A Novel Spore Collection Device for Sampling Exposure Pathways: A Case Study of *Puccinia psidii*

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Abstract

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A device comprising a filter attached to a vacuum cleaner was purpose-built to sample rust spores from three potentially high-risk pathways in Australia: passengers, fresh flowers, and sea cargo. The proportion of spores recovered from eight surfaces comparable with those on each pathway (cotton, denim, roses, carnations, chrysanthemums, wood, plastic, and metal) was estimated in the laboratory. Spore recovery percentages were highest for denim clothing (61% *Puccinia triticina* Erikss. and 62% *Uromyctadium tepperianum*) and lowest for carnations (4% *P. triticina* Erikss. and 5% *U. tepperianum*). Subsequently, the device was tested at several locations on the Central Coast of New South Wales, Australia, recently affected by a "myrtle rust" outbreak. Symptomatic and asymptomatic myrtle rust hosts, myrtle rust non-hosts, and inanimate objects (e.g., clothing and vehicles) were sampled

in conjunction with the emergency response to the outbreak. A polymerase chain reaction (PCR) assay developed for *P. psidii* established the presence of myrtle rust, and visual inspections provided spore count estimations. All samples from symptomatic myrtle rust hosts produced positive PCR results and spore count estimations were generally much greater. Several samples from asymptomatic myrtle rust hosts, myrtle rust nonhosts, and inanimate objects also produced positive PCR results; however, there were discrepancies between PCR results and spore count estimations in some of these samples, all of which had <100 spores. This study highlights the utility of the device and analytical methodology, especially during the early stages of a disease outbreak when infection symptoms on plants and contamination on objects is not visible upon gross examination.

Australia is in a unique position with respect to biosecurity because of its remoteness, unique biodiversity, low-density population, and relatively recent settlement by Europeans. Strict quarantine measures have been effective in protecting Australian agriculture, horticulture, and forestry from many pests and diseases that affect trading partners and competitors (23). Recent changes to open up and promote world trade, combined with an increase in the volumes and speed of international tourism, are now increasing the challenges to quarantine and biosecurity (1,8,28,34). The Beale Review of Australia's biosecurity system recommended that risk-profiling methods should focus on risk-return profile analysis (1). Accordingly, the Department of Agriculture, Fisheries and Forestry, in collaboration with the Australian Centre of Excellence for Risk Analysis, initiated a research project to develop a risk analysis framework for pests and pathogens that have multiple pathways for entry into Australia. Specifically, the project explored the use of pathway modeling and spatial habitat analyses to evaluate the risks of entry, establishment, and spread of a group of closely related fungal pathogens with broad host ranges and with hypothesized multiple entry pathways. As a case study, the project focused on multiple exposure pathways for

pathogens in the *Puccinia psidii* sensu lato complex, guava rust, into Australia.

The pathogens in the *P. psidii* sensu lato complex (hereafter *P. psidii*) cause a disease variously known as guava rust, eucalyptus rust, 'ohi'a rust, and myrtle rust which typically affects young, actively growing tissues in susceptible hosts within the family Myrtaceae (6,21,32). Symptoms of the disease typically include leaf lesions, leaf and shoot distortion, and shoot dieback following the emergence of uredinia that produce yellow masses of urediniospores. In severe outbreaks, extensive defoliation, flower blight, fruit infection, and dieback can manifest in young trees of highly susceptible species (9,32).

Guava rust is native to South and Central America, where it was first described on guava (*Psidium guajava*) in Brazil in 1884 (38). Subsequent to the first record on guava, the reported host range of guava rust has extended and presently includes 129 species in 33 genera, all in the Myrtaceae family (5). The geographic range of guava rust has also expanded to include North America (Mexico and the United States, including Hawaii), most countries of South America, the Caribbean, and, more recently, Japan and China (12,25,31,32,39).

Quarantine measures have been in place in Australia since the mid-1980s to minimize risk of introduction of guava rust, which could cause major damage to host plant populations, because members of the Myrtaceae family dominate many natural plant communities in Australia and include plants that sustain economically important industries (12,13). However, with increasing volumes and speed of international trade and tourism, and with the pathogen moving geographically closer to Australia in recent years, there had been increasing concern about the potential of an incursion to Australia. In April 2010, this potential was realized when an outbreak of an exotic rust that resembled *P. psidii* was detected on the Central Coast of New South Wales (NSW), Australia (6). The fungus was identified as *Uredo rangellii*, an anamorph species in the

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guava rust complex, based on urediniospore morphology (6,31) and given the common name “myrtle rust”. However, it is now believed that the Australian rust is a strain of *Puccinia psidii* based on observations of teliospores that match the description of *P. psidii* sensu stricto (4,5).

Like many exotic rust incursions, it is unclear when and where *P. psidii* entered Australia (3). In 2004, the Australia Quarantine and Inspection Service detected spores resembling *P. psidii* on sawn *Eucalyptus* timber imports from Brazil, including the plastic wrapping and the external surfaces of shipping containers. Molecular analyses confirmed that the fungus was *P. psidii*, and viability testing confirmed that the spores were viable after the 2-month journey at sea (13). Although trade in timber of *Eucalyptus* spp. from countries where guava rust is established was suspended in response to this detection, the finding suggests that the external surfaces of shipping containers and their contents (whether timber or other commodities) can become contaminated by airborne spores if the port of origin is located in close proximity to areas where guava rust is established (13).

Humans can also transport rust fungi, either deliberately or inadvertently (36). Spores adhering to clothing and personal effects of passengers arriving from countries where the pathogen is established is a known mode of introduction (29,33,35,36). In the early 1980s, Sheridan detected approximately 70,000 viable rust urediniospores of diverse species present on clothing and baggage of Australian passengers arriving into Wellington airport, New Zealand (29). Furthermore, Wellings et al. (36) investigated the viability of *P. striiformis* f. sp. *tritici* urediniospores present on clothing. Contaminated clothing was stored at room temperature for 1 week before being shaken over susceptible seedlings. After 2 weeks, seedlings were heavily infected with uredinia and sporulation was abundant. To minimize the risk of pathogen introductions via this pathway, they recommended washing clothing and personal effects suspected of being contaminated. However, a study by Sheridan and Nendick (30) found that one machine wash of contaminated clothing removed only half of the spores present, and many may have remained viable after washing.

This study describes the design and testing of a collection device for rust spores that was developed to sample three potentially high-risk, border-crossing pathways relevant to guava rust: passengers arriving by air, fresh cut flowers arriving as air cargo, and sea cargo. To extract spores from commodities and surfaces on the putative pathways, a device comprising an open-face filter housing connected to a hand-held vacuum cleaner was purpose-built, and was tested in the laboratory and the field to establish its utility as a spore collection tool. The outcomes of these experiments are reported here. Possible applications of the device in wider contexts and recommendations for further experimentation are discussed.

Materials and Methods

Spore collection device and components. A hand-held and fully portable Dyson DC31 vacuum cleaner (model number DC31DMMHSP; Dyson Appliances Limited), measuring 320 by

Fig. 1. Spore collection device comprising a modified filter housing connected to a Dyson DC31 vacuum cleaner.

110 by 200 mm, was modified to function as a spore collection device (Fig. 1). A 47-mm-diameter circular aluminum open-face filter housing (Pall Life Sciences, Pall Corporation) was adapted to screw on to the original hose that connects to the vacuum outlet via a threaded plastic block that was permanently fixed to the hose (Fig. 2).

Single 5- μ m pore-size, 47-mm-diameter PVC filter membranes (Pall Life Sciences, Pall Corporation) were placed in the filter housing and used to trap spores and debris. A permeable stainless-steel mesh screen covering a 3-mm-pore-size stainless steel backing plate supported the filter membrane during vacuuming. An o-ring (Viton) was locked around the outer 6 mm of the filter membrane to fasten it during vacuuming, leaving a total area of 9.6 cm² available to trap spores and debris. All components of the filter housing were periodically removed (by unscrewing it from the plastic block permanently fixed to the hose) for steam sterilization in an autoclave at 121 to 123°C (250 to 253°F) and 1.0 bar (100 kPa, 15 psi) for 15 to 20 min.

A digital motor powered the vacuum and centrifugal forces spun dirt and debris at an airflow rate of 24.4 CFM and a velocity of 12 m/s. The vacuum's standard power mode delivered continuous suction for 15 min, corresponding to a suction power of 337 mm H₂O.

Laboratory experiments. Laboratory testing commenced in July 2010. Eight surface types comparable with those on the target pathways were tested: cotton and denim (passenger pathway); roses, carnations, and chrysanthemums (fresh cut flower pathway); and wood, plastic, and metal (sea cargo pathway). Each “surface” was defined as a pair of jeans (denim); a t-shirt (cotton); a 10-stem bunch each of roses, carnations, and chrysanthemums (wrapped in paper or plastic); and a single 50-by-50-cm sheet of wood (rough-sawn treated pine), plastic (flexible PVC), and metal (stainless steel).

Plant material. Quarantine restrictions in Australia precluded use of *P. psidii* urediniospores in the laboratory experiments. *Uromycladium tepperianum* (Sacc.) McAlpine and *P. tritricina* Erikss., two common species of rust fungi in Australia, were used as model pathogens because they are pervasive in Australia and their spore morphologies are similar to urediniospores of *P. psidii* (10,14,15).

Fig. 2. Detachable filter housing that connects to the vacuum and traps spores and debris during vacuuming.

Teliospores of the Acacia gall rust fungus, *U. tepperianum*, were harvested from a diseased *Acacia saligna* tree located at the far northeastern corner of the Murdoch University campus (Western Australia, Australia). Heavily affected sections were cut and teliospores were harvested from the galls by scraping the surface with a clean scalpel. The spores were air dried at room temperature for 24 h and stored in 1.7-ml microcentrifuge tubes (Axygen Incorporated). Urediniospores of the wheat leaf rust pathogen, *P. tritici* Erikss., were harvested and supplied by the Western Australian Department of Agriculture and Food.

Inoculation protocol. Surfaces were inoculated by spraying spores using a Preval Sprayer Unit (Precision Valve Corporation) that comprised an atomizer attached to a glass reservoir. This application method is widely reported in other similar contexts (7,22,24) and allowed spores to disperse and lightly adhere to the surfaces, thereby mimicking natural dispersal mechanisms.

One Preval Sprayer Unit was used for each species' suspension and each was prepared by vortexing 1 mg of spores in 100 ml of deionized water for approximately 30 min. The concentration of spores in each suspension was standardized at 1,000 spores ml⁻¹, which was verified by spraying three replicate PVC filter membranes with 1 ml of suspension at a distance of 50 mm and counting the number of spores present within the entire area of the filter membrane using a dissecting microscope (×70 magnification). (The diameter of the spray "fan" was approximately the same diameter as the filter membrane at a distance of 50 mm; hence, all spores sprayed at this distance landed within the filter membrane.) The mean spore count across the three replicates for each suspension was used in the subsequent spore recovery calculation.

The eight surface types were prepared for each model pathogen. Each surface was partitioned into three discrete areas to give three replicates per surface, with the exception of the flowers, where a 10-stem bunch wrapped in paper or plastic was one replicate. Each replicate was directly sprayed with 1 ml of suspension at a distance of 50 mm (as described above). Due to the structural complexity of the flowers, and to ensure that all spores sprayed onto the flowers actually landed within the bunch, each flower bunch was unwrapped and the individual flowers laid out in a uniform layer on their paper or plastic wrapping prior to inoculation.

Quantifying spore recovery. Once dry, each replicate of each surface, including all parts of the flowers and their wrapping, was vacuumed for 30 s using a single filter membrane. This length of time was anticipated as the maximum allowable time to sample passengers (the most time-sensitive pathway) in the pathway sampling. Between each replicate, the filter housing and tools (e.g., tweezers) were thoroughly cleaned with alcohol swabs (Medi-Swabs, saturated with 70% [vol/vol] isopropyl alcohol) to minimize the risk of cross-contamination. Using a dissecting microscope (×70 magnification), the number of spores present within the entire area of each filter membrane was counted. The number of spores recovered from each replicate was calculated as a percentage of the suspensions' mean spore counts.

Field experiments. Field testing commenced in September 2010 and was carried out in collaboration with the NSW state government which, at the time, was implementing surveillance, tracing, quarantine, and destruction activities as part of the emergency response to an outbreak of "myrtle rust" on the Central Coast of NSW, Australia (3).

Sampling protocol. The selection of sites, or infected premises (IPs), for sampling was based largely upon accessibility and the

number of symptomatic myrtle rust host plants detected at each IP. The numbers and types of samples collected at each IP could not be predetermined because the exact nature of the IPs (i.e., the number of symptomatic and asymptomatic myrtle rust host plants) was unknown prior to arrival. The objective was to take samples directly from myrtle rust host plants with visible symptoms of disease (e.g., leaf and shoot lesions that, when young, are distinct yellow spots). Myrtle rust host plants with no visible symptoms of disease (i.e., either healthy or diseased but undetectable), myrtle rust nonhost plants, and inanimate objects (e.g., clothing and vehicles of surveillance personnel, and objects located within IPs) located in close proximity to symptomatic myrtle rust host plants were also sampled. Details of each IP targeted, including the number of symptomatic myrtle rust host plants detected and the number of samples collected at each, are shown in Table 1.

The accessible leaves, shoots, and (where applicable) fruit of target plants were vacuumed, with particular focus on the sporulating uredinial pustules that are present on young, actively growing leaves and shoots of susceptible myrtle rust host plants. For the inanimate objects, all accessible areas were vacuumed (where the objects were too large to be vacuumed entirely, representative areas were vacuumed). Samples were collected in batches at each IP to minimize the risk of cross-contamination. That is, symptomatic myrtle rust host plants were sampled in one batch and asymptomatic myrtle rust host plants, myrtle rust nonhost plants, and inanimate objects were sampled in another batch. In addition, the sampler wore gloves and thoroughly cleaned the vacuum, filter housing, and tools with alcohol swabs between each sample. A single filter membrane was used for each plant or inanimate object, and each was stored in individual paper envelopes.

Sample handling and analysis. Samples were gamma irradiated at 25 kGray and packaged according to state biosecurity procedures before they were dispatched to a laboratory in Tasmania, Australia, for analysis. Each filter membrane was cut into one half and two quarters. One arbitrarily selected quarter was analyzed for the presence of *P. psidii* using a DNA extraction protocol followed by a nested polymerase chain reaction (PCR) assay developed for *P. psidii* (20), which also detects the Australian strain (6). The DNA extraction protocol was adapted to accommodate the filter membrane samples, and an initial amplification of the ribosomal DNA internal transcribed spacer (ITS) region using the fungal-specific primers ITS1-F (11) and ITS4 (37) was carried out before the specific assay in order to enhance the sensitivity to within the detection thresholds reported in the original publication (M. Glen, *unpublished*).

To estimate the numbers of spores present in each sample, and as a basic crosscheck for potential false negatives from the PCR tests, another quarter of each sample was systematically visually inspected using a dissecting microscope (×70 magnification). Each filter membrane was stained with Melzer's reagent to highlight fungal material. Many samples, particularly those from symptomatic myrtle rust host plants, were expected to contain large numbers of *P. psidii* spores. For these samples, a wedge approximately one-fifth the size of the filter membrane quarter was cut and the number of spores present on the wedge was estimated by visually scanning the entire area of the wedge. The count estimation was multiplied by five and categorized as follows: A, 0 spores; B, ≥1 but <20 spores; C, >20 but <100 spores; D, >100 but <500 spores; E, >500 but <2,000 spores; and F, >2,000 spores.

Table 1. Details of the infected premises sampled during the field experiments

Type of infected premises	Number of symptomatic myrtle rust host plants detected (at the time of sampling)	Number of samples
Nursery 1	Approximately 10 <i>Gossia inophloia</i> (syn. <i>Austromyrtus inophloia</i>) potted juvenile plants	10
Private residence	1 native <i>Syzygium jambos</i> tree (approximately 2 m tall)	3
Commercial cut-flower property	>1,000 <i>Agonis flexuosa</i> 'Afterdark' plants and several native plants within and immediately surrounding the property, including <i>Syncarpia glomulifera</i> (windbreak) trees	7
Nursery 2	>300 <i>G. inophloia</i> (syn. <i>A. inophloia</i>) potted juvenile plants	23
State institution	4 native <i>Rhodamnia rubescens</i> trees (approximately 6–8 m tall)	17

Results

Laboratory experiments. The mean spore counts in the suspensions were 1,068 spores ml⁻¹ (standard deviation [SD] ± 72, n = 3) for *U. tepperianum* and 1,113 spores ml⁻¹ (SD ± 122, n = 3) for *P. triticina*. Consistently similar spore counts between replicates were observed for several batches of suspensions that were prepared over the course of the laboratory experiments (*data not shown*).

The highest percentage of spores was recovered from denim clothing for *U. tepperianum* (mean = 62%, SD ± 8.4, n = 3) and *P. triticina* (mean = 61%, SD ± 7.2, n = 3) (Fig. 3). The percentage of spores recovered from the hard surfaces (i.e., wood, plastic, and metal) and all three kinds of flowers was comparatively lower for both species. There was no significant difference in spore recovery percentages between species for the various surfaces, with the exception of the wood surface, where *P. triticina* had a significantly (*P* = 0.03) higher percentage recovery (Fig. 3).

Fig. 3. Percentage of *Uromyces tepperianum* (white) and *Puccinia triticina* Erikss. (black) spores recovered from each of the eight different surfaces. Error bars are standard errors of the means and the asterisk denotes a statistically significant difference (*P* = 0.03).

Field experiments. In total, 60 samples were collected across the five IPs; 21 of these were from symptomatic myrtle rust host plants (Table 2). Field observations indicated that many of these samples had thousands of *P. psidii* spores because the (white) filter membranes were distinctly yellow after vacuuming just a few sori. All of these samples produced positive PCR results, with the exception of three that remain unknown because they were inadvertently destroyed during the DNA extraction process. Furthermore, visual inspections confirmed the presence of thousands of urediniospores in many of these samples (Table 2; Fig. 4). They also revealed the distribution of spores on the filter membrane, which was useful when deciding on how to section each for analyses. Spores (and debris) collected during vacuuming were most densely deposited around the outer perimeter of the filter membrane where the oring was positioned, creating a discernible ring (Fig. 5).

Positive PCR results were also produced for five samples from asymptomatic myrtle rust host plants (sample IDs 007, 008, 011, 013, and 014), one sample from a myrtle rust nonhost plant (sample ID 054), and one sample from a 6-week-old mulch heap of *Syncarpia glomulifera* and *Agonis flexuosa* ‘Afterdark’ (sample ID

Fig. 4. Masses of *Puccinia psidii* urediniospores (arrows) vacuumed from the leaves and shoots of *Gossia inophloia* (syn. *Austromyrtus inophloia*) (sample ID 021). Bar = 0.1 mm.

Table 2. Details of each sample collected from symptomatic myrtle rust host plants, including the results of spore count estimates and polymerase chain reaction (PCR) tests

Sample ID	Type of infected premises	Description of sample ^x	Spore count estimate ^y	PCR result ^z
001	Nursery 1	Leaves and shoots of <i>Gossia inophloia</i>	D	Positive
002	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	D	Positive
003	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	E	Positive
004	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	E	Positive
005	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	D	Positive
006	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	D	Positive
010	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	C	Unknown
021	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	F	Positive
022	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	E	Positive
023	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	F	Unknown
024	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	E	Positive
025	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	F	Positive
026	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	C	Positive
027	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Positive
028	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Positive
029	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	C	Positive
030	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Unknown
044	State institution	Leaves, shoots, and fruit of <i>Rhodamnia rubescens</i>	F	Positive
045	State institution	Leaves, shoots, and fruit of <i>R. rubescens</i>	E	Positive
046	State institution	Leaves, shoots, and fruit of <i>R. rubescens</i>	F	Positive
047	State institution	Leaves, shoots, and fruit of <i>R. rubescens</i>	D	Positive

^x Spore count estimates: A, 0 spores; B, ≥1 but <20 spores; C, >20 but <100 spores; D, >100 but <500 spores; E, >500 but <2,000 spores; and F, >2,000 spores.

^y *Gossia inophloia* (syn. *Austromyrtus inophloia*).

^z Unknown = samples were inadvertently destroyed during the DNA extraction process.

018) (Table 3). All of these samples were either adjacent to or within a few meters of symptomatic myrtle rust host plants, with the exception of the last sample (sample ID 018), where all remaining myrtle rust host plants on the premises had been removed and destroyed during the 3 months preceding the field experiments. Up to 100 spores were observed in most of these samples; however, no spores were observed in one of the samples from an asymptomatic

myrtle rust host plant (sample ID 014) and the sample from the 6-week-old mulch heap (sample ID 018) that was visibly contaminated with soil (Table 3).

More surprisingly, positive PCR results were produced for four samples from inanimate objects: one sample from clothing worn underneath disposable overalls worn onsite (sample ID 019), two samples from the outer surface of disposable overalls worn onsite (sample IDs 041 and 042), and one sample from the wheel of a vehicle parked near symptomatic myrtle rust host plants (sample ID 058) (Table 4). These samples were in close proximity to symptomatic myrtle rust host plants, with the exception of the clothing worn underneath the overalls (sample ID 019), which was always covered by the full-body-length overalls worn onsite by surveillance personnel. Up to 100 spores were observed in the two samples from the outer surface of disposable overalls (sample IDs 041 and 042); however, no spores were observed in the dirtier samples from clothing worn underneath disposable overalls (sample ID 019) and the wheel of a vehicle (sample ID 058) (Table 4).

Discussion

Although there are many examples of commercially available sampling devices for the collection of fungal plant pathogens, their size and functionality are such that it was not feasible to collect samples from pathway surfaces and commodities. This led to the development of a novel spore collection device for our particular application, and we found several examples in the literature of purpose-built vacuum devices for rust spore sampling (27,30). To be effective, the device had to recover spores from a range of surfaces and, for operational reasons, it also had to be portable, be able to be cleaned between samples to minimize cross-contamination, and use sample media suitable for molecular analyses.

The device was evaluated in the laboratory and the field to establish its usefulness as a spore collection tool. In the laboratory, spore recovery percentages were highest for the pliable clothing compared with the rigid surfaces and structurally complex flowers

Fig. 5. Sample from the outer surface of clothing worn underneath disposable overalls by surveillance personnel during inspections at an infected premises on the Central Coast of New South Wales, Australia (sample ID 043). Bar = 10 mm.

Table 3. Details of each sample collected from asymptomatic myrtle rust host and myrtle rust nonhost plants located near symptomatic myrtle rust host plants, including the results of spore count estimates and polymerase chain reaction (PCR) tests

Sample ID	Type of infected premises	Description of sample ^w	Spore count estimate ^x	PCR result ^y
007	Nursery 1	Leaves and shoots of <i>Gossia inophloia</i>	C	Positive
008	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	C	Positive
009	Nursery 1	Leaves and shoots of <i>G. inophloia</i>	C	Unknown
031	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	A	Negative
032	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	A	Negative
033	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Negative
034	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Negative
035	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	A	Negative
036	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	A	Negative
037	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	B	Negative
038	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	C	Negative
039	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	C	Negative
040	Nursery 2	Leaves and shoots of <i>G. inophloia</i>	C	Negative
011	Private residence	Leaves and shoots of <i>Syzygium jambos</i>	C	Positive
012	Private residence	Leaves and shoots of <i>S. jambos</i>	C	Negative
013	Private residence	Leaves and shoots of <i>S. jambos</i>	B	Positive
014	Commercial	Leaves and shoots of <i>Leptospermum rotundifolium</i>	A	Positive
015	Commercial	Leaves and shoots of <i>L. rotundifolium</i>	A	Negative
016	Commercial	Leaves and shoots of <i>Regelia velutina</i>	A	Negative
017	Commercial	Leaves and shoots of <i>R. velutina</i>	C	Negative
048	State institution	Leaves and shoots of <i>S. smithii</i>	B	Negative
053	State institution	Leaves and shoots of <i>S. smithii</i>	A	Negative
055	State institution	Leaves and shoots of <i>S. smithii</i>	B	Negative
050	State institution	Leaves and shoots of <i>Hakea salicifolia</i>	A	Negative
054	State institution	Leaves and shoots of <i>H. salicifolia</i>	B	Positive
018 ^z	Commercial	Approximately 1 m ² of the top of <i>Syncarpia glomulifera</i> and <i>Agonis flexuosa</i> 'Afterdark' mulch heap (6 weeks old)	A	Positive

^w *Gossia inophloia* (syn. *Austromyrtus inophloia*); *Syzygium smithii* (syn. *Acmena smithii*); and *Hakea salicifolia* (syn. *H. saligna*), myrtle rust nonhost (family Proteaceae).

^x Spore count estimates: A, 0 spores; B, ≥1 but <20 spores; C, >20 but <100 spores; D, >100 but <500 spores; E, >500 but <2,000 spores; and F, >2,000 spores.

^y Unknown = samples were inadvertently destroyed during the DNA extraction process.

^z Sample was covered with a layer of soil similar to that shown in Figure 5, which interfered with visual inspections and may have caused inhibition in the PCR test.

for both model pathogens tested. The device was able to maintain greater suction on clothing compared with the other surfaces, which might explain the relatively high number of spores recovered from clothing. Variations in the surface ornamentation of spores and the physical and chemical nature of the surfaces (i.e., porosity, hydrophobicity, and electrostatic charge), which are known to play important roles in the adherence of spores (2,19), might also explain the differences in spore recovery percentages between surfaces and model pathogens.

Although the proportion of spores recovered could not be estimated in the field experiments, the results provide additional data that support the effectiveness of the device. In all instances, samples from symptomatic myrtle rust host plants produced positive PCR results. Visual inspections agreed with the PCR results, where spores were observed in all of these samples (the majority of which had many more than 100 spores). A few samples from asymptomatic myrtle rust host plants, myrtle rust nonhost plants, and inanimate objects that were located within close proximity to symptomatic myrtle rust host plants also produced positive PCR results. Visual inspections did not always agree with the PCR results for these samples, all of which had less than 100 spores.

At relatively low spore concentrations, some discrepancies between PCR results and spore count estimations are to be expected. First, the PCR testing and visual inspections were performed on different quarters of the filter membrane. Therefore, the quarter that was PCR tested may have had more or less spores than the quarter that was visually inspected. Further, we expected that it would be difficult to visually detect spores on at least a few samples, particularly those with relatively low spore numbers. This was especially the case for samples from inanimate objects because a great deal of soil and debris was often collected in addition to spores, making it very difficult to identify spores. This extraneous material might also have inhibited the PCR assay, making it less sensitive (18). In addition, the gamma irradiation treatment that the samples were subjected to prior to DNA extraction may have degraded the DNA enough to reduce the sensitivity of the PCR assay (17).

In addition to generating false negatives, false-positive results may also have arisen during PCR testing. As well as being highly sensitive and specific to the target pathogen, the triple-nested design of the PCR assay makes it prone to cross-contamination, which increases the rate of false positives. Although we cannot discount this explanation, all internal controls included in the DNA extraction and PCR stages produced negative results, indicating that the positive PCR results are genuine (M. Glen, *unpublished*).

False-positive results may have arisen during the field sampling as a result of insufficiently decontaminating the device between samples. The field strategy was to sample in batches: symptomatic myrtle rust host plants (known positives) in one batch and asymptomatic myrtle rust host plants, myrtle rust nonhost plants, and inanimate objects (expected negatives) in another batch. If cross-contamination between samples did occur, it would be most evident in the first few samples between batches. This pattern, or any other pattern, is not apparent in the field results, suggesting that cross-contamination did not occur.

The objective of this study was to experimentally test the device in the laboratory and the field to generate data that evaluate its effectiveness. The results demonstrate that the device can effectively recover different types of plant pathogens from surfaces comparable with the pathways that were the subject of the project. In addition to sampling pathways, the study demonstrates that the device is particularly useful when spores from symptomatic host plants deposit on nearby host and nonhost plants, people, and inanimate objects by airborne dispersal or physical contact transfer, thereby contaminating them. The device might also be useful during the early stages of disease when infection symptoms are not visible upon gross examination. These types of contamination are likely to be missed in current surveillance for plant pathogens because inspections rely on visual identification of infection symptoms, which might not be apparent at the time of surveillance, or at all, in the case of nonhost plants, people, and inanimate objects.

The implication of failing to detect early disease on host plants is obvious but failing to detect spore contamination on inanimate objects such as clothing, vehicles, and other mobile objects is potentially more serious because disease can be spread to previously unaffected areas. This can lead to an increase in the geographic extent of diseases and makes containment, monitoring, and eradication activities considerably more difficult. The inclusion of a portable spore-sampling device in surveillance programs, together with a quick and reliable diagnostic methodology, could facilitate early disease detection in plants and contamination on surveillance personnel and objects, which might improve plant biosecurity outcomes (16). The device can also be used to gather data about the effectiveness of quarantine measures used during containment and eradication activities and, thus, provide input to risk analysis of spread mediated by such activities and support the design and testing of more effective quarantine measures.

The ability to collect spores in samples from objects on unregulated pathways—either on arrival at the receiving country or,

Table 4. Details of each sample collected from inanimate objects located near symptomatic myrtle rust host plants, including the results of spore count estimates and polymerase chain reaction (PCR) tests

Sample ID	Type of infected premises	Description of sample	Spore count estimate ^y	PCR result
019 ^z	Commercial	Outer surface of clothing worn underneath disposable overalls (arms, shoulders, collar, cuffs of shirt; pockets, front and back of lower legs, cuffs of trousers)	A	Positive
020 ^z	Commercial	Outer surface of clothing worn underneath disposable overalls (arms, shoulders, collar, cuffs of shirt; pockets, front and back of lower legs, cuffs of trousers)	A	Negative
043 ^z	Nursery 2	Outer surface of clothing worn underneath disposable overalls (arms, shoulders, collar, cuffs of shirt; pockets, front and back of lower legs, cuffs of trousers)	B	Negative
060 ^z	State institution	Outer surface of clothing worn underneath disposable overalls (arms, shoulders, collar, cuffs of shirt; pockets, front and back of lower legs, cuffs of trousers)	B	Negative
041	Nursery 2	Outer surface of disposable overalls (arms, shoulders, legs, head, cuffs)	C	Positive
042	Nursery 2	Outer surface of disposable overalls (arms, shoulders, legs, head, cuffs)	C	Positive
056	State institution	Outer surface of disposable overalls (arms, shoulders, legs, head, cuffs)	B	Negative
057	State institution	Outer surface of disposable overalls (arms, shoulders, legs, head, cuffs)	B	Negative
049 ^z	State institution	Doormat located near building entrance and almost directly underneath sample ID 044	B	Negative
051	State institution	Wooden pallets positioned directly underneath sample ID 046	A	Negative
052	State institution	Sandstone bricks located approximately 10 m north of sample ID 046	B	Negative
058 ^z	State institution	Accessible parts of front tire and wheel arch of vehicle parked approximately 4 m east of sample ID 047	A	Positive
059 ^z	State institution	Driver's front seat and area under steering wheel of vehicle in sample ID 058	A	Negative

^y Spore count estimates: A, 0 spores; B, ≥ 1 but < 20 spores; C, > 20 but < 100 spores; D, > 100 but < 500 spores; E, > 500 but $< 2,000$ spores; and F, $> 2,000$ spores.

^z Sample was covered with a layer of soil similar to that shown in Figure 5, which interfered with visual inspections and may have caused inhibition in the PCR test.

potentially, on departure in the source country—makes it possible to estimate the approach rate of contaminated objects on those pathways, and assess relative risk of entry for the pathogen. Furthermore, the ability to collect samples from surfaces that cannot be transported to a laboratory (e.g., a shipping container) makes it possible to conduct surveillance for regulated pathways and pathogens. Collectively, this information can both inform risk analysis and support decision making about the allocation of surveillance resources and the regulation of trade (26).

To supplement the data that support the outcomes of this study, we recommend further experimental work that might include evaluating the effectiveness of the device against a larger group of plant pathogens, testing more surface types to infer effectiveness on additional pathways, and investigating different types of vacuum and filter housing designs and filters.

Possible improvements to the design of the device might include the following. (i) Use of a higher-powered vacuum to improve the effectiveness of spore extraction. (ii) Use of a filter (with a larger pore size) between the vacuumed surface and the filter membrane to reduce the amount of extraneous material collected in the sample. This could improve the sensitivity of the PCR testing but may also reduce the effectiveness of spore extraction (if spores are bound up in the matrix of extraneous material). (iii) Use of an enclosed filtration vessel that can be sealed after sample collection, detached from the vacuum, and sent to the laboratory for testing without the need for handling and packaging at the sample collection location. This would reduce the likelihood of contamination of the filter membrane sample prior to receipt by the laboratory.

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