Ultraviolet-C (UV-C) for disease and pest management and automating UV-C delivery technology for strawberry

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Ultraviolet-C (UV-C) for disease and pest management and automating UV-C delivery technology for strawberry

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Abstract

Alternative disease and pest management strategies are needed due to increased resistance by strawberry pathogens and arthropod pests to currently used synthetic pesticides and increasing consumer demands for fruit without pesticide residues. Thus, novel approaches that are ecologically sound, sustainable and do not rely on the application of synthetic chemicals are urgently needed. Prior research on ultraviolet (UV) light to control fungal diseases was hampered by high plant damage. In 2011, we developed a method for controlling diseases of strawberry with night-time conventional UV-C (254 nm) irradiation treatment, which resulted in lowering of UV-C doses required to kill fungi (*Botrytis cinerea*, *Podosphaera aphanis*, and *Colletotrichum acutatum* and *gloeosporioides*) and subsequently this work was extended to control arthropod pests (e.g., two-spotted spider mite) with no adverse effects on the strawberry plant. Here we report on further progress made on field UV-C application technology (e.g., robotics and UV-C light array), control of spotted wing *Drosophila* with UV-C light, effect of UV-C irradiating on fungal pathogens causing anthracnose (*Colletotrichum* spp.) with low UV-C doses (≤36 J m^{-2}) of 254 nm and farUV at 220 nm and induction of fungal resistance in strawberry plants with UV-C irradiation.

Keywords: *Fragaria*, anthracnose, two-spotted spider mite, *Colletotrichum*, robotics, dark period, automation, robot

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is cultivated under diverse environmental conditions using different production systems to make fruit available year-round. This creates challenges for controlling pathogens and pests. Gray mold (*Botrytis cinerea*), anthracnose (*Colletotrichum* spp.) and powdery mildew (*Podosphaera aphanis*), and arthropod pests (e.g., two-spotted spider mite and spotted wing drosophila) can cause severe crop losses if not managed during production and after harvest. Currently, growers in California apply more than 20 fungicide/insecticide/miticide sprays to control fungal diseases and arthropod pests (Strand, 2008) and use myriads of biological and mechanical measures each year. Fungicide applications begin in the field prior to bloom to protect flowers because infection at this stage accounts for as much as 80% of fruit decay at and after harvest (Bulger et al., 1987), and insecticides and miticides are applied as needed during the growing season. Frequent applications of pesticides have led to resistance buildup in targeted and non-targeted strawberry pathogens and arthropod pests. The development of resistance makes chemical control of diseases and pests unsustainable.

Ultraviolet (UV-C) light (100 to 285 nm) kills microorganisms, plants, and animals. In Belgium, Van Delm et al. (2014) demonstrated the killing power of UV-C on powdery mildew fungus and other fungal pathogens in glasshouse strawberry system, but leaf burns also occurred. Thus, UV-C light has not been widely used in crop production due to its damaging effects to plants at the doses required to kill microorganisms and arthropod pests with daytime UV-C application. In Japan, Kanto et al. (2009) reported the promotion of resistance induction in strawberry plants against fungal pathogens by nightly 30-min UV-B irradiation treatments. Janisiewicz et al. (2016a, b), Short et al. (2018) and Leskey et al. (2020) reported that fungi that cause gray mold, anthracnose, and powdery mildew, and two-spotted spider mite and greenhouse whitefly, respectively, were controlled with low doses of UV-C (≤72 J m^{-2}), if darkness followed immediately after irradiation. The darkness prevented activation of the light-induced photolyase DNA repair mechanism of DNA damaged by UV-C irradiation (Pathak et al., 2020; Xu et al., 2019; Zhu et al., 2018) and greatly increased the lethality of UV-C irradiation treatment. The inclusion of a specific dark period after UV-C (254 nm) irradiation allowed for a substantial reduction in exposure time (s) and doses (J m^{-2}) (Janisiewicz et al., 2016a, b) compared to effectives doses reported by Van Delm et al. (2014). At these low doses, UV-C had no negative effects on strawberry plant phytotoxicity, pollen germination, tube growth, leaf chlorophyll content, photosynthetic activity, fruit set and fruit quality (Janisiewicz et al., 2016a, b). Recently, shorter 222 nm UV-C has been reported to kill viruses and bacteria (Buonanno et al., 2017; Kim et al., 2020). Here we describe the recent advances in UV-C application technology (e.g., robotics and UV-C light array for field application at night, management of spotted wing drosophila (*Drosophila suzukii*), uses of 254 and 222 nm UV-C spectral band for killing fungal pathogens, and elevation of induction of resistance to *Colletotrichum* and *Botrytis* infection in strawberry plants.

MATERIALS AND METHODS

Autonomous vehicle for field application of UV-C technology

In 2018, the design work was initiated to develop an autonomous vehicle capable of treating field-grown strawberry with UV-C light and the following steps were taken: 1) develop a robotic frame and drivetrain with geometry capable of navigating on strawberry farms with velocity control, fail-safe override in software architecture; and 2) develop a UV-C light array that can be used on the autonomous platform with height sensing, a modular mechanism for mounting light array and integrated to autonomous control/power system.

The UV-C light array for this study was designed for one-row configuration. The array comprised multiple 55 W 254 nm UV-C lamps and reflectors mounted under a shield. A unique flexible canopy reflective surfaces was designed to provide >80% UV-C reflectivity. The modularity of the robot allowed adjustability in UV-C light array configuration for treatment in increments of 0.9 m width and for treating 0.2 to 1.4 ha farms during a 5-h run. To ensure that the robot platform could provide adequate treatment under a variety of field conditions using proper mobility and navigation systems, three robots were built and evaluated at three sites. Two robots were operated at night. The third robot was operated during the day due to work restriction imposed by the COVID-19 pandemic. At all three sites, the robots were programmed to operate three times per week beginning at pre-bloom stage for 10 weeks. Records were maintained on robot performance and the plots were checked weekly for disease, arthropod pests, and plant development.

Management of spotted wing drosophila (SWD)

Strawberries from California were purchased at a local supermarket and then rinsed thoroughly with water to remove any lingering pesticide residues. Three strawberries of similar weight were suspended equidistant among the leaves of 16 potted 'Chandler' strawberry plants placed on two shelves in a walk-in growth chamber. Eight plants were placed under the UV-C irradiation apparatus (Takeda et al., 2019) and eight plants were placed on the second shelf without the irradiation apparatus. A black-cloth curtain was placed between the two shelves to prevent indirect irradiation of strawberry plants on the second shelf (non-irradiated). UV-C irradiation was administered for 24 h in 15 s "on" and 15 s "off" cycles. Twenty adult SWD females were released on each plant immediately following the initiation of the light cycles, but only during the "off" portion of the irradiation cycles. The release was repeated on non-irradiated strawberry plants. After 24 h, strawberries were removed from the plant and each fruit was placed in a sealed, screened cup and held for ~17 days at 24°C after which adult SWD in each cup were counted. Additionally, other purchased strawberries were subjected to both positive and negative control treatments (10 fruit for each treatment) in a controlled-environment laboratory. Strawberries were held under the same conditions as the previously described irradiated fruit trial. The positive control fruit were placed individually into sealed, screened containers with two mated SWD females to ensure that the fruit were suitable for oviposition and there was no limitation of oviposition by mated females. The negative control fruit were placed individually into sealed, screened containers with no SWD. This was done to ensure that fruit were not already infested upon purchase from the supermarket. The percentage of fruit with emerged SWD adults and the number of SWD fruit^{-1} were determined.

Evaluation of different UV-C spectral band on *Colletotrichum*

Germicidal UV-C lamp (GermAway; CureUV, Delray Beach, FL 55-W Phillips Model TUVPL-L, Phillips North America Corp., Andover, MA USA) with maximum emission peak at 254 nm was used. Krypton Chloride excimer lamp (250W) with a maximum emission peak at 222 nm (MicroBuster; Sterilary Inc., Somersworth, NH) was also used for UV-C irradiation treatments. The lamps were mounted similarly on a rack and adjusted to 30 cm from the targeted irradiation surface frame (Takeda et al., 2019) and were enclosed with black cloth to confine the UV-C light. A calibrated spectrometer (Model EPP2000, StellarNet Inc., Tampa, FL) was used to obtain light intensity measurements per 0.5 nm bandwidth. The total irradiation (W m^{-2}) between 245 and 265 nm for the conventional germicidal lamp was 1.2 W m^{-2}. Therefore a 15, 30, 45, and 60 s exposure delivered 18, 36, 54, 72 J m^{-2}, respectively. The irradiance from the 222 nm Krepton Chloride excimer lamp between 210 and 235 nm at 30 cm distance was 2.95 W m^{-2}. Therefore, a 5, 30, 45, and 60 s exposure corresponded to an irradiance of 15, 90, 135, 180 J m^{-2}, respectively.

Conidia of *Colletotrichum gloeosporioides* (isolates 162) and *C. acutatum* were harvested from 5- to 10-day-old cultures grown on potato dextrose agar (PDA) with 0.05% Tween 20 (Sigma Aldrich, St. Louis, MO) by gently scraping culture surfaces with a glass rod and filtering through two layers of cheese cloth. The pathogen suspension was adjusted with sterile distilled water to 10^3 and 10^4 conidia mL^{-1} using hemacytometer and aliquots of 100 µL spread onto 10-cm diameter Petri plates with PDA. Plates were randomized within each set of concentrations then placed on trays by treatment (UV-C sources and exposure duration), and after 20 min the lids were removed and the plates were placed under UV-C (254 nm) or farUV (222 nm) lights at a distance of 30 cm. The plates were exposed to irradiation from 254 nm UV-C for 15, 30, 45, or 60 s or from 222 nm farUV for 5, 15, 30, 45, or 60 s after which the lids were placed back on the plates. One set of plates was placed immediately in light and the other was held in dark for 4 h before placing under light. All covered plates were sealed with parafilm, and incubated at 25°C. The number of colonies that emerged from the conidia were counted after four days of incubation. The counts from 10^3 and/or 10^4 dilutions, whichever was countable and not overgrown were used and the results were extrapolated to single original concentration and expressed as relative CFU plate^{-1}.

Induced resistance

Potted 'Chandler' strawberry plants were irradiated nightly for 60 s with 254 nm germicidal UV-C light for 4 weeks. Nine leaflets (3 replicates of 3 leaflets) were harvested from UV-C treated and from control plants and challenged with *Colletotrichum gloeosporioides* (isolates 162). Two agar plugs cut from the actively growing margin of a *Colletotrichum* culture grown on PDA were placed with mycelial side facing the leaf on nine leaflets and two plugs from a blank PDA were placed on nine leaflets harvested from UV-C treated and control plants. Two agar plugs cut from actively growing margins of *Botrytis cinerea* culture grown on PDA were placed on strawberry leaflets with mycelial side facing the leaf. Over a period of 7 to 10 days infection (black and yellow lesion) development was observed for both *Colletotrichum* and *Botrytis* assays and lesion size (cm) was recorded after a 7-day incubation and the leaflets were photographed after 10 days.

RESULTS AND DISCUSSION

Development of field-ready autonomous vehicle for UV-C application

A robotic frame and drivetrain with geometry capable of navigating on strawberry farms was constructed for operating in research and commercial fields (Figure 1). Operating the platform through the 2020 season has given important design insights for every aspect of the robot's capabilities and of the challenges of autonomous vehicles in open fields. The three-wheel configuration maintained all wheels in contact with the ground and did not require suspension to maintain traction. Specifically, a tricycle style frame was adapted to reduce the width of the platform and overall adjustability requirement needed for different row geometries. A motorized front wheel allowed the robot to travel up and down rows and its steering mechanism kept the raised bed centered under the robot even in situations with uneven or curved rows and terrain changes within the field. For additional mobility in rough terrains, rear-wheel drive capability will be considered necessary to assist the front drive in wet conditions or for fields for >6% grade. Initially a chain and sprocket drive drove the front wheel, but a right-angle motor/gearbox expedited assembly, improved safety and ground-speed control. The cabling and electrical components were protected by internalizing them in the frame, however, an impact resistant plastic shields were added that offered extra water resistance and robustness to the design. The robot used an Intel i5 processor to fuse sensor feedback from motor encoders, camera, PIR sensors, inertial measurement units, RTK GPS, and other low bandwidth modalities which ensured adequate UV-C dosing. Beyond navigation and control, the robot prioritized safety and robustness, with failsafe features that protected users, checked system faults and prevented runaway risks. As part of field automation, redundancy in the system was important and necessary consideration.

Figure 1. A second-generation autonomous UV-C application platform undergoing trials at the Appalachian Fruit Research Station, in Kearneysville, WV in a research plot established to study the infestation of strawberry plants by pestiferous arthropods. The robot has a computer vision system to simultaneously capture images for later analysis. The person in the background is manually capturing images of strawberry plants with a camera mounted on a pushcart.

The initial robot platform was powered with two 24-V marine batteries with separate lithium-ion batteries powering the robot sensing and navigation systems and its UV-C treatment array. The pre-programmed system was able to travel over multiple rows of strawberries autonomously at constant velocity, exiting each row, and making a turn into an adjacent row and then traveling in the opposite direction, etc. For operating in larger fields in the future, a hybrid battery/propane generator is under consideration to provide higher output. However, this would necessitate refueling and complicates the power management system. Testing of such hybrid power systems is planned for future trials when higher acreage plantings will be treated with larger, multi-row UV-C treatment array. In 2020, three robots treated strawberry plots for 12 weeks on three dedicated experimental sites. At one of these sites, an operator was required to be present at the start of treatments to ensure that the robot was receiving an adequate GPS signal before the robot was run. Overall, one of the technical challenges was maintaining consistent signal with a low-cost RTK GPS system. This challenge has motivated the development of proprietary localization solution that will be validated in future literation of the robot.

Specialized UV-C light arrays were also developed by TRIC Robotics to maximize the platform treatment capability. The light arrays ranged from one to four germicidal lamps per raised bed depending on the number of plant rows per bed. Increased lamp numbers can allow for shorter treatment times but can also damage plants if left uncontrolled. Additionally, the robots featured a manual adjustment mechanism that provided users to adjust the distance between the lamps and plant surfaces. It is important that lamps are not placed too close to plants as that can potentially damage leaves and their photosynthetic apparatus. Automating the height adjustment features is one way to reduce manual labor for operating the robot and can ensure proper treatment is applied to plants without human error. Future versions of the robot will focus heavily on development of UV-C system with specific design attributes that will improve treatment of strawberry plants.

Management of spotted wing drosophila

Positive and negative tests of store purchased strawberries indicated that the fruit used in the study were free of SWD prior to irradiation trials and laboratory reared female SWD were able to lay eggs in the strawberry fruit (data not reported). Egg-laying activity on non-irradiated strawberries over the 24-h period from release of adult SWD to fruit harvest was high. SWD emerged from about 35% of non-irradiated strawberries compared to just 2.5% (one out 40 fruit was infested) of irradiated strawberries. Of those fruit that were infested with SWD, the average number of SWD emergence per fruit was 10 (Figure 2) in non-irradiated fruit and 0.2 in UV-C treated fruit (statistically significant at p=0.05). Additional studies are needed to examine the impact of reduced frequency and varying intensity levels of UV-C irradiation on the plant and SWD. Also, studies are planned to elucidate the mechanism by which UV-C irradiation treatments protected the strawberry fruit from SWD oviposition (e.g., repellency, direct mortality on adults or eggs).

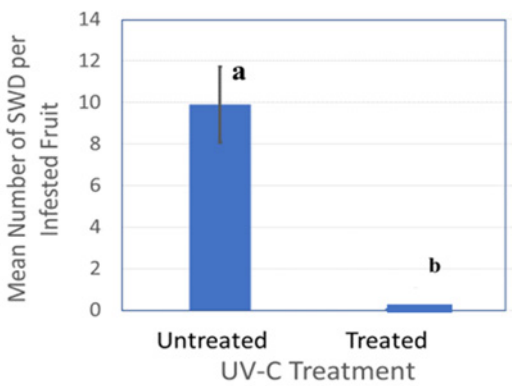

Figure 2. Effect of pulsed UV-C irradiation of strawberry fruit for 24 h on SWD emergence during 20-day incubation.

Killing power of other UV-C spectral wavelength

Spectral properties of conventional (254 nm) and farUV Krypton Chloride excimer (222 nm) UV-C lamps are presented in Figure 3. There were differences in the effects of conventional UV-C and farUV treatments on survival of all the pathogens evaluated in the study. FarUV was more effective in killing conidia of *C. gloeosporioides*-162 (Figure 4). Exposure to farUV irradiation for only 5 s effectively achieved 100% kill of *C. gloeosporioides* while 60 s exposure to conventional UV-C was required to achieve nearly 100% kill. Among other *Colletotrichum* species investigated, *C. acutatum* was the most susceptible to both conventional UV-C and farUV-C irradiation. FarUV-C irradiation was effective in killing *Colletotrichum* conidia in light and dark incubation conditions and other fungal pathogens including *Botrytis cinerea* and *Penicillium expansum* (data not reported).

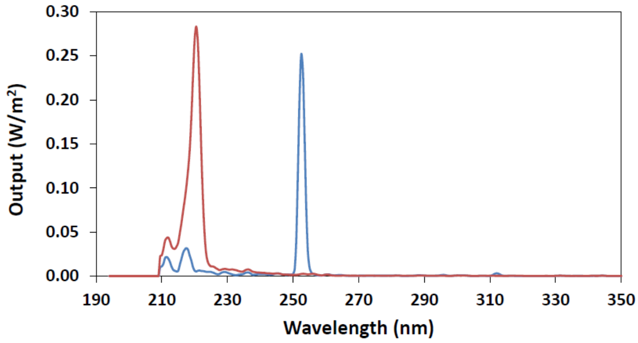

Figure 3. Spectral discharge (W m^{-2}) from a 55-W UV-C (254 nm, low-pressure mercury lamp (blue line) and 250 W 222 nm Krypton Chloride excimer lamp (red line) measured with a fiber optics StellarNet spectrometer at 30-cm distance from the lamp. The 254 nm mercury lamp also emitted 3% of its total irradiance in 210 to 215 nm range.

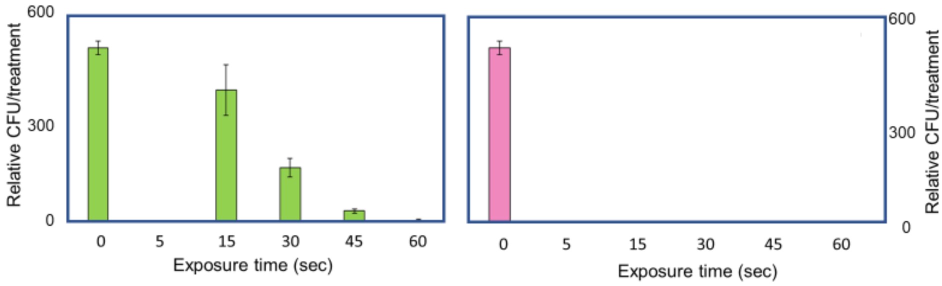

Figure 4. Survival of *Colletotrichum gloeosporioides*-162 conidia and production of fungal colonies on Petri plates with PDA medium (CFU = colony forming units) is affected by UV-C wavelength (254 nm, green bars, left; 222 nm pink bars, right) and exposure time (s). Note that farUV-C irradiation has a significantly greater killing power on *C. gloeosporioides*-162.

Induction of resistance to fungal infection

For this test, we harvested leaves from strawberry plants that had been treated with brief (15 to 60 s), nightly irradiation with UV-C (254 nm) for about a month and then inoculated each leaflet with 2 agar plugs with actively growing fungus causing anthracnose (*Colletotrichum*) (Figure 5). On the leaves taken from UV-C irradiated plants, the dark area was confined to the agar plug. However, leaflets taken from untreated plants contained black and yellow discolored lesions radiating out over a large area from the bed out from the plug. The growth of *Botrytis cinerea* on UV-C-treated leaflets was inhibited (Table 1). Our research has shown multiple effects of the UV-C treatments including direct effects on disease-causing pathogens as well as increasing resistance to fungal infection on plants exposed to night-time UV-C irradiation.

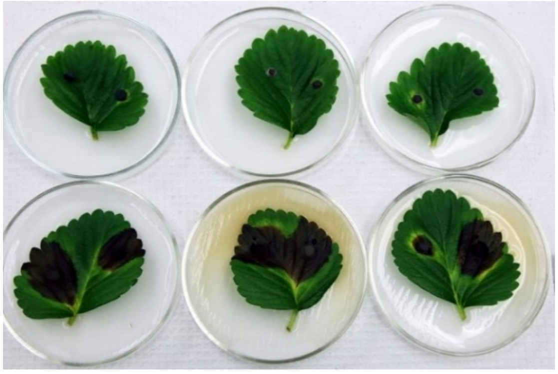

Figure 5. Induction of resistance to *Colletotrichum gloeosporioides*-162 infection by night-time UV-C irradiation treatment. Top row: leaflets harvested from strawberry plants irradiated with UV-C light. Bottom row: leaflets harvested from non-UV-C irradiated plants. Small, round dark area on the leaflets in the top row show the location of mycelial plug placement. Note that on irradiated leaflets the development of fungal lesion is indicated by a narrow yellow band around each mycelial plug. On non-irradiated leaflets the lesion(black) caused by *C. gloeosporioides*-162 infection has expanded >1 cm from inoculation sites.

Table 1. Effect of nightly UV-C irradiation of strawberry plants on subsequent infection by *Botrytis cinerea*. Two agar plugs (0.7 cm diameter) with *B. cinerea* mycelia were placed on each leaflet and incubated for 7 days. Lesion diameter measurements are the average of lesion growth on 3 leaflets.

Treatment	Mean lesion diameter (cm)	Std. error (cm)
Irradiated with UV-C (254 nm)	8.3	1.7
Control	24.6	2.1

Plants exposed to a variety of environmental or biotic stresses respond by producing more phenolic and other chemical compounds such as jasmonic acid to resist infection by a pathogen or attacks from insects and mites. The infection of strawberry leaves by *Colletotrichum gloeosporioides* was manifested as black and yellow lesion around the agar plugs (Figure 5). On the leaflets taken from UV-C irradiated plants, the dark area was limited to the agar plug area while on the leaflets taken from untreated plants the lesion (e.g., discolored area) expanded outward from the plugs and caused large areas of the leaflet to turn black and yellow. UV-C treatments affect gene expression in strawberry leaves and lead to overexpression of a set of pathogenesis-related genes (Xu et al., 2019). Previously, we reported that cumulative UV-C dose of 1.4 kJ m^{-2} over 30-day period had no effect on fruit phenolic contents (Sun et al., 2020). However, Xu et al. (2019) showed that in strawberry leaves exposed to cumulative UV-C dose of 12 kJ m^{-2} the total phenolics, volatile terpenes, reactive oxygen species, abscisic acid, salicylic acid, and jasmonic acid increased significantly.

CONCLUSIONS

UV-C light can become a viable option for managing diseases and pests for small and large growers. The irradiation of plants at night has enabled an effective control of three major fungal pathogens (e.g., *Botrytis*, *Colletotrichum*, and *Podosphaera*) and arthropod pests (e.g., two-spotted spider mite and greenhouse whitefly) without damaging plants or fruit (Janisiewicz et al., 2016a, b; Short et al., 2018; Leskey et al., 2020). Night-time application of UV-C delivering a dose ranging from 75 to 100 J m^{-2} two or three times per week should be adequate for managing these pests in either greenhouse or outdoor production system. Multiple lamps and use of reflective surfaces are necessary to make certain that illumination of abaxial surfaces is adequate when the primary target organism is either the two-spotted spider mite or powdery mildew. Development of a functional autonomous vehicle for night-time application of UV-C technology can cover a large treatment area while providing good control with short exposure times. Advances are being made to the robotic system to make this technology useful and affordable for small-scale strawberry growers as well as for the rapidly expanding organic-certified farms that are generally ≤2 ha in size.

The management of disease-causing fungal pathogens and arthropod pests is a complex task for specialty crop producers. Failure to manage *Botrytis cinerea* at bloom and SWD as fruit nears harvest can greatly impact marketability of fruit to consumer. The occurrence of latent infection and the highly perishable nature of fruits and berries, and the need for a complete control of diseases and pests in the berry field is a challenge especially when attempting to develop an effective non-chemical crop protection system. An effective control of these diseases and pests requires a comprehensive knowledge of disease etiology, host plant and pest biology, and the physical environment determining the severity of infection and infestation. Future research will focus on multi-prong strategies instead of searching for a single approach to produce effective, long-term solutions for specialty crop growers. Technological advances in autonomous vehicles, light optics, and UV-C light arrays combined with biological control and genetic improvements, and good sanitation practices are needed to reduce disease and pests in a range of cropping and “clean” nursery systems.

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