n	27.34 e

Plant organs×Silicon×Time <0.001
l.s.d. 0.821
s.e.d. 0.412

Means with the same letter in the same column are not significantly different at P≤0.05.

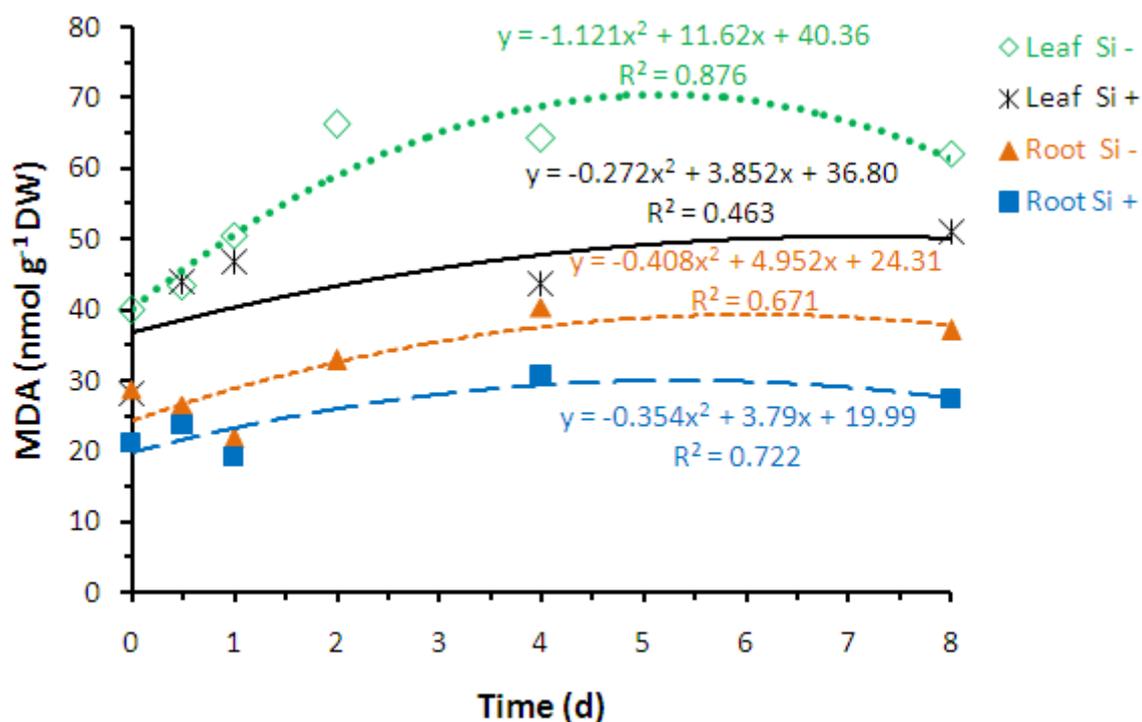


Figure 7.3 Lipid peroxidation of banana leaf and root subjected to chilling temperature

7.3.6 Sugars

The banana leaves and roots of silicon-treated and untreated plants accumulated different levels of sucrose, glucose, fructose and raffinose after they were treated to different periods of cold stress. Sucrose and raffinose levels were generally higher in silicon-treated plants than silicon-untreated plants, while glucose and fructose levels were lower.

Sucrose levels increased with the increase in cold stress on silicon-amended plants. However, on silicon-untreated plants, after an initial increase in the first 2 d at 4°C, it steadily decreased as the stress level increased. Before plants were subjected to chilling temperatures there was no significant difference in sucrose level between silicon-treated or untreated plants, and between leaves and roots. After cold treatment, the sucrose levels were higher in the leaves than in the roots of silicon-treated and untreated plants, and the difference was bigger in silicon-treated plants.

Glucose was higher in the roots than in the leaves of banana plants. Cold stressed plants had higher levels of glucose than unstressed plants. The leaves of silicon-treated plants had lower levels of glucose than those of untreated plants. Glucose levels in silicon-treated plants were below detection level before cold treatment. It increased during the first few days. However, after the stress levels increased, after 4 d, sucrose levels decreased to undetectable levels.

The roots of banana plants contained higher levels of fructose than the leaves, and were significantly higher in silicon-untreated plants than silicon-treated plants ($P < 0.001$). Fructose levels in the leaves of silicon-treated plants were below detection level before the plants were stressed with chilling temperatures. Cold stress increased the fructose level in the root and leaves of silicon-treated and untreated banana plants. However, the fructose level decreased as the plants continued to be exposed to chilling temperatures.

At room temperatures, there was no significant difference in the level of raffinose between silicon-treated and untreated banana plants. On the other hand, the roots contained significantly higher levels of raffinose than the leaves. After 4 d and 8 d at 4°C, the leaves of silicon-untreated plants contained a 42% and an 80% lower level of raffinose than the untreated plants, respectively. Similarly, the roots of silicon-untreated banana plants had

24% and 38% less raffinose at 4 d and 8 d at 4°C than silicon-treated banana plants, respectively.

Table 7.4 Sucrose content of roots and leaves of banana plants kept at 4°C

Silicon (mg ℓ^{-1})	Time (d)	Sucrose (mg g^{-1} DW)			
		Leaf	Root		
0	0	9.66	bcd	11.25	de
	0.5	8.87	abcd	10.19	cd
	1	21.14	g	9.23	bcd
	2	21.87	gh	8.26	abc
	4	17.98	f	18.24	f
	8	6.56	a	7.34	ab
	1000	0	10.42	cd	13.08
0.5		23.99	hi	10.82	de
1		22.03	gh	10.62	cd
2		21.13	g	9.93	cd
4		24.79	i	10.44	cd
8		29.35	j	22.98	ghi
Plant organ×Silicon×Time		<0.001			
l.s.d.		2.415			
s.e.d.		4.862			
cv%		19.7			

Means with the same letter in the same column are not significantly different at $P \leq 0.05$.

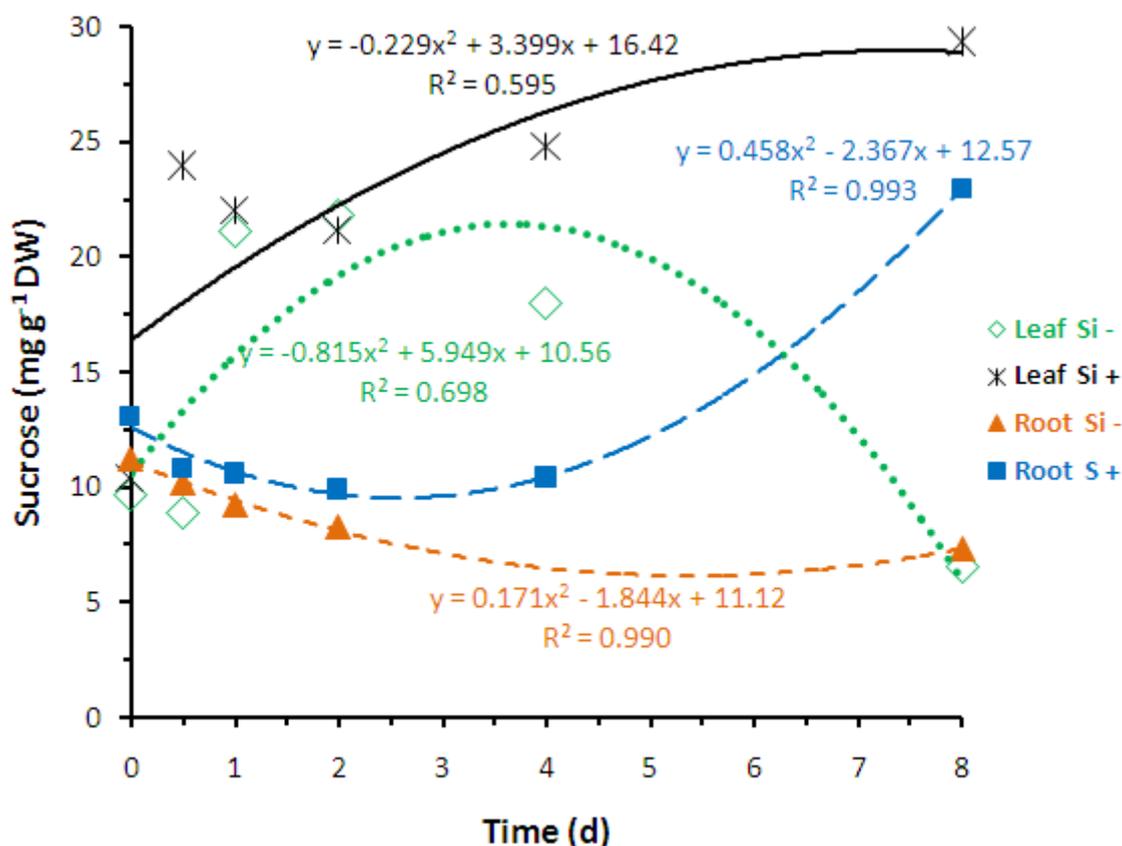


Figure 7.4 Sucrose content of roots and leaves of banana plants kept at 4°C

Table 7.5 Raffinose content of roots and leaves of banana plants kept at 4°C

Silicon (mg l ⁻¹)	Time (d)	Raffinose (mg g ⁻¹ DW)	
		Leaf	Root
0	0	25.34 bc	71.52n
	0.5	36.57 bcdefg	60.31 klmn
	1	38.66 cdefgh	29.54bcde
	2	45.02 fghij	54.03 ijklm
	4	26.11 bcd	48.88ghijk
	8	11.40 a	43.83 fghi
1000	0	24.64 bcdef	66.34 lmn
	0.5	33.77 efgh	50.53 hijkl
	1	39.79 defgh	49.68 ghijk
	2	39.51 fghij	67.68 mn
	4	45.33 jklmn	64.22 lmn
	8	58.18 bc	70.62 n
Plant organ×Silicon×Time		0.040	
l.s.d.		13.843	
s.e.d.		6.885	
cv%		18.4	

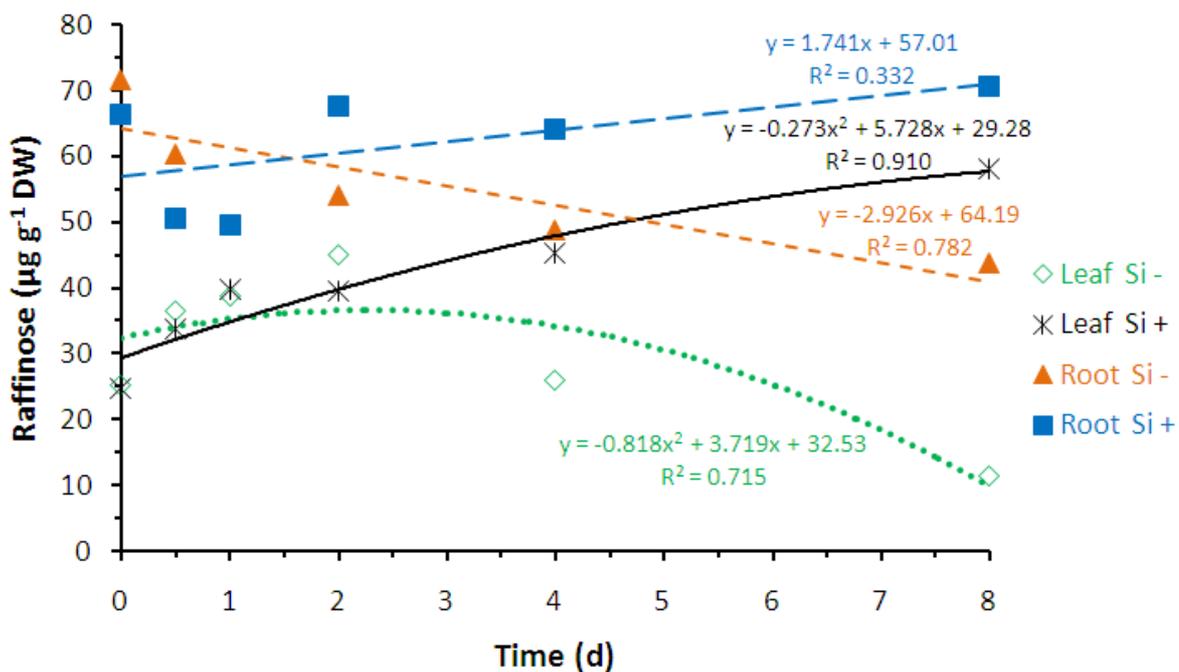


Figure 7.5 Raffinose content of roots and leaves of banana plants kept at 4°C

Table 7.6 Glucose content of roots and leaves of banana plants kept at 4°C

Silicon (mg l ⁻¹)	Time (d)	Glucose (mg g ⁻¹ DW)			
		Leaf		Root	
0	0	5.14	fg	5.95	hi
	0.5	6.80	jk	5.57	gh
	1	10.29	m	7.15	k
	2	8.52	l	6.50	ij
	4	10.23	m	12.27	n
	8	0.84	b	10.11	m
1000	0	0.00 ND	a	3.80	e
	0.5	3.01	d	2.14	c
	1	5.33	g	4.69	f
	2	4.09	e	13.55	o
	4	0.00 ND	a	9.79	m
	8	0.00 ND	a	0.00 ND	a

Plant organ×Silicon×Time <0.001

l.s.d. 0.577

s.e.d. 1.165

cv% 12.5

Means with the same letter in the same column are not significantly different at P≤0.05.

ND = Not detected

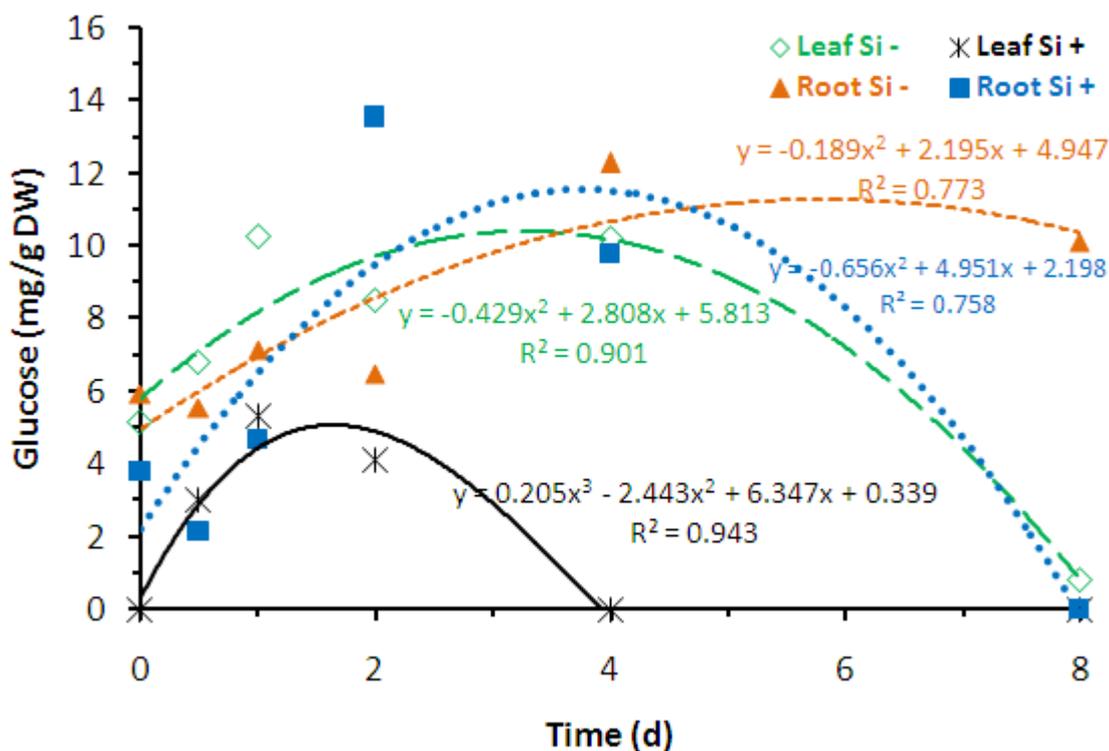


Figure 7.6 Glucose content of roots and leaves of banana plants kept at 4°C

Table 7.7 Fructose content of roots and leaves of banana plants kept at 4°C

Silicon (mg l ⁻¹)	Time (d)	Fructose (mg g ⁻¹ DW)	
		Leaf	Root
0	0	3.40 ef	3.85 f
	0.5	5.60 gh	3.41 ef
	1	6.12 hi	7.80 k
	2	5.17 g	7.09 jk
	4	8.98 l	9.55 l
	8	0.00 ND a	11.06 m
1000	0	0.00 ND a	2.24 cd
	0.5	0.00 ND a	1.99 bc
	1	2.84 de	1.87 bc
	2	1.44 b	10.34 m
	4	3.06 e	6.53 ij
	8	2.86 de	1.88 bc
	Plant organ×Silicon×Time		<0.001
l.s.d.		0.747	
s.e.d.		1.51	
cv%		20.5	

Means with the same letter in the same column are not significantly different at P≤0.05.

ND = Not detected

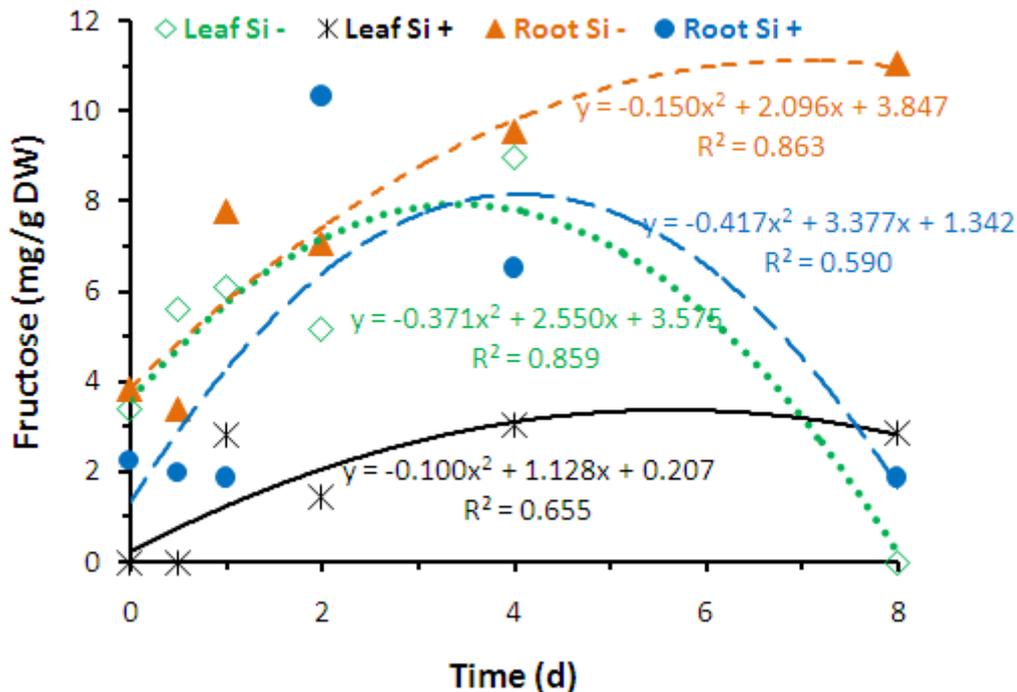


Figure 7.7 Fructose content of roots and leaves of banana plants kept at 4°C

7.4 Discussion

Increased tolerance to chilling stress in banana plants in response to silicon amendments may be associated with the induction of antioxidant responses and the protection of membranes that increased the tolerance of plants to damage. Low temperature damage to cell membrane of susceptible plants causes desiccation or dehydration of cells (Beck et al., 2007). Cellular membrane damage caused by different types of abiotic stresses is largely mediated through membrane lipid peroxidation (Mishra and Choudhuri, 1999). According to Jain et al. (2001) free-radical-induced peroxidation of lipids of membranes as a result of stress is an indicator of damage at cellular level. “Therefore, the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage” (Alpaslan and Gunes, 2001; Demiral and Türkan, 2005; Inal and Tarakcioglu, 2001; Karabal et al., 2003). In this study, we showed that cold damage, membrane permeability and lipid peroxidation (MDA level) increased with the increased exposure of banana plants to low temperatures. Such parameters were higher in silicon-untreated plants than the silicon-treated plants, which indicated the stress alleviating effect of silicon. Application of silicon to decrease MDA level in barley was reported by Liang

Plants produce proline under stress conditions. Proline levels were significantly higher in silicon-untreated plants than silicon-treated plants. In our experiments, silicon-untreated banana plants sustained significantly higher level of cold injury than silicon-amended plants. Increased proline levels is common responses of plants to chilling, drought and heavy metal toxicity (Karabal et al., 2003). The protective role of silicon in reducing low temperature damage was matched by decreases in levels of proline and lipid peroxidation concentrations in banana plants. The ability of silicon to provide protection from oxidative damage is consistent with the findings of Gunes et al. (2007) and Al-Aghabary et al. (2005), who reported that application of silicon reduced H₂O₂ production because of boron-induced and salt-induced toxicity, respectively.

The functional role of various sugars to minimize cellular desiccation in many organisms is through their stabilizing effect on biological molecules, cells and organisms. In our study, levels of sucrose and raffinose were significantly higher in silicon-amended banana plants than silicon-untreated plants.

On the other hand, although there was initial increase in the production of glucose and fructose in silicon-treated plants, it declined later. Plants translocate sugars such as sucrose, stachyose and/or sugar alcohols. Glucose and fructose are not translocated. Leaves are sinks when they are young but become sources as they mature. This means glucose and fructose are present in higher concentrations in young leaves. Under stressful conditions plants prioritize their supply of nutrients. Young shoots or first leaves get first priority. In our experiment, cold damage on banana plants was more noticeable on older leaves than young leaves. The increase in glucose and fructose levels in silicon-untreated plants could be because the mature leaves were not properly functional, whereas cold stressed plants supply higher levels of glucose and fructose to young leaves in order to protect them. On the other hand, the silicon-treated plants, due to their stronger leaf structure were able to slowly acclimate to the cold and make physiological adjustments by slowing down their metabolisms. This may have resulted in the accumulation of higher levels of sucrose and raffinose, which are also better cryoprotectants. By assessing the damage as a loss of the soluble electron-transport protein, plastocyanin, after thawing thylakoids frozen to -20°C, Hinch et al. (2006) reported that sucrose and raffinose function as cryoprotectants. Raffinose, however, was superior to sucrose. Lineberger and Steponkus (1980) reported similar finding when a functional assay was used to determine freezing tolerance in

thylkaoids. However, as Hinch et al. (2006) noted, "...the natural stress tolerance of plants can never be explained by the action of just one compound and that in addition to sugar synthesis, many other physiological adaptations have to take place to allow an organism to survive extremes of stress such as freezing or desiccation". Silicon clearly mediates a number of the abiotic stress tolerance mechanisms in banana.

7.5 References

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CHAPTER EIGHT

General Overview

Banana production is a major source of income and is an essential source of food for more than 70 million people in Africa (Rutherford and Viljoen, 2003). Africa grows 34% of world banana production (Lescot, 2000). Although only less than 15% of the banana fruit produced are exported, it is a crop of great economic, environmental, social and political interest (Anania, 2006; Josling and Taylor, 2003). On the other hand, common bean (*Phaseolus vulgaris* L.) is the most important legume food for direct consumption in the world (Jones, 2007). It is also considered to be the most important field crop in relation to its high protein content and dietary benefits (du Plessis et al., 2002).

These and many other important field, horticultural, ornamental and forest crops are affected by pathogenic species from the fungal genus *Fusarium*. It is one of the most ubiquitous fungi in the terrestrial ecosystem (Ploetz, 2006), and based on the diversity of hosts they attack, the number of pathogenic taxa and range of habitats in which they cause disease, are the greatest in plant pathology (Booth, 1971; Gerlach and Nirenberg, 1982; Leslie and Summerell, 2006; Nelson et al., 1983; Ploetz, 2006). However, this genus also consists saprophytes and non-pathogenic strains that can be used to reduce the impact of the pathogenic strains. The role of this non-pathogenic *Fusarium* spp. is well documented in soils known to suppress *Fusarium* wilt diseases (Alabouvette and Couteaudier, 1992; Smith and Snyder, 1971; Tamietti et al., 1993).

In this study, the value of non-pathogenic *Fusarium oxysporum* strains, soluble silicon and their combination in suppressing pathogenic forms of *F. oxysporum* on two important crops, banana and beans, were assessed. The ability of these crops to take up and accumulate silicon in their organs, and the effects of silicon on biotic and abiotic stresses was also investigated.

In this overview, the important findings of this study and the questions that need to be answered in future research are presented:

Choice and screening of biocontrol organisms:

Within the context of using biocontrol as an alternative strategy to reduce the impact of Fusarium wilt diseases, the research emphasis was focused on endophytic non-pathogenic *Fusarium* spp. These organisms share the same niche as the pathogens giving them an opportunity to compete directly with the pathogens. There is a wealth of literature showing the role of non-pathogenic *F. oxysporum* strains to suppress their closely related pathogenic counterparts. Another important property, of these biocontrol organisms at least in commercial application of a biocontrol product, is their ability to colonize and suppress Fusarium wilt diseases of multiple crops. The two non-pathogenic *F. oxysporum* strains, Strain N7 and Strain 16, isolated from banana plants, were found to colonize and suppress Fusarium wilts of both banana and beans. They were found superior to a rhizosphere biocontrol agent, *T. harzianum* Eco-T[®], which is effective against several soilborne pathogens such as *Rhizoctonia*, *Pythium* and *Sclerotinia*.

In this study, all screening trials were conducted on target plants. Such procedures, though effective, limited the number of organisms that could be tested due to constraints of time space and budget. Therefore, there is a need for simpler and easier method of screening. In addition, it would be most valuable to clearly understand the evolutionary relationship between non-pathogenic and pathogenic forms of *F. oxysporum*.

Silicon uptake and deposition:

The relationship between plant mineral nutrition and plant diseases has been reported by several authors. The most studied nutrients are nitrogen, in its cationic (NH_4^+) and anionic (NO_3^-) forms, K^+ , Ca^{++} and several minor elements (Schneider, 1990). Silicon is the only element to have no known phytotoxicity effect on plants regardless of the amount taken up (Ma and Takahashi, 2002). Plants vary in the amount of silicon they take up, accumulate and deposit it unevenly into different organs. In this study, we showed that silicon was mainly accumulated in the root and leaves of banana and bean plants. The corm and pseudostem, and stem of banana and beans, respectively, accumulated lower levels of silicon. The fact that high levels, relative to the other organs, of silicon was deposited in the roots of banana and beans may be significant in preventing root infection from soilborne pathogens such as Fusarium wilt fungi. However, mechanisms by which silicon helps plants suppress disease are not well understood. Also, how does silicon concentration help?

It is important to understand why plants differ in their ability to take up silicon. Identification of the genes, if any, responsible for this difference may help breed plants with the ability to take up higher levels of silicon. It is also equally important to clearly understand how silicon suppresses plant diseases. The role of silicon in enhancing plant defense responses and as a physical barrier needs to be fully investigated.

pH effects:

Bean and banana plants grown in composted pine bark showed yellowing of the leaves and retarded growth at high silicon fertilization levels. Applications of silicon in the form of soluble silicates raise soil pH. In acidic soils, as in much of Africa (Anonymous, 2004), this may not be a problem. However, plants grown in hydroponic solutions or in alkaline soils may suffer from lack of nutrients that are unavailable at high soil pHs. Addition of nitric acid to lower pH in a hydroponic solution turns the potassium silicate solution into a gel of polymerized polysilicic acid. Hence the buffering of soluble silicon remains an unresolved technical problem.

In order to utilize silicon in hydroponic solutions there is a need for a buffering solution to lower soil pH without precipitating the silicon itself. Development of formulations of silicon fertilizers in the form of “slow release” formulations is crucial in order to apply silicon in field crops such as beans.

Silicon toxicity to fungi:

There are several reports on the effect of silicon on plant disease through a variety of mechanisms. However, there are no, few if any, reports on the toxicity of silicon to Fusarium wilt fungi. In this study, toxicity responses such as inhibition of fungal growth, spore germination and sporulation (Bhuiyan et al., 2000; Damicone, nd; Iacomi-Vasilescu et al., 2004) of pathogenic and non-pathogenic *F. oxysporum* strains were noted. Silicon concentrations $>3.9 \text{ g } \ell^{-1}$ significantly inhibited Fusarial hyphal growth, spore germination, sporulation and reduced disease severity. However, the fungi also showed significantly faster hyphal growth at $0.49 \text{ g } \ell^{-1}$ silicon compared to the control. This indicates that the fungi need silicon for growth and development but at high concentrations it becomes toxic, as do elements such as Cu and Mn. Although pH is an important factor in the growth and development of many fungi, the increase in pH due to the addition of potassium silicate to

PDA did not make any contribution to the inhibition of fungal growth. This was clearly shown by the uninhibited growth of the Fusarial strains at pH as high as 11.5.

The amount of silicon required in order to cause toxicity effects is high. However, if such levels of soluble silicon could actually be available in the soil or hydroponic solution it might adversely affect beneficial organisms such as the non-pathogenic *F. oxysporum* strains. It is, therefore, useful to investigate the effect of silicon on beneficial organisms to avoid non-target effects.

Synergy between biocontrol organisms and silicon:

The non-pathogenic *F. oxysporum* strains suppressed Fusarium wilt of banana and bean plants. However, they could not provide complete protection against the Fusarium wilt pathogens in beans and banana plants. Similarly, silicon fertilization could not prevent infection of these plants by their wilt fungi. Combinations of silicon and the non-pathogenic *F. oxysporum* strains significantly reduced disease severity in bananas but not in beans. Banana plants were stressed using low temperature to induce quicker disease expression. The improvement obtained in banana plants may be associated with ability of silicon to alleviate cold stress in banana plants (Chater 6).

To our knowledge there is no report on the effect of silicon on beneficial microorganisms. There is a need to understand how silicon fertilization affects soil microbial activity, and determine if the composition and amount of plant root exudates change with silicon fertilization.

Low temperature stress and Fusarium wilt of banana:

In the subtropical regions, banana production often suffers from chilling injury (Ganjun and Chunyu, 2008) which exacerbates the severity of Fusarium wilt disease (Buddenhagen, 1990). In this study, silicon fertilization of banana plants significantly reduced chilling stress when plants were exposed to 4°C. Banana plants amended with silicon showed lower levels of lipid peroxidation, membrane damage and proline levels, and higher levels of sucrose and raffinose. Similar findings were also reported from plants treated with silicon under other abiotic stresses (Al-aghabary et al., 2005; Gunes et al., 2007; Liang, 1999). Such findings are of great significance to banana growers from these regions and other low temperature sensitive plants grown in this region. However, the mechanism by which

silicon increases plant's resistance to a variety of abiotic stress factors is not well understood. It would be of great values to determine the effect of silicon in alleviating heat stress on heat sensitive plants.

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