Effectiveness of Fungicides and Biopesticides in Controlling *Botrytis* Gray Mold on Western Hemlock Nursery Stock

Gary Chastagner, Marianne Elliott, and Joseph M. Hulbert

Professor, Plant Pathology, Washington State University (WSU), Research and Extension Center, Puyallup, WA; Senior Research Associate, WSU Research and Extension Center, Puyallup, WA; Postdoctoral Research Associate, WSU Research and Extension Center, Puyallup, WA

Abstract

Botrytis gray mold is a disease that impacts conifer seedling production and causes postharvest losses during storage. This disease is difficult to control and often persists after common fungicide applications. Many new products exist with the potential to control *Botrytis* diseases, but little research has been conducted to determine their efficacy on conifers. Through support provided by the Washington State Department of Agriculture Specialty Crop Block Grant, USDA IR-4 Environmental Horticulture programs, and the USDA NIFA McIntire-Stennis program, research was conducted to evaluate the effectiveness of 31 fungicide and biopesticide products to control gray mold caused by *Botrytis cinerea* on 2-year-old container-grown western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) seedlings. Although several effective products were identified for controlling gray mold on hemlock seedlings, additional research is needed to determine the optimal application rates and timing of these products to maximize disease control on a broad range of conifer hosts under nursery production conditions.

Introduction

It is estimated that nearly 62 million conifer seedlings are produced annually in Washington nurseries (Haase et al. 2020). These nurseries fall into two broad groups: those that produce bareroot seedlings and those that produce container stock (and some that grow both). Container production, especially of species that are difficult to germinate and grow, has been increasing over the past two decades and now accounts for about 26 million trees grown annually in Washington (Haase et al. 2020, Trobaugh 2012).

Botrytis gray mold is a chronic disease of conifer nursery stock, particularly in container-grown production systems (Haase and Taylor 2012, Lanthier and Watts 2020, Mittal et al. 1987). Although most *Botrytis* species are host-specific pathogens, *Botrytis cinerea* causes gray mold on many crops, including conifer nursery stock (Elad et al. 2007). In conifers, *Botrytis* primarily infects needle tissues (Haase and Taylor 2012). Symptoms appear as browning of needles, followed by development of masses of gray spores and mycelium (figure 1). The airborne spores are easily dislodged and can rapidly spread the disease, particularly in greenhouses. Conditions with high humidity and poor air circulation are especially conducive to disease development. Under these conditions, *Botrytis* can spread into stem tissues and kill seedlings (Haase and Taylor 2012). Additional losses can occur when seedlings are lifted, packed in boxes or bags, and then held in cold storage for several months prior to shipping and transplanting to the field. *Botrytis* can also cause a shoot blight of conifers in Christmas tree plantations and landscape plantings (Chastagner and Talgo 2018).

While most conifer seedlings are susceptible to gray mold, Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), spruce (*Picea* spp.), western hemlock (*Tsuga heterophylla* [Raf.] Sarg.), coast redwood (*Sequoia sempervirens* [D. Don] Endl.), and giant sequoia (*Sequoiadendron giganteum* [Lindl.] Buchholz) are among the species that are very susceptible to attack by *Botrytis* (Haase and Taylor 2012, Lanthier and Watts 2020, Mittal et al. 1987).

A recent survey of Canadian forest seedling nurseries indicated that *Botrytis* gray mold was the major disease of concern in forest seedling nurseries across

Figure 1. *Botrytis* gray mold on Douglas-fir seedlings. Under high humidity, grayish-colored mycelium and fruiting structures often appear on the foliage at the base of seedlings. Infection leads to discoloration and death of the needles. (USDA photo)

Canada (Lanthier and Watts 2020). Prior to the start of this project, we solicited input from growers in Washington conifer bareroot and container nurseries where *Botrytis* is an ongoing challenge. Some growers with new greenhouses and improved environmental control systems indicated that they could limit crop losses to 5 to 10 percent. Other growers, however, indicated that even with attempts to implement recommended best management practices, gray mold continues to elude reliable control, often resulting in crop losses of 10 to 50 percent, particularly on highly susceptible species grown in containers.

Management of *Botrytis* on conifer nursery stock relies on a combination of cultural practices, such as sanitation and the management of irrigation and ventilation to reduce periods of high relative humidity that favor infection (Dumroese and Haase 2018). Growers also typically make one or more application of fungicides, particularly in the fall when the weather is more conducive for disease development (Haase and Taylor 2012, Lanthier and Watts 2020). *Botrytis* can rapidly develop resistance to some classes of fungicide, however, which reduces the effectiveness of nursery disease management programs (Elad et al. 2007, James et al. 1982, Leroux 2007, Ogawa, et al. 1976, Stremeng et al. 2015).

Recently, several new reduced-risk fungicides and biopesticides that are potentially very effective against *Botrytis* have become available. Many of these products have been tested on a number of horticultural crops (Vea and Palmer 2017 and 2020). The identification of new fungicides and biopesticides that are effective in controlling *Botrytis* on conifer nursery stock would enable growers to integrate new classes of products into their disease management program to potentially improve the control of gray mold and reduce the buildup of fungicide-resistant strains of the pathogen in conifer seedling production systems. The objective of our study was to conduct an initial screening of fungicides and biopesticides for their ability to control gray mold on western hemlock seedlings.

Table 1. Products included in the *Botrytis* western hemlock trial (biopesticides are highlighted in bold).

Product and formulation	Percent active ingredient and common name	FRAC Code[1]
Affirm™ WDG	11.3% Polyoxin D zinc salt	19
Astun® (IKF-5411)	36% isofentamid	7
Botector®	*Aureobasidium pullulans* strains DSM 14940 + DSM 14941	NC
Broadform™ SC500	25% fluopyram + 25% trifloxystrobin	7 + 11
BW165N	*Ulocladium oudemansii* strain U3	NC
Chipco® 26019 F	50.0% iprodione	2
Cleary's 3336®	41.25% thiophanate-methyl	1
Daconil WeatherStik® SC	54% chlorothalonil	M5
Decree® 50WDG	50% fenhexamid	17
EcoSwing®	82% Extract of *Swinglea glutinosa*	P05
Empress®	23.3% pyraclostrobin	11
Fame™ SC	40.3% fluoxastrobin	11
Fore® 80WP	80% mancozeb	M3
Heritage®	50% azoxystrobin	11
MBI 110	96.4% *Bacillus amyloliquefaciens* strain F727	44
Medallion® WDG	50% fludioxonil	12
Mural® WDG	15% benzovindiflupyr + 30% azoxystrobin	7+11
Orkestra® Intrinsic	21.3% fluxapyroxad + 21.3% pyraclostrobin	7+11
OxiPhos®	17.7% phosphorus acid + 14% hydrogen peroxide	P07
Pageant® 38WG	25.2% boscalid + 12.8% pyraclostrobin	7 + 11
Palladium® 62.5WG	37.5% cyprodinil + 25% fludioxonil	9+12
Picatina™ Gold (A20808C)	7% pydiflumetofen + 9.3% azoxystrobin + 11.6% propiconazole	7+3+11
Proud 3®	5.6% Thyme oil	NC
Regalia®	5% Extract of *Reynoutria sachalinensis* (giant knotweed)	P05
Regime™ (F9110)	Extract of *Lupinus*	P
SP2480	Experimental	-
Spectro® 90WDG	78% chlorothalonil + 12% thiophane-methyl	M5+1
Triathlon®	98.85% *Bacillus amyloliquefaciens* strain D747	P
Trinity®	19.2% triticonazole	3
Tourney®	50% metconazole	3
ZeroTol® 2.0	27.1% hydrogen peroxide + 2.0% peroxyacetic acid	NC

[1] Fungicide Resistance Action Committee Code List 2019. http://www.frac.info/ (accessed May 2019)

NOTE: Some of the pesticides discussed in this paper were tested under an experimental use permit granted by WSDA. Application of a pesticide to a crop or site that is not on the label is a violation of pesticide law and may subject the applicator to civil penalties. It is your responsibility to check the label before using products to ensure lawful use and obtain all necessary permits in advance.

Methods

The study evaluated the effectiveness of 31 fungicide and biopesticide products (table 1) in controlling gray mold on 2-year-old western hemlock seedlings. Seedlings were initially grown in Superblock™ 112/95 Styroblocks® at a commercial container nursery for one year and then transplanted into D16H Deepot™ cells in Deepot™ D50 trays and maintained at Washington State University, Research and Extension Center (Puyallup, WA).

Product Applications

On October 1, 2019, each product was applied at a single rate except for BW 165N and SP2480 which were applied at two rates (table 2). Each treatment was applied to 10 seedlings with a handheld sprayer. The foliage on each seedling was sprayed until wet. Two sets of 10 seedlings were designated as untreated positive (inoculated) and negative (non-inoculated) controls and were sprayed with an equivalent amount of water alone.

Fungal Inoculation and Incubation

One day after fungicide application, all 10 seedlings from each treatment group and 10 untreated positive control seedlings were placed in separate 5-gal (19 L) buckets and the foliage was sprayed until wet with a suspension of *Botrytis cinerea* conidia (14.8×10^6 conidia per oz [5.0×10^5 conidia per ml]). The negative controls were sprayed with an equivalent amount of water alone. To ensure the foliage on the seedlings remained wet, 2 L of hot (125 to 136 °F [52 to 58 °C]) water was poured below the bases of the D16H Deepot™ cells in the bottom of each bucket and the inside of another bucket was sprayed with water, inverted over the top of the bottom bucket, and sealed with tape to create a moist incubation chamber. Seedlings were incubated in these chambers for 5 days at 68 to 72 °F (20 to 22 °C). After 5 days, the seedlings were removed and placed in Deepot™ D50 trays on benches in a greenhouse that was maintained at 59 to 68 °F (15 to 20 °C). Seedlings were overhead irrigated twice daily.

Disease Assessments

Disease symptoms on the shoot tips (figure 2) were assessed upon removal from the incubation chambers, which was 5 days post inoculation (5 dpi) and again

Table 2. Rates of products tested.

No.	Treatment	Product rate/100 gal
1	Non-inoculated check	-
2	Inoculated check	-
3	Affirm™	8 oz
4	Astun®	13.5 fl oz
5	Botector®	10 oz
6	Broadform™	8 fl oz
7	BW165N (3 lbs)	3 lbs
8	BW165N (4 lbs)	4 lbs
9	Chipco® 26019	16 fl oz
10	Cleary's 3336®	16 fl oz
11	Daconil WeatherStik®	2 3/4 pts
12	Decree®	1.5 lbs
13	Fore®	2 lbs
14	EcoSwing®	2 pts
15	Empress®	6 fl oz
16	Fame™ SC	8 oz
17	Heritage®	4 oz
18	MBI 110	6 qts
19	Medallion®	4 oz
20	Mural®	7 oz
21	Orkestra®	8 fl oz
22	OxiPhos®	1 gal
23	Pageant®	14 oz
24	Palladium®	6 oz
25	Picatina™ Gold	13.7 fl oz
26	Proud 3®	1 gal
27	Regalia®	1 gal
28	Regime™	45.7 fl oz
29	SP2480 (20 fl. oz.)	20 fl oz
30	SP2480 (30 fl. oz.)	30 fl oz
31	Spectro® 90	5.7 lbs
32	Triathlon®	6 qts
33	Trinity®	12 fl oz
34	Tourney®	4 oz
35	ZeroTol® 2.0	2 gal

Metric conversions: 1 oz. = 28.4 g; 1 lb = 453.6 g;
1 fl oz. = 29.57 ml; 1 gal = 3.8 L.

Figure 2. *Botrytis* shoot tip blight symptoms on western hemlock due to infection of the needles by Botrytis spores. (Photo by Gary Chastagner 2019)

29 dpi. On these dates, the incidence of diseased shoot tips was rated on a scale of 0 to 10 based on the percentage of tips that were blighted (0=none; 1=1 to 10 percent; 2=11 to 20 percent; 3=21 to 30 percent;… and, 10=91 to 100 percent). The severity of disease was also assessed 29 dpi to determine the percentage of shoots where disease had spread from the blighted tips down into the shoots while the plants were in the greenhouse (figure 3). Disease severity was rated on a scale of 0 to 10 (0=no spread, symptoms restricted to shoot tips; 1= spread into 1 to 10 percent; 2=spread into 11 to 20 percent; 3= spread into 21 to 30 percent;… and, 10=spread into 91 to 100 percent of the shoots) An overall disease level on seedlings at 29 dpi was calculated by multiplying the incidence rating by the severity rating, resulting in a disease index that ranged from 0 to 100.

Experimental Design and Statistical Analysis

Following incubation, seedlings were placed on greenhouse benches in a completely randomized design with one seedling per treatment in each of 10 blocks. Differences among treatment groups were analyzed with one-way ANOVA followed by Tukey's HSD test if results were significantly different at $p = 0.05$. All data analysis was done in R v. 4.0.01 (R Core Team 2020).

Results

Free moisture was observed on all seedlings when removed from the incubation chambers at 5 dpi,

Figure 3. *Botrytis* disease symptoms worsen after progression down the shoots of the branch. (Photo by Gary Chastagner 2019)

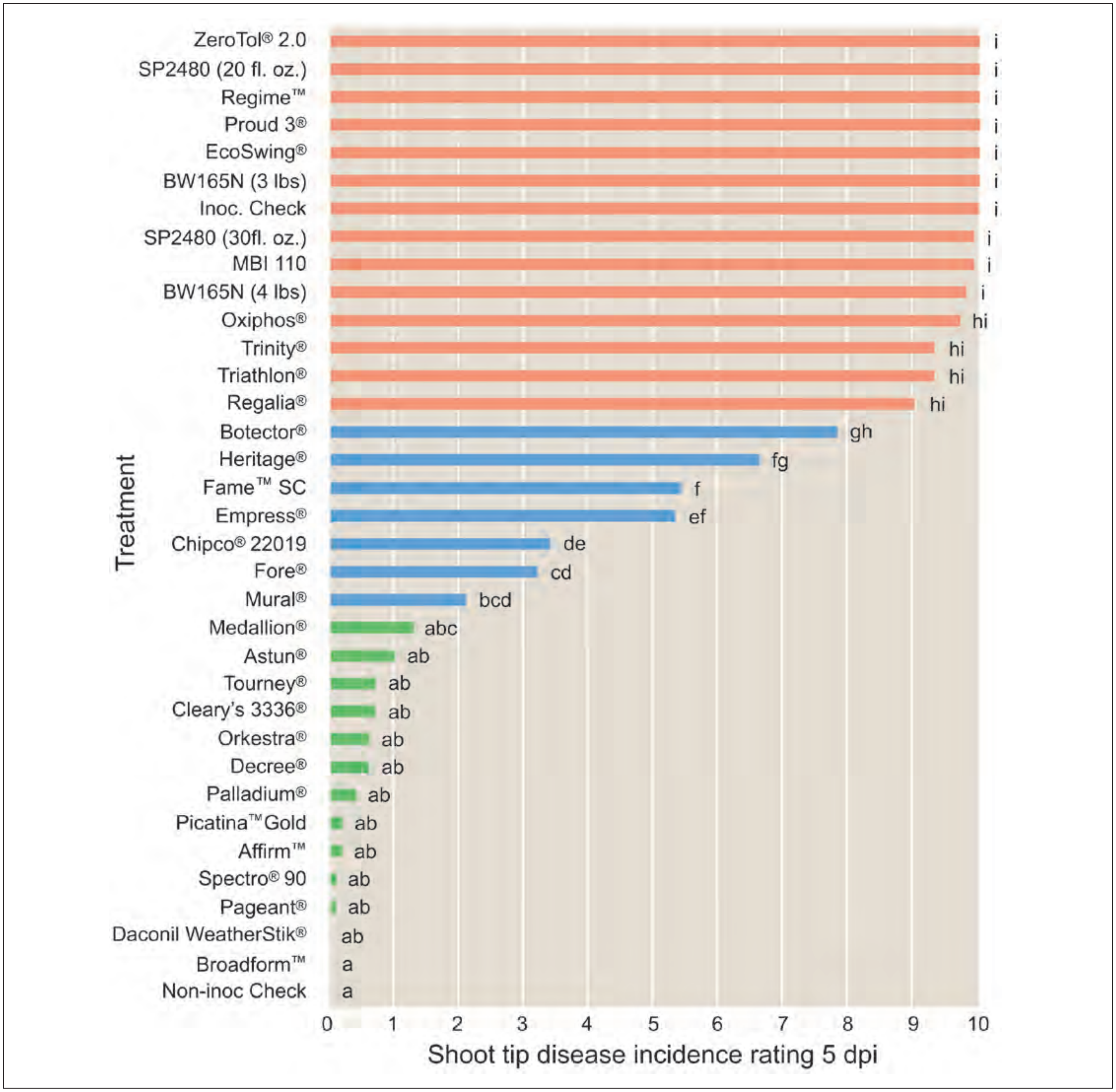

Figure 4. Shoot tip *Botrytis* disease incidence rating 5 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

indicating that conditions were optimal for infection. Upon removal of the seedlings from the buckets, symptoms were restricted to blighted shoot tips. The inoculated positive controls had an average disease incidence rating of 10, while no disease was evident on the non-inoculated negative controls. Statistical analysis of the 5 and 29 dpi disease incidence ratings indicated that the treatment ratings fell into three groups (figures 4 and 5): those that were not significantly different from the inoculated check (red bars), those that were not significantly different than the non-inoculated checks (green bars), and those that had intermediate disease ratings (blue bars).

Overall, there was very little increase in the incidence of blighted shoot tips during the time seedlings were maintained in the greenhouse. The overall average disease incidence rating only increased from 5.0 at 5 dpi to 5.4 at 29 dpi. Blight severity,

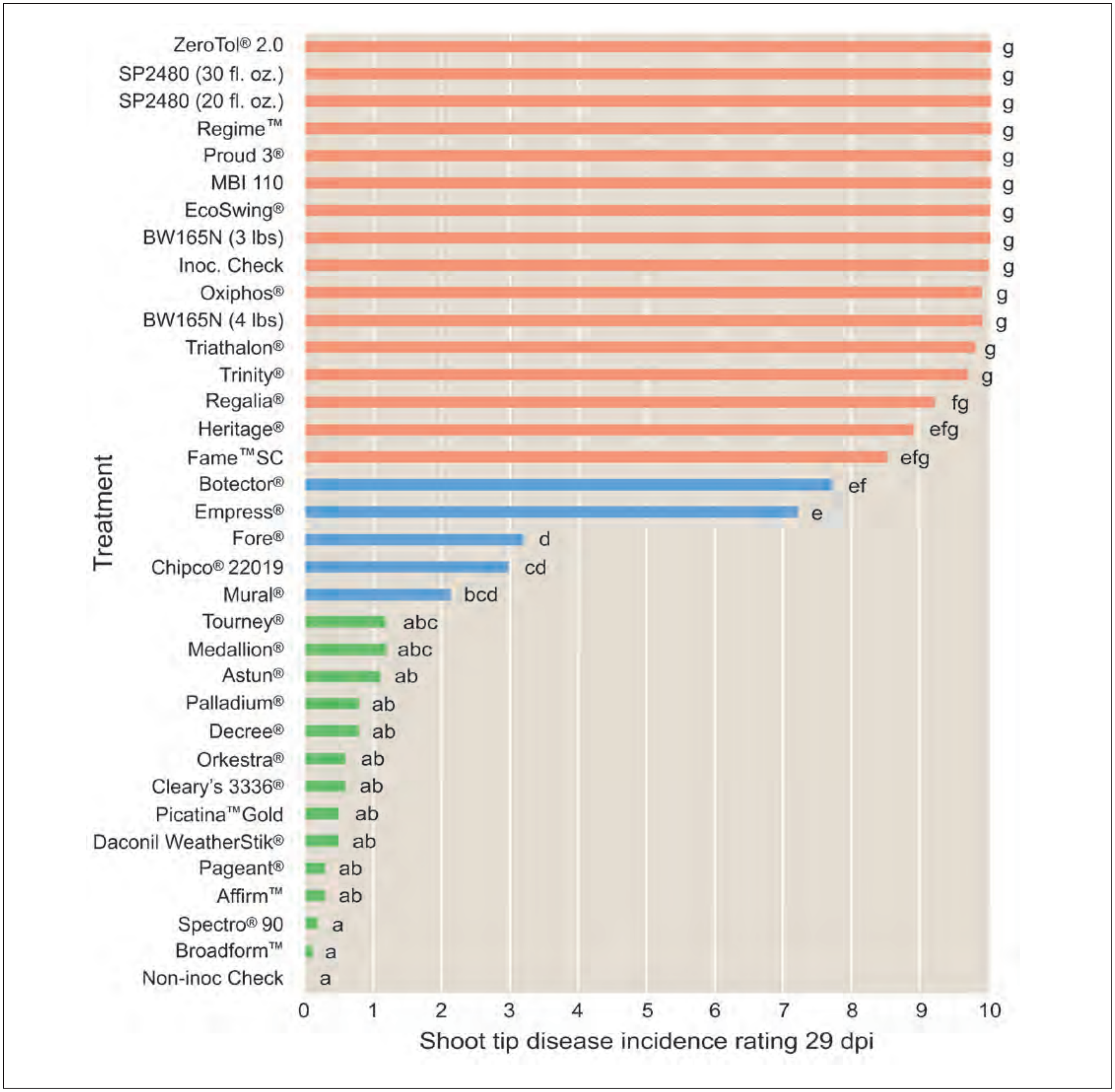

Figure 5. *Botrytis* shoot tip disease incidence rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

however, increased on some seedlings as the disease spread down the shoots. Analysis of disease severity and disease index data at 29 dpi indicated that treatments fell into two groups (figures 6 and 7): those that were not significantly different from the inoculated checks (red bars) and those that were not significantly different from the non-inoculated checks (green bars), which continued to have no disease symptoms after nearly 1 month.

Discussion

Thirteen products (Broadform™, Spectro® 90, Affirm™, Pageant®, Daconil WeatherStik®, Picatina™ Gold, Cleary's 3336®, Orkestra®, Decree®, Palladium®, Astun®, Medallion®, and Tourney®) were very effective in reducing the incidence of blighted shoot tips. These products, along with Chipco® 26019, Botector®, Empress®, Fore® and Mural® also were

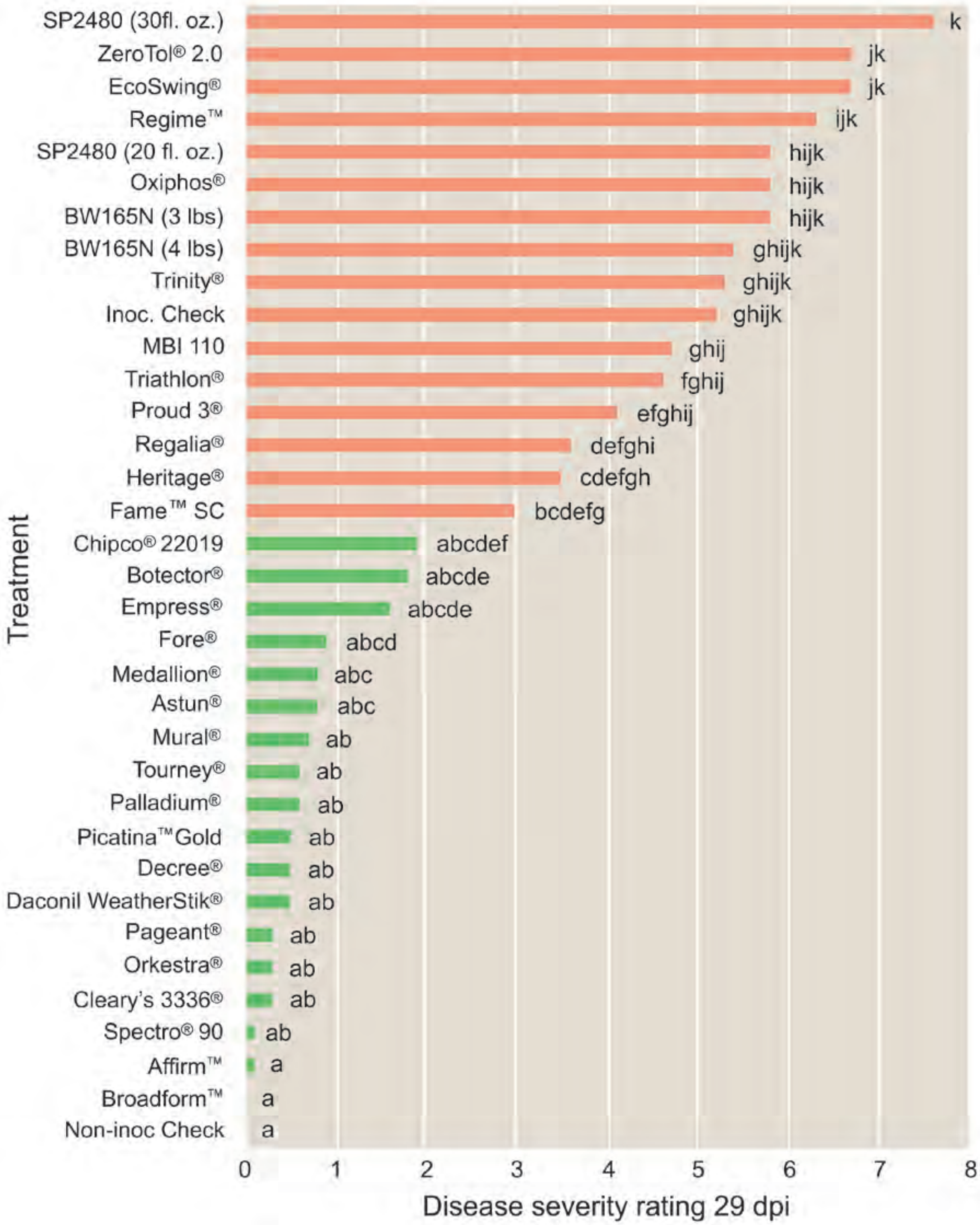

Figure 6. *Botrytis* disease severity rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

the most effective products in reducing the disease severity and disease index ratings at 29 dpi. These products consist of a mix of standard fungicides used to control *Botrytis* on conifers and several newer products shown to provide good control of *Botrytis* on a number of ornamental crops (James et al. 1982, James and Woo 1984, Lanthier and Watts 2020, McCain and Smith 1978, Vea and Palmer 2017 and 2020). Although Capieau et al. (2004) demonstrated that applications of *Streptomyces*, *Trichoderma*, and *Gliocladium*-based biopesticides were potentially as effective as standard fungicides in controlling *Botrytis* on Scots pine (*Pinus sylvestris* L.) seedlings, none of the biopesticides and oxidizers (ZeroTol® and

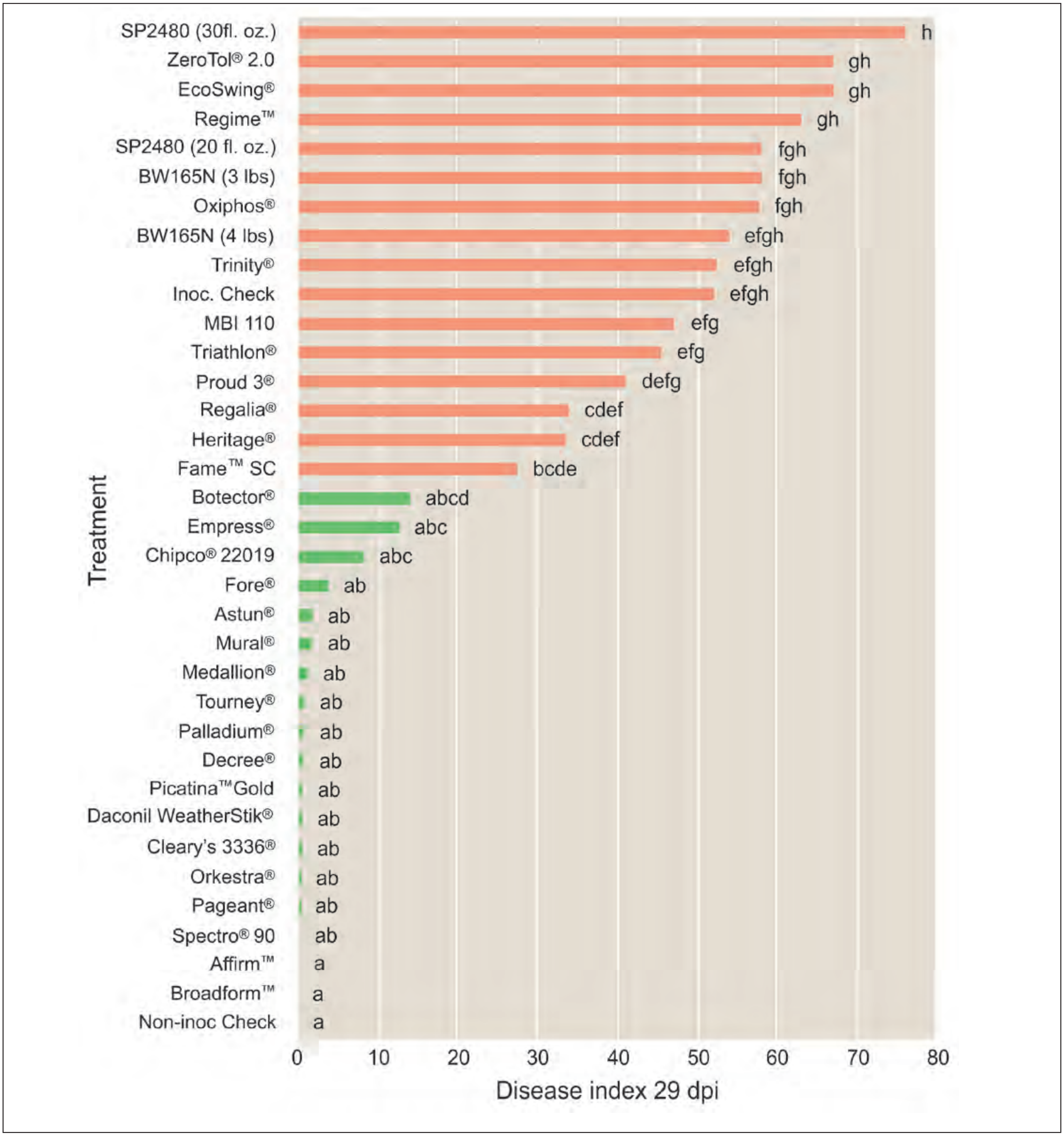

Figure 7. *Botrytis* disease index rating 29 days after inoculation. Columns followed by the same letter are not significantly different (p=0.05, ANOVA, Tukey test).

OxiPhos®) tested except Botector® were effective in reducing the incidence and severity of disease under our test conditions. Many of these products were also ineffective in controlling *Botrytis* development on a number of other ornamental hosts (Vea and Palmer 2017 and 2020).

The methods used in this trial were designed to efficiently screen a large number of products for their potential efficacy in controlling gray mold. Although several potentially effective products in controlling gray mold on western hemlock seedlings were identified, additional research is needed to determine optimal application rates and application timing for these products to maximize disease control on a broad range of conifer hosts under nursery production conditions. The effectiveness of these products under production conditions is likely to be affected by disease pressure; application methods, coverage, and timing; residual activity; and mix of products used. The potential adverse effects of products on seedlings also needs to be determined (James and Woo 1984).

Ultimately, an integrated approach that includes cultural practices, such as sanitation, promoting good air circulation, and reducing humidity in addition to the application of fungicides will increase the effectiveness of gray mold disease management programs (Dumroese and Haase 2018, Haase and Taylor 2012, Lilja et al. 2010, Pscheidt and Ocamb 2020). Fungicide resistance is a major problem in *Botrytis* disease management programs, particularly when products within high-risk FRAC (Fungicide Resistance Action Committee; https://www.frac.info/) groups, such as benzimidazoles and thiophanates (FRAC group 1), dicarboximides (FRAC group 2) and strobilurins (FRAC group 11), are applied multiple times during a growing season. Resistance to some high-risk fungicides such as benomyl has been detected in nurseries within a few years of introduction into nursery disease management programs (Gillman and James 1980). Rotating products with different FRAC codes or using products that contain a mixture of active ingredients with multiple FRAC codes is an important strategy to minimize the risk of fungicide resistance problems (Elad et al. 2007, Leroux 2007, Ogawa, et al. 1976). The highly effective products identified in our tests belong to nine unique FRAC groups or were mixtures of active ingredients with more than one FRAC code. If registered, these products would provide growers with multiple options to reduce the risk of fungicide resistance limiting the effectiveness of their gray mold disease management programs.

Address correspondence to—

Gary Chastagner, Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA 98371; email: chastag@wsu.edu

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