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Evaluation of the Efficacy of Commercial Disinfectants Against *Fusarium oxysporum* f. sp. *cubense* Race 1 and Tropical Race 4 Propagules

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Abstract

Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* has devastated banana production worldwide. This work aimed to determine effective disinfectants against two races of *F. oxysporum* f. sp. *cubense*, race 1 and tropical race 4 (TR4), for implementation with on-farm biosecurity procedures against this disease following the outbreak of TR4 in North Queensland in 2015. A total of 32 commercial disinfectants were screened and their activity was assessed after ≤30 s, 5 min, 30 min, and 24 h of contact with an *F. oxysporum* f. sp. *cubense* suspension containing 10^5 chlamydospores/ml without and with soil added (0.05 g/ml). Of the disinfectants tested, the quaternary ammonium compounds containing ≥10% active ingredient were found to be the most effective against both *F. oxysporum* f. sp. *cubense* races. These products, when used at a 1:100 dilution, completely inhibited the survival of all *F. oxysporum* f. sp. *cubense* propagules across all the contact times regardless of the absence or presence of soil. The bioflavonoid product EvoTech 213 and bleach (10% sodium hypochlorite) used at a 1:10 dilution also eliminated all *F. oxysporum* f. sp. *cubense* propagules across all the contact times. None of the detergent-based or miscellaneous products tested were completely effective against both *F. oxysporum* f. sp. *cubense* races even used at a 1:10 dilution. Soil decreases the efficacy of disinfectants and therefore must be removed from contaminated items before treatments are applied.

Fusarium oxysporum f. sp. *cubense* is an important soilborne fungus that causes an onset of symptoms in banana cultivars, including wilting and yellowing of leaves, browning of the vascular system, splitting of pseudostems, and eventually plant death (Pegg et al. 1996; Ploetz 2006). The disease, also known as Fusarium wilt or Panama disease, is one of the most destructive plant diseases and has devastated banana production worldwide (Ploetz 2015a). Based on their specific pathogenicity to different cultivars, the pathogenic strains of *F. oxysporum* f. sp. *cubense* have been classified into four races. Race 1 (R1) infects Gros Michel (AAA), Maqueño, Silk, Pome (AAB), and Pisang Awak (ABB) cultivars. Race 2 (R2) infects ABB cooking bananas such as Bluggoe, whereas race 4 is pathogenic to the Cavendish cultivar and to all banana cultivars susceptible to R1 and R2. Race 3 was previously reported to affect *Heliconia* spp. (tropical American banana relatives) but is no longer considered as being pathogenic to bananas. Race 4 is further divided into subtropical race 4 (STR4) and tropical race 4 (TR4). STR4 strains only infect Cavendish in the subtropics, where the plants are more susceptible as a result of predisposing factors such as low temperature, while TR4 strains infect Cavendish in both the subtropics and tropics (Perez-Vicente and Dita 2014; Ploetz 2015b).

During the first half of the 20th century, strains of R1 caused major devastation to the banana industry in Central America following their detection on Gros Michel cultivars. As a consequence, the industry recovered with the introduction of R1-resistant Cavendish cultivars in the international banana export trade (Ploetz et al. 2003; Stover 1990). However, since the early 1990s, banana production worldwide has faced a major threat from TR4 because of its ability to infect Cavendish cultivars (Ploetz 2015a). Despite various measures implemented to manage the spread of TR4, the pathogen has been identified in many countries (Ploetz 2015b). In Australia, TR4 was first detected in 1997 in the Northern Territory (Conde and Pitkethley 2001) and subsequently detected in Queensland in 2015. The most common route of *F. oxysporum* f. sp. *cubense* transmission is through the use of infected rhizomes or suckers for planting material. However, humans are also responsible for the spread of *F. oxysporum* f. sp. *cubense* by moving contaminated footwear and vehicles and using contaminated equipment for farm work. In addition, the release of untreated wastewater infested with *F. oxysporum* f. sp. *cubense* also allows dissemination of the pathogen from infested areas to noninfested regions (Ploetz 2015b). The spread of *F. oxysporum* f. sp. *cubense* can be minimized by using disinfectants to decontaminate footwear, vehicles, farm equipment, and machinery or to treat wastewater on farms. Therefore, disinfectants with efficacy against *F. oxysporum* f. sp. *cubense* are essential to the implementation of effective on-farm biosecurity procedures.

In Australia, the detergent-based product, Castrol Farmcleanse (active ingredients: 5 to 10% alkali metal salts of alkylbenzene sulfonic acid, 1 to 5% coconut diethanolamide, and 0.1 to 1% pyridine-2-thiol 1-oxide, sodium salt; Castrol Australia Pty Ltd., Docklands, VIC, Australia), has been proposed for use as a disinfectant against *F. oxysporum* f. sp. *cubense* in the banana industry based on research conducted on *F. oxysporum* f. sp. *vasinfectum*, the cause of Fusarium wilt in cotton (Moore et al. 2001). This product was found to be the most effective disinfectant for reducing colony numbers of *F. oxysporum* f. sp. *vasinfectum*. However, later studies demonstrated that Castrol Farmcleanse failed to prevent germination or subsequent growth of an *F. oxysporum* f. sp. *cubense* STR4 isolate (Nel et al. 2007) and was also not effective in inhibiting the germination of microconidia of an *F. oxysporum* f. sp. *cubense* TR4 isolate (Meldrum et al. 2013). However, both of these studies found that a quaternary ammonium (QA)-based product, Sporekill (active ingredient: 12% didecyldimethyl ammonium chloride [DDAC]; Nufarm Australia Ltd., Laverton North, VIC, Australia), was the most effective disinfectant against *F. oxysporum* f. sp. *cubense*

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compared with other fungicides and surface sterilants. Sporekill was also found to maintain its effectiveness against TR4 microconidia after 6 months of exposure to sunlight and diurnal temperature variation in the field (Meldrum et al. 2013; Nel et al. 2007). In addition to microconidia and macroconidia, *F. oxysporum* f. sp. *cubense* isolates also produce thick-walled structures (chlamydospores). Chlamydospores are usually formed in the dead host plant tissues in the final stages of the disease cycle and once they are returned to the soil, they can survive for more than 20 years in plant debris and readily germinate and infect new banana plants (Perez-Vicente and Dita 2014). Despite their extended survival and resilience compared with conidia, *F. oxysporum* f. sp. *cubense* chlamydospores have not been used in previous disinfectant studies. Also, although Sporekill has been demonstrated to be effective against *F. oxysporum* f. sp. *cubense*, its activity has only been tested on conidia in aqueous solution in the absence of soil. The presence of soil or organic matter may reduce the efficacy of disinfectants against *F. oxysporum* f. sp. *cubense*, since soil and organic matter have been shown to decrease the efficacy of antifungal cleansers against conidia and chlamydospores of *F. oxysporum* f. sp. *vasinfectum* (Bennett et al. 2011). However, the effect of soil on the activity of disinfectants against *F. oxysporum* f. sp. *cubense* has been neglected.

This study was undertaken to evaluate the efficacy of commercial disinfectants available in the Australian market on the survival of *F. oxysporum* f. sp. *cubense* R1 and TR4 propagules (in particular, chlamydospores) at four contact times (≤30 s, 5 min, 30 min, and 24 h) and in the absence and presence of soil. This work aimed to determine (i) which disinfectants were effective against *F. oxysporum* f. sp. *cubense* R1 and TR4 to form part of a practical on-farm decontamination procedure against Panama disease and (ii) whether treatment times and the presence of soil affected the activity of disinfectants against *F. oxysporum* f. sp. *cubense*.

Materials and Methods

Isolates. A total of four *F. oxysporum* f. sp. *cubense* R1 and four *F. oxysporum* f. sp. *cubense* TR4 isolates were used in this study (Table 1). Three of the R1 isolates (BRIP 23760, 24258, and 24295) were obtained from the Queensland Plant Pathology Herbarium (BRIP) culture collection at the EcoSciences Precinct (ESP) in Brisbane, Australia. The R1 isolate BRIP 63370 was isolated from fresh material of Ducasse plants on the Atherton Tablelands in Rocky Creek, Queensland, Australia, and subsequently forwarded to the ESP for confirmation of species and vegetative compatibility group determination. All the TR4 cultures were obtained from the culture collection at the Department of Primary Industry and Resources, Northern Territory, Australia. Prior to experiments, all *F. oxysporum* f. sp. *cubense* isolates from long-term storage were revitalized on Spezieller Nahrstofarmer agar (SNA), followed by incubation at 25°C for 7 days. These SNA cultures were then subcultured on carnation leaf agar (CLA) under black light to allow them to produce conidia (Leslie and Summerell 2006), which were subsequently used to inoculate soil broth to produce chlamydospores.

Disinfectant products. A total of 32 disinfectant products received from chemical companies, resellers, and local banana producers were assessed (Table 2). They included 19 QA compounds, three bioflavonoid products, two oxidizing agents, six detergent-based products, and two miscellaneous products. Products were either used undiluted or diluted with sterile distilled water (SDW) to the rates of 1:10, 1:100, and 1:1,000 and suspensions were made up fresh prior to each experiment.

Preparation of soil broth for chlamydospore production. Searles Superior Garden Soil Mix (Searles Pty Ltd., Kilcoy, QLD, Australia) was used to prepare soil broth following a modified protocol described by Bennett and Davis (2013). Air-dried soil mix (125 g) was mixed with 500 ml of reverse osmosis water in 2-liter flasks and agitated on an orbital shaker at 90 rpm for 1 h. Following agitation, the soil-water mixture was poured through a 1.7-mesh sieve to remove large particles and then filtered through eight layers of cheesecloth. The flow-through was filtered again through filter paper discs to achieve clear broth. The broth was autoclaved at 121°C for 20 min and then placed on the bench overnight to allow sediments to settle completely. The top clear soil broth was decanted into 250-ml bottles (50 ml of soil broth per bottle) and again autoclaved.

Chlamydospore production. To stimulate *F. oxysporum* f. sp. *cubense* to produce chlamydospores, each bottle of clear sterile soil broth (50 ml) was inoculated with 400 µl of 2.5×10^6 conidia/ml suspension prepared from 8-day *F. oxysporum* f. sp. *cubense* cultures grown on CLA under black light. The inoculated soil broth was agitated at 90 rpm on an orbital shaker at room temperature for at least 5 days to allow *F. oxysporum* f. sp. *cubense* to produce chlamydospores. To harvest chlamydospores, the soil broth cultures were passed through a layer of sterile cheesecloth to trap mycelia clumps containing chlamydospores. The mycelia clumps were rinsed with SDW over the cheesecloth to reduce the number of conidia and then transferred to a small sterile beaker using a volume of approximately 10 ml of SDW. Mycelium clumps were stirred using a sterile stirring bar to release chlamydospores, and stirring continued until the mycelial clumps were no longer visible. The spore suspension was passed through a layer of sterile cheesecloth to remove remaining clumps and mycelia. The chlamydospore concentration was determined using a Neubauer hemocytometer and adjusted to 10^5 chlamydospores/ml before use in disinfectant experiments.

Disinfectant experiments. In this study, *F. oxysporum* f. sp. *cubense* propagules were treated with disinfectants for ≤30 s, 5 min, 30 min, and 24 h in the absence and presence of soil. The contact times of ≤30 s and 5 min were designed to simulate the length of time to walk through a footbath or drive vehicles or farm machinery through a drive-through dip containing a disinfectant. The longer contact times of 30 min and 24 h were designed to simulate the length of time required for treatment of runoff water in a holding tank or for soaking boots or farm implements such as secateurs or cane knives. Treatments without soil were made by adding 100 µl of the chlamydospore suspension, as described above, into 2-ml tubes containing 900 µl of disinfectants diluted to the required rates. Tubes were

Table 1. *Fusarium oxysporum* f. sp. *cubense* race 1 and tropical race 4 (TR4) isolates used in this study

Isolate	Race	Vegetative compatibility group	Isolation source
BRIP 23760[a]	1	0124	Ingham, Queensland, Australia
BRIP 24258	1	0125	Yarrabah, Queensland, Australia
BRIP 24295	1	0124	Atherton, Queensland, Australia
BRIP 63370[b]	1	0124	Rocky Creek, Queensland, Australia
NTP-Dc 34438[c]	TR4	01213/16	Rapid Creek, Northern Territory, Australia
NTP-Dc 35521	TR4	01213/16	Lambells Lagoon, Northern Territory, Australia
NTP-Dc 35667	TR4	01213/16	Yirrkala, Northern Territory, Australia
NTP-Dc 35673	TR4	01213/16	Middle Point, Northern Territory, Australia

[a] Race 1 isolates BRIP 23760, 24258, and 24295 were obtained from the culture collection at the Queensland Plant Pathology Herbarium, Australia (BRIP).
[b] Race 1 isolate BRIP 63370 was obtained from Ducasse plants exhibiting typical vascular symptoms of Panama disease on the Atherton Tablelands, Rocky Creek, Queensland, Australia.
[c] TR4 isolates NTP-Dc 34438, NTP-Dc 35521, NTP-Dc 35667, and NTP-Dc 35673 were obtained from the Northern Territory culture collection at the Department of Primary Industry and Resources, Northern Territory, Australia (NTP-Dc).

either briefly vortexed or pipetted a few times to ensure the solution was well mixed. After the desired exposure times, a 100-µl aliquot was plated on half-strength potato dextrose agar plates supplemented with 30 IU/ml (0.042 mg/ml) of streptomycin sulfate (Sigma, St. Louis, MO). All the plates were then incubated at 25°C for 48 to 72 h, after which the number of *F. oxysporum* f. sp. *cubense* colonies was recorded. Where "zero" colony counts were recorded at 72 h, further assessments were conducted at either day 6 or 7 postincubation to confirm whether the disinfectant products effectively eliminated *F. oxysporum* f. sp. *cubense* or just delayed fungal colony development. Treatments resulting in no colony formation after 6 or 7 days of incubation were considered to be effective against *F. oxysporum* f. sp. *cubense*. Treatments with soil were performed in the same process as stated above for treatments without soil, except that one part of soil was added and mixed with 20 parts of disinfectant solutions prior to initiating the contact times.

The R1 trial was conducted in a laboratory in Mareeba, Queensland, and the TR4 trial was conducted in a separate laboratory in

Table 2. Disinfectants assessed in this study

Category	Disinfectant trade name	Manufacturer	Active ingredient (%)[a]
Quaternary ammonium	Agriquat	SeaJay Industries Pty Ltd., Chipping Norton, NSW, Australia	BC (25%)
	Algacide 1200/9/2015	Minehan Agencies Pty Ltd., Townsville, QLD, Australia	DDAC (12%)
	Anti-Fungal SS No. 1 Cleaner	Taipan Traders, Mudgeeraba, QLD, Australia	DDAC (<40%), ABDAC (<40%)
	Atmosphere Forte Blue Concentrate	A1 Chemicals/Industries, Milperra, NSW, Australia	BC (<10%)
	Bactex CF	Whiteley Industrial, North Sydney, NSW, Australia	BC (10%)
	Banana Basher Cleaner	Taipan Traders	DDAC (10 to 30%), ABDAC (10 to 30%), ethyl alcohol (<10%), 2-butoxyethanol (<10%)
	Banana Buster Cleaner	Taipan Traders	DDAC (10 to 30%), ABDAC (10 to30%), ethyl alcohol (10 to 30%)
	Banana Disease Blockbuster	Taipan Traders	DDAC (10 to 30%), ABDAC (10 to 30%), BC (10 to 30%), terpene hydrocarbons (<10%)
	Banana Disease Buster	Taipan Traders	DDAC (10 to 30%), ABDAC (10 to 30%), BC (10 to 30%)
	Banana Shed/Equipment Cleaner No. 1	Taipan Traders	ABDAC (30 to 60%)
	Banana Shed/Equipment Cleaner No. 2	Taipan Traders	ABDAC (30 to 60%), phosphoric acid (<20%)
	Banana Vehicle Truck Wash Cleaner	Taipan Traders	DDAC (<20%)
	F10SC Disinfectant	Health and Hygiene Pty Ltd., Florida Hills, South Africa	BC (5.4%), polyhexamethylene biguanide hydrochloride (0.4%)
	Farm Cleaner Detergent	Taipan Traders	ABDAC (<10%), 2-(2-butoxy) ethanol (<10%)
	Organic Food Fungal Cleaner	Taipan Traders	DDAC (<20%), ABDAC (<20%)
	Path-X	Nutri-Tech Solutions Pty Ltd., Yandina, QLD, Australia	DDAC (12%)
	Sporekill	Nufarm Australia Ltd., Laverton North, VIC, Australia	DDAC (12%)
	Steri-maX	Agricrop, Hindmarsh, SA, Australia	DDAC (12%)
	Super Cleaner Anti-Fungal	Taipan Traders	ABDAC (<60%)
Bioflavonoid	Citran 1	Organic Farming Systems, Cottesloe, WA, Australia	Bitter orange extract (8 to 12%), octanoic acid (2 to 4%), lactic acid (2 to 4%)
	Citran 2	Organic Farming Systems	Bioflavonoid mixture (1 to 5%), glycerine (2 to 4%), Citran 1 (2 to 10%)
	EvoTech 213	Evolution Organics, Cannonvale, QLD, Australia	Bioflavonoid mixture (5 to 10%), organic acid blend (15 to 30%), glycerine (30 to 60%), ethanol (5 to 10%), essential oil blend (1 to 5%)
Oxidizing agent	Bleach	DP Cleaning, Mareeba, QLD, Australia	Sodium hypochlorite (10%)
	Anosan	Ecabiotec Pty Ltd., Morningside, QLD, Australia	Electrochemically activated table salt solution (400 to 900 ppm of active chlorine)
Miscellaneous	Antisept E12	Fuchs Lubricants (Australasia) Pty Ltd., Sunshine, VIC, Australia	2,2′,2″-(hexadydro-1,3,5-triazine-1,3,5-triyl) triethanol (70 to 80%), 2-aminoethanol (1 to <3%)
	Bactofix P	Fuchs Lubricants (Australasia) Pty Ltd.	*N*,*N*′-methylenebismorpholine (>60%)
Detergent based	Bio-Cleanse	Queensland Cleaning Solutions, Little Mountain, QLD, Australia	Pyridine-2-thiol 1-oxide, sodium salt (0 to 5%)
	Bio-Cleanse Plus	Queensland Cleaning Solutions	Pyridine-2-thiol 1-oxide, sodium salt (0 to 5%)
	Castrol Farmcleanse	Castrol Australia Pty Ltd., Docklands, VIC, Australia	Alkali metal salts of alkylbenzene sulfonic acid (5 to 10%), coconut diethanolamide (1 to 5%), pyridine-2-thiol 1-oxide, sodium salt (0.1 to 1%)
	Fleetmaster Harvest Kleen	Minehan Agencies Pty Ltd.	Sodium dodecylbenzenesulphonate (5 to 15%), cocoampho disodium dipropionate (5 to 10%), sodium tripoly phosphate (5 to 10%), sodium xylene sulphonate (1 to 5%)
	H.D. Gensolve	Custom Chemicals International Pty Ltd., Narangba, QLD, Australia	Sodium hydroxide (2.4%), butoxyethanol (10 to 30%)
	Spore Attack	Queensland Cleaning Solutions	Pyridine-2-thiol 1-oxide, sodium salt (0 to 5%)

[a] BC = benzalkonium chloride, DDAC = didecyldimethyl ammonium chloride, ABDAC = alkyl benzyl dimethyl ammonium chloride, and ppm = parts per million.

Berrimah, Northern Territory. The original intent of the study was for the TR4 trial to be conducted in Queensland and validated in the Northern Territory. However, because of the quarantine restrictions placed on research work on TR4 in Queensland following the TR4 outbreak in this state at the time of conducting this study, *F. oxysporum* f. sp. *cubense* R1 was initially used as a proxy for TR4 in the trial in Queensland. The disinfectant bioassay was then validated against TR4 in the Northern Territory, where there are no quarantine restrictions for research work on this race. However, *F. oxysporum* f. sp. *cubense* R1 is not present in the Northern Territory; therefore, R1 work could not be replicated in the Northern Territory. Also, because of quarantine restrictions on moving soil interstate between Queensland and the Northern Territory, the soil types used for the R1 and TR4 trials were different. However, the soils were typical in relation to banana production in each state. In particular, the soil used for the experiments on *F. oxysporum* f. sp. *cubense* R1 is referred to as red ferrosol, which was sourced from a residential property on the Atherton Tablelands, whereas the soil used for the trials on *F. oxysporum* f. sp. *cubense* TR4 is referred to as red kandosol, which was collected from the Coastal Plains Research Station in Middle Point, Northern Territory. The soil was air dried, sieved (2 mm) to remove any large rocks or clods, placed into glass Petri dishes, moistened, mixed, and autoclaved twice before use.

Because of the substantial number of treatments, multiple experiments were conducted. For every experiment, the control treatments were included and consisted of water alone, water with soil, water plus inoculum with and without soil, and Castrol Farmcleanse (1:10 dilution). This allowed all treatments to be combined in a single multiexperiment analysis. All experiments included three replicates of each treatment.

Statistical analysis. Each individual experiment was analyzed separately before being combined in a multiexperiment residual maximum likelihood (REML) analysis. The R1 and TR4 experiments were combined in separate REML analyses and only the results from these two combined analyses are presented. The fixed terms included in the REML models were the main effects and interactions of treatment (product × dilution), contact time, and the presence or absence of soil. A backward stepwise selection approach was used to determine the most parsimonious fixed-effects model. The experiment effects and interactions between experiments and treatment factors were included as random terms in each multiexperiment REML model and separate residual terms were fitted for each experiment. A $\log_{10}$ transformation was required for the combined analysis of the R1 experiments in order to satisfy the underlying assumptions. No transformation was required for the TR4 data. The mean colony counts presented for R1 are on the original count scale.

Table 3. Maximum mean final counts of *Fusarium oxysporum* f. sp. *cubense* race 1 colonies per plate and exposure periods resulting in no growth for a range of product dilutions and in the presence and absence of soil

		No soil		Soil added	
Disinfectant trade name	**Dilution**	**Maximum**	**No growth**	**Maximum**	**No growth**
Quaternary ammonium					
Agriquat	1:100	0	1, 2, 3, 4[a]	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	6.7	
Algacide 1200/9/2015	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800[b]	
Anti-Fungal SS No. 1 Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.1	2, 3, 4	594.5	
Atmosphere Forte Blue Concentrate	1:100	0	1, 2, 3, 4	0.2	
	1:1,000	7.5	2, 3, 4	≥800	
Bactex CF	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.1	2, 3, 4	≥800	
Banana Basher Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	626.7	
Banana Buster Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	561.3	
Banana Disease Blockbuster	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	66.2	
Banana Disease Buster	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	65.2	
Banana Shed/Equipment Cleaner No. 1	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.1	2, 3, 4	443.4	
Banana Shed/Equipment Cleaner No. 2	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	40.9	
Banana Vehicle/Truck Wash Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	251.9	
F10SC Disinfectant	1:100	0	1, 2, 3, 4	10	2, 3, 4
	1:1,000	46.7	2, 3, 4	594.5	
Farm Cleaner Detergent	1:100	2.4	2, 3, 4	49.2	3, 4
	1:1,000	245.2	3, 4	≥800	
Organic Food Fungal Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	516.2	
Path-X	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	786.7	
Sporekill	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.2	2, 3, 4	594.5	

(*Continued on next page*)

[a] No colony growth was observed after the following time periods: 1 = ≤30 s, 2 = 5 min, 3 = 30 min, and 4 = 24 h.
[b] More than 800 colonies per plate.
[c] NT = not tested.

Where the number of colonies developed on each plate was too large to identify individual colonies, a count of 800 is used in the analyses. This will potentially result in an underestimate of the mean colony count for these treatments. However, as the objective is to identify disinfectants that are effective against *F. oxysporum* f. sp. *cubense* R1 and TR4, this will not affect the overall conclusions.

In all analyses, the 95% protected least significant difference was used to make pairwise comparisons. All tests of significance were performed at the 0.05 level. All analyses were performed in the 18th edition of Genstat for Windows (VSN International 2015).

Results

In the analysis of both *F. oxysporum* f. sp. *cubense* R1 and TR4 survival, values for the highest-order interaction of disinfectant treatment, contact, time, and the presence or absence of soil were highly significant (*F. oxysporum* f. sp. *cubense* R1: $\chi^2_{213} = 427.64$, $P < 0.001$; TR4: $\chi^2_{159} = 611.82$, $P < 0.001$); hence, these results focus on the interaction means. The effects of disinfectant treatments on the survival of *F. oxysporum* f. sp. *cubense* R1 and TR4 propagules in aqueous suspensions with and without soil added, based on the number of *F. oxysporum* f. sp. *cubense* colonies that developed following treatments, are presented in Tables 3 and 4 and Supplementary Table S1. In general, with the exception of Anosan, results were similar regardless of *F. oxysporum* f. sp. *cubense* race type. Of the five categories of disinfectants assessed, the QA compounds were the most effective in reducing the survival of propagules of both *F. oxysporum* f. sp. *cubense* R1 and TR4. In particular, no colonies were detected in the 1:100 dilution treatments both with and without soil and across all contact times for 16 of 19 QA products tested. For the remaining QA products (F10SC Disinfectant, Farm Cleaner Detergent, and Atmosphere Forte Blue Concentrate), although treatments at 1:100 dilution significantly ($P < 0.001$) reduced *F. oxysporum* f. sp. *cubense* colony numbers compared with inoculated controls, zero colony counts were only achieved at 5 min of contact in treatments without soil or longer in treatments with soil added. When used at 1:1,000 dilution, all QA products were less effective, with zero colony counts across all four contact times only achieved in treatments without soil for 10 of 19 QA products when tested against R1 and for 12 of 18 QA products when tested against TR4. In the presence of soil, zero colony counts across all four contact times were not achieved with any QA product when tested against either R1 or TR4.

Compared with QA compounds, the bioflavonoid products, oxidizing agents, miscellaneous products, and detergent-based products were less effective against propagules of both *F. oxysporum* f. sp. *cubense* races. Of the three bioflavonoids tested, zero colony counts were observed at all contact times for EvoTech 213 but only at a 1:10 dilution. At this dilution, Citran 1 only achieved zero colony counts after 5 min of contact in all treatments both with and without soil. This was not the case for Citran 2, as R1 and TR4 colonies were

Table 3. (*Continued from previous page*)

		No soil		Soil added	
Disinfectant trade name	**Dilution**	**Maximum**	**No growth**	**Maximum**	**No growth**
Steri-maX	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.1	2, 3, 4	52.8	
Super Cleaner Anti-Fungal	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.1	2, 3, 4	712.6	
Bioflavonoid					
Citran 1	1:10	0.1	2, 3, 4	0.3	2, 3, 4
	1:100	446.8	4	776.6	
Citran 2	1:10	≥800		≥800	
EvoTech 213	1:10	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:100	0.7	2, 3, 4	2.9	2, 3, 4
	1:1,000	660.7	4	639.9	
Oxidizing agent					
Anosan	1:10	333.1	2, 3, 4	195.2	3, 4
	1:100	≥800		≥800	
	Neat	0	1, 2, 3, 4	2.4	4
Bleach	1:10	NT[c]	NT	NT	NT
	1:100	2.9	3, 4	1.6	3, 4
	1:1,000	103	4	7.6	4
Miscellaneous					
Antisept E12	1:10	28.7	3, 4	70.8	3, 4
	1:100	≥800	4	≥800	4
Bactofix P	1:10	47.9	2, 3, 4	39.9	2, 3, 4
	1:100	≥800	4	≥800	4
Detergent based					
Bio-Cleanse	1:10	20.4	4	8.5	4
	1:100	581.4		605.9	
Bio-Cleanse Plus	1:10	43.9	4	26.6	
	1:100	604.6		238.6	
Castrol Farmcleanse	1:10	12.2		13.7	
Fleetmaster Harvest Kleen	1:10	4.5	4	1.5	3, 4
	1:100	357.8		322.7	
H.D. Gensolve	1:10	≥800		847.2	4
Spore Attack	1:10	85.6		16.5	
	1:100	≥800		≥800	
Control					
Water only	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
Water plus inoculum	1:100	734.2		≥800	

detected at all four contact times at the 1:10 dilution both with and without soil present. For the oxidizing agents, bleach containing 10% sodium hypochlorite diluted to 1:10 was found to completely inhibit the colony formation of *F. oxysporum* f. sp. *cubense* TR4 propagules. However, when used at 1:100 dilution, bleach only inhibited *F. oxysporum* f. sp. *cubense* colony formation after 30 min of contact with R1 propagules and after 5 min of contact with TR4 propagules. When used at the 1:1,000 dilution, bleach required longer contact times to achieve zero colony counts: 24 h for R1 and 30 min for TR4. Anosan, another oxidizing agent, achieved zero colony counts against R1 when used undiluted across all contact times and at ≥5 min when used at the 1:10 dilution. The results against TR4 were completely the opposite, with no reduction in colony numbers observed at any of the product application rates. The two miscellaneous products, Antisept E12 and Bactofix P, used at 1:10 and 1:100 dilutions were also not effective against R1 until 30 min and 24 h of contact, respectively; therefore, testing of their efficacy against TR4 was not conducted.

For the six detergent-based products, activity was primarily assessed against R1 and colony development was observed across all four contact times in treatments at 1:100 dilution both with and without soil. With R1, zero colony counts were mostly achieved after 24 h of contact at 1:10 dilution for Bio-Cleanse, Bio-Cleanse Plus, and Fleetmaster Harvest Kleen. Because of their poor performance against R1, only Castrol Farmcleanse and Fleetmaster Harvest Kleen were tested on TR4 and it was found that neither product inhibited TR4 colony formation at any contact time when used at 1:10 dilution, with or without soil present. The detergent-based products did not effectively eliminate colony formation of *F. oxysporum* f. sp. *cubense* R1 or TR4 propagules, and they were found to delay the development of colonies of both *F. oxysporum* f. sp. *cubense* races until 6 or 7 days postincubation. This result was consistent across all separate experiments.

With respect to interactions between disinfectant treatments and contact times, the statistical analysis showed that colony counts of R1 and TR4 were significantly ($P < 0.001$) lower at 24 h of contact than at shorter contact times. This result was consistent for nine QA products used at the 1:1,000 dilution and for most of the bioflavonoid products, oxidizing agents, miscellaneous products, and detergent-based products, both with and without soil. For interactions between disinfectant treatments and soil, significantly ($P < 0.001$) higher colony counts for both R1 and TR4 were recorded in treatments with soil added than those without for QA products at the 1:1,000 dilution and Citran 1 at the 1:100 dilution. These

Table 4. Maximum mean final counts of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 colonies per plate and exposure periods resulting in no growth for a range of product dilutions and in the presence and absence of soil

		No soil		Soil added	
Disinfectant trade name	**Dilution**	**Maximum**	**No growth**	**Maximum**	**No growth**
Quaternary ammonium					
Agriquat	1:100	0	1, 2, 3, 4[a]	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800[b]	4
Algacide 1200/9/2015	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
Anti-Fungal SS No. 1 Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	316	
Atmosphere Forte Blue Concentrate	1:100	0.7	2, 3, 4	61	2, 3, 4
	1:1,000	48.3	2, 3, 4	638.7	
Bactex CF	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	1	2, 3, 4	701.3	
Banana Basher Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
Banana Buster Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
Banana Disease Blockbuster	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	105.7	2, 3, 4
Banana Disease Buster	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	3, 4
Banana Shed/Equipment Cleaner No. 1	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.3	2, 3, 4	≥800	
Banana Shed/Equipment Cleaner No. 2	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
Banana Vehicle/Truck Wash Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
F10SC Disinfectant	1:100	NT[c]		NT	
	1:1,000	NT		NT	
Farm Cleaner Detergent	1:100	94	2, 3, 4	≥800	4
	1:1,000	≥800	4	799.9	
Organic Food Fungal Cleaner	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	
Path-X	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.3	2, 3, 4	≥800	
Sporekill	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	≥800	

(*Continued on next page*)

[a] No colony growth was observed after the following time periods: 1 = ≤30 s, 2 = 5 min, 3 = 30 min, and 4 = 24 h.
[b] More than 800 colonies per plate.
[c] NT = not tested.

results suggest that both contact time and the presence of soil affect the activity of certain disinfectant treatments against both *F. oxysporum* f. sp. *cubense* R1 and TR4.

Discussion

In assessing the efficacy of commercial disinfectants against the survival of *F. oxysporum* f. sp. *cubense* R1 and TR4 propagules, it was found that QA compounds were the most effective against the survival of propagules of both *F. oxysporum* f. sp. *cubense* R1 and TR4 compared with bioflavonoid products, oxidizing agents, miscellaneous products, and detergent-based products. Our findings were consistent with those from previous studies, which also reported that the QA product Sporekill was more effective in inhibiting germination and mycelial growth of *F. oxysporum* f. sp. *cubense* TR4 and STR4 isolates than other products, including sodium hypochlorite and the detergent-based product Farmcleanse (Meldrum et al. 2013; Nel et al. 2007). In our study, 16 of 19 QA products, including Sporekill, were completely effective against both *F. oxysporum* f. sp. *cubense* R1 and TR4 across all contact times when used at 1:100 dilution both with and without soil. However, this was not the case for F10SC Disinfectant, Farm Cleaner Detergent, or Atmosphere Forte Blue Concentrate, which did not eliminate all *F. oxysporum* f. sp. *cubense* propagules at ≤30 s of contact or at longer contact times in treatments with soil present. This could be attributed to the relatively low concentrations of active ingredient in these three products as compared with other QA products. More specifically, these products contain less than 10% of the active ingredient benzalkonium chloride (BC) or alkyl benzyl dimethyl ammonium chloride (ABDAC), while other more effective QA products contained at least 10% active ingredient. For example, Bactex CF contains 10% BC, Banana Shed/Equipment Cleaner No. 1 has at least 30% ABDAC, and 12% DDAC is present in Sporekill. Despite showing complete effectiveness against both *F. oxysporum* f. sp. *cubense* races at 1:100 dilution, all the QA products lost their efficacy when diluted to 1:1,000. Based on these findings, our study suggests that when QA products containing ≥10% active ingredient are diluted to 1:100, they could potentially be implemented in on-farm biosecurity procedures. Usage could include footbaths, drive-through dips, decontamination of farm machinery and equipment, and treatment of wastewater. If QA products are not available, 1:10 dilutions of the bioflavonoid product EvoTech 213 and bleach (10% sodium hypochlorite) could also be used to form on-farm biosecurity procedures against Panama disease, owing to their effectiveness in completely inhibiting the survival of propagules of both *F. oxysporum* f. sp. *cubense* races.

With regard to the detergent-based products, our study showed that all six products, including Castrol Farmcleanse, only delayed fungal colony development and did not completely eliminate growth

Table 4. (*Continued from previous page*)

		No soil		Soil added	
Disinfectant trade name	**Dilution**	**Maximum**	**No growth**	**Maximum**	**No growth**
Steri-maX	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0.3	1, 2, 3, 4	480	
Super Cleaner Anti-Fungal	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:1,000	0	1, 2, 3, 4	424	4
Bioflavonoid					
Citran 1	1:10	0.7	2, 3, 4	2.3	2, 3, 4
	1:100	384	3, 4	481.3	
Citran 2	1:10	≥800		≥800	
EvoTech 213	1:10	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:100	47	2, 3, 4	46.3	2, 3, 4
	1:1,000	346.7	4	328.7	4
Oxidizing agent					
Anosan	1:10	≥800		≥800	
	1:100	≥800		≥800	
	Neat	≥800		≥800	
Bleach	1:10	0	1, 2, 3, 4	0	1, 2, 3, 4
	1:100	12.7	2, 3, 4	1.3	2, 3, 4
	1:1,000	356	3, 4	40.7	3, 4
Miscellaneous					
Antisept E12	1:10	NT		NT	
	1:100	NT		NT	
Bactofix P	1:10	NT		NT	
	1:100	NT		NT	
Detergent based					
Bio-Cleanse	1:10	NT		NT	
	1:100	NT		NT	
Bio-Cleanse Plus	1:10	NT		NT	
	1:100	NT		NT	
Castrol Farmcleanse	1:10	407.1		367.5	
Fleetmaster Harvest Kleen	1:10	139.7		204	
	1:100	≥800		≥800	
H.D. Gensolve	1:10	NT		NT	
Spore Attack	1:10	NT		NT	
	1:100	NT		NT	
Control					
Water only	1:100	0	1, 2, 3, 4	0	1, 2, 3, 4
Water plus inoculum	1:100	794.7		792	

of R1 or TR4 in most of the treatments. Such results suggest that these products should not be applied to decontaminate against Panama disease as previously proposed, but they could be used to assist in the removal of soil and organic material prior to the application of a QA product. Castrol Farmcleanse was also found to deactivate the activity of QA products against R1 propagules (P. Trevorrow and K. Grice, *personal communication*); therefore, it is recommended that this product should not be incorporated or combined with QA products in decontamination procedures against Panama disease.

Our study also showed that an increase in contact time to 24 h enhanced the efficacy of most disinfectant treatments against both *F. oxysporum* f. sp. *cubense* R1 and TR4 compared with shorter contact times. However, the presence of soil decreased the efficacy of disinfectants against both *F. oxysporum* f. sp. *cubense* races. This supports the findings of Bennett et al. (2011), who reported that soil or organic matter decreased the efficacy of antifungal cleansers against conidia and chlamydospores of *F. oxysporum* f. sp. *vasinfectum*. Based on this result, soil adhered to items (boots, equipment, or farm machinery) must be removed before disinfectants are applied in order to maintain efficacy. In addition to contact time and the presence of soil, other factors such as water temperature, pH, and mineral content were reported to affect the germicidal properties of disinfectants including QA compounds and chlorine against a number of microorganisms (Copes et al. 2004; Ridenour and Armbruster 1948; Scarlett et al. 2016). The above factors were not considered in the present study but may influence the disinfectant activity. Additional studies to investigate these effects on the performance of disinfectants, particularly QA products, against *F. oxysporum* f. sp. *cubense* are needed.

In selecting a suitable disinfectant for on-farm decontamination procedures, aside from product efficacy, other factors such as product cost, corrosiveness, longevity, and environmental impact should be considered. For example, the use of bleach as a disinfectant against *F. oxysporum* f. sp. *cubense* can be a cost-effective option because it is inexpensive; however, bleach is known to be highly corrosive and the active ingredient, sodium hypochlorite, quickly degrades. This is also the likely reason for the inconsistent results for the Anosan product between R1 and TR4, given that the active chlorine concentration ranged from 400 to 900 parts per million (ppm). However, further investigation as to why Anosan did not work for TR4 compared with R1 is required. For QA products, Veivers et al. (2017) reported that in the absence of soil or organic matter, the active ingredient DDAC in three QA products (Sporekill, Path-X, and Steri-maX) diluted at a 1:100 rate remained at its original concentration of 1,200 ppm when exposed to field conditions for 4 months. Also, there was no significant difference between these products and water alone in degrees of corrosiveness to different metal types, including aluminum, galvanized, painted, unpainted, and stainless steel (Veivers et al. 2017). However, detailed investigations on the corrosiveness and longevity of the QA products are still in progress.

In conclusion, this study evaluated the efficacy of commercial disinfectants against propagules including chlamydospores of *F. oxysporum* f. sp. *cubense* R1 and TR4 for the first time and determined that the QA products were the most effective disinfectants. These products may be implemented in on-farm biosecurity procedures against Panama disease for use in footbaths, drive-through dips, decontamination of farm machinery or equipment, and treatment of wastewater, provided they contain at least 10% active ingredient and are not diluted more than a 1:100 rate before application. The presence of soil or organic matter decreases the efficacy of disinfectants; therefore, soil on footwear or farm equipment should be completely removed before disinfectant treatments are applied. However, in order to assist with selecting suitable products for on-farm decontamination procedures, in addition to the current investigation on the corrosiveness of the QA products on materials used in farm equipment and machinery and their longevity, additional studies on the effects of other factors such as water temperature, pH, and mineral content on their activity against *F. oxysporum* f. sp. *cubense* are required.

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