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Preventing spread of Fusarium wilt of Hiemalis begonias in the greenhouse

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Abstract

To develop strategies to manage Fusarium wilt of Hiemalis begonias (*Begonia* × *Hiemalis*), caused by *Fusarium foetens*, studies were conducted to understand how the disease might be spread in the greenhouse. Inoculum density studies showed that as few as 100 conidial ml^{-1} were sufficient to cause significant disease indicating that shared irrigation systems need strict sanitation along with bench tops, pots, and trays. The role of fungus gnats (*Bradysia* spp.) in vectoring *F. foetens* within a greenhouse was studied using nylon tent cages with diseased and healthy Hiemalis begonias. Fungus gnats were released into half of the cages. In cages where fungus gnats were present only healthy plants became diseased, indicating the effectiveness of fungus gnats in spreading Fusarium wilt. The pathogen was also isolated from adult fungus gnats. *In vitro* studies showed that two commercially available H_2O_2-based compounds, ZeroTol® (2.0% peroxyacetic acid and 27.0% hydrogen peroxide), and SaniDate® (12.0% peroxyacetic acid and 18.5% hydrogen peroxide), were effective in causing 100% spore mortality at rates that would allow their use in irrigation water. Seven cultivars of Hiemalis begonias (*Begonia* × *Hiemalis*) grown in soil infested with *F. foetens* were highly susceptible. No other Begonia species showed typical symptoms of chlorosis and wilt, but two cultivars of Rex begonia (*Begonia rex*) exhibited significant stunting in repeated trials.

Keywords: *Fusarium foetens*; Fungus gnats (*Bradysia* spp.); Hydrogen peroxide

1. Introduction

Fusarium wilt of Hiemalis begonias (*Begonia* × *Hiemalis* Fotsch) was not seen in North America until 2003 (Elmer et al., 2004). The disease had appeared 2 years earlier in the Netherlands and quickly spread to England and Germany (Schmalstieg et al., 2003; Schrage, 2003). The pathogen was initially described as *Fusarium oxysporum* (Neubauer and Nirenberg, 2002), but new observations indicated that some conidia were produced in polyphialides, which would distinguish it morphologically from *F. oxysporum* (Schroers et al., 2004). The new species was named *Fusarium foetens* in response to the pungent odours that were produced on potato dextrose agar *in vitro*. The odours were caused by volatile sesquiterpene compounds that are constitutively produced by this fungus *in vitro* (Tschoepe et al., 2007).

The new wilt disease was easily distinguished from another Fusarium stem rot disease reported in the 1990s that also attacked Hiemalis begonias. That disease, caused by *Fusarium begoniae* (Nirenberg and O'Donnell) [syn = *Fusarium sacchari* (Butler) W. Gams var. *elongatum*] (Cavat, 1993), caused a dry rot canker on stems of begonias and was not associated with root damage or vascular discolouration.

In Germany, Wohanka (2003) showed that *F. foetens* could spread rapidly in irrigation water in ebb and flow systems. Levels as low as 100 conidia ml^{-1} could incite the disease, but filtration and the use of chlorine dioxide applied at 1.5–1.7 ug ml^{-1} was effective in eliminating *F. foetens* from recycled water (Wohanka et al., 2005). Chlorine dioxide has also been used effectively in the US, but many operations have instead adopted H_2O_2 disinfectants for algae control because these products can be

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applied in irrigation lines. At present, information on what rates would be necessary to eliminate *F. foetens* in irrigation water is not available.

In Connecticut, the disease was observed to develop rapidly on benches sharing the same irrigation water, but disease spread was also observed to plants that did not share irrigation water. We recognized that another way *Fusarium* spp. can spread in greenhouses is by insects, most notably fungus gnats (*Bradysia* spp.). Fungus gnats have been implicated as vectors of *Fusarium* in conifer nurseries (Keates et al., 1989), and in greenhouses planted with tomato (Gillespie and Menzie, 1993) or lisianthus (El-Hamalawi and Stanghellini, 2005). Their role in disseminating *F. foetens* is not known.

The disease has only been observed on *Begonia* × *Hiemalis* cultivars in Connecticut. Other begonia types, such as Rex begonia (*Begonia* × *rex-cultorum* L. H. Bailey) and angel wing begonias (*Begonia coccinea* Hook), are also commonly grown in these operations, but their susceptibility was not known. In Germany, Wohanka (2003) demonstrated that *Begonia semperflorens-cultorum* Hook and *Begonia* × *tuberhybrida*, poinsettias (*Euphorbia pulcherrima*) and zonal geraniums (*Pelargonium zonale*) were not susceptible to the disease.

The objectives of this study were to determine what inoculum levels are necessary to incite severe disease in irrigation water, to determine if fungus gnats were important in vectoring the pathogen, to determine the efficacy of different sanitizing agents on eradicating the conidia of the pathogen, and to assess whether other commercially grown begonia species could serve as susceptible hosts.

2. Materials and methods

2.1. Effect of inoculum density on disease

Conidia were harvested from potato carrot agar (Dhingra and Sinclair, 1985) cultures grown for 5 d, then washed from the plates, and adjusted to the proper concentration with a haemacytometer. Seedling plugs of Hiemalis begonias cv. 'Emma' were transplanted into 10 cm pots filled with potting mix (Promix BX, Premier Brand, New Rochelle, NY) and allowed to grow for 1 week to produce new feeder roots. Pots were set into trays containing water supplemented with 0, 10, 100, 1,000, 10,000, and 100,000 conidia ml^{-1} of the begonia pathogen. About 15 min later, after the soilless mix had become saturated, pots were removed and set on greenhouse benches. There were five replicate plants per treatment. Plants were treated with 1.5 g of Marathon 1% G (imidacloprid) to inhibit insects and were watered as needed. Plants were rated for disease severity after 6 weeks using a scale of 1–5 in which 1 = no symptoms, 2 = slight dull-green coloration of the foliage, 3 = dull-green wilted foliage, 4 = stunted and wilting plants with distinct darkened lesions on basal stem sections, and 5 = dead or near death. Plants were destructively harvested for above-ground dry weights.

2.2. Efficacy of sanitizing agents on F. foetens

Varying concentrations of four disinfectants were examined for their ability to kill conidia of *F. foetens* in irrigation water. The disinfectants were household bleach (5.25% sodium hypochlorite), GreenShield® CA (quaternary ammonium compounds), ZeroTol (2.0% peroxyacetic acid and 27.0% hydrogen peroxide), and SaniDate® (12.0% peroxyacetic acid and 18.5% hydrogen peroxide). A range of concentrations was prepared in 5 ml quantities. A conidial suspension of *F. foetens* was added to the solution to give 500 spores ml^{-1}. The final concentrations of the disinfectants bracketed commercial rates and ranged from 50 to 10,000 µl l^{-1}. Sterile, distiled water served as the control. Spores were exposed to the disinfectants for 15 min, then three aliquots of 0.1 ml were spread onto minimal media (Correll et al., 1987). Plates were incubated at 22–25 °C for 3 d, and the number of colonies was counted. The experiment was repeated twice.

2.3. Transmission by fungus gnats

Inoculum was prepared from isolate O-2348 that was deposited at Fusarium Research Center, Pennsylvania State University, State College, PA. Twice-autoclaved millet and water (1:1 v/v) was seeded with a colonized agar plug and incubated for 10 d, dried, ground and passed through a 0.5 mm sieve. Seedling plugs of susceptible Hiemalis begonia 'Emma' were transplanted (one plant per pot) into pots filled three-quarters full with potting mix infested with *F. foetens* inoculum (1 g millet inoculum l^{-1} potting mix). The top quarter layer of each pot was filled with non-infested potting mix to reduce the possibility of airborne spore dispersal from infested pots to non-infested pots. Four plants that had been put into infested soil were placed into PVC cages (0.8 × 0.6 m) that had been enclosed in nylon mesh (100 µm) fabric. Six healthy seedlings of 'Emma' were transplanted into non-infested potting mix and placed in each cage, but kept approximately 0.3 m away from plants that were in infested soil. All plants were kept in saucers to prevent the sharing of irrigation water. Two cages were infested with 75 adult *Bradysia* spp. per cage. Adult fungus gnats were collected off the surface of healthy Easter lily pots by gently vacuuming them into collection vials and releasing them inside the cage. To check against any contamination, 25 adult fungus gnats were collected at the same time, briefly frozen, and placed onto Komada's selective media. After 5 d, the fungus gnats did not yield any *Fusarium* spp. In another set of control cages, no fungus gnats were released, and all plants were treated with 1.5 g of Marathon 1% G (imidacloprid) to inhibit any fungus gnat larvae that might have been present in the potting mix. Plants were grown within the cages for 2 months, whereupon the study was terminated. This

amount of time was long enough for fungus gnats to have completed their life cycles from adult to adult at least twice (Gillespie, 1986). Plants were destructively harvested and rated for disease using the scale described above. Twenty-five adult fungus gnats were captured, frozen, and placed on Komada's selective media and single conidia were sub-cultured from colonies onto carnation leaf agar for identification. Nit mutants (Correll et al., 1987) were selected and compared with tester strains of known pathogens to confirm the identity of the pathogen on the fungus gnat. The study was repeated. In the course of the study, we found that we could identify *F. foetens* by its characteristic pungent odour when colonies were grown on PDA for 5–7 d.

2.4. Susceptibility of begonia species

In three series of experiments, the susceptibility of four begonia species was evaluated in the greenhouse. The first series of experiments examined the susceptibility among seven cultivars of Hiemalis begonias to the new *Fusarium* pathogen. One dozen healthy seedlings (6-week-old) of each of the cultivars 'Bente,' 'Berseba,' 'Camilla,' 'Dina,' 'Emma,' 'Nadine,' and 'Tess' were obtained from a commercial producer and placed into 10 cm pots filled with potting mix infested with ground millet colonized by *F. foetens* (1 g millet inoculum l^{-1} potting mix). An equal number of plants were potted into potting mix infested with sterile, ground millet. Plants were randomly arranged on a greenhouse bench. The experiment was repeated the following year with six replicates.

A second set of studies examined the susceptibility of 12 cultivars of Rex begonia seedlings (10-week-old) to the pathogen. Cultivars 'Coral Sands,' 'Devils Paradise,' 'Flamingo Shoals,' 'Hurricane Bay,' 'Rainbow Reed,' 'Reggae,' 'Rum Painkiller,' 'Savannah Bay,' 'Silver Sands,' 'Trade Winds,' 'Tropical Breeze,' and 'White Caps' were obtained from a commercial producer and transplanted into infested mix as described above. There were 12 replicate pots filled with infested soil and 12 pots filled with non-infested soil. Hiemalis begonia 'Emma' was included as a susceptible control. The study was repeated with 10 replicates the next year.

The last series of experiments examined the susceptibility of tuberous begonias (*Begonia* × *tuberhybrida* Voss), angel wing begonias (*Begonia coccinea* Hook), and seedling begonias (*Begonia* × *semperflorens-cultorum*) to *F. foetens*. The tuberous begonias and the seedling begonias were provided as seedlings by a commercial producer whereas the angel wing begonias were rooted from 6 to 12 cm stem cuttings in potting mix. Twelve plants of each species were grown in infested potting mix and control plants were placed into non-infested potting mix. The experiment was repeated with four replicate plants.

All greenhouse experiments were conducted in 10 cm diam. plastic pots filled with ProMix BX potting mix. Unless noted, all pots were treated with 1.5 g of Marathon 1% G (imidacloprid) to inhibit insects. All plants were watered as needed and sharing of irrigation water was not allowed. Plants were rated for disease severity after 6 weeks using the scale of 1–5 described above. Plants were destructively harvested for above-ground weights that were colleted after tissue was air-dried. Samples of basal stem tissue were removed from any plant that showed symptoms of stunting or poor health, surface disinfested in 10% household bleach as described above, and placed onto Komada's selective medium (Komada, 1975) to determine if the pathogen could be recovered.

3. Results

3.1. Effect of inoculum density on disease

A polynomial fit best described the relationship between the log inoculum density and disease severity (Fig. 1). Symptoms appeared in a few plants treated with the 100 conidia ml^{-1} (log 2), and all plants showed high severe disease severity when inoculated with 100,000 conidia ml^{-1} (log 5). Similarly, a negative polynomial fit described the relationship between the log inoculum density and the dry weights of the plants. Disease severity and dry weights were inversely correlated ($R = 0.73$, $P < 0.001$).

3.2. Efficacy of sanitizing agents on F. foetens

The H_2O_2 product, ZeroTol®, was toxic at concentrations greater than 500 µl l^{-1} and had a LD_{50} of 258.0 µl l^{-1} (Fig. 2). The other H_2O_2 product, SaniDate, was more toxic to *F. foetens* than ZeroTol. Concentrations greater than 200 µl l^{-1} were 100% toxic to the conidia and the LD_{50} was 66.7 µl l^{-1}. The lowest concentrations (50 µl l^{-1}) of sodium hypochlorite (household bleach) and the quaternary ammonium compound (Greenshield®) used in these studies were still 100% toxic to *F. foetens* indicating

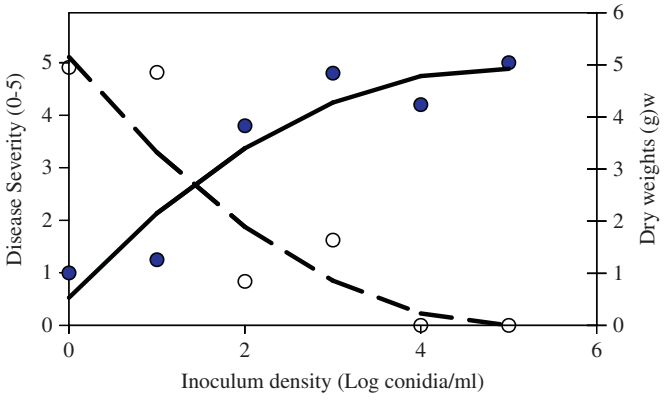

Fig. 1. Effect of inoculum density (ID) of *Fusarium foetens* on the disease severity rating (DR) ● and dry weights (DW) ○ of above-ground tissue of Hiemalis begonia 'Emma' after 6 weeks ($DR = -0.18\,ID^2 + 1.79ID + 0.53$; $R^2 = 0.68$; $P < 0.001$; $DW = 0.20ID^2 - 2.01ID + 5.11$; $R^2 = 0.54$; $P < 0.001$).

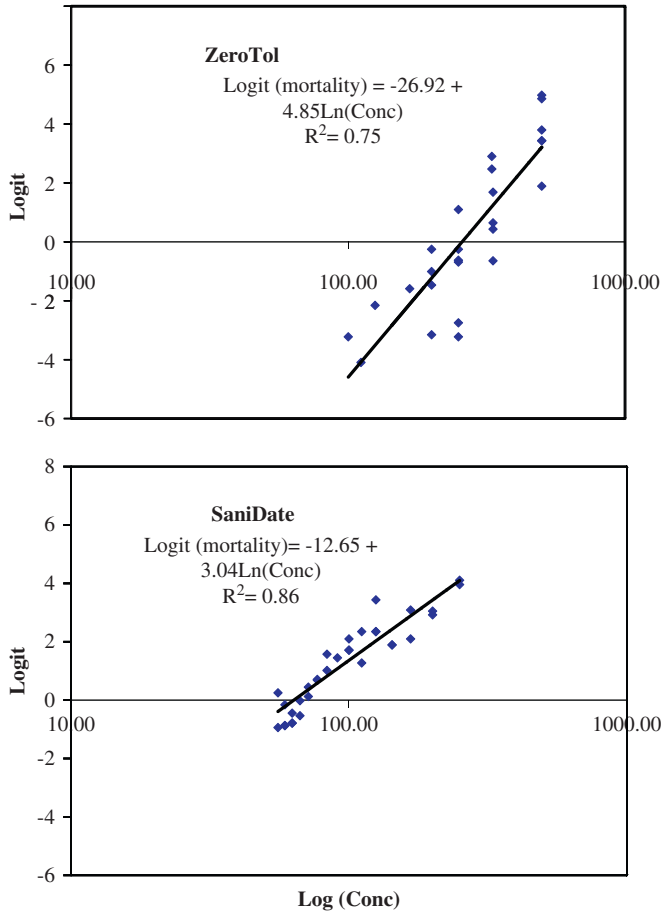

Fig. 2. Logit analysis of Zerotol® and SaniDate® for lethal dosage of *Fusarium foetens*.

that these products remain very powerful sterilants for use in the greenhouse.

3.3. Transmission by fungus gnats

When healthy begonias were caged with fungus gnats and symptomatic, infected begonias, symptoms began to appear in the healthy plants approximately 1 month later. Between 66% and 83% of the healthy plants had developed typical symptoms of Fusarium wilt after 2 months. Fungus gnats that were captured on sticky cards and placed onto Komada's selective media had yielded colonies of *F. foetens* 9% and 11% of the time (Table 1). Healthy plants grown in potting mix treated with the slow-release, granular insecticide Marathon 1% G (imidacloprid) and caged with symptomatic plants remained asymptomatic (Fig. 3). Surface disinfested stem and crown tissues from these plants were placed onto Komada's selective media and yielded no colonies of *Fusarium* spp.

3.4. Susceptibility of begonia species

There was no interaction between the repetition and cultivars so values were combined. All Hiemalis begonia cultivars began to show the dull-green coloration in the foliage after 2 weeks. After 6 weeks, all of the cultivars had mean disease ratings between 4 and 5 (data not shown). Plants were badly stunted, but rarely died (Fig. 4).

There was no interaction between repetition of the experiment and the Rex begonia cultivars, so the values were combined. None of the cultivars exhibited any sign of chlorosis or wilt, but the data indicated that two cultivars—'Hurricane Bay' and 'White Caps'—showed a significant reduction in dry weights when grown in soil infested with *F. foetens* (Fig. 5). None of the other cultivars showed reduction in dry weights when compared to controls. Similarly, there were no symptoms and no reduction in the dry weight of the tuberous begonias, angel wing begonias, or the seedling begonias when grown in soil infested with *F. foetens*.

Table 1
Result of studies on the transmission of *Fusarium foetens* by fungus gnats (*Bradysia* spp.)

Treatment	% plants symptomatic[a]	Mean disease rating[b]	% fungus gnats with *F. foetens*[c]
Experiment 1			
Control	0	0	0
Fungus gnats	83	3.4	12
Experiment 2			
Control	0	0	0
Fungus gnats	66	3.2	4

[a]Out of six plants.
[b]Based on a scale of 1–5 where 1 = no symptoms, 2 = slight dull-green colouration of the foliage, 3 = dull-green wilted foliage, 4 = stunted and wilting plants with distinct darkened lesions on basal stem sections, and 5 = dead or near death.
[c]Isolations were made on Komada's selective media and species identified using VCG tests with known pathogens and by the pungent odour that appeared on potato dextrose agar after 5–7 d incubation.

4. Discussion

Fusarium wilt of Hiemalis begonias has the potential to be a devastating disease for begonia growers. Its appearance in 2001 in the Netherlands was rapidly followed by reports in Germany, England (Schmalstieg et al., 2003; Schrage, 2003) and, by 2003, had reached the United States (Elmer et al., 2004). Infected transplants appear to be the main vehicle for long distance transport and their dissemination in horticultural trade may explain the simultaneous appearance of the disease in Europe and New England, USA. Spread within the greenhouse was first shown in Germany to be through irrigation water in ebb and flow systems (Wohanka, 2003). The current study supports Wohanka's results that levels as low as 100 conidia ml^{-1} can cause significant disease. These findings explain the rapid spread of the disease on plants sharing irrigation water. However, it is not known if conidia are produced on infected roots in the soil and then released into the irrigation water as is commonly found with

Fig. 3. (A) Nylon mesh cages where adult fungus gnats were released along with Hiemalis begonias; plants in back had been grown in non-infested potting mix and kept separate from plants in the front that had been grown in potting mix infested with *Fusarium foetens*. (B) Healthy Hiemalis begonias that had been grown in potting mix that was treated with Marathon 1% G (imidacloprid) and caged with Hiemalis begonias that had been artificially infested with *Fusarium foetens*; no fungus gnats were added (infected plants not shown).

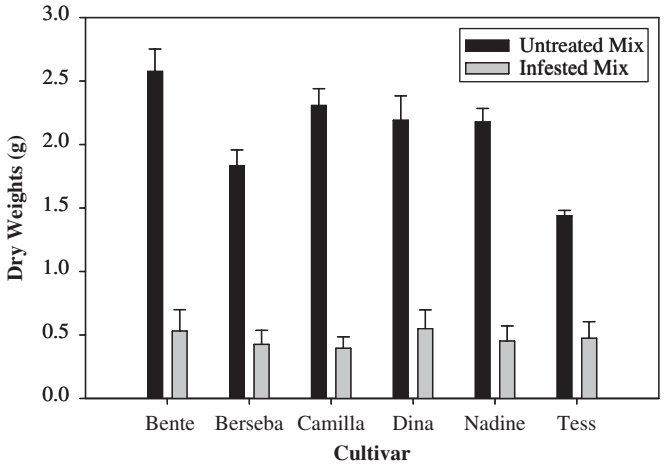

Fig. 4. Dry weights of Hiemalis begonias when grown in potting mix infested with *Fusarium foetens* compared to plants grown in non-infested potting mix. Error bars represent the standard errors of the means from two repetitions.

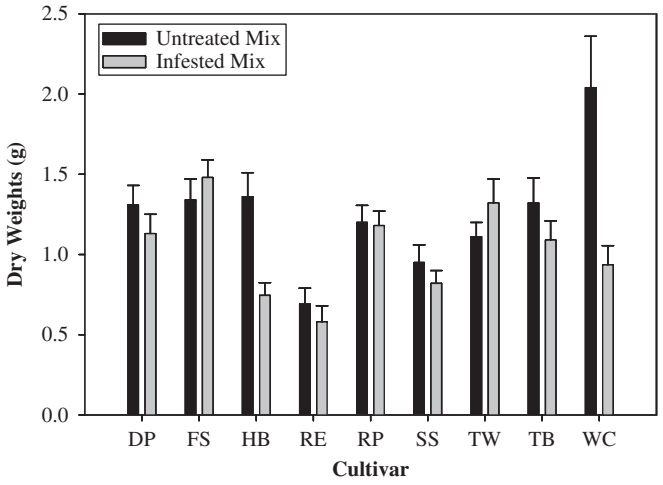

Fig. 5. Dry weights of Rex begonias when grown in potting mix infested with *Fusarium foetens* compared to plant grown in non-infested potting mix; DP = Devil's Paradise, FS = Flamingo Shoals, HB = Hurricane Bay, RE = Reggae, RP = Rum Painkiller, SS = Silver Sands, TW = Trade Winds, TB = Tropical Breeze, WC = White Caps; error bars represent the standard errors of the means from two repetitions.

inoculum produced by species of *Pythium* or *Phytophthora*. More likely, a sporulating stem from an infected plant might wither and fall either onto the greenhouse bench or floor. Shared irrigation water would allow quick dispersal of the conidia, and it has been shown that low levels of this inoculum could incite disease. Equally as important was the finding that fungus gnats were very efficient in acquiring the fungus and spreading it to healthy transplants. Although this project did not study whether fungus gnats were specifically attracted to diseased begonia, the volatile sesquiterpenes reported from *F. foetens* could have served as an attractant for fungus gnats (Kaiser, 2006) and may have enhanced their likelihood of vectoring the disease.

As expected, chlorine bleach was a highly effective sanitizing agent at very low concentrations. Chlorine bleach is corrosive to metals, and a non-corrosive alternative quaternary ammonia product, Greenshield®, is registered in the US and Europe as a non-corrosive sanitizing agent. The label rate for Greenshield® is approximately 4000 $\mu l\,l^{-1}$. The lowest concentration tested in this study was 50 $\mu l\,l^{-1}$ (80 times lower) and it still resulted in 100% mortality. These high labelled rates are very phytotoxic, so the material is labelled for use on inanimate surfaces only. ZeroTol®, on the other hand, can be used in irrigation water or in mist application water at low rates of approximately 1000 $\mu l\,l^{-1}$ depending on the crop and mode of irrigation. At this rate, we found ZeroTol® provided 100% mortality (LD_{50} = 258.0 $\mu l\,l^{-1}$). SaniDate® has a higher toxicity than ZeroTol®; it is currently advertised as a disinfectant that can also be used in irrigation water at much lower rates, around 100 $\mu l\,l^{-1}$. Accordingly, SaniDate®, was more toxic to *F. foetens* than ZeroTol® with 100% mortality observed at 200 $\mu l\,l^{-1}$ (LD_{50} was 66.7 $\mu l\,l^{-1}$).

In all reports to date, symptoms were reported only on Hiemalis begonias. Wohanka (2003) reported seedling begonias (*Begonia* × *semperflorens-cultorum* and

Begonia × *tuberhybrida* were not susceptible. The current study also concluded that tuberous begonias (*Begonia* × *tuberhybrida*), seedling begonias (*Begonia* × *semperflorencultorum*), and angel wing begonias (*Begonia coccinea* Hook) were not susceptible. Although Rex begonias did not exhibit the typical wilt or chlorosis symptoms, a significant decrease in dry weights of two cultivars was observed in two separate trials. The fungus was re-isolated from the asymptomatic roots of these stunted plants. Since survival of related species in the *F. oxysporum* complex are known to occur on symptomless roots of non-host plants, it is not surprising to find that *F. foetens* can persist on the roots of related begonia species. However, the ability to incite stunting is not commonly found on non-host plants. Given these findings, one might expect other species and hybrids of begonia to exhibit symptoms if inoculum densities were high enough and conditions strongly favoured disease development. This information would be useful to growers attempting to eradicate *F. foetens* from their greenhouses. However, given that there are well over 1000 species of begonias (Hortus Third, 1976), determining the specific pathogenicity within the *Begonia* genus becomes a daunting task.

5. Conclusions

Since no resistance to Fusarium wilt has yet been identified in Hiemalis begonias, growers that encounter this disease must immediately initiate strict sanitation procedures. Benches, walkways, trays, and pots must be treated with appropriate disinfectants. The current study found that the H_2O_2 products were effective at rates that are reported to be non-phytotoxic. Strict fungus gnat control is also necessary to minimize spread. Other begonia species like Rex and Angel wings do not develop typical wilt symptoms, but some Rex begonia cultivars can be stunted and can harbour the pathogen.

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