THE TREATMENT OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* IN SOIL AND WATER

by

SAHABNE ULLAH

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Supervisor: Prof A. Viljoen

Co-supervisor: Dr G. Mostert

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SUMMARY

Banana is an economically important food crop globally. It is, however, also severely affected by biotic and abiotic constraints, including banana Fusarium wilt (Panama disease). The causal agent of banana Fusarium wilt is the fungus *Fusarium oxysporum* f. sp *cubense* (Foc), which can survive as chlamydospores in soil for several years, making it difficult to control. The only effective way to manage Foc is by planting resistant cultivars and by preventing the fungus from being introduced into new areas. Resistant cultivars are often not acceptable to local and international markets, which makes disease prevention and reducing the spread of Foc to new areas an important disease management strategy.

Foc can easily spread with contaminated soil and water. Contaminated soil attaches to boots, vehicles, plantation tools and machinery, and transfers the fungus over short or long distances. Heavy rainfall and typhoons may also carry infested soil with run-off water from infested to non-infested areas. The run-off water further contaminates irrigation sources such as rivers, dams and ponds. To prevent Foc from spreading, it is important to implement strict quarantine measures that prevent the movement of the pathogen on field equipment, shoes, vehicles and in contaminated water. Measures to do this include the use of footbaths and disinfestation areas, as well as the treatment of water used for irrigation. Limited studies, however, have been done on the survival and treatment of water contaminated with Foc.

In this study, the sanitation products Sporekill, Saniwash, Farmcleanse, HTH and HyperCide were tested for efficacy against Foc race 1, STR4 and TR4 at their recommended dose and a 0.1 x dilution in the presence and absence of soil in water. The sanitisers were all tested at an exposure time of 0 s, 30 s and 2 min. All sanitisers were effective at both concentrations, but not in the presence of soil. Sporekill and Saniwash were the most effective sanitisers at the recommended dose upon contact (0 s) in the absence of soil and in the presence of soil after 2 min. This indicated that soil needs to be removed from shoes, field equipment and vehicles before being treated with Sporekill and Saniwash.

The survival of Foc in 20-L buckets filled with water was determined in the presence and absence of soil. The water was either stirred or left stagnant. Water samples were extracted from the top, middle and bottom of the buckets 1, 7, 14, 30, 60 and 120 days after inoculation. The survival of Foc decreased over time, and the spores sunk to the bottom of water that remained stagnant. The contaminated water was also treated with ozone, UV radiation, HTH and the peracetic acid products (HyperCide and Tsunami 100) to determine the survival of Foc colony forming units in the presence and absence of soil. Ozone, HTH and HyperCide were only effective in the absence of soil, while UV radiation was ineffective. Thus, it is important that soil be removed for the treatment of irrigation water since Foc was able to survive for 4 months in water without agitation.

OPSOMMING

Piesang is wêreldwyd ‘n ekonomies-belangrike voedselgewas. Dit word egter ook ernstig deur biotiese en abiotiese beperkings geaffekteer, insluitende piesang Fusarium-verwelk (Panama-siekte). Die veroorsakende agent van piesang Fusarium-verwelk is die swam *Fusarium oxysporum* f. sp *cubense* (Foc), wat as klamydospore in die grond vir verskeie jare kan oorleef, wat sodoende die beheer daarvan baie bemoeilik. Die enigste effektiewe manier vir die bestuur van Foc is deur die plant van weerstandbiedende kultivars en deur te voorkom dat die swam in nuwe areas ingebring word. Weerstandbiedende kultivars is dikwels nie vir plaaslike en internasionale markte aanvaarbaar nie, wat siekte-voorkoming en vermindering van die verspreiding van Foc na nuwe areas ’n belangrike siektebestuurstrategie maak.

Foc kan maklik met gekontamineerde grond en water versprei. Gekontamineerde grond sit aan skoene, voertuie, gereedskap en masjinerie vas, en dra die swam oor kort of lang afstande oor. Hewige reënval en tifone kan ook besmette grond met afloopwater vanaf besmette na onbesmette areas oordra. Die afloopwater kontamineer verder besproeiingsbronne soos riviere, damme en poele. Ten einde die verspreiding van Foc te voorkom, is dit belangrik om streng kwarantynmaatreëls te implementeer wat die beweging van die patogeen op veldgereedskap, skoene, voertuie en in gekontamineerde water verhoed. Maatreëls om dit te doen, sluit die gebruik van voetbaddens en ontsmettingsareas, asook die behandeling van water wat vir besproeiing gebruik word, in. Beperkte studies is egter al op die oorlewing en behandeling van water wat met Foc gekontamineer is, gedoen.

In hierdie studie is die saniteerprodukte Sporekill, Saniwash, Farmcleanse, HTH en HyperCide vir effektiwiteit teen Foc ras 1, STR4 en TR4 getoets, teen hul aanbevole dosis en ’n 0.1 x verdunning, in die aanwesigheid en afwesigheid van grond in water. Die saniteerders is almal by ’n blootstellingsperiode van 0 s, 30 s en 2 min getoets. Al die saniteerders was teen beide konsentrasies effektief gewees, maar nie in die aanwesigheid van grond nie. Sporekill en Saniwash was die effektiefste saniteerders teen die aanbevole dosis gewees, op kontak (0 s) in die afwesigheid van grond en in die aanwesigheid van grond ná 2 min. Dit dui daarop dat grond vanaf skoene, veldgereedskap en voertuie verwyder moet word voordat dit met Sporekill en Saniwash behandel word.

Die oorlewing van Foc in 20-L houers gevul met water, is in die aanwesigheid en afwesigheid van grond bepaal. Die water is óf geroer of staande gelos. Watermonsters is vanuit die boonste, middelste en onderste gedeelte van die houers 1, 7, 14, 30, 60 en 120 dae ná inokulasie geneem. Die oorlewing van Foc het oor tyd afgeneem, en die spore het na die bodem van water gesak wat staande gebly het. Die gekontamineerde water is ook met osoon, UV-bestraling, HTH en perasynsuur behandel ten einde die oorlewing van Foc kolonie-vormende eenhede in die aanwesigheid en afwesigheid van grond te bepaal. Perasynsuur was die effektiefste behandelingsmetode en het Foc in die aanwesigheid en

afwesigheid van grond uitgewis. Osoon en HTH was slegs in die afwesigheid van grond effektief, terwyl UV-bestraling oneffektief was. Perasynsuur moet dus vir die behandeling van besproeiingswater gebruik word aangesien Foc in staat is om vir 4 maande in water wat nie geroer word nie, te oorleef.

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CONTENTS

CHAPTER 1

The survival and spread of *Fusarium oxysporum*, with special reference to *F. oxysporum* f. sp. *cubense*

INTRODUCTION

Fusarium oxysporum is a soil-borne fungus known to cause several diseases of plants, including cortical rot, root rot, fruit rot, head blight, leaf spot, fruit rot, cankers, dieback and vascular wilt (Nelson, 1981). Of these, vascular wilt is the most important. The disease is caused by approximately 120 different *formae speciales* of *F. oxysporum*, each affecting a specific crop (Booth, 1971; Nelson, 1981), which makes the fungus one of the most important plant pathogens worldwide (Dean *et al*., 2012). Vascular wilt diseases affect crops such as bananas (*F. oxysporum* f. sp. *cubense* or Foc), cotton (*F. oxysporum* f. sp. *vasinfectum*), carnation (*F. oxysporum* f. sp. *dianthi*), cabbage (*F. oxysporum* f. sp. *conglutinans*), flax (*F. oxysporum* f. sp. *lini*), muskmelon (*F. oxysporum* f. sp. *melonis*), onion (*F. oxysporum* f. sp. *cepae*), pea (*F. oxysporum* f. sp. *pisi*), watermelon (*F. oxysporum* f. sp. *niveum*), gladioli (*F. oxysporum* f. sp. *gladioli*), tulip (*F. oxysporum* f. sp. *tulipae*), china aster (*F. oxysporum* f. sp. *callistephi*), chrysanthemum (*F. oxysporum* f. sp. *chrysanthemi*) and tomato (*F. oxysporum* f. sp. *lycopersici*) (Armstrong and Armstrong, 1981; MacHardy and Beckman, 1981; Beckman, 1987). Some *F. oxysporum* isolates are non-pathogens of plants, while others might cause human diseases (Rebell, 1981).

Fusarium wilt is mainly managed by planting resistant cultivars in pathogen-infested soils. Breeding for resistance, however, can be complex when plants do not produce many seeds, or when a dominant gene is unknown, like in bananas (Stover and Buddenhagen, 1986). It also becomes difficult when new races of pathogens emerge that can overcome the plant resistance. Success with resistance for successful breeding against *F. oxysporum* has been achieved though. This include the breeding of tomato and melon cultivars against races of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *melonis*, respectively (Ori *et al*., 1997; Joobeur *et al*., 2004). When resistant plants are not available, Fusarium wilt needs to be prevented by the exclusion of the pathogen, or managed by the elimination of diseased plants and by isolating the pathogen in one area (Simone and Cashion, 1996).

Fusarium wilt of banana, caused by Foc, is one of the most destructive diseases of agricultural crops (Stover, 1962). The disease is difficult to control since the fungus can survive in soil for many years. It can spread with infected planting material, by soil attached to boots, machinery, vehicles and tools, as well as in water used for irrigation (Dita *et al*., 2018). It is, therefore, imperative to find methods to prevent the spread of the fungus in soil

and water. The aim of this review, thus, is to discuss the survival and spread of *F. oxysporum*, as well as methods to kill the fungus, with special reference to Foc.

BIOLOGY OF *FUSARIUM OXYSPORUM*

Taxonomy of *Fusarium oxysporum*

*Fusarium oxysporum*s is an asexual fungal species defined by its morphological structures. Wollenweber and Reinking (1935) first described *F. oxysporum*, and grouped it with eight other *Fusarium* spp. in the section Elegans. Snyder and Hansen (1940), however, described *F. oxysporum* and divided it into *formae speciales*. Nelson *et al* (1983) modified the classification system by Snyder and Hansen (1940) and described 30 *Fusarium* spp. based on morphological and cultural difference. Leslie and Summerell (2006) divided the genus *Fusarium* into 70 species based on their morphology, genetics and phylogeny.

Fusarium oxysporum produces asexual spores known as microconidia, macroconidia and chlamydospores (Nelson *et al*., 1983). The microconidia are single-celled and kidney-shaped and are produced in false heads on monophialides. Macroconidia are sickle-shaped and have multiple cells. Both micro- and macroconidia contribute to the spread of the fungus inside as well as outside the plant (Nelson, 1981). Chlamydospores are survival spores that are spherical in shape and are often produced either singly or in pairs in macroconidia or attached to hyphae (Nelson *et al*., 1983). Chlamydospores have a thick cell wall that contains lipid-rich cytoplasm that makes it compatible to several environments. It can remain in soil without a host for several years (Schippers and Van Eck, 1981).

Pathogenic members of *F. oxysporum* are divided into *formae speciales* according to the host it attacks (Armstrong and Armstrong, 1981). For example, bananas are affected by Foc and cotton by *F. oxysporum* f. sp. *vasinfectum*. The genetic basis of host specificity is unclear. The *formae speciales* are further divided into races based on pathogens specificity on the cultivars of that host. For example, in bananas, Foc race 1 affects Gros Michel cultivars but not Cavendish cultivars (Kristler, 1997).

Life cycle of *F. oxysporum*

The majority of the *formae speciales* of *F. oxysporum* survives as dormant saprophytes on decaying plant tissue in the soil (Beckman and Roberts, 1995). Once a susceptible host is planted on infested soils, nutrients released by the roots of the host allow the germination of chlamydospores (Stover, 1962; Griffin, 1981; Beckman and Roberts, 1995). The pathogen can either penetrate plant roots directly or through wounds (Nelson, 1981). Penetration mostly occurs at the tip of the tap and lateral roots of the plant (Griffin, 1981; Lucas, 1998).

Fusarium oxysporum gains access to the primary and secondary xylem via the root cortex by inter- and intracellular movement through the pits (Bishop and Cooper, 1983). Microconidia are produced abundantly within the xylem vessel elements, and are then transported in the sap stream until they reach a perforation plate (Griffin, 1981; Bishop and Cooper, 1983). The spores start to germinate and newly produced mycelium penetrate the perforation plate. Once this occurs, more microconidia are produced and the process is repeated (Beckman *et al*., 1961; Beckman *et al*., 1962).

The intensity of *F. oxysporum* invading the vascular system, along with the host defence responses, influence symptom development (Beckman *et al*., 1961; Beckman *et al*., 1962). The pathogen releases cell wall-degrading enzymes like pectin methylesterase, polygalacturonase and cellulose, and triggers the host to produce vascular gels, tyloses and gums that block the xylem vessels (Agrios, 1997; Beckman *et al*., 1962; Davis, 1966). Cellulose have been shown to enhance the wilting of tomatoes by providing carbohydrates to *F. oxysporum* f. sp *lycopersici* that increased disease development and spread of the pathogen *in planta* (Husain and Dimond, 1960). Symptom development is also influenced by the number of infection sites, plant age, and period of pathogen interaction with the root surface. Inoculum density also plays an important role in infection and symptom development (MacHardy and Beckman, 1981).

Wilting, as a symptom of Fusarium wilt, first becomes visible with the yellowing of the lower leaves or shoots of plants. This is followed by the chlorosis and wilting of the other leaves, which results in the eventual death of plants (Nelson, 1981). Symptom development might differ on different hosts. *F. oxysporum* f. sp. *lycopersici*, for instance, causes vein clearing and dropping in tomato plants grown in the greenhouse (Walker, 1971). In the field, however, initial symptoms are yellowing on lower leaves, followed by yellowing of the younger leaves. Branches of plants may display symptoms or remain symptomless. Vascular browning can also be seen when the petiole or stem of an infected plant is cut in half (Nelson, 1981). Flax seedlings wilt from the top and die, while some plants show yellowing symptoms of the upper leaves that eventually turns brown and drops. Leaves of carnations first become yellow and then chlorotic on one side before the plant wilts and die (Nelson, 1981). Vascular discolouration start off with a pale yellow colour and then changes to dark red or black. Chlamydospores are formed after the invasion of the parenchyma tissue just before plant death and in decaying plant tissue. Once the decaying plant tissues collapses, the chlamydospores are released into the soil where it can survive for more than 40 years (Stover and Waite, 1954).

Diversity in the banana Fusarium wilt pathogen

The *F. oxysporum* species complex (FOSC) comprises pathogenic and non-pathogenic strains. The pathogenic strains affect a range of hosts to form *formae speciales*, of which between 120 and 150 are known. The *formae speciales* are further divided in races that affect different cultivars. In Foc, for instance, three different races are recognised that infect a set of differential banana cultivars (Ploetz *et al*., 2015; Stover, 1990; Stover and Buddenhagen, 1986; Stover and Simmonds, 1987). Foc race 1 affects Gros Michel bananas and Foc race 2 affects Bluggoe and other cooking bananas. Foc race 4 is pathogenic to Cavendish bananas. *Fusarium oxysporum* can further be divided into vegetative compatibility groups (VCGs). A VCG include individuals that form a heterokaryon when nitrogen-deficient mutants are paired on a nutrient-poor agar (Kristler, 1997). Classification using VCGs is dependent on common alleles in the loci of both isolates (Correll, 1991). The classification of VCGs follow a numbering system whereby the first three numbers indicate the *forma specialis*, and the remaining numbers indicate the VCG (Puhalla, 1985).

EPIDEMIOLOGY OF *FUSARIUM OXYSPORUM*

The effect of the environment on *F. oxysporum*

Temperature

Favourable conditions for the growth of *F. oxysporum* ranges from 25ºC to 28ºC. Growth of the fungus, however, ceases at temperatures above 33ºC and below 17ºC (Cook and Baker, 1983). Nelson (1964) reported that symptom and disease development of carnation Fusarium wilt was influenced by soil and air temperature. Symptoms developed and were severe when the air temperatures were 23-26ºC, but not when it was 18-20ºC (Fletcher and Martin, 1972; Harling *et al*., 1988). *Fusarium oxysporum* f. sp. *tulipae* caused severe wilting of tulips at warm temperatures but at cooler temperatures, the severity of the pathogen decreased (Bergman and Noordermeee-Luyk, 1973).

Temperature of the soil may affect the resistance and susceptibility of banana plants (Beckman *et al*., 1962). From October to January when temperatures were cooler in Honduras, bananas were able to live longer after wilt symptoms had appeared than in warmer temperatures. At temperatures of 15ºC, it was also noticed that the incidence of the pathogen was reduced in Jamaica (Stover, 1962). A study showed that the Gros Michel cultivar was able to resist Foc for a longer time when the roots were at a temperature of 21ºC. The temperature caused the mycelial growth to slow down and the spores were unable to spread due to the formation of temporary gels and tyloses by the plant. It was also observed that temperature influenced disease development of Foc in the subtropical region where Cavendish bananas are severely affected by Foc STR4 (Ploetz *et al*., 1990).

Water

Water affects disease development in *F. oxysporum* during infection, growth, colonisation as well as symptom development. The fungus thrives in dry soil conditions as there is less competition with other microorganisms (Sung and Cook, 1981). Dry soils and a low evaporative demand can also slow down the transportation of microconidia in the xylem vessels. Tomatoes have been reported to be more resistant to the Fusarium wilt pathogen when the soil is dry (Clayton, 1923; Cook and Papendick, 1970). In hot, humid conditions, the fungus can sporulate on the surface of plants, allowing conidia to spread with wind.

Fusarium oxysporum readily spreads with contaminated irrigation water in a greenhouse, nursery and field. This is most often the case when irrigation water is recycled and reused. In the field, flooding and run-off water can spread the pathogen to disease-free areas, rivers and dams (Stewart-Wade, 2011). Not much research has been done on the survival of *F. oxysporum* in water, but a lack of oxygen can suffocate the pathogen over time as elevated levels of carbon dioxide decrease the formation of chlamydospores (Stover, 1962).

Soil

Acid soil favours the growth of *F. oxysporum* compared to alkaline soils, which are favourable for bacterial growth (Scher and Baker, 1980; Dominguez *et al*., 2001). The reduction of nutrients in soil, and the suppressive ability of some microbes, may reduce disease incidence (Jones *et al.,* 1989; Alabouvette *et al*., 1993). These microbes include *Bacillus* spp*., Trichoderma* spp*., Pseudomonas* spp*.,* actinomycetes and some non-pathogenic strains of *F. oxysporum* (Baker, 1987). High bacterial population in the soil were able to suppress Fusarium wilt in flax (Hoper *et al*., 1995).

A pathogen requires sufficient nutrition to germinate and spread. This includes a carbon source for energy, inorganic compounds (Woltz and Jones, 1981) as well as nutrient elements like carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, magnesium, sulphur, iron, manganese, molybdenum and zinc (Steinberg, 1950). Iron in acidic soils were found to increase disease development of Foc (Dominguez *et al*., 2001), as did nitrogen and ammonium (Woltz and Jones, 1981). An increase in nitrogen and decrease in potassium increased disease development whereas a decrease in nitrogen and increase in potassium reduced disease development (Walker, 1971). A low level of calcium and boron increases the rate of disease (Edgington and Walker, 1958; Corden, 1965; Keane and Sackston, 1970).

Survival

Microconida and macroconidia are fragile spores and not as resilient as chlamydospores (Newcombe, 1960). Chlamydospores are formed in dead and dying plant tissue, and released into the soil when the plant tissue decays.

Fusarium oxysporum can also survive on other hosts. Many weeds that grow in soil infested with *F. oxysporum* are infected but do not show wilt symptoms. *F. oxysporum* f. sp. *vasinfectum* infected weeds belonging to the genera *Oryzopsis, Digitaria, Amaranthus* and *Malva* (Katan, 1971). A study found that three grass species and one herb out of several grass and weed species that were tested were colonized by Foc. The grass species were *Paspalum fasciculatum*, *Panicum purpurascens* and *Ixophorus unisetus* and the herb was *Commelina diffusa* (Waite and Dunlap, 1953).

DISSEMINATION OF *FUSARIUM OXYSPORUM*

Plant material

Certain *formae speciales* of *F. oxysporum* are able to spread to uninfested fields with seeds. These include *F. oxysporum* f. sp. *pisi* on peas (Snyder, 1932), *F. oxysporum* f. sp *phaseoli* on beans (Kendrick, 1944), *F. oxysporum* f. sp. *asparagi* on asperagus (Grogan and Kimble, 1959), *F. oxysporum* f. sp. *betae* on sugarbeet (MacDonald and Leach, 1976), *F. oxysporum* f. sp. *callistephi* on aster and *F. oxysporum* f. sp. *elaeidis* on oil palm (Locke and Colhoun, 1973). In tomato, however, *F. oxysporum* f. sp. *lycopersici* can colonise the fruit and then spread with the seed (Kendrick, 1944), in other cases *F. oxysporum* infects injured seed through wounds. These include *F. oxysporum* f. sp. *lini* in flax seeds (Nair and Kommedahl, 1957), *F. oxysporum* f. sp. *vasinfectum* in cotton seed (Elliot, 1923), *F. oxysporum* f. sp. *mathioli* in garden stock (*Mathiola incana)* (Baker, 1948) and *F. oxysporum* f. sp. *perniciosum* in mimosa seed (Gill, 1968). *Fusarium oxysporum* f. sp. *lini* can also be disseminated with infected plant parts. Spores of the pathogen often travels on the seed coat. The pathogen attaches itself to withered seed more readily than on a smooth seed coat (Nelson, 1981).

Fusarium wilt pathogens can also be disseminated with vegetative propagation material. This is usually seen with Fusarium wilt pathogens of carnation and chrysanthemum since they are propagated from vegetative cuttings. *F. oxysporum* f. sp. *dianthi* can be moved with carnation cuttings from plants that have shown no symptoms (Bickerton, 1942; Nelson, 1964), similar to Foc-contaminated banana suckers (Stover, 1962). In addition, banana leaves can spread the pathogen when they are used to wrap and pack bananas in resource poor areas and on-farm (Dita *et al*., 2018). Fusarium wilt pathogens, therefore, can be

disseminated with many plant parts, from seed, cuttings, stems and leaves, regardless of whether symptoms are visible (Engelhard and Woltz, 1973).

Soil

F. oxysporum in soil is not likely to travel long distances unless it is by infected plant material, weeds or dispersal agents like wind and rain (Trujillo and Snyder, 1963). Contaminated soil is mostly disseminated by humans. Fusarium wilt pathogens can spread with soil attached to shoes, machinery, vehicles and tools (Dita *et al*., 2018).

Water

Water is a major source of dissemination of *F. oxysporum.* The pathogen contaminates dams, lakes, rivers and ponds from where irrigation water is moved or sprayed onto clean fields with susceptible crops (Hong *et al*., 2014).

Fusarium wilt in date palm trees caused by *F. oxysporum* f. sp. *albedinis* was readily spread during heavy rainfall and when irrigated frequently (Louvet and Toutain, 1981). Park (1959) also speculated that *F. oxysporum* f. sp. *elaeidis* is spread during flooding in oil palm trees. In bananas, it was observed that Foc was spread by contaminated irrigation water in the Philippines (Trueggelmann, Unifrutti/Bloom Agro, personal communication).

In greenhouses and nurseries, water is often recycled and reused, and if infected, this can lead to the contamination of disease-free plants (Stewart-Wade, 2011). Agricultural and horticultural systems use massive amounts of water, and due to increasing cost and environmental advantages of re-using water, recycling of nursery water has become necessary (Norman *et al*., 2003; Schnitzler, 2004).

MANAGEMENT OF FUSARIUM WILT

Prevention of spread

Exclusion can prevent the spread of *F. oxysporum* to uninfected areas. It involves good quarantine measures, exclusion, and the use of pathogen-free planting materials (Agrios, 1997). These measures are dependent on awareness, contingency plans and a legal framework supported by the National and Regional Plant Protection Organizations (N/RPPOs) (Dita *et al*., 2018). Growers should implement biosecurity measures at farm gates to assist in preventing the introduction of the Fusarium wilt pathogen onto farms. Certification agencies like Global Gap can also play a role in limiting the spread of diseases by implementing biosecurity measures on farm (Globalgap, 2018).

Foc is impossible to eradicate once the pathogen has entered a banana field. The only option, then, is to reduce the amount of inoculum in the soil and to limit the spread of the

pathogen. This can be achieved by destroying infected plants, and by isolating these plants to limit the movement of people and animals and prevent run-off water to spread the fungus outside the area (Stover, 1962; Gullino *et al*., 2015; Dita *et al*., 2018). Footbaths at entrances of farms, greenhouse and nurseries can also limit the spread of *F. oxysporum* (Gullino *et al*., 2015). Tools and shoes used in infested areas should remain on the farm, and effective sanitation products should be used at sanitary stops to disinfect vehicles and shoes (Dita *et al*., 2018). In greenhouse and nurseries, trays, pots and other equipment may also carry inoculum. It is therefore important to also treat them with disinfectants.

In Australia, Farmcleanse (alkali metal salts of alkylbenzene sulfonic acid and coconut diethanolamide) was reported as an effective disinfectant against Foc used in footbaths to disinfect equipment and vehicles (Moore *et al*., 1999; Moore and O'Neill, 2000). Sporekill® and Prozin®, both quaternary ammonium compounds, were subsequently shown to be more effective (Nel *et al*., 2007), but Sporekill's activity was reportedly neutralised when exposed to sunlight (Meldrum *et al*., 2013). More recently, Nguyen *et al*. (2018) showed that diluted quaternary ammonium compounds were effective as disinfectants both in the presence and absence of soil. Sporekill® was able to inhibit microcondial germination immediately after application.

Organic matter has the ability to reduce the efficacy of disinfectants by limiting direct contact with the pathogen (Russell, 1983). Bennett *et al*. (2011) demonstrated that Simple Green d Pro 3 (a quaternary ammonium compound), Formula 409 Antibacterial All Purpose cleaner (a quaternary ammonium compound) and Zep Industrial Purple cleaner and degreaser (sodium hydroxide, 2-butoxyethanol) were less effective against *F. oxysporum* f. sp. *vasinfectum* when the disinfectants were diluted in water with soil compared to clean water. It is, therefore, important to first clean field tools, vehicles and shoes contaminated with *F. oxysporum* from soil before treatment with disinfectants.

Plant resistance

Plant resistance is the most effective means to manage Fusarium wilt diseases. In bananas, for instance, Gros Michel cultivars affected by Foc race 1 was replaced by the Cavendish cultivars because of their resistance (Ploetz and Pegg, 2000). Cavendish bananas, however, are susceptible to Foc race 4. Plant breeding has been used to develop resistant banana cultivars. Breeding of bananas is complex since it is slow due to the low amount of fertile seeds found in the fruit (Ortiz *et al*., 1995). Resistant hybrids are also not always commercially acceptable to the consumer (Stover and Buddenhagen, 1956). Fusarium wilt resistance can also be introduced with genetic engineering (Agrios, 1997), but such bananas are not accepted in most countries. Resistance can also be achieved by somaclonal variation or genetic transformation (Hwang and Ko, 2004).

Integrated disease management

An integrated disease management strategy involves combining several disease management options. These include biological control, chemical control and cultural control, which can be combined with resistant varieties. From 1923 to 1927, Knudson combined crop rotation with potassium and phosphate fertilizers in Guatemala to control Foc. This combination was, however, ineffective when Gros Michel was replanted in infested fields (Stover, 1962). The combination of flood fallowing and fungicides reduced soil inoculum levels, but did not eradicate them. Saravanan *et al*. (2003) found that *P. fluorescens* in combination with organic amendments was able to suppress Foc race 1 in both the greenhouse and the field. In the greenhouse, a combination of *Trichoderma* and benomyl drenches were able to control Fusarium wilt of chrysanthemums (Locke, 1985), and combination of non-pathogenic isolates of *F. oxysporum* with the fungicide, benzimidazole, improved the control of Fusarium wilt of cyclamen (Minuto *et al*., 1995).

The use of fungicides in the benzimidazole group have shown some effectiveness against Fusarium wilt diseases. These fungicides include benomyl, carbendazim, thiabendazole, and thiophanate. Benomyl controls Fusarium wilt of tomato (Thanassoulopoulos *et al*., 1970), chrysanthemum (Engelhard and Woltz, 1973) and sweet potato when used as a spray or suspension (Nielsen, 1977). Carbendazim, thiabendazole and thiophanate were effective in the control of Fusarium wilt in cucurbits (Li and Liu, 1990).

Biological control can be an alternative method to control the disease. Reports have shown the efficacy of *Trichoderma* to control Fusarium crown rot of tomato (Sivan, 1987). *Gliocladium* is another biocontrol available to control soil borne diseases and has been effective in the control of Fusarium wilt on cotton (Zhang *et al*., 1996).

Cultural control methods like crop rotation reduces the inoculum level of *F. oxysporum* but is unable to eradicate the fungus in the soil (Baker, 1981). Crop rotation is not effective in controlling *Foc* because of its ability to survive without a host for several years (Stover, 1962). A study done in Taiwan found that crop rotation with sugarcane had no effect on the pathogen population of Foc (Hwang, 1985).

FUSARIUM OXYSPORUM IN WATER

Occurrence and dissemination of *Fusarium oxysporum* in water

Water bodies like lakes, rivers and dams often becomes infected with plant pathogens (Hong *et al*., 2014). This usually happens after flooding caused by typhoons and hurricanes (Dita *et al*., 2018). Heavy rain and excess irrigation water in the field, known as tailwater, may come into contact with *F. oxysporum* in infested fields. The excess water in the field is then run

back to the main water source. This can result in the redistribution and spread of the pathogen in a field (Rattink, 1977). In Puerto Rico, for instance, the number of banana plants affected by Foc increased rapidly after the rainy season (Fawcett, 1913).

Greenhouses irrigation water is often recycled and reused too (Stewart-Wade, 2011). This may result in significant losses of crops and seedlings if the irrigation water is not properly treated (Elmer, 2008; Hong *et al*., 2014). Shokes and McCarter (1979) found that fumigated vegetable transplants were affected by *F. oxysporum* due to its presence in irrigation ponds.

Survival in water

The survival of *F. oxysporum* in water has been poorly studied. Newcombe (1960) and Stover (1962) both showed that Foc chlamydospores survives longer in water that is continuously agitated than in standing water. This is due to the necessity of oxygen to the spores for survival. A laboratory study showed that the survival of Foc microconidia and macroconidia were reduced after 10 days, while chlamydospores were able to survive for 40 days (Stover, 1962). In soil, however, Foc was able to survive for a longer period without oxygen. A lack of oxygen and wet soil led to a lower rate of survival (Stover, 1962; Newcombe, 1960).

An increase in carbon dioxide favours chlamydospore germination but the production of more chlamydospore, however, eventually ceases in water (Stover, 1962). Newcombe (1960) tested the effect of gases on Foc in soil and water and found that carbon dioxide caused a major reduction of viable Foc spores.

MANAGEMENT OF *F. OXYSPORUM* IN WATER

Chemical and non-chemical methods can be used to eradicate *F. oxysporum* in water. The chemical methods include ozone, chlorine, peracetic acid and hydrogen peroxide, and their effectivity often depends on the absence of soil in water. Thus, water first needs to be filtered before it can be treated with chemicals or when using non-chemical treatments such as UV.

Filtration techniques

Membrane filtration

Membrane filtration consists of filters with a pore size that separates organic material from liquid. Microfiltration removes particles ≥50 nm, ultrafiltration ≥3 nm and hyperfiltration ≥0.1 nm (Stewart-Wade, 2011). A high pressure filtration system against a membrane can be effective at eliminating pathogens in irrigation water and in the desalination of water. However, high installation costs, pumping costs and expensive filters make membrane

filtration often unaffordable for the eradication of pathogens in water (Runia, 1995; Rolfe *et al*., 2000).

Slow sand filtration

Slow sand filtration (SSF) can be used to filter out soil-borne pathogens from water, but *Fusarium* spp. are only partially removed (Minuto *et al*., 2008). The efficacy of this method depends on the sand used. The finer the sand, the more efficient the filter is (Barth, 1998). SSF can remove *Fusarium oxysporum* at a low concentration but it is unable to remove the pathogen at a higher concentration (Pettitt, 2002). SSF depends on the flow rate, the propagules of the pathogen and particle size (Barth, 1998). A flow rate of 300 L/h/m^2 is best to remove plant pathogens from water (Barth, 1998, 1999). After a month the microbes colonises the surface and blocks the sand filter (Barth, 1998). Disadvantages of SSFs include the inability to move the filter system due to its weight and the fact that SSFs are easily clogged by inorganic particles (Wohanka and Helle, 1996).

Chemical treatment

Ozone

Ozone has been used to treat water since its discovery in 1840 (Hong *et al*., 2014), mostly for the treatment of wastewater, drinking water, water in swimming pools as well as for the postharvest treatment of several crops (White, 1992; Graham, 1997). The production of ozone involves the installation of an oxygen accumulator, an ozone generator, an ozone dissolution apparatus and apparatus for the elimination of excess ozone. Oxygen gas contained in a tank is passed into an ozone generator that splits oxygen molecules (O_2) into atoms (O^-) and then binds to other oxygen molecules to produce ozone (O_3). It is then injected into water with a Venturi injector that ensures that sufficient ozone is deposited into the water by adhering to the flow rate and the gas rate (Hong *et al*., 2014).

Ozone has the highest oxidation potential of all chemical treatments, and can be used to effectively eradicate bacteria, fungi and viruses from water (Venosa *et al*., 1984; Newman, 2004; Hunter, 2008). It oxidizes the cell membrane, DNA, RNA and proteins of the organism; eventually causing cell death. Once ozone decomposes in water it forms hydroperoxyl and hydroxyl radicals which are strong oxidizers that further eliminate the remaining organisms (Zheng *et al*. 2018). Ozone was effective against conidia of *F. oxysporum* at 0.6 ppm for 3 min (Igura *et al*., 2004). In dam water, however, 1.1 ppm was required for 4 min for complete disinfection (Beardsell and Bankier, 1996).

A major advantages of ozone is its high oxidation potential, which means it requires a lower dosage and a shorter exposure time to eliminate pathogens (Voigt *et al*., 2013). It easily degrades into oxygen and does not leave any residue on crops during postharvest

treatment (Zheng *et al*., 2007; Stewart-Wade, 2011; Zheng *et al*., 2018). However, the installation and maintenance of the ozone production system in the field is expensive due to its instability (Masten and Davies, 1994). Health problems can occur from leakages if not handled well. Disinfection by-products (DBP) that are formed in reaction to organic matter in water and can also be toxic to plants and humans (Bouwer, 2002; Freese *et al*., 2003).

Chlorine

Chlorine is a disinfectant used worldwide because it is effective and inexpensive (Van Haute *et al*., 2013). It is commonly used to disinfect drinking water and to eliminate plant pathogens in water (Thompson, 1965; Datnoff *et al*., 1987; Kong *et al*., 2004). Chlorine is comprised of three forms: chlorine gas, sodium hypochlorite and calcium hypochlorite (Newman, 2004; Ivey and Miller, 2013). Hypochlorite is safer than chlorine gas and is commercially available (Lewis, 2010). Sodium hypochlorite is the active ingredient of liquid bleach (Newman, 2004). When mixed with water it produces hypochlorous acid, depending on the pH of the water (Hong *et al*., 2014). Hypochlorous acid is a strong and fast oxidizer that is often used for water disinfection in the greenhouse. Calcium hypochlorite is a dry form of chlorine that comes in a powder, tablet or granules. It is effective in eliminating bacteria, algae, slime, fungi and other microorganisms. It is safe to handle, easier to store and less harsh on equipment (Newman, 2004).

Chlorine eliminates pathogens by penetrating their cell walls and disrupting their metabolic activity (Bitton, 2011). They also damage membranes and critical proteins, which eventually leads to cell death. Nucleic acids and enzymes such as catalase and dehydrogenases are also destroyed by chlorine (Bitton, 2011). Chlorine can also disrupt nutrient transport, cell respiration and sulfhydryl groups, resulting in cell death (Leyer and Johnson, 1997).

Although chlorine is effective against some microorganisms, a high concentration of chlorine forms DBP, which is toxic. In the presence of organic and inorganic matter in water, by-products like trihalomethanes (e.g. chloroform) and haloacetic acids can cause health problems in humans (Hong *et al*., 2014). A low concentrations of chloroform poses a lesser risk (Kobylinski and Bhandari, 2010). A chlorine concentration limit has therefore been established by the Department of Water Affairs (South Africa) of 0.25 mg/L for the disinfection of wastewater used for irrigation to reduce the production of DBP (DWA, 2013). However, the South African water guidelines suggest that <140 mg/L of chlorine is ideal to prevent toxicity on crop yield and quality (DWAF, 1996).

Several factors can influence chlorine activity (Hong *et al*., 2014). These include temperature, pH, organic matter and turbidity. At lower temperatures, chlorine disinfection is reduced (Pickard, 2006). Hypochlorous acid performs best at a pH of 6.5-7, but a higher pH

converts it to hypochlorite, making it a weaker disinfectant. The pH also influences the oxidation reduction potential (ORP). The ORP indicates the oxidative potential of a chemical. The higher the ORP, the greater the oxidizing potential of the chemical (Park *et al*., 2004). It was found that an increase in ORP readings is due to an increase in the chlorine concentration which results in a lower pH (Hong *et al*., 2014).

The presence of organic matter greatly influences the efficacy of chlorine. It induces the formation of DBP and increases turbidity. It further provides a shield to microorganisms from chlorine treatment (Pickard, 2006; Bitton, 2011). Thus, a filtration method needs to be used before chlorine disinfection. A study that investigated the contact time required to control *F. oxysporum* found that an application of 8-10 ppm for 0.5-1.5 min was effective (Cayanan *et al*., 2009), and another that 5 ppm for 20 min was effective in killing chlamydospores of *F. oxysporum* in deionized water. In dam water, however, 30 min were required (Scarlett *et al*., 2015). It is important to realise that the exposure time and concentration of chlorine depends on the pathogen species and spore type targeted (Raudales *et al*., 2011).

Advantages of the use of chlorine is its low cost and availability. It is simple to use and requires no installation equipment (Van Haute *et al*., 2013). It is also effective against several pathogens and can be used for the control of algae (Chase and Conover, 1993). The main disadvantage of chlorine that it produces DBP, which poses a health risk to humans. Once decomposition of hypochlorites occurs, it can be combustible due to the combination of heat and light (Newman, 2004).

Peracetic acid

Peracetic acid (PAA) is a chemical disinfectant consisting of acetic acid and hydrogen peroxide (Alasri *et al*., 1992). PAA's germicidal properties were reported in 1902, but it became commercially available much later (Freer and Novy, 1902; Block, 1991). In 1949, PAA was the most effective of 23 germicides against *Bacillus thermoacidurans* (Hutchings and Xezones, 1949). Its bactericidal, fungicidal and sporicidal concentration was later confirmed as 0.001%, 0.003% and 0.3%, respectively. PAA is often used in gnotobiotics, for wastewater treatments, the disinfection of environmental applicators such as cooling towers, and as ion exchangers and combined sewer overflows. Industrial applications as a disinfectant include the pharmaceutical, beverage, paper and food industries (Kitis, 2004). Due to its efficacy PAA was tested in the treatment of wastewater since the 1980s (Gehr *et al*., 2002). PAA was effective against *F. oxysporum* f. sp. *narcissi* at 80 minutes with a concentration of 0.5% (Hanks and Linfield, 1999). Another study found PAA effective against *F. oxysporum* on seeds of watermelon at a concentration of 80 µg/ml in less than 5 minutes (Hopkins *et al*., 2003). No studies on the efficacy of Foc have been done.

The mode of action of PAA is poorly understood still. It is speculated to act similar to peroxides and oxidizing agents (Block, 1991). Oxidation of proteins, enzymes and metabolites occur by affecting sulfhydryl and sulfur bonds when oxygen is present (Liberti and Notarnicola, 1999). Intracellular PAA also oxidizes vital enzymes that disrupts active transport between membranes, biochemical pathways and solute levels (Fraser *et al*., 1984). Cell wall rupture and dislocation disrupts the chemiosmotic function of lipoprotein cytoplasmic membrane as a result of PAA (Baldry and Fraser, 1988; Leaper, 1984). Its efficacy in the lipoprotein cytoplasmic membrane make it also effective in the outer membranes of lipoproteins (Leaper, 1984). It can also act as a sporocide due to its ability to denature proteins (Block, 1991) and is known to affect the bases of DNA (Tutumi *et al*., 1973).

PAA is preferred over chlorine as disinfectant as it does not produce DBP (De Luca *et al*., 2008). Harmless by-products of PAA are oxygen, hydrogen peroxide, and acetic acid (Wagner *et al*., 2002; Koivunen and Heinonen-Tanski, 2005b; Kobylinski and Bhandari, 2010). Mutagenic by-products produced by PAA are limited following contact with organic materials (Baldry and Fraser, 1988; Monarca *et al*., 2002). High dosages of PAA, however, can result in the formation of harmful DBP (Zanetti *et al*., 2007). Therefore lower dosages of PAA that are still effective for treatment should be used (Koivunen and Heinonen-Tanski, 2005a). The efficacy of PAA can be affected by several factors, including temperature, pH and presence of organic matter (Kitis, 2004). PAA is effective at a broad range of temperatures, but its effectivity increases at higher temperatures (Baldry *et al*., 1995; Profaizer et *al*., 1997; Stampi *et al*., 2001). A pH of 5-8 does not affect the efficacy of PAA but at pH of 9 the efficacy decreases (Block, 1991; Baldry *et al*., 1991; Sanchez-Ruiz *et al*., 1995). More suspended solids decreases the efficacy of PAA in wastewater (Gehr *et al*., 2002; Kitis, 2004; Koivunen and Heinonen-Tanski, 2005b), and organic matter in biofilms significantly decreases its disinfectant potential (Akinbobola *et al*., 2017). Conversely, Julio *et al*. (2014) reported that PAAs disinfection capabilities before filtration was only 8% lower than after filtration, whereas others reported that its disinfection rates were constant in the presence and absence of suspended solids (Lazarova *et al*., 1998; De Luca *et al*., 2008; Stampi *et al*. 2001).

Advantages of PAA are their limited production of DBP compared to other chemical disinfectants and their low residue levels (Freese *et al*., 2003; Kitis, 2004). It is stable when handled properly and is easy to use (Freese *et al*., 2003). Lower concentration of PAA and shorter exposure times make the disinfectant economically feasible. However, the decomposition of PAA leads to the production of acetic acid, which can result in the increase in organic content leading to microbial regrowth (Kitis, 2004).

Hydrogen peroxide

Hydrogen peroxide is a versatile disinfectant that can be used alone or in combination with other disinfectants (Vargas *et al*., 2013). It is used to increase the efficacy of any disinfectant solution with a dosage of 0.010% against *Fusarium* spp. (Runia, 1995). It is used for wastewater treatment and can be applied to water and soil (Ronen *et al*., 2010; Vargas *et al*., 2013; LENNTECH, 2014) to eradicate bacteria, algae, yeast and fungi in irrigation water (McDonnell and Russell, 1999; Newman, 2004). Hydrogen peroxide and PAA were used in the disinfection of irrigation water contaminated with *F. foetens* (Elmer *et al*., 2014). Hydrogen peroxide was shown to be effective against *F. circinatum* spores at an oxidation reduction potential (ORP) of 400 mV and an exposure time of 6 hrs (Van Wyk *et al*., 2012).

Hydrogen peroxide consist of the radicals hydroxyl and superoxide, which enter the cell membrane. These radicals can destroy the cell membrane, proteins and DNA of an organism (McDonnell and Russell, 1999; Vargas *et a*l., 2013; LENNTECH, 2014). The disinfection process depends on the concentration, water quality and exposure time (Labas *et al*., 2008). It has a high oxidation potential and leaves no residue. Hydrogen peroxide is unstable and corrosive, and high concentrations may lead to phytotoxicity (Newman, 2004; Sichel *et al*., 2009).

Non-chemical treatments

Ultraviolet (UV) radiation

One of the most common non-chemical treatments is the use of ultra-violet (UV) lights for disinfection. Its wavelengths are divided into UV-A (315-400 nm), UV-B (290-315 nm) and UV-C (220-280 nm) (Oppenländer, 2003; Hunter, 2008). A wavelength of 200-280 nm is most lethal (Hunter, 2008). UV-A is used for skin tans, for fading of paints and fabrics (Buonocore, 1970; Zeman, 2011). UV-B can cause harmful radiation at high doses that results in health risks to humans and animals, and crop losses in plants. It is often used in plastics and the testing of fades (OAR, 2010). UV-C lamps generate a wavelength of 254 nm (Burgener, 2006; Zheng *et al*., 2014) and is used for disinfection of bacteria, viruses, algae, fungi, molds and protozoa by a photochemical reaction (Wong, 2002; Hijnen *et al*., 2006; Meunier *et al*., 2006; Trombert *et al*., 2007; Bolton and Cotton, 2008). The photochemical reaction causes the pathogens inactivity and does not produce any DBP (Newman, 2004; Hijnen *et al*., 2006).

When UV light is absorbed by the DNA and RNA of an organism, the pyrimidine bases form dimers between the two nucleotides and ceases the production of new DNA and RNA chains (Poepping *et al*., 2014; Bolton and Linden, 2003). High, medium and low pressure lamps are used to provide the required intensity for disinfection (Eischeid and Linden, 2007).

A wavelength of 260 nm is able to react with DNA, which makes the use of a medium or low pressure lamps ideal for disinfection (Bolton and Linden, 2003).

In laboratories a collimated beam is utilized to provide UV doses, but in the field it requires professional installation (Bolton and Linden, 2003). In the field, water passes through a cylinder and is treated at the required UV dose (Zheng *et al*., 2014). UV dosages can be calculated by finding the product of the intensity and time. UV is an ideal treatment to eradicate pathogens in water (Fynn *et al*., 2011). Dosages commonly used to eliminate pathogens are 16 $mJ.cm^{-2}$, 30 $mJ.cm^{-2}$ and higher. For fungi, a dosage of 30-300 $mJ.cm^{-2}$ is required, (Shama, 2007; Koutchma *et al*., 2009). A dosage of 250 $mJ.cm^{-2}$ kills *F. oxysporum* (Scarlett *et al*., 2015). Runia (1995) showed that 90% of conidia of *F. oxysporum* f. sp. *melongenae* was reduced at 70 $mJ.cm^{-2}$, while UV-C light completely eradicated *Fusarium* spp. in drain water (Adrados *et al*., 2005).

Certain factors affect the efficacy of UV treatment. The rate of water flow affects the eradication of pathogens with UV. High rates of water flow results in a lower exposure to UV light than when water flows at a low rate (EWP, 2014). Its efficacy is also dependent on water quality and turbidity (Freese and Nozaic, 2004). Water that needs to be treated should be as clean as possible to avoid turbidity (Linden *et al*., 2006). It is, therefore, important that a pre-treatment method, like filtration, is available to remove organic matter prior to UV disinfection (Linder, 2007). A study found that sand filtration prior to UV disinfection increased the rate of efficacy in bacteria (Jolis *et al*., 2001).

One of the main advantages of UV treatment is that it does not produce DBP. It is also non-corrosive and not dependent on pH. Its disadvantages are high installation costs. Operational costs are fairly low, but lamps need to be monitored and replaced frequently. UV disinfection is reduced in the presence of organic matter, thus filtration methods need to be installed which results in additional costs (Zheng *et al*., 2014).

Heat treatments

Heat treatments are commonly used by subjecting the pathogen to high temperatures that can kill it. For example, in fungi, temperatures of 40-70ºC can be lethal at an exposure time of 10 sec (Runia *et al*., 1988). *Fusarium* spp. can be disinfected at a temperature of 85ºC for 30 sec.

CONCLUSION

Banana is the eighth most important crop worldwide and the fourth most in developing countries (Ploetz, 2015). Cooking and dessert bananas are the two types of bananas consumed worldwide. Cooking bananas are a staple food crop in Africa, Asia and tropical

America (Karamura *et al*., 2012). Cavendish bananas, a dessert banana, is the main export banana. Bananas, however, are severely affected by pathogens, of which Foc is considered the most important.

There are limited measures to control banana Fusarium wilt. One of the only effective ways to control the disease is by planting resistant cultivars. If such plants are not readily available, Fusarium wilt can be managed by slowing down the spread of the pathogen. Foc spreads readily in soil and water, which makes it difficult to manage. Heavy rain often lead to run-off water from infested plantations contaminating sources used for irrigation.

Few studies have investigated the survival, or treatment, of Foc in water. Infected water is an important means to rapidly contaminate banana fields. Foc-infested soil can also spread the fungus if it is attached to shoes, vehicles and plantation tools. The objective of **Chapter 2**, therefore, is to test the efficacy of five commercial disinfectants (Sporekill, Saniwash, Farmcleanse, HyperCide and HTH) to eradicate Foc at different concentrations and exposure times in the presence and absence of soil in water. In **Chapter 3**, the survival of Foc in water at different depths, agitation, and in the presence and absence of soil is determined over 120 days. Treatment of Foc in water with ozone, UV radiation, HTH and peracetic acid products (HyperCide and Tsunami 100®) was also investigated.

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CHAPTER 2

The efficacy of five commercial sanitisers on disinfecting water contaminated with *Fusarium oxysporum* f. sp. *cubense*

ABSTRACT

Fusarium wilt of banana, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most devastating constraints to banana production worldwide. Once introduced into plantations the pathogen is impossible to eradicate as it produces survival structures, known as chlamydospores, which can remain in the soil for many years. From infested fields the pathogen is dispersed with infected planting material, run-off water and soil attached to shoes, machinery and vehicles. The dissemination of Foc with infected plants can be prevented by planting tissue culture bananas, but limited options exist to reduce its spread with soil and water. Proper biosecurity measures and the effective use of sanitisers, therefore, are required to prevent its spread to non-infested areas. This study investigated the efficacy of five commercial sanitisers (Sporekill, Saniwash, Farmcleanse, HyperCide and HTH) against Foc conidia and chlamydospores in clean and muddy water. The Foc suspensions included isolates representing Foc race 1, subtropical race 4 (STR4) and tropical race 4 (TR4). Foc conidia and chlamydospores were generated using a soil broth technique, and their survival was tested against the sanitisers at the recommended dose and a 1:10 dilution thereof. The Foc spores were exposed to the sanitisers for 0 s, 30 s and 2 min, where after the effect of the product was stopped with an inactivator medium that inactivates the active compounds. The survival of Foc spores was assessed by dilution plating of fungal spores on potato dextroxe agar amended with streptomycin sulfate. Foc spores not treated with sanitisers served as controls. All the disinfectants effectively killed Foc conidia and chlamydospores in the absence of soil at the recommended dose. However, in the presence of soil, only Sporekill and Saniwash were effective. Sporekill and Saniwash were the only sanitisers effective at all exposure times in the absence of soil, but in the presence of soil, Sporekill and Saniwash were effective only after 2 min, while the other sanitisers took longer to kill Foc spores. Sporekill and Saniwash could therefore be used to disinfect shoes, field equipment and vehicles. It must, however, be emphasised that all soil needs to be removed prior to disinfection.

INTRODUCTION

Fusarium oxysporum f. sp. *cubense* (Foc) is a soil-borne pathogen that threatens banana production globally. The pathogen comprises three races, called Foc races 1, 2 and 4. Foc race 1 causes disease to Gros Michel bananas and was responsible for a devastating epidemic in Latin America during the 1890s (McKenny, 1910; Brandes, 1919; Stover, 1962; Ploetz, 2005). Foc race 2 affects Bluggoe banana, a cooking variety, and Foc race 4 affects Cavendish bananas and many Foc race 1 and 2 susceptible varieties. Foc race 4 is further divided into subtropical (STR4) and tropical race 4 (TR4) isolates based on their ability to affect Cavendish bananas in the subtropics and tropics, respectively (Ploetz, 2015). Foc races has a global distribution, and was responsible for one of the worst plant disease epidemics of the 1900s when it destroyed Gros Michel bananas, on which the international banana export industry was built (Ploetz, 2005). Gros Michel was eventually replaced by Cavendish, which is now affected by Foc STR4 in countries like Australia, Canary Islands, China and South Africa (Ploetz *et al*., 1990) and Foc TR4 in Asia, the Middle East and Mozambique in Southern Africa (Pérez-Vicente *et al*., 2014; Ploetz, 2015).

Foc spreads to uninfected areas with infected planting material, soil attached to shoes and vehicles, and in run-off water after irrigation and flooding (Rishbeth, 1955; Stover, 1962). Once it is introduced into a plantation, the fungus becomes almost impossible to control because of its soil-borne nature (Ploetz and Pegg, 1999). Prevention of entry and the planting of disease-resistant varieties, therefore, are the only strategies available to successfully control Fusarium wilt. To prevent its introduction, strict quarantine and biosecurity measures need to be applied. This involves practises such as farm gate control, the use of foot- and wheel baths for workers, visitors and vehicles, and the use of clean planting material and irrigation water (Dita *et al*., 2018). To disinfect shoes and vehicles at farm gates, and for the cleaning of field equipment in plantations, sanitation products need to be selected that are effective, safe to humans and the environment, and that are affordable.

Several factors may influence the efficacy of sanitation products. Organic matter can hinder the ability of the chemical to successfully disinfect contaminated objects since it prevents direct contact with the pathogen (Russell, 1983). The presence of soil also deactivates quaternary ammonium compounds and iodophors (Berchieri and Barrow, 1996; Dvorak, 2008). Therefore, it is imperative to remove soil from shoes, plantation tools and vehicles as it affects the sanitation product's ability to disinfect. The on-farm dilution of a product may also reduce its efficacy. Bennett and Davis (2011) tested the efficacy of three commercial products at five dilutions against *F. oxysporum* f. sp. *vasinfectum*, a fungus causing Fusarium wilt of cotton, and found that a higher dilution decreased the effectiveness of the disinfectant. Exposure time is another important factor that determines the

effectiveness of a disinfectant, as it ensures longer contact with the pathogen. This was shown in a study that found that an exposure time of 15 min significantly increased the efficacy of Farmcleanse compared to 30 s (Meldrum *et al*., 2013).

Quaternary ammonium compounds are one of the most effective disinfectants available (Ridenour and Armbruster, 1948; Dixon *et al*., 1976). Their formulation can, however, influence their efficacy as two hydrophilic head groups and two hydrophilic alkyl chains in quaternary ammonium compounds contribute to its disinfection capabilities (Merianos, 2001; Prince and Prince, 2001). Sporekill is a quaternary ammonium compound that effectively kills micro- and macroconidia produced by Foc (Nel *et al*., 2007; Meldrum *et al*., 2013). Microconidia and macroconidia are, however, the more vulnerable stages of the pathogen (Leslie and Summerrell, 2006), whereas the more hardy chlamydospores can survive for decades (Nelson, 1981; Bennett and Davis, 2011; Ploetz, 2015). A recent study by Nguyen *et al* (2018) tested the efficacy of 32 sanitation products against chlamydospores and found that quaternary ammonium compounds with ≥10% of the active ingredient were effective in killing Foc chlamydospores in the presence and absence of soil at 30 s, 5 min, 30 min and 24 hrs.

Disinfectants used in the past in footbaths and for disinfection of equipment on banana farms included copper oxychloride, methylated spirits and chlorine bleach (Moore *et al*., 1999). This was replaced by Farmcleanse in Australia (Moore and O'Neill, 2000) and later with Sporekill in South Africa and Australia (Nel *et al*., 2007; Meldrum *et al*., 2013). Other products effective against Foc in laboratory and field trials included Prazin (polymeric biquanidine, hydrochloride and quaternary ammonium compound), Jik (sodium hypochloride) (Nel *et al*., 2007), Domestos (sodium hypochlorite) (Meldrum *et al*., 2013), Agriquat (benzalkonium chloride), Algacide (quaternary ammonium compound) and most quaternary ammonium compounds at a 1:100 dilution (Nguyen *et al*., 2018). Other disinfectants shown to be effective against *F. oxysporum* f. sp. *vasinfectum* included Simple Green d Pro 3 (quaternary ammonium compound), Trewax Nature's Orange (unknown), Formula 409 (quaternary ammonium compound, n-propoxypropanol, monoethanolamine) Formula 409 Orange (quaternary ammonium compound) and Lysol Disinfectant Antibacterial Kitchen Cleaner Citrus Scent (quaternary ammonium compound), even when they were diluted to 1:10 and 1:100 of their recommended dosages (Bennett *et al*., 2011).

Sanitisers are usually applied as a liquid in foot and wheel baths at banana farm entrances, or sprayed onto vehicles. The exposure time to potentially disinfect Foc-contaminated surfaces when people and vehicles enter banana farms is often less than 10 s. Early attempts to test such a short contact time required that sanitation products were either diluted or washed with sterile water to stop the contact between product and pathogen (James *et al*., 2007). Often, however, the sanitiser remained in contact with the fungus after

the exposure time, making results imprecise. It is, therefore, important that the sanitisers be inactivated fully when the exposure time is tested to accurately reflect their effectiveness.

In this study, five commercial products were tested for effectiveness against conidia and chlamydospores of three pathogenic races of Foc. These products were selected due to their safety to humans and animals, their non-corrosiveness, and their availability in the target country, South Africa. To stop the activity of sanitation products, an inactivator media was used after different exposure times. Due to the regular pollution of foot and vehicle baths with soil and organic material, the effectiveness of sanitisers was tested in both the presence and absence of soil.

MATERIALS AND METHODS

Isolates used

Isolates of Foc representative of Foc race 1, STR4 and TR4 were selected for the production of chlamydospores and for the evaluation of sanitation products (Table 1). Each isolate was grown on carnation leaf agar (CLA) for 2 months at room temperature. They were then viewed under a compound microscope (Eclipse E100, Nikon, New York, USA) at a 40× magnification, and the abundance of chlamydospores produced scored on a scale of 1-5. On this scale, 1 was consider as absent, 2 as few, 3 as intermediate, 4 as many and 5 as abundant. Three CLA plates were used to evaluate chlamydospore production for each isolate. Based on the abundance of chlamydospores produced, two representative isolates of each race were selected for the testing of the commercial sanitiser products. These isolates are stored at the Fusarium culture collection of the Department of Plant Pathology, Stellenbosch University, South Africa.

Mass-production of Foc chlamydospores

For the production of chlamydospores, 1 mL of sterile distilled water was added to CLA plates with actively growing Foc colonies, the spores loosened with an L-shaped plastic stick, and the spores transferred to a soil suspension for chlamydospore production.

The soil suspension was prepared by adding 62.5 g of potting soil (DoubleGrow, Cape Town, South Africa) to 250 mL of distilled water in 250-mL Erlenmeyer flasks. The soil suspension was then agitated at 90 revolutions min^{-1} (rpm) overnight, followed by the straining of the suspension through a 2-mm mesh sieve. The suspension was then filtered through eight layers of cheesecloth, and 100 mL of the filtrate was decanted into 250-mL Erlenmeyer flasks. An amount of 0.025 g glucose was added to each suspension, and the pH adjusted to 7 using 1 M HCl and 1 M NaOH. The suspensions were then autoclaved for

20 min at 121ºC, which was repeated the next day. Once the suspensions had cooled down, 4 mg of streptomycin sulfate was added to each flask and left to settle for 3 hrs.

Fifty mL of the soil suspension was poured into sterile 250-mL tissue culture flasks (Greiner Bio-One GmbH, Cellstar, Johannesburg, South Africa). Three flasks were then inoculated with a 1-mL Foc spore suspension of two representative isolates each of Foc race 1, STR4 and TR4 at a concentration of 10^4 spores mL^{-1} (Table 1). The control treatment contained only the sterile soil suspension in three flasks. The flasks were shaken under dark conditions at 90 rpm at 25ºC using a shaking incubator (Labcon, California, USA). Chlamydospore production was monitored regularly by aseptically transferring 20 µL of the soil solution to a haemocytometer every 5 days for viewing under a compound microscope. After 1 month, 40 mL of the soil suspension with Foc was transferred into 50-mL Falcon tubes and centrifuged for 10 min at 4 000 rpm. The supernatant was discarded, leaving only the pellet, which was suspended in 40 mL of sterile distilled water before being centrifuged again for 10 min at 4 000 rpm. This step was repeated twice. Chlamydospores were then counted using the haemocytometer, and concentrations of 10^5, 10^4, 10^3 and 10^2 spores mL^{-1} were prepared in 40 mL stock solutions for the evaluation of sanitisers.

Testing of disinfectants

The sanitation products tested for effectivity against Foc included Sporekill (ICA International Chemicals, Stellenbosch, South Africa), Saniwash (ICA International Chemicals, Stellenbosch, South Africa), Farmcleanse (Castrol, Australia), HTH (HTH, Lonza, Johannesburg, South Africa) and HyperCide (ICA International Chemicals, Stellenbosch, South Africa) (Table 2). Ten mL of Sporekill, Saniwash, Farmcleanse and HyperCide was added to 990 mL of sterile, distilled water in 1-L Schott bottles. An amount of 500 mg of HTH was dissolved in a 1 L of sterile distilled water in a 1-L Schott bottle. The pH of HyperCide and HTH was adjusted to 7 with 1 M HCl and 1 M NaOH. A second set of Schott bottles had 50 g of potting soil added to 1 L of distilled water, which was autoclaved at 121ºC for 15 min before sanitisers were added. To obtain a 0.1 x recommended dose (RD), 30 mL of the sanitisers were diluted with 270 mL sterile distilled water. Eighteen mL of each of the sanitisers at their recommended dose was transferred into 50-mL Falcon tubes. Using the 10^4 and 10^3 stock solution for each race, 2-mL of each concentration was added to the 50-mL Falcon tubes containing the 18 mL of the sanitisers to give a final concentration of 10^3 and 10^2 spores mL^{-1}. Positive controls of the fungus in the absence of a sanitiser were included in the experiment, with concentrations of 10^5, 10^4, 10^3, 10^2 spores $mL^{-1}$with five replicates for each concentration. A negative control consisted of sterile distilled water only. The solutions were then mixed by hand for 3 sec, and 500-µL aliquots were plated out immediately onto potato dextrose agar (PDA) amended with 0.04 g/L of streptomycin sulfate

(PDA^+), with five replicates. Plates were incubated for 2 days at room temperature, and were examined by counting the colony forming units (CFU's) that developed. The experiment was repeated once.

Effect of exposure times

To determine the effect of sanitiser exposure time, 500 µL of the Foc suspension, at a concentration of 10^5 spores mL^{-1}, was added to 4.5 mL of each sanitiser at their RD in clean water and in water mixed with soil. After 0 s, 30 s and 2 min, the spore suspensions were mixed with 5 mL of an inactivator media. The inactivator media consisted of 0.5 g of monopotassium phosphate (KH_2PO_4), 0.5 g of sodium citrate ($Na_3C_6H_5O_7.3H_2O$), 8 g of sodium taurocholate ($C_{26}H_{44}NNaO_7S$), 1.5 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) and 8 g of polyoxyethylene sorbitan mono-oleate in 1 L of distilled water (Erwin, 1999). Inactivator media stops the activity of most quaternary ammonium compounds, iodophors and organic halogen compounds (Erwin, 1999). Its effectivity in inactivating the sanitisers used in the current study was confirmed by treating the disinfectant solutions with the neutraliser before inoculation with a fungal spore suspension, and then demonstrating that the fungus was not killed by the sanitisers.

Once the sanitiser reactions were inactivated with the neutraliser, 500 µL of the Foc-sanitiser mixture was plated out immediately onto PDA^+ dishes, with five replicates per treatment. To ensure that the inactivator had no influence on fungal growth, Foc was also added to the inactivator at a final concentration of 10^3 spores mL^{-1}, and 500 µL of the mixture plated onto PDA^+ plates to serve as the positive control. A second positive control included a spore suspension without the inactivator at a final concentration of 10^3 spores mL^{-1}. Sterile distilled water served as the negative control. Plates were examined for CFU's after 2 days incubation at room temperature, and the experiment was repeated.

Statistical analysis

SAS® version 9.4 software (SAS Institute Inc., Cary, North Carolina, USA) was used for Leven's test for homogeneity, Shapiro-Wilk's test for normality and ANOVA to check for significant differences. A 95% least significant difference was used to make pairwise comparisons using Tukey's test. For testing the efficacy of disinfectants, ANOVA was performed to determine whether the different factors (sanitiser, race, sanitiser concentration, the presence and absence of soil) had a significance on each other. For the exposure time experiment, ANOVA was also performed to determine whether the different factors (sanitiser, race, exposure time, the presence and absence of soil) had a significance on each other.

When colonies were too many to count, a maximum count of 300 CFUs was used for the analysis. This resulted in an underestimation of the mean colony counts for the treatments, but did not influence the deductions formed since the aim was to identify effective disinfectants against Foc races.

RESULTS

Chlamydospore production by Foc isolates

The isolate that produced most chlamydospores was CAV 2123 (Table 1). This Foc race 1 isolate was also the only isolate among all races to have received a rating of 5. Isolates of Foc race 1 that followed with a rating of 4 were CAV 986, CAV 1990, and CAV 2260, and those with a rating of 3 were CAV 623, CAV 941, CAV 2241 and CAV 2400. The Foc race 1 isolates that produced the least amount of chlamydospores were CAV 967 and CAV 2282, with a rating of 2. Foc STR4 isolates with a rating of 4 were CAV 95, CAV 115 and CAV 117, and those with ratings of 3 were CAV 24 and CAV 980, whereas the least chlamdospores were produced by CAV 105, CAV 191 and CAV 289. The Foc TR4 isolate that produced most chlamydospores was CAV 2307, which had a rating of 4, followed by CAV 789, CAV 1185, CAV 3049 and CAV 3157 with a rating of 3. Isolates CAV 610, CAV 852, CAV 2346 and CAV 3491 scored a rating of 2, and CAV 604 produced no chlamydospores. Thus, the isolates that were selected for the testing of commercial sanitisers were isolates CAV 2123 and CAV 2260 for Foc race 1, CAV 95 and CAV 115 for Foc STR4, and CAV 2307 and CAV 3049 for Foc TR4 (Table 1).

Effectivity of disinfectants

Significant interactions were obtained between trials, sanitisers, the sanitiser concentrations, spore concentrations, the presence and absence of soil, and the Foc race used ($P \leq 0.05$) (Table 3). For ease of comparison, the effect of sanitisers at different sanitiser concentrations on the Foc races in the presence and absence of water was observed (Fig. 1). The five sanitisers all reduced the survival of Foc spores significantly compared to the non-treated control (Fig. 1). Sporekill and Saniwash were most effective, and reduced the survival of Foc spores significantly more than the other sanitisers. They were the only sanitisers to have significantly killed Foc in the presence of soil at the RD. When the other sanitisers were used in the presence of soil at both concentrations, their effectiveness significantly decreased compared to the absence of soil (Fig. 1). The dilution of the sanitisers in the absence of soil, however, did not significantly affect their effectivity except for Farmcleanse and HyperCide (Fig. 1).

There was no significant difference in the effectiveness of the sanitisers to the three Foc races in the absence of soil except for Farmcleanse and HyperCide when diluted (Fig. 1). When Sporekill was diluted in the presence of soil, Foc race 1 had significantly more colony forming units than Foc TR4 but not Foc STR4. When Saniwash was diluted in the presence of soil, the colony forming units of Foc STR4 was significantly reduced compared to Foc race 1 and Foc TR4 (Fig. 1). Farmcleanse reduced the colony forming units of Foc race 1 significantly more than Foc STR4 and TR4 in the presence of soil at the RD. However, at 0.1 x RD of Farmcleanse, Foc race 1 and TR4 had significantly more colony forming units than Foc STR4. At the 0.1 x RD of Farmcleanse and HyperCide in the absence of soil, there was significantly more colony forming units in Foc STR4 and TR4 when compared to Foc race 1 (Fig.1). HyperCide at the RD and 0.1 x RD reduced the colony forming units of Foc STR4 significantly more than Foc race 1 and TR4 (Fig. 1). HTH at the RD and 0.1 x RD also significantly reduced colony forming units of Foc STR4 more than Foc race 1 and TR4 (Fig. 1).

Effect of exposure times

Significant interactions were obtained between the trials, sanitisers tested, the exposure time, Foc race and the presence and absence of soil (Table 4). For ease of comparison, the effect of sanitisers at different exposure times on the Foc races in the presence and absence of water was observed (Fig. 2). The sanitisers most effective against all Foc races over the three time points, both in the presence and absence of soil, were Sporekill and Saniwash (Fig. 2). Farmcleanse was ineffective at all time points in the presence and absence of soil, and had no significant differences between the positive controls. Sporekill and Saniwash were effective against Foc in the absence of soil at 0 s, 30 s and 2 min (Fig. 2B). All sanitisers were ineffective against Foc in the presence of soil at 0 s, 30 s and 2 min, however, Saniwash and Sporekill significantly reduced Foc at 2 min (Fig. 2A).

Soil in water significantly reduced the efficiency of the sanitisers (Fig. 2A). Sporekill and Saniwash were effective against Foc spores in the absence of soil at first contact (0 s) and showed no significant difference between each other, while HTH was only effective after 30 s (Fig. 2B). Farmcleanse was ineffective at all the time points (Fig. 2). In the presence of soil, however, none of the sanitisers killed Foc upon contact (0 s), but Sporekill and Saniwash reduced the survival of the fungus after 2 min (Fig. 2A). Farmcleanse, HTH and HyperCide were ineffective in the presence of soil, and did not reduce Foc survival after 2 min (Fig. 2A). HyperCide was only effective after 2 min in the absence of soil (Fig. 2A). When disinfectants were treated with the neutraliser and inoculated with Foc their survival was similar to that of the untreated positive Foc control (Fig. 2).

DISCUSSION

Two sanitisers, Sporekill and Saniwash, have been identified in this study as effective commercial products to be used by banana growers to disinfect shoes, vehicles and farm equipment contaminated with Foc. Sporekill has been identified as an effective disinfectant of Foc before, but at the time the product was either not evaluated against chlamydospores (Nel *et al*., 2007; Meldrum *et al.,* 2013) or its action on fungal surfaces had not been stopped with inactivators (Nguyen *et al*., 2018). Inactivators prevent disinfectants to affect fungal spores beyond the contact time measured, which allows accurate information to be collected on the rapidity of sterilant action. Contact time is an important factor during field application of disinfectants. In foot and vehicle baths at farm entrances, sanitisers seldom remain in contact with surfaces for longer than 10 s. It is, therefore, important that sanitisers be used that can eradicate plant pathogens in such a short time. Temperature and pH may also affect the efficacy of the disinfectant (Ridenour and Armbruster, 1948; Copes *et al*., 2004; Scarlett *et al*., 2016). Their effect on Sporekill and Saniwash should therefore be further investigated.

Sporekill and Saniwash contain the active ingredient didecyldimethyl ammonium chloride (DDAC). DDAC is a typical quaternary ammonium biocide used to disinfect hard surfaces. Once the product comes into contact with the pathogen, it penetrates into the cell wall by binding to the proteins and lipids of the membrane and results in the destruction of the cytoplasmic membrane. This causes membrane leakage and damages vital proteins and nucleic acids, which results in cell lysis (Salton, 1968). It can be used in footbaths and on contaminated tools and vehicles. Quaternary ammonium compounds such as Sporekill, Path-X and Steri-max have been effective against Foc under field conditions for 4 months (Nguyen *et al*., 2018), while Sporekill was stable when exposed to sunlight for 6 months (Meldrum *et al*., 2013). It can also be used in irrigation water to ensure that it is pathogen-free and it is effective even at a 1-in-10 dilution in the absence of soil. Saniwash differs from Sporekill as it is sold commercially as a household product and has not been tested for agricultural use. It not only contains DDAC, but also glutaraldehyde. Glutaraldehyde is commonly used for disinfection and sterilisation purposes (McDonnell and Russell, 1999). It can be used the same as Sporekill as it also contains a similar formulation. Quaternary ammonium compounds are inexpensive, non-corrosive and are not pH dependent, which makes it ideal for disinfecting footwear, machinery and vehicles contaminated with Foc. Sporekill and Saniwash are also environmentally friendly products. However, one of the main disadvantages of using these products is that its efficacy decreases in the presence of soil.

Sporekill and Saniwash eradicated Foc in the absence of soil when used at the recommended dose. However, the sanitisers were only able to completely eradicate Foc in the presence of soil after 2 min. Meldrum *et al*. (2013) tested the efficacy of Sporekill at 0.25, 0.5, 1 and 2 times the recommended dosage, while Nel *et al*. (2007) tested it at 0.1, 1 and 10 times the recommended dose. Both found Sporekill to be effective at all concentrations in the absence of soil. Nguyen *et al*. (2018), however, found that Sporekill was effective at a 1:100 dilution in the presence of soil. Organic matter prevents contact by creating a physical barrier which essentially deactivates the sanitiser (Merianos, 2001; Amass, 2004). Based on this study, it is evident that soil has a major impact on the efficacy of the sanitation products tested. Therefore, removal of soil and organic matter from vehicles, tools, machinery and boots prior to disinfection is essential in order to provide direct contact of the product on the fungus. They can be diluted for disinfection in the absence of soil, but in the presence of soil, it is advised that the recommended concentrations of Sporekill or Saniwash be used. The effect of pH also plays a role in the efficacy. Unlike the rest of the disinfectants, HTH is effective when the pH is approximately at 7. HyperCide loses its effectiveness over time as it oxidises. It was found that HyperCide would lose effectiveness when added to water a few days prior to the disinfection of Foc. More field studies should, therefore, be done to investigate the effect of temperature, oxidative potential, pH and soil on the disinfectants at a longer period.

HyperCide, HTH and Farmcleanse cannot be recommended for use as disinfectants at farm entrances and in plantations. HyperCide eradicated Foc in water in the absence of soil, but required more than 2 min to eliminate the fungus in the presence of soil. HyperCide is also environmentally friendly, but when in contact with water at a higher dose it can become toxic for aquatic animals. HyperCide, together with Sporekill and Farmcleanse, are products that had been used in agriculture before. HTH is commonly used for disinfection because it is inexpensive, but its inefficacy, corrosiveness and environmental impact makes it a poor choice for the disinfection process. Its effectiveness is also highly dependent on pH.

In conclusion, the efficacy of commercially used disinfectants were tested against Foc propagules, including chlamydospores, by using an inactivator media to stop contact time after 0, 30 and 2 min. This is the first time HyperCide and Saniwash have been tested against Foc. Sporekill or Saniwash are recommended for use in footbaths, for the disinfection of tools, footwear, machinery and vehicles. Since these products are also able to disinfect clean surfaces at a 10 times dilution, it makes them cost effective to farmers. Sporekill and Saniwash are environmentally friendly and inexpensive. It is important to remove organic material prior to disinfection for maximum efficacy of the product. Further investigations should include the efficacy of disinfectants on farm equipment or footbaths to test the longevity of the disinfectants as well as the effects of temperature and pH on it.

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Table 1. Chlamydospore production by *Fusarium oxysporum* f. sp. *cubense* isolates grown on carnation leaf agar plates.

[1]CAV	Race ID	VCG	Mean ratings[2]
*95	STR4	0120	4
289	STR4	0120	2
980	STR4	0120/15	3
191	STR4	0120/15	2
105	STR4	0120	2
24	STR4	0120	3
*115	STR4	0120	4
117	STR4	0120	4
*2260	Race 1	0124	4
*2123	Race 1	0124	5
626	Race 1	0124/5	3
968	Race 1	0124/5	4
967	Race 1	0124/5	2
2282	Race 1	0125	2
941	Race 1	0125	3
2241	Race 1	0124	3
1990	Race 1	0124	4
2400	Race 1	0124	3
610	TR4	01213	2
2346	TR4	01213	2
789	TR4	01213/16	3
1185	TR4	01213/16	3
604	TR4	01216	1
852	TR4	01216	2
*2307	TR4	01216	4
*3049	TR4	01213/16	3
3491	TR4	01213/16	2

3157	TR4	01213/16	3

[1]CAV – Culture collection of the Fusarium group at Stellenbosch University.

[2]The average chlamydospore ratings of three plates for each isolate of Foc on a scale of 1-5, where 1 was considered as absent, 2 as few, 3 as intermediate, 4 as many and 5 as abundant.

*****Isolates that were selected for mass production.

Table 2. Sanitation products selected for testing against *Fusarium oxysporum* f. sp. *cubense* with their active ingredients and recommended concentration.

Sanitation product	Active ingredients	Recommended concentration
Sporekill	Didecyldimethyl ammonium chloride 120 g/L	10 mL/1 L
Saniwash	Generic quartenary ammonium compounds 60 g/L Glutaraldehyde 70 g/L	10 mL/1 L
Farmcleanse	Alkali metal salts of alkylbenzene sulfonic acid 5-10% Coconut diethanolamide 1-5%	10 mL/1 L
HTH	Calcium hypochlorite 60-70% Calcium chloride 0.1-5% Calcium hydroxide 0.1-4%	500 mg/1 L, pH 7
HyperCide	Peracetic acid 14% Hydrogen peroxide 22%	10 mL/1 L, pH 7

Table 3. Analysis of variance representing the sum of squares of all the interactions between sanitation products, sanitiser concentration, race and the presence and absence of soil when tested for activity against *Fusarium oxysporum* f. sp. *cubense.*

Source	DF	Sum of squares	Mean squares	F	Pr > F
Race	2	112756.822	56378.411	2594.75	<.0001
SpConc	1	974244.053	974244.053	44838.4	<.0001
RacexSpConc	2	177322.412	88661.206	4080.52	<.0001
San	4	4548509.89	1137127.47	52334.9	<.0001
RacexSan	8	290337.737	36292.217	1670.3	<.0001
SpConcxSan	4	18029.922	4507.48	207.45	<.0001
RacexSpConcxSan	8	111415.263	13926.908	640.97	<.0001
SanConc	1	2224479.63	2224479.63	102379	<.0001
RacexSanConc	2	10058.685	5029.343	231.47	<.0001
SpConcxSanConc	1	272706.75	272706.75	12551	<.0001
RacexSpConcxSanConc	2	29646.915	14823.457	682.23	<.0001
SanxSanConc	4	696285.045	174071.261	8011.41	<.0001
RacexSanxSanConc	8	92765.39	11595.674	533.68	<.0001
SpConcxSanxSanConc	4	110773.592	27693.398	1274.55	<.0001
RacexSpConcxSanxSanC	8	70052.943	8756.618	403.01	<.0001
SoilH20	1	6001299.2	6001299.2	276202	<.0001
RacexSoilH20	2	161373.852	80686.926	3713.51	<.0001
SpConcxSoilH20	1	395670.083	395670.083	18210.2	<.0001
RacexSpConcxSoilH20	2	124220.662	62110.331	2858.55	<.0001
SanxSoilH20	4	942072.438	235518.11	10839.4	<.0001
RacexSanxSoilH20	8	141845.907	17730.738	816.03	<.0001
SpConcxSanxSoilH20	4	241081.692	60270.423	2773.87	<.0001
RacexSpConcxSanxSoil	8	112601.613	14075.202	647.79	<.0001
SanConcxSoilH20	1	950.52	950.52	43.75	<.0001
RacexSanConcxSoilH20	2	218543.255	109271.627	5029.09	<.0001
SpConcxSanConcxSoilH	1	33665.613	33665.613	1549.42	<.0001
RacexSpConcxSanConcx	2	22322.732	11161.366	513.69	<.0001
SanxSanConcxSoilH20	4	2107858.02	526964.505	24252.9	<.0001
RacexSanxSanConcxSoi	8	319770.553	39971.319	1839.63	<.0001
SpConcxSanxSanConcxS	4	398528.562	99632.14	4585.44	<.0001
RacexSpConcxSanxSanC	8	60756.393	7594.549	349.53	<.0001

Trial	1	255.763	255.763	11.77	0.0006
RacexTr	2	213.082	106.541	4.9	0.0076
SpConcxTr	1	244.803	244.803	11.27	0.0008
RacexSpConcxTr	2	54.752	27.376	1.26	0.2841
SanxTr	4	787.362	196.84	9.06	<.0001
RacexSanxTr	8	1382.793	172.849	7.96	<.0001
SpConcxSanxTr	4	324.822	81.205	3.74	0.005
RacexSpConcxSanxTr	8	1618.873	202.359	9.31	<.0001
SanConcxTr	1	52.92	52.92	2.44	0.1189
RacexSanConcxTr	2	309.305	154.653	7.12	0.0009
SpConcxSanConcxTr	1	5.88	5.88	0.27	0.603
RacexSpConcxSanConcx	2	223.895	111.947	5.15	0.0059
SanxSanConcxTr	4	407.438	101.86	4.69	0.0009
RacexSanxSanConcxTr	8	1342.487	167.811	7.72	<.0001
SpConcxSanxSanConcxT	4	238.878	59.72	2.75	0.0272
RacexSpConcxSanxSanC	8	1218.447	152.306	7.01	<.0001
SoilH20xTr	1	61.653	61.653	2.84	0.0924
RacexSoilH20xTr	2	322.212	161.106	7.41	0.0006
SpConcxSoilH20xTr	1	149.813	149.813	6.89	0.0088
RacexSpConcxSoilH20x	2	388.182	194.091	8.93	0.0001
SanxSoilH20xTr	4	972.638	243.16	11.19	<.0001
RacexSanxSoilH20xTr	8	1559.797	194.975	8.97	<.0001
SpConcxSanxSoilH20xT	4	809.212	202.303	9.31	<.0001
RacexSpConcxSanxSoil	8	1386.043	173.255	7.97	<.0001
SanConcxSoilH20xTr	1	187.23	187.23	8.62	0.0034
RacexSanConcxSoilH20	2	306.215	153.107	7.05	0.0009
SpConcxSanConcxSoilH	1	60.75	60.75	2.8	0.0948
RacexSpConcxSanConcx	2	279.585	139.793	6.43	0.0017
SanxSanConcxSoilH20x	4	348.528	87.132	4.01	0.0031
RacexSanxSanConcxSoi	8	2032.577	254.072	11.69	<.0001
SpConcxSanxSanConcxS	4	264.175	66.044	3.04	0.0167
RacexSpCxSanxSanCxSo	8	1489.29	186.161	8.57	<.0001

Table 4. Analysis of variance representing the sum of squares of all the interactions between sanitation products, time points, race and the presence and absence of soil when tested for activity against *Fusarium oxysporum* f. sp. *cubense*.

Source	DF	Sum of squares	Mean squares	F	Pr > F
Race	2	616.167	308.083	127.45	<.0001
San	4	5687743	1421936	588252	<.0001
RacexSan	8	2175.37	271.921	112.49	<.0001
SoilH20	1	5186551	5186551	2145666	<.0001
RacexSoilH20	2	1399.73	699.863	289.53	<.0001
SanxSoilH20	4	1859130	464783	192280	<.0001
RacexSanxSoilH20	8	1391.81	173.976	71.97	<.0001
ExpTime	2	1739824	869912	359881	<.0001
RacexExpTime	4	2648.51	662.128	273.92	<.0001
SanxExpTime	8	882313	110289	45626.4	<.0001
RacexSanxExpTime	16	3665.49	229.093	94.78	<.0001
SoilH20xExpTime	2	90037.6	45018.8	18624.2	<.0001
RacexSoilH20xExpTime	4	992.647	248.162	102.66	<.0001
SanxSoilH20xExpTime	8	2532099	316512	130941	<.0001
RacexSanxSoilH20xExp	16	5321.35	332.585	137.59	<.0001
Trial	1	368.64	368.64	152.51	<.0001
RacexTr	2	179.527	89.763	37.13	<.0001
SanxTr	4	327.493	81.873	33.87	<.0001
RacexSanxTr	8	1175.61	146.951	60.79	<.0001
SoilH20xTr	1	59.804	59.804	24.74	<.0001
RacexSoilH20xTr	2	516.429	258.214	106.82	<.0001
SanxSoilH20xTr	4	636.329	159.082	65.81	<.0001
RacexSanxSoilH20xTr	8	838.704	104.838	43.37	<.0001
ExpTimexTr	2	202.907	101.453	41.97	<.0001
RacexExpTimexTr	4	702.327	175.582	72.64	<.0001
SanxExpTimexTr	8	303.493	37.937	15.69	<.0001
RacexSanxExpTimexTr	16	1645.81	102.863	42.55	<.0001
SoilH20xExpTimexTr	2	105.849	52.924	21.89	<.0001
RacexSoilH20xExpTime	4	920.478	230.119	95.2	<.0001
SanxSoilH20xExpTimex	8	400.551	50.069	20.71	<.0001
RacexSanxSoilH20xExp	16	1427.66	89.228	36.91	<.0001

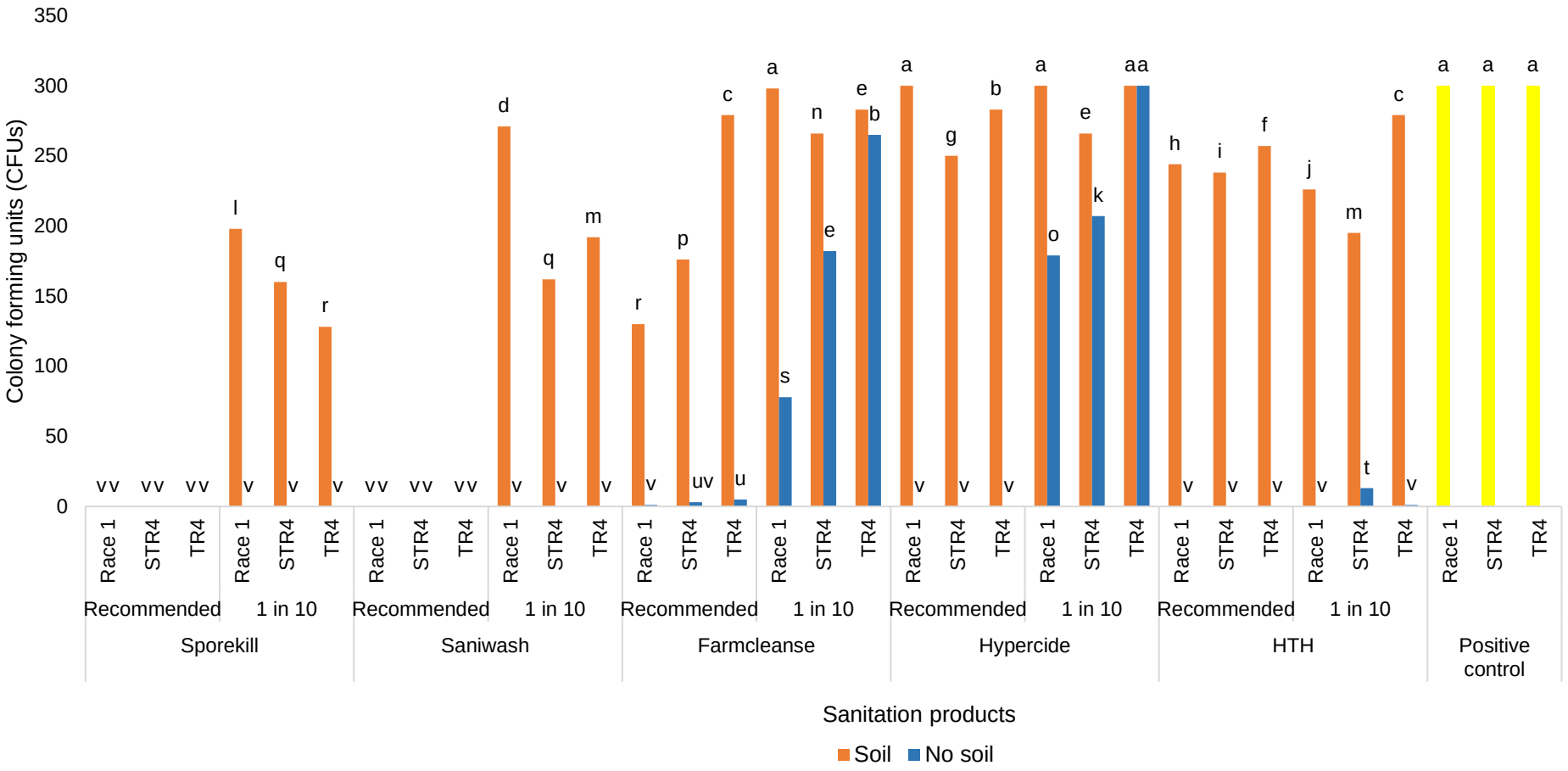

Figure 1. The efficacy of sanitation products at the recommended dose (RD) and 0.1 x RD in the presence (brown bar) and absence (blue bar) of soil against three *Fusarium oxysporum* f. sp. *cubense* races.

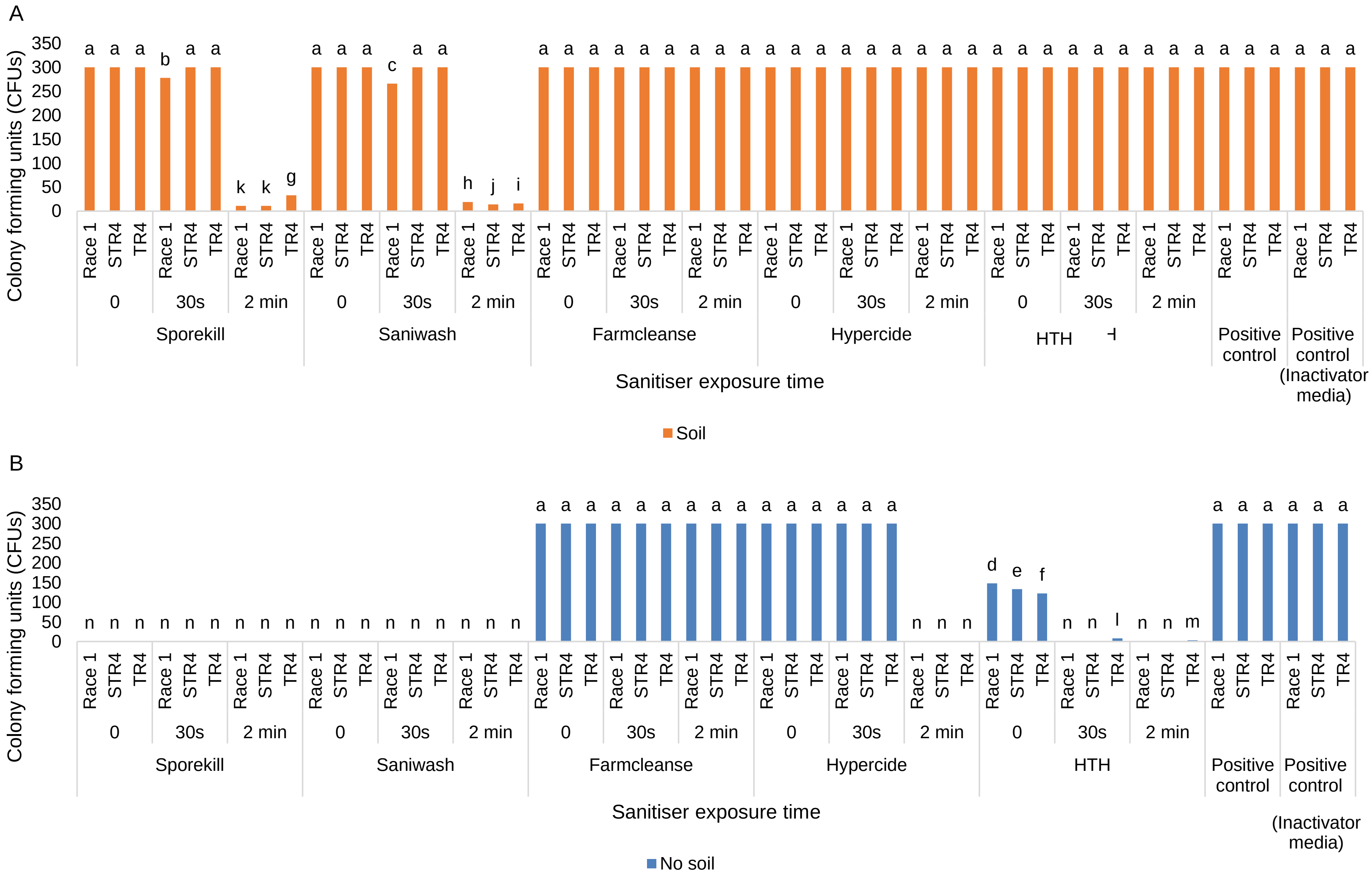

Figure 2. The efficacy of sanitation products at an exposure time 0 s, 30 s and 2 min against three *Fusarium oxysporum* f. sp. *cubense* races in the presence (A) and absence (B) of soil in water.

CHAPTER 3

The survival and treatment of *Fusarium oxysporum* f. sp. *cubense* in water

ABSTRACT

Fusarium oxysporum f. sp. *cubense* (Foc) is a soil-borne fungus that is considered one of the most destructive pathogens of banana. The fungus spreads with infected planting material, soil attached to shoes, vehicles and plantation tools, and in water. Spread in water is particularly concerning, as contaminated water can rapidly contaminate disease-free areas in plantations. It is unclear how long Foc survives in water, and how the fungus can be treated in irrigation and flood water. The objective of this study, therefore, was to investigate the survival of Foc in still and agitated water, both in the presence and absence of soil. To mimic stagnant and flowing water in the field, buckets were filled with 20 L distilled water and inoculated with Foc spores (conidia and chlamydospores) to a final concentration of 10^3 spores L^{-1}. Half of the buckets were fitted with air pumps to simulate flowing water, and the other half were undisturbed. Soil was added to the buckets to represent muddy water. Water samples were collected at the top, middle and bottom of buckets 1, 7, 14, 30, 60 and 120 days after inoculation to determine the survival of Foc colony forming units. Survival of the fungus decreased over time in absence of soil that remained stagnant. The spores also sunk to the bottom when the water was not stirred. To limit the spread of plant pathogens in water, chemical and non-chemical treatments can be used. In this study we evaluated the potential of ozone, ultraviolet (UV) radiation, HTH and peracetic acid (PAA) products (HyperCide and Tsunami 100®) to eradicate Foc in water. UV was ineffective and unable to kill Foc at a UV dose of 300 $mJ.cm^{-2}$ in clean water. Ozone, HTH and HyperCide effectively killed Foc in clean water, but not in the presence of soil. These methods, therefore, would require filtration prior to disinfection. This study demonstrated that Foc can survive in still water for longer than 4 months, and that ozone, HTH and HyperCide can be used effectively to limit its spread in the absence of soil. The use of ozone, HTH and HyperCide should be further tested for treating irrigation and flood waters on-farm, and the practicality and affordability determined.

INTRODUCTION

Fusarium wilt of banana is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), a plant pathogen considered to be one of the most destructive in agricultural history (Stover, 1962a). The pathogen is dispersed with infected planting material and in infested soil attached to shoes, wheels and plantation tools. An important means of dissemination of Foc in banana plantations is with contaminated water used for irrigation, in run-off water from infested fields, and after flooding (Coates and Pegg, 2016). Once introduced into banana fields, the fungus can cause massive losses and is almost impossible to eradicate (Stover, 1962b; Ploetz, 2005). Certain strategies have been used to prevent its spread which include the use of plants produced in tissue culture, clean irrigation water, and the proper sanitation of shoes, vehicles and field equipment. Foc can survive in soil for decades, and the only method to manage Fusarium wilt in infested fields is to replace susceptible with resistant banana varieties (Ploetz, 2015).

Water is an essential resource for productive banana farming. A banana plant obtains water from natural precipitation in the wet tropics, and from sprayer or flood irrigation in dryer areas (Dita *et al*., 2018). Tropical storms, cyclones and typhoons are common events that swamp banana fields, causing damage to plants and infrastructure. It also contributes to the spread of diseases such as banana Fusarium wilt. Contaminated irrigation water from the Pearl River and infected planting material were reported to be the cause of spread of Foc TR4 in China (Xu *et al*., 2003). Epp (1987) also noted that disease occurrence increased after heavy rainfall in the Philippines. In addition, typhoons in the Philippines and flooding in Mozambique resulted in the spread of Foc with contaminating water running off infested banana fields (Trueggelmann, personal communication; Viljoen, personal communication).

In the 1950s, flood fallowing was used in an attempt to eradicate Foc from infested soils (Stover, 1962a). The technique involved the flooding of infested soils with water to create anaerobic conditions to reduce the survival of the pathogen. Flood fallowing reduced the survival of Foc after approximately 40-50 days of flooding (Stover, 1979), and decreased inoculum levels to a depth of 2-5 ft for a period of 18 months. This allowed producers to regrow bananas in such fields. However, 2 years after treatment losses increased again to levels that prevented further banana production (Wardlaw, 1961; Stover, 1962a). Foc was also reduced by applying fungicides such as ferrous sulphate and calcium cyanide as a drench on the surface of soil after flooding (Wardlaw, 1961).

Laboratory tests in glass vessels done by Stover (1954) showed that Foc was able to survive for 45-165 days in soil submerged under 2.5 cm of water. It was also reported that flowing water provided aeration for the pathogen, enabling it to survive 70 days longer than in stagnant water (Stover, 1954). Chlamydospores were produced when soil was flooded

with water and subjected to an atmosphere of carbon dioxide (Newcombe, 1960). These studies concluded that oxygen was needed for Foc to survive in flooded soils.

To reduce the spread of plant pathogens in water, chemical and non-chemical treatments can be used. These treatments may include the use of ozone, ultraviolet light, chlorine and peracetic acid (PAA). These treatment methods are often used in greenhouse systems and for wastewater management (Hong *et al*., 2014).

Studies on ozone have shown its efficacy against conidia of *F. oxysporum* with a concentration of 0.6 ppm for 3 mins (Igura *et al*., 2004). However, when applied to dam water contaminated with *F. oxysporum*, a concentration of 1.1 ppm was required at 4 min for disinfection (Beardsell and Bankier, 1996). Ozone is advantageous to use as it requires lower dosages and shorter exposure times for disinfection due to its high oxidation potential (Voigt *et al*., 2013). It is also environmentally friendly as it leaves no residue (Zheng *et al*., 2018). Disadvantages of using ozone include its high installation and maintenance costs (Hong *et al*., 2014).

For UV radiation, fungi generally requires a dosage of 30-300 $mJ.cm^{-2}$ for disinfection. A study found that *F. oxysporum* requires a dosage of 250 $mJ.cm^{-2}$ (Scarlett *et al*., 2015). Runia (1995) showed that 90% of the conidia of *F. oxysporum* f. sp. *melongenae* was eliminated at 70 $mJ.cm^{-2}$. Advantages of UV radiation include its non-corrosiveness which makes it environmentally friendly while its disadvantages include the high installation and maintenance costs. It is also ineffective in the presence of soil and therefore, requires filtration before treatment resulting in additional costs (Zheng *et al*., 2014).

Chlorine treatment was effective at a concentration of 8-10 ppm for an exposure time of 0.5-1.5 min against *F. oxysporum* (Cayanan *et al*., 2009). Scarlett (2015) showed that chlamydospores of *F. oxysporum* were eliminated at a concentration of 5 ppm for 20 min when treated in deionized water, however, in dam water, an exposure time of 30 min was required. Advantages of chlorine include that it is readily available and relatively cheap. It is also easy to use and requires no installation or maintenance (Van Haute *et al*., 2013). Chlorine, however, is not environmentally friendly and factors like pH and organic matter can influence its efficacy.

PAA is a combination of acetic acid and hydrogen peroxide. Studies on the efficacy of PAA against Foc have not been tested. PAA is, however, environmentally friendly (Freese *et al*., 2003). Its main disadvantage is that it is not readily available and is expensive.

The objective of this study was to determine the survival of Foc in still and agitated water, both in the presence and absence of soil. Ozone, UV, HTH, and PAA were investigated for potential to decontaminate water infected with Foc.

MATERIALS AND METHODS

Fungal inoculum used

Foc isolates used for the production of inoculum were selected based on their ability to produce chlamydospores readily (Chapter 2). These include the Foc race 1 isolates CAV 2123 and CAV 2260, the Foc STR4 isolates CAV 95 and CAV 115, and the Foc TR4 isolates CAV 2307 and CAV 3049. The isolates are all stored at the Fusarium culture collection of the Department of Plant Pathology, Stellenbosch University, South Africa.

Foc chlamydospore production

Foc chlamydospores were produced in 250-mL Erlenmeyer flasks. Each flask was first filled with 62.5 g potting soil and 250 mL of distilled water, which was agitated overnight at 90 revolutions min^{-1} (rpm). The soil suspensions were then filtered through a 2-mm mesh sieve to remove large soil particles, and the filtrate strained through eight layers of cheesecloth to separate fungal spores from hyphae and small soil particles. One hundred mL of each filtrate was then transferred to 250-mL Erlenmeyer flasks. The pH of the filtrate was adjusted to 7, and 2.5 mg glucose was added to each flask. The soil substrate was then autoclaved for 20 min at 121ºC on 2 consecutive days. When the soil substrate cooled down, 4 mg of streptomycin sulfate was added to each flask. After 3 hrs, 50 mL of the soil suspension was poured into sterile 250-mL tissue culture flasks (Greiner Bio-One GmbH, Cellstar, Johannesburg, South Africa). The soil suspension was then inoculated with 1-mL of the representative isolates of Foc race 1, STR4 and TR4. Each isolate was added to three flasks, and three flasks containing a sterile soil suspension was regarded as the control. The flasks were then incubated in the dark for 1 month at 25ºC and shaken at 90 rpm in a shaking incubator (Labcon, California, USA).

Survival of Foc

To determine the survival of Foc in water, four buckets were filled with 20-L distilled water and kept at 25ºC in an incubation room in the dark. One kg of soil was added to two of the four buckets, and the water stirred to suspend and equally distribute the soil (Fig. 1). An air pump (ViaAqua VA-130A, Cape Town, South Africa) was placed at the centre of two of the four buckets, one with soil and the other without soil, and switched on to continuously stir the water in these buckets.

An equal amount of each Foc race was added to 50-mL Falcon tubes. The spores were washed by centrifuging the suspension for 10 min at 4 000 rpm. The supernatant was then discarded, and the spores washed again by suspending the pellet in 50-mL water that was vortexed and again centrifuged. The pellet was then suspended in 50-mL of sterile distilled

water, and the number of Foc chlamydospores counted using the haemocytometer. The spore suspensions were then diluted to obtain a final spore concentration of 10^3 spores mL^{-1} when added to each of the four buckets.

The survival of Foc in water was determined at 1, 7, 14, 30, 60 and 120 days after inoculation. Samples were collected by extracting 100 mL of water from the top, the centre and the bottom of each bucket with a fast pipette controller (Fastpette pro, Labnet Interbational, Inc., Poland), and transferring these into 250-mL sterile Erlenmeyer flasks. From each flask, 500 µL aliquots of the spore suspensions were then plated onto 10 Petri dishes with potato dextrose agar (PDA) modified with 0.04 g/L of streptomycin sulfate (PDA^+). The PDA^+ plates were incubated at room temperature, and the number of colony forming units (CFU) counted after 2 days. The experiment was repeated.

Water treatments

For the water treatment experiment the Foc isolates were washed as described above, mixed at equal amounts, and then diluted with distilled water, in the presence and absence of soil, to obtain a concentration of 10^3 spores mL^{-1} for treatment with ozone, UV, HTH and peracetic acid.

Ozone

The effect of ozone on Foc was tested using an ozone generator (Del Ozone Genesis, California, USA). The fungus was added to water in the presence and absence of soil to obtain 40-mL samples at a spore concentration of 10^3 spores mL^{-1} in 50-mL Falcon tubes. The tubes were then exposed to 3 g hr^{-1} of ozone for 10, 30 and 60 min. Positive controls comprising water samples in the presence and absence of soil were not treated with ozone. The tubes were left open for 1 hr after treatment to allow the ozone to dissipate, and 500 µL of the suspensions were then pipetted onto five PDA^+ plates. The plates were incubated for 2 days and the number of CFU's counted as described earlier. The experiment was repeated.

Ultraviolet light radiation

Water with and without soil, containing a Foc spore concentration of 10^3 spores mL^{-1}, was treated with UV by using a low pressure UV lamp (Berson, Nuenen, Netherlands) at a dose of 100, 200 and 300 $mJ.cm^{-2}$. The 40-mL samples were first transferred into 50-mL beakers with a sample depth of 1 cm, and the water quality measured using an UV transmission (UVT) meter (Berson, Nuenen, Netherlands). Before treatment, the UV light was switched on for 10 min to measure the wavelength using a radiometer (International light technologies ILT1400, USA). Once the UVT and wavelength were measured, the exposure time was calculated with the equation below.

$$I_{avg,\lambda\ (mW.cm^{-2})} = I_0\lambda \left[\frac{1- e^{d\ln(UVT(\lambda))}}{-d\ln(UVT(\lambda))}\right]$$

$$\text{Desired dose } (mJ.cm^{-2}) = \text{Average intensity } (mW.cm^{-2}) \times \text{Exposure time (s)}$$

In the above equation, $I_{(avg,\lambda)}$ refers to the average intensity of UV light over the sample depth (d) in cm; UVT(λ) refers to the UV transmission of the sample at a wavelength (λ) of 254 nm, determined by using an optical path length of 1 cm; $I_0(\lambda)$ is the intensity of UV light measured at the surface of the sample. The exposure time was calculated by dividing the desired dose by the average intensity.

After the exposure time was calculated for each dose, a stirrer bar was added into the beaker with the Foc suspension and placed on a stirrer under UV light. Once the exposure time was reached, 500 µL from each beaker was pipetted onto five PDA^+ dishes. The positive control sample was not treated with UV radiation. The Petri dishes were incubated at room temperature, and the CFU counted after 2 days. The experiment was repeated.

HTH

The effect of HTH (granular pool chlorine) on Foc at a concentration of 10^3 spores mL^{-1} was tested by adding 0.1 g of granular pool chlorine (HTH, Lonza, Johannesburg, South Africa) to 20 L of distilled water in the presence and absence of soil with the pH adjusted to 7 using 1 M HCl and 1 M NaOH in buckets that were kept undisturbed. After 10, 20 and 30 min, 100 mL of the water was collected from the top of the bucket using a fast pipette controller and immediately treated with 100 mL of inactivator media in a 250-mL Erlenmeyer flask. Aliquots of 500 µL from each sample were then transferred onto five PDA^+ dishes. Non-treated water with Foc was used as the positive control, and the experiment was repeated. The Petri dishes were incubated as described before, and CFU counted after 2 days.

Peracetic acid

A commercial PAA product, called Tsunami 100® (Ecolab, Cape Town, South Africa) and HyperCide (ICA International Chemicals, Stellenbosch, South Africa), was used to treat Foc water in the presence and absence of soil. Tsunami 100® contains 15.2% PAA and 11.2% hydrogen peroxide as active ingredients, and was used at its recommended dosage of 523 µl/L while HyperCide contains 14% PAA and 22% hydrogen peroxide as active ingredients, and was used at its recommended dosage of 5 mL. Samples were prepared by transferring 36 mL of each PAA product into 50 mL Falcon tubes, and by adding 4 mL with a spore concentration of 10^4 spores mL^{-1}. This resulted in a final concentration of 10^3 spores mL^{-1}. After 10, 20, 30, 60 and 120 min, the treatments were inactivated by decanting the solution into 40 mL of the inactivator media in 250-mL Erlenmeyer flasks. Water that was not treated

with the PAA products was used as the positive control. From each sample, 500 µL was then pipetted out onto five PDA^+ plates, the plates incubated and the CFU counted as described above. The experiment was repeated.

Statistical analysis

SAS® version 9.4 software (SAS Institute Inc., Cary, North Carolina, USA) was used for Leven's test for homogeneity, Shapiro-Wilk's test for normality and ANOVA to check for significant differences. A 95% least significant difference was used to make pairwise comparisons using Tukey's test. For testing the survival of Foc experiment, ANOVA was initially performed to determine whether the different factors (day, motion, the presence and absence of soil, depth) had a significance on each other. The different factors were then analysed separately. For the water treatment experiments, ANOVAs were performed for each treatment separately.

Colonies that were too many to count were given a value of 300 CFUs for the analysis, which resulted in an estimate of the mean colony count. However, this did not affect the conclusions formed as the experiment aims to track the survival of Foc and to identify effective water treatments.

RESULTS

Survival of Foc in water

A significant interaction was obtained for the number of days that Foc survived in water, the agitation of water, the depth and the presence and absence of soil in water ($P \leq 0.05$) (Table 1). Significantly more Foc survived in water that was agitated than in water that was not (Fig. 2). There was also significantly less colony forming units recorded at the top and middle of the buckets on day 30, 60 and 120 compared to day 1, 7 and 14 with the exception of day 14 at the top when water was stagnant (Fig. 2), meaning that fewer colony forming units survive in water over time. When water was agitated in the presence and absence of soil at all depths, more colony forming units survived on day 7 and day 14, compared to later dates (Fig. 2A and 2C). The surviving Foc collected from the top and middle levels of the buckets when agitated were significantly reduced after 30 days (Fig. 2A and 2C). The colony forming units at the bottom of the buckets did not decrease when water was agitated from day 7-120 when compared to buckets that were not agitated (Fig. 2A and 2C). The number of colony forming units surviving at the top and in the middle of the buckets that were not agitated in the presence and absence of soil was reduced from day 7 onwards, but those surviving at the bottom of the buckets were high at day 60, whereafter it dropped at day 120 (Fig. 2B and 2D).

Water treatments

Ozone

Ozone significantly reduced Foc numbers in clean water after 0 min when compared to water where soil was present, and completely eradicated the pathogen in clean water after 10 min (Table 2). This, however, was not the case in water containing soil. Although eradication was not complete in the presence of soil, the ozone significantly reduced the number of surviving colony forming units in the presence of soil after 30 min to numbers that did not differ significantly from the treated clean water (Table 2). Interestingly, significantly more Foc survived in the untreated control in the absence of soil (control) than in the untreated control with soil (Table 2).

Ultraviolet light radiation

UV was not able to eradicate Foc in water, even at a dosage of 300 $mJ.cm^{-2}$ (Table 3). It did, however, significantly reduce the number of viable colony forming units as dosages were increased. Significantly more colony forming units survived in the presence of soil compared to its absence at doses 100 $mJ.cm^{-2}$ and 200 $mJ.cm^{-2}$ (Table 3). A higher dose in the presence of soil reduced the number of colony forming units significantly (Table 3), but no significant differences in survival was obtained for clean water treated at a dosage of 200 and 300 $mJ.cm^{-2}$. The number of CFUs in the absence of soil at 200 $mJ.cm^{-2}$ and 300 $mJ.cm^{-2}$ did not differ significantly from the treatment in the presence of soil at 300 $mJ.cm^{-2}$ (Table 3). The untreated control with the Foc contaminated water also was significantly different with the untreated control containing the Foc contaminated water with soil (Table 3).

HTH

HTH eradicated Foc in clean water after 10 min, but did not affect the fungus in water in the presence of soil (Table 4). The survival of Foc in the presence of soil in water was similar to that in the control treatments (Table 4).

Peracetic acid

HyperCide eradicated Foc at 20 min while Tsunami 100® eradictated Foc at 30 min in the absence of soil. In the presence of soil, the PAA products was ineffective.There was no significant difference in the survival of Foc in water containing soil when compared to the control (Table 5).

DISCUSSION

Foc survived for 120 days in water in this study. Agitation of the water significantly affected their survival, most likely due to an increased aeration. Oxygen is required by most fungi to survive, and a lack thereof will ultimately kill Foc spores (Newcombe, 1960). The agitation dispersed the Foc spores evenly in water, unlike when they sink to the bottom in standing water. Stover (1954) reported that a lack of oxygen in stagnant water, like in dams, will cause chlamydospores to die, as was demonstrated in the current study. Soil significantly increased the survival of Foc in water, but reasons for this finding require further investigation. Newcombe (1958) reported that Foc can live in soil submerged in water for 3 months, and that chlamydospores survive significantly longer in soil than microconidia. In flooded soil viable Foc spores are also reduced by an increase in carbon dioxide that causes the germination of chlamydospores, but that also prevents the formation of new chlamydospores (Newcombe, 1960; Stover, 1962b).

More viable Foc CFUs were found in the upper level of water in the buckets 1 day after inoculation than after 7 days. This may be due to the floating of the fungus before it started to sink to the bottom. The inoculum used for the experiment consisted of chlamydospores, microconidia and macroconidia, which might have resulted in this finding. Microconidia and macroconidia are fragile spores and are not adapted for long-term survival like chlamydospores, which could have resulted in their early death, unless they were converted into chlamydospores (Coates and Pegg, 2016). Newcombe (1960) previously reported that the survival of microconidia and macroconidia gradually declined after 10 days and chlamydospores only later.

Most Foc colony forming units in this study sunk to the bottom of water over time. This may be due to the weight, as Rattink (1990) also reported that spores of *F. oxysporum* f. sp. *cyclaminis* sunk to the bottom of water containers within 24 hrs. Dams that are located below infested fields are likely to receive contaminated run-off water. This finding prompted Deacon (1984) to suggest that irrigation water should be taken from the surface of dams rather than their bottom 2 days after heavy rainfall. According to this study, a number of Foc colony forming units were still in the upper layers of water 7 days after they were added. Therefore, if water is collected for irrigation from dams that are potentially contaminated with Foc, it is advised that the water be treated before it is sprayed back onto plantations.

The eradication of plant pathogens in irrigation water is not a simple task. Rain water is regarded as pathogen-free, but rivers, dams and ponds used for sprinkler and flood irrigation may be contaminated if the water had been in contact with Foc-infested land. Using water from such sources, therefore, can result in the contamination of disease-free areas. Similarly, drainage and flood waters flowing from Foc-contaminated fields onto disease-free plantations can also introduce the pathogen into clean plantations. Irrigation and flood water, therefore, has to be carefully managed to prevent the introduction and spread of Foc in

disease-free areas. For sprinkler irrigation this means the treatment of water before being sprayed onto banana fields, and for flood irrigation and flooding it means the proper planning of slopes and drainage channels on banana farms.

Based on this study, soil reduced the efficacy of all the treatments evaluated. Organic matter in the soil consists of iron and manganese, which reduces the efficacy of ozone, UV and the peracetic acid products. This then requires higher dosage and longer exposure times for the eradication of pathogens in water (Portjanskaja, 2010). Sand filtration could be used to first remove organic matter in order for UV to properly disinfect contaminated water (Jolis *et al*., 2001). HTH was completely ineffective against Foc in the presence of soil, and should not be recommended for the treatment of Foc-contaminated irrigation water. It is not environmentally friendly and highly dependent on pH for its efficacy.

A method to clean irrigation water should not only be effective against the target pathogen, but should also be practical and affordable. Ozone and UV radiation require installation of treatment standsFor large scale use, a corona discharge ozone generator can be used to covert oxygen into ozone by splitting O_2 molecules into O^- atoms to create ozone (Hong *et al*., 2014). Disinfection by UV radiation requires a low pressure (290-315 nm) or medium pressure (220-280 nm) lamp, which provides intensities that can kill bacteria and fungi (Hunter, 2008). HTH and the PAA products require no treatment facilities. HTH is often used for disinfection because it does not require any installation, is readily available and is inexpensive (Kitis, 2004; Van Haute *et al*., 2013), however, it is not effective in the presence of soil. PAA products require no machinery for its application, and it does not release harmful disinfection by-products (Koivunen and Heinonen-Tanski, 2005).

PAA products and HTH both require continuous purchases of the chemicals, whereas ozone and UV treatment require a once off payment for installation. UV is impractical for field application, as doses required to kill Foc need a minimum waiting time of 45 min. Ozone and UV treatment require installation that may cost more than US $10 000 and an operating cost of less than US $5 000 (Hong *et al*., 2014). Nevertheless, no special training is required for the operation of these machines. A filtration method also needs to be installed to first remove organic matter that interferes with the efficacy of the treatment. This results in additional costs (Hong *et al*., 2014).

In conclusion, Foc is able to survive in water for more than 120 days. Aeration and agitation appear to play a big part in its survival, which means that running water allows Foc to survive longer than in farm dams where water is stagnant. Foc spores tend to sink in water due to its weight, and water should therefore be pumped from surface water to reduce spread of the pathogen and then treated. It is important to completely disinfect irrigation water to prevent the dispersal of Foc in the field (Ploetz, 2015). HyperCide would be

recommended to treat Foc-contaminated water on banana farms, compared to HTH, as it does not require any installation costs and is safe for use.

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Table 1. Analysis of variance representing the sum of squares with all the interactions between days, motion, water quality and depth for the survival of *Fusarium oxysporum* f. sp. *cubense*.

Source	DF	Sum of squares	Mean squares	F	Pr > F
Day	5	3058630.058	611726.012	247.991	<0.0001
Stirred/still	1	2831048.163	2831048.163	1147.694	<0.0001
Dirty/clean	1	33475.203	33475.203	13.571	0.000
Depth	2	2107444.155	1053722.077	427.174	<0.0001
Day*stirred/still	5	636349.162	127269.832	51.595	<0.0001
Day*dirty/clean	5	106371.105	21274.221	8.624	<0.0001
Day*depth	10	2113620.512	211362.051	85.685	<0.0001
Stirred/still*dirty/clean	1	40577.070	40577.070	16.450	<0.0001
Stirred/still*depth	2	191541.022	95770.511	38.825	<0.0001
Dirty/clean*depth	2	13180.102	6590.051	2.672	0.070
Day*stirred/still*dirty/clean	5	42135.588	8427.118	3.416	0.005
Day*stirred/still*depth	10	793946.278	79394.628	32.186	<0.0001
Day*dirty/clean*depth	10	40448.365	4044.836	1.640	0.090
Stirred/still*dirty/clean*depth	2	40237.715	20118.858	8.156	0.000
Day*stirred/still*dirty/clean*depth	10	46587.252	4658.725	1.889	0.043

Table 2. The survival of *Fusarium oxysporum* f. sp. *cubense* (Foc) in the presence and absence of soil in water after treatment with ozone.

Treatment time (min)	Mean colony forming units of Foc after ozone treatment	
	Absence of soil	**Presence of soil**
Control (non-treated)	297a[a]	275b[b]
0	179d	239c
10	0f	27e
30	0f	3f
60	0f	2f

[a]Different letters indicate significant differences at P ≤ 0.05 using Tukey's least significant difference.

Table 3. The treatment of *Fusarium oxysporum* f. sp. *cubense* (Foc) contaminated water in the presence and absence of soil with ultraviolet radiation.

Dose (mJ.cm^{-2})	Mean colony forming units of Foc after ultraviolet radiation	
	Absence of soil	**Presence of soil**
Control (non-treated)	294a[a]	254b
100	72d	112c
200	5f	60e
300	3f	13f

[a]Different letters indicate significant differences at P ≤ 0.05 using Tukey's least significant difference.

Table 4. Treatment of *Fusarium oxysporum* f. sp. *cubense* (Foc) contaminated water in the presence and absence of soil with HTH.

Treatment time (min)	Mean colony forming units of Foc after HTH treatment	
	Absence of soil	Presence of soil
Control (non-treated)	300a[a]	300a
10	0b	300a
20	0b	300a
30	0b	300a

[a]Different letters indicate significant differences at P ≤ 0.05 using Tukey's least significant difference.

Table 6. Treatment of *Fusarium oxysporum* f. sp. *cubense* (Foc) contaminated water in the presence and absence of soil with peracetic acid.

Peracetic acid product	Treatment time (min)	Mean colony forming units of Foc after treatment	
		Absence of soil	Presence of soil
	Control (non-treated)	300a[a]	300a
HyperCide	10	1c	300a
	20	0d	300a
	30	0d	300a
	60	0d	300a
	120	0d	300a
Tsunami 100®	10	300a	300a
	20	274b	300a
	30	0d	300a
	60	0d	300a
	120	0d	300a

[a]Different letters indicate significant differences at P ≤ 0.05 using Tukey's least significant difference.

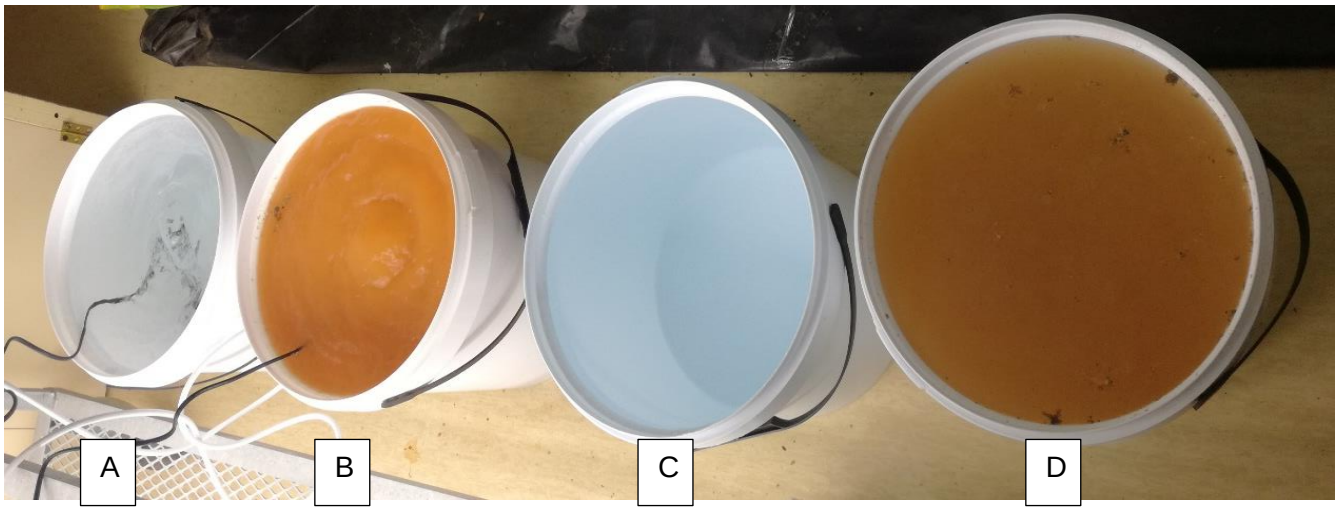

Figure 1. The experimental setup to test for the survival of *Fusarium oxysporum* f. sp. *cubense* in water. (A) Water agitated in the absence of soil in water, (B) Water agitated in the presence of soil, (C) Still water in the absence of soil and (D) Still water in the presence of soil in stagnant water.

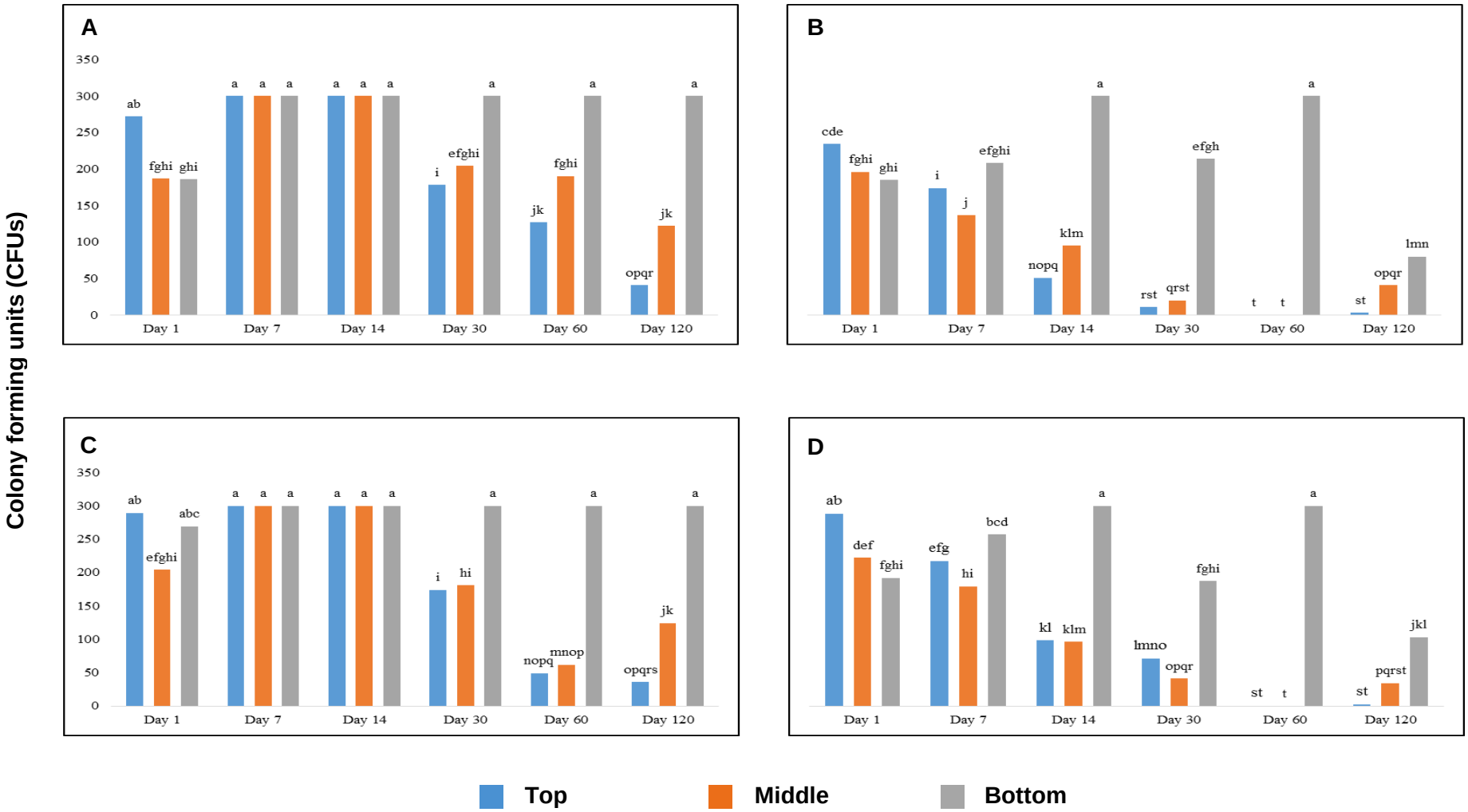

Figure 2. The effect of sampling depth, motion and the presence and absence of soil on the survival of *Fusarium oxysporum* f. sp. *cubense* in water over a period of 120 days in buckets. (A) Agitated water in the absence of soil, (B) Still water in the absence of soil, (C) Agitated water in the presence of soil and (D) Still water in the presence of soil.