TECHNICAL WHITE PAPER: BOTRYSTOP® WP

MICHAEL BROWNBRIDGE
BIOLOGICAL PROGRAM MANAGER – PLANT DISEASES, BIOWORKS INC.

Effective *Botrytis* management now comes in a new, room-temperature stable formulation

Botrytis is a tough disease to manage at the best of times but its resistance to many conventional fungicides adds another layer of complexity. This has focused attention on the use of cultural and biological techniques as the primary, and sometimes only, lines of defense against this pathogen. BotryStop WP allows growers to bring a new mode of action (MOA) into their *Botrytis* management programs. Re-formulated so it can be stored at room temperature and is easier to prepare and apply, this biofungicide will suppress *Botrytis* on indoor and outdoor crops, and can be used in organic and conventional production. The new formulation label has also been expanded to include bacterial leaf spot (*Xanthomonas* sp.), and wording allows use on a broader range of crops. With proven efficacy against *Botrytis* on ornamental, nursery, food and medicinal crops, BotryStop WP is a valuable tool and rotational partner for resistance management.

BACKGROUND

Know your enemy: *Botrytis cinerea*

Although many *Botrytis* species have a narrow host range, *B. cinerea* is a generalist, causing grey mold diseases in over 200 fruit, vegetable, ornamental and medicinal plant species. Most severely affected agricultural crops include vegetables (e.g., cucumber, tomato, zucchini) and fruit-bearing plants, such as strawberry, grape and raspberry. In the fungal world, *B. cinerea* would be considered a success story – a highly successful pathogen due to its diverse modes of infection, high reproductive output, wide host range, ability to survive for extended periods as conidia and/or sclerotia, and its capacity to rapidly develop fungicide resistance. Unfortunately for us, that makes it a hard pathogen to manage without utilizing a range of strategies targeting crop health and pathogen suppression.

Invasion of host plants by *B. cinerea* invariably starts with the fungus colonizing dead, senescing or damaged tissues or flowers. The fungus utilizes the sugars and amino acids released by damaged cells and can multiply rapidly in these initial infection sites, forming large quantities of grey conidia, which allows the fungus to spread through the crop.

At first, *Botrytis cinerea* survives as a biotroph within or on the plant, deriving nutrients and energy from living cells but transitions to a necrotrophic lifestyle, killing the host cells and obtaining nutrients released by the dead or dying cells. During this stage, the fungus secretes various compounds (known as virulence factors) that disrupt the host plant's metabolism, immune system and cellular structure, resulting in flower or fruit decay.

Like other fungi, *Botrytis* has specific temperature and relative humidity requirements that are necessary for spore germination, infection, and disease development. Germination of spores and infection of the host is dependent on a film of moisture on plant surfaces for 8 to 12 hours, relative humidity of 85% or greater, and temperatures between 55 to 75 °F (12 to 24 °C). Colonization of plant tissue can occur over a wide range of temperatures, but 60 to 75 °F (15 to 24 °C) is optimal. *Botrytis* blight is more prevalent in the spring and fall months when weather conditions tend to be cooler and overcast. Humid conditions can result from improper irrigation practice, plants placed too close together, or the structure of the greenhouse not allowing for efficient ventilation and air flow. Spores are easily disseminated by air currents and splashing water. The fungus may also produce chlamydospores and/or microsclerotia, both of which can survive in soil for several years, depending on soil conditions.

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In many horticultural crops, gray mold is the most serious of all fungal diseases, and repeated fungicide sprays are necessary to control the pathogen especially in mild and humid climates. Chemical control of *Botrytis* spp. almost solely relies on fungicides which have specific modes and sites of action, inhibiting fungal growth by interfering with critical cellular processes. Consequently, fungicide resistance in *B. cinerea* can develop quickly in the field and within the past few years, reports of *Botrytis* strains which are resistant to multiple fungicide classes have become increasingly common. Resistance has become particularly problematic in fruits which are prone to *Botrytis* infection, such as strawberries raspberries, grapes, and tomatoes. Resistance management in a chemical program requires rotation of fungicides in different FRAC (Fungicide Resistance Action Committee) Mode of Action groups*, but today we are better served by using an integrated plant health management strategy that includes cultural, biological, and chemical components.

Integrated disease management

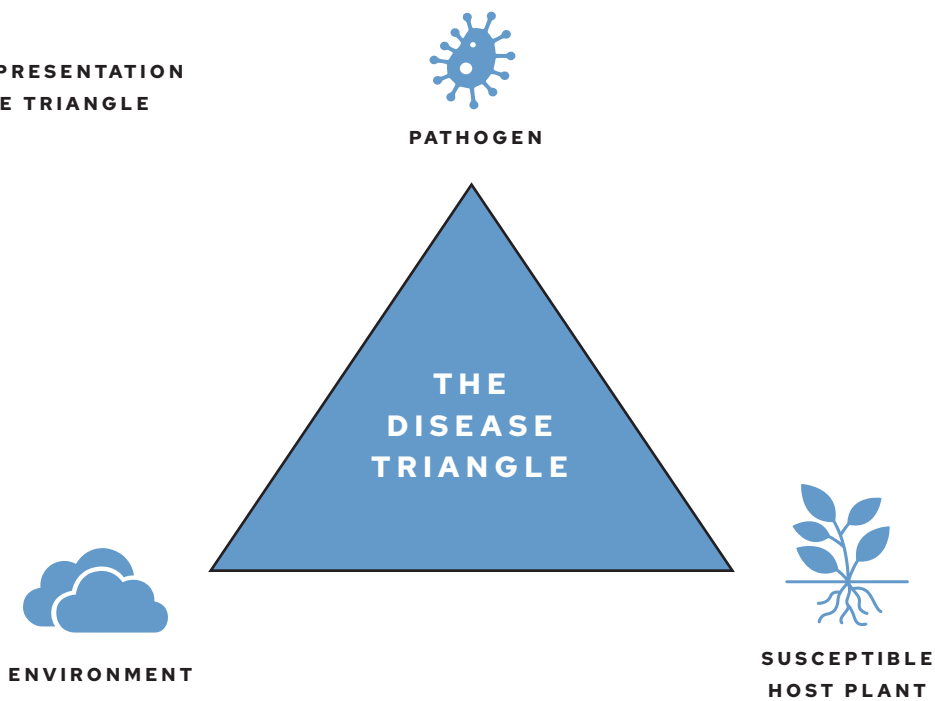
Let's consider *Botrytis* through the lens of the disease triangle (Fig 1).

The disease triangle is a simple way to present the factors required for plant infection to occur:

1. Environmental conditions must be favorable for disease development.
2. A susceptible plant host must be accessible to the pathogen.
3. Pathogen levels in the proximity of the plant must be high enough to infect healthy plants.

Infection will only occur if all three of these elements are satisfied concurrently. If we can eliminate or reduce effects of any one of these factors, we significantly reduce the likelihood of disease developing and spreading within the crop. Given the common occurrence of *Botrytis* in greenhouses and the relative ease with which it spreads, avoiding conditions that are conducive to disease development will have a significant impact on disease risk. Creating a growing environment that does not cause plant stress is also beneficial as stressed plants are more susceptible to infection. Host plant susceptibility can be further reduced by using resistant varieties. Finally, pathogen levels can be reduced by using strict sanitation practices in production and through use of products to manage diseases.

FIGURE 1 :
GRAPHICAL REPRESENTATION OF THE DISEASE TRIANGLE



* The FRAC Mode of Action (MOA) classification provides growers, advisors, extension staff, consultants and crop protection professionals with a guide to the selection of fungicides for use in an effective and sustainable fungicide resistance management strategy.

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Cultural Management Strategies

Cultural strategies form the foundation of a successful *Botrytis* management program. They include the following actions:

- Control weeds, remove and dispose of plant debris and old flowers during production and between crop cycles before they become infected and function as spore sources
- Space plants in a way that allows for good air circulation through the crop. This reduces the level and duration of leaf wetness as free moisture is necessary for germination and infection of *Botrytis*
- Avoid overhead watering during blooming. If this is the only method of irrigation available, irrigate early in the day so that the foliage can dry as rapidly as possible
- Maximize the period between irrigations to further enhance drying of foliage and flowers
- Manage the growing environment (temperature, relative humidity, pH of the growing medium, avoiding overwatering) to create conditions which are not conducive to growth of the pathogen, and to reduce plant stress
- Manage crop nutrition levels and avoid over-application of N-fertilizers which can lead to overproduction of succulent tissues which are more susceptible to infection
- Manage insect pests proactively, as they can passively vector *Botrytis* spores between plants or cause injuries that serve as infection sites for the pathogen

The importance of air movement and managing the growing environment

Moving air, even in a closed greenhouse, helps to reduce moisture on plant surfaces. Horizontal airflow (HAF) produces more uniform temperatures and reduces the likelihood that cool areas develop within the greenhouse where the risk of condensation on leaves increases. Free moisture is necessary for germination of *Botrytis* spores and for growth within plant tissues; low humidity may slow the growth of the fungus.

In-crop humidity can also be reduced by appropriate heating and ventilating. Warm air holds considerably more moisture than cool air. In the evening, as warm air cools, RH rises in the crop canopy and water vapor will condense on leaves. This resulting moisture film is essential for *Botrytis* (and other fungal pathogens) to germinate and cause infection. One way in which excessive humidity and condensation can be prevented is by turning on the heat and opening the roof vents. The warm humid air is ventilated to the outside and cooler outside air is drawn into the greenhouse, leading to a net reduction in RH. This air exchange takes 5 or 10 minutes and may have to be repeated several times during the evening.

Integrated management strategies and where BotryStop WP fits

So, *Botrytis* can be prevented by managing environmental conditions, use of sound cultural practices, and fungicides. But given the resistance challenges, this is where biologicals like BotryStop WP play an important role. BotryStop WP can be used alone or, owing to its compatibility with many standard foliar fungicides, in rotation with traditional disease-management products, bringing an alternative MOA into the program.

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BOTRYSTOP WP, A TRULY UNIQUE BIOLOGICAL CONTROL AGENT

What is BotryStop WP?

BotryStop WP is based on the fungus, *Ulocladium oudemansii* U3 strain. *Ulocladium oudemansii* is a naturally occurring saprophyte found in soils worldwide. *Ulocladium* spp. are typically associated with decaying plant matter, which provides a clue as to its unique mode of action.

How BotryStop WP works

Like *Botrytis*, *Ulocladium* colonizes dead, damaged and senescing plant tissues. When spores of *Ulocladium oudemansii* are deposited on dead or decaying plant debris and environmental conditions are suitable, they will germinate and colonize the necrotic plant tissue. If decayed vegetative matter is not available, or becomes exhausted, the fungus will not survive. Additional sporulation can occur on the developing mycelium and these new spores may colonize remaining debris. *Ulocladium* aggressively out competes *Botrytis* spp. and *Sclerotinia* spp. at these sites for the physical space and nutrients released by the dead and senescing plant tissue, preventing the disease from establishing on the plant and building inoculum levels in the crop. The mode of action exhibited by *Ulocladium oudemansii* is technically known as antagonistic competition and unlike other biofungicides, it does not produce toxins which inhibit or kill pathogenic microbes. The beneficial fungus is non-invasive and causes no damage to living plant tissue. With this mechanism of action, it is highly unlikely that resistance to BotryStop WP will develop. *Ulocladium* species do not produce mycotoxins or enzymes associated with mammalian toxicity, which makes the product very safe for humans and non-target organisms.

Ulocladium oudemansii grows at temperatures from ~10-26 °C (50-80 °F), with optimal growth occurring from 20-24 °C. It does not grow above 30 °C (86 °F).

The new BotryStop has been formulated to remove insoluble inert ingredients and is shelf-stable at room temperature for >12 mo (the label will guarantee a concentration of $8x10^7$ CFU/g product for 12 months). The label provides a formulation description as follows:

ACTIVE INGREDIENT:

Ulocladium oudemansii (U3 Strain)[1].....45.00%

OTHER INGREDIENTS:.....55.00%

TOTAL:.....100.00%

[1]Contains not less than $8X10^7$ CFU/g of product

Use rates remain the same as the previous WDG formulation (2 to 4 lbs / 100 gals) as the WP has been formulated with the same ratio of active (spores) to inert ingredients.

History of development and use

Ulocladium oudemansii strain U3 was originally isolated from kiwifruit leaf litter collected from a Massey University kiwifruit research plot in New Zealand in 1995. The fungus produces asexual spores, or conidiospores, on artificial substrates and these are the active ingredients in the biopesticide product.

Following several years of evaluation and research on the organism, a commercial product BOTRY-Zen® was developed in New Zealand as a biological control agent for *Botrytis cinerea* in grapes, blackcurrants, kiwifruit and ornamental flowers, and for *Sclerotinia sclerotiorum* in kiwifruit. Trials in wine grapes have also been conducted in the United States, France, Germany, Greece, Slovakia and Spain. The results of these trials show that when BOTRY-Zen® is applied correctly, it provides protection against *Botrytis* and *Sclerotinia* infections comparable to a standard fungicide program. The active was commercialized in the US by BioWorks as BotryStop® and is labelled for use on various edible and ornamental crops grown in the greenhouse, nursery and outdoors.

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Advantages of the new BotryStop WP formulation

1. Stable at room temperature for more than 12 mo

+ Product can be shipped to customers without ice packs and using standard shipping methods; reduces shipping costs
+ Can be stored at room temperature, no longer has to be refrigerated
+ Twelve months shelf life give our customers more flexibility in purchasing and using the biofungicide in their operation

2. Contains no insoluble inerts

+ The WP does not contain any clay or other insoluble inert ingredients in the formulation
+ The WP is easier to prepare and apply
+ Eliminates issues with clumping and significantly reduces risks of clogging in low-volume sprayers

3. More diseases and crops included on the label

+ Now includes *Xanthomonas* for ornamental and bedding plants (nursery and greenhouse), in addition to all those previously listed (*Botrytis cinerea*, *B. squamosa, Monilinia* spp., *Sclerotinia* spp. and *Erwinia* sp.)
+ Crop use has been expanded to include edible flowers and hemp

4. Can be used on indoor and outdoor crops

+ Label includes greenhouse and nursery ornamentals
+ Indoor and outdoor food crops, including asparagus, berries and small fruits, Cucurbits, fruiting vegetables, leafy vegetables, strawberries, tobacco, hemp and edible flowers
+ The stability of the formulation at room temperature makes it easier to use the product for outdoor applications, enabling spray mixes to be prepared on-site without transporting the product in a cooler

5. Outperforms other biopesticides labeled for *Botrytis* control (Ecoswing, Botector, Serenade) and performs as well as or better than broad-spectrum fungicides, e.g., Captan

+ See selected trial summaries in following 'Proven performance' section

TIPS AROUND PREPARATION AND APPLICATION

Mixing guidelines

For conventional hydraulic sprayers, the following steps should be followed:

1. Ensure that spray tank is clean
2. Fill tank halfway and begin agitation; the agitator should be in constant operation throughout to prevent settling of the spores in the spray tank
3. Add desired amount of BotryStop WP and fill tank. At this stage, a non-ionic surfactant can be added but is not necessary for many crops as the formulation already contains a wetting agent. NOTE: i. Do not use surfactants that claim to be "penetrants" or "stomatal flooders or infiltrators"; ii. check for crop safety if using a wetting agent, particularly on sensitive crops
4. Use spray mixture immediately. Do not allow spray mixture to stand overnight or for prolonged periods
5. Apply to leaf wet, but NOT runoff

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For low-volume sprayers, ensure the rate is adjusted accordingly for that equipment and the area to be treated. The *Ulocladium oedemansii* spores in BotryStop WP are relatively large and sometimes form clumps. These typically dissociate during mixing in conventional sprayers which also have larger nozzle apertures. However, the following steps are recommended for low-volume applications where fine nozzle apertures are used to create the tiny spray droplets, e.g., AutOFOG® and TracFOG® devices. This will ensure preparation of a homogenous suspension, thereby avoiding clogging of nozzles.

1. Thoroughly pre-mix (pre-slurry) BotryStop WP with water in a separate container to ensure full dispersion and suspension of the spores. The new formulation enhances this step.
2. Fill the tank halfway and turn on the agitator.
3. Pour the pre-slurry through a filter into the partially filled tank (with the agitator running) to remove any remaining particulates.
4. Add a recommended wetting agent or carrier (such as Dramm's NutriFog™ carrier) and fill the spray tank to the desired final volume. For low volume sprayers, use the maximum amount of water that a given machine allows to help ensure material is completely mixed and flows through the nozzle more easily. For some spray equipment, e.g., TracFOG sprayer, in-line filters may have to be removed to avoid clogging and allow free movement of the spray suspension to the nozzle.

NOTE: Addition of a wetting agent (see recommendations below) is not essential but will improve leaf contact and spreading, especially when fine mist or low volume sprays are made. Use of a non-ionic surfactant or spreader sticker is recommended, and use of compatible organosilicone surfactants is beneficial when spraying low water volumes. Do not use surfactants that claim to be "penetrants" or "stomatal flooders or infiltrators"

DO NOT APPLY THROUGH A THERMAL FOGGER

ADJUVANT RECOMMENDATIONS FOR BOTRYSTOP WP		
SURFACTANT	**RECOMMENDED RATE / 100 GALS**	**RECOMMENDED RATE / L**
Capsil	4 fl oz	0.3 mL
Silwet L-77	0.015%	0.15 mL
Silwet ECO	0.025%	0.25 mL
MilStop®	1.25 lb	1.25 g

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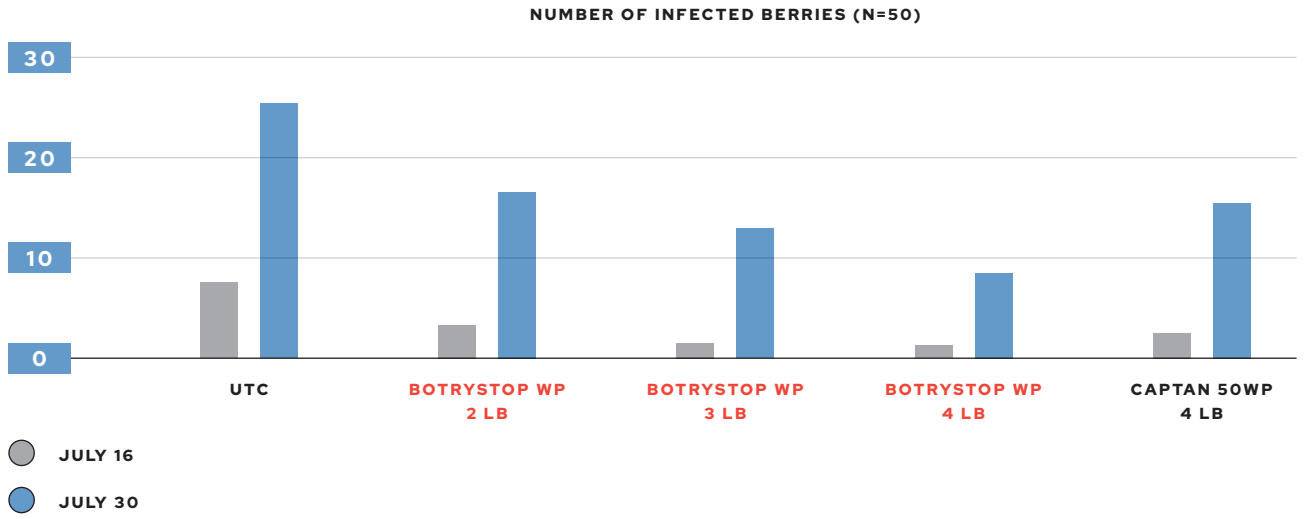
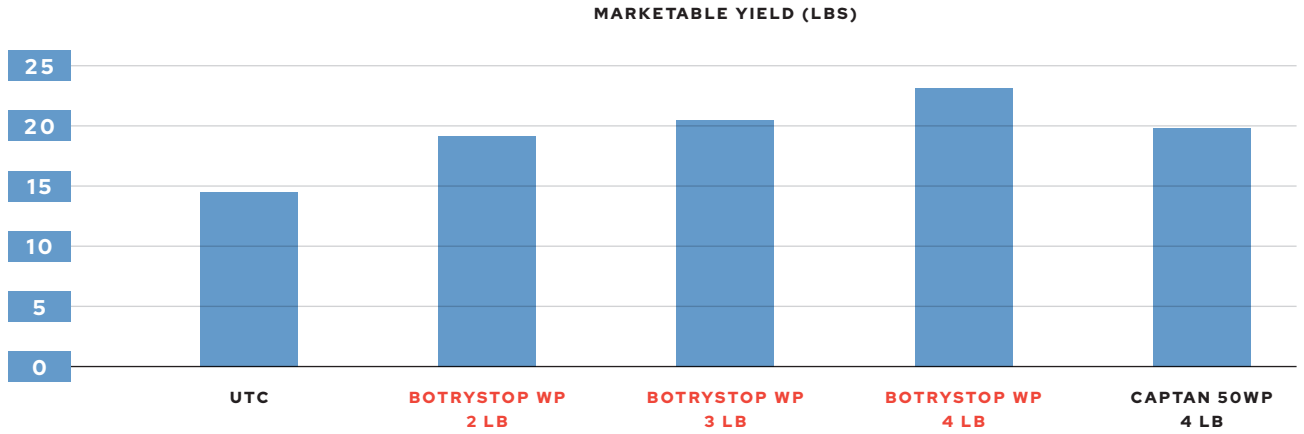
BotryStop WP: proven performance

BotryStop WP's unique fungal active ingredient surpasses the performance of other biopesticides and provides efficacy that is on par with or superior to several chemical fungicides. Extensive trialing of the BotryStop WP formulation has demonstrated its effectiveness and plant safety in a diverse range of crop settings, indoors and outdoors, as shown in the following examples from third-party independent trials.

I. BOTRYSTOP WP VS *BOTRYTIS* FRUIT ROT IN RASPBERRIES (2021)





Key takeaways:

+ Disease severity was significantly lower in the BotryStop WP and Captan treatments compared to the untreated control (UTC)
+ BotryStop WP at 4 lbs provided the greatest increase in berry yield over the UTC
+ Performance at 3 and 4 lbs was as good as Captan

The trial was carried out on 10-year-old "Meeker" Raspberry bushes, Lyndon WA; AgroSci Agriculture Consulting.

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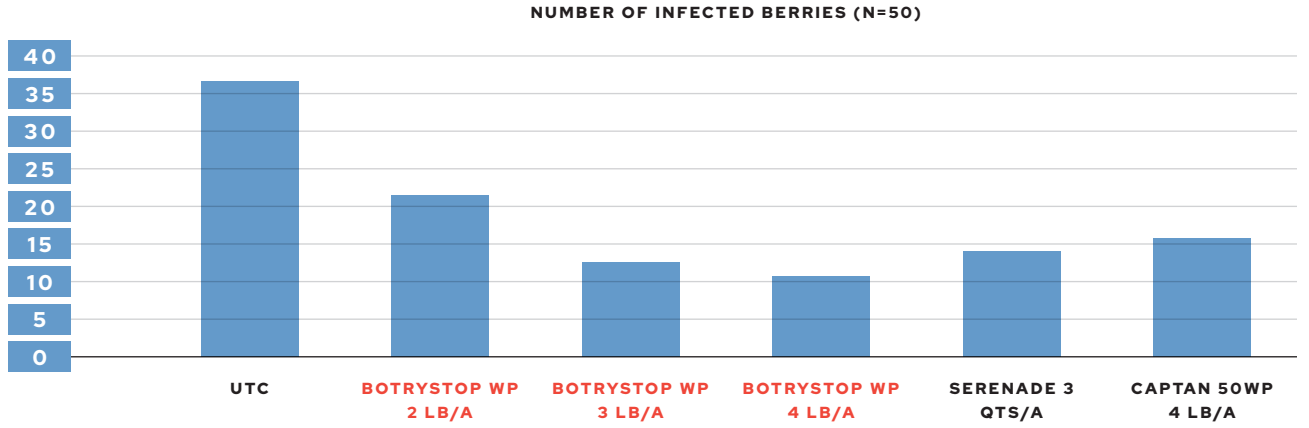
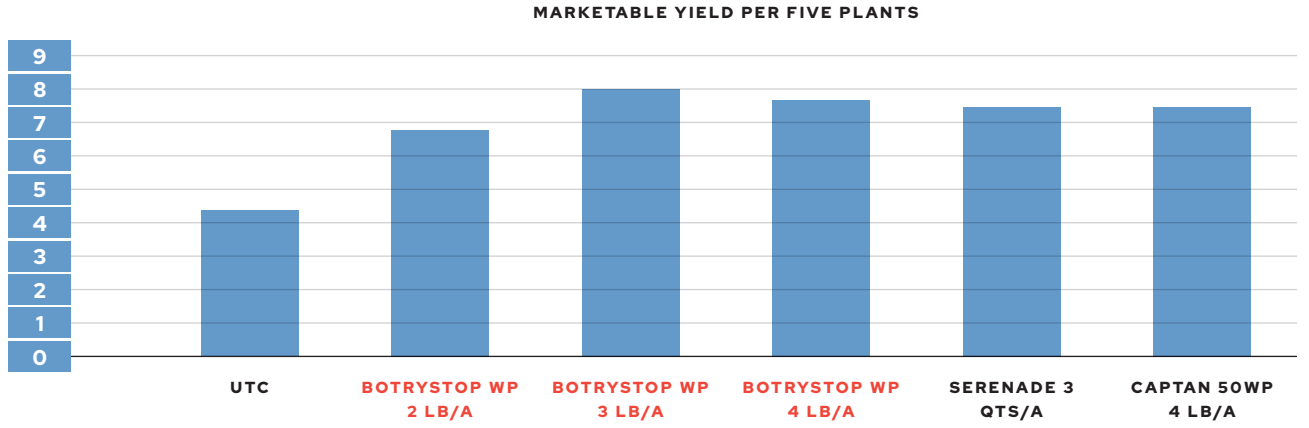
II. BOTRYSTOP WP VS *BOTRYTIS* FRUIT ROT IN BLUEBERRIES (2022)





Key takeaways:

+ BotryStop WP at 4 lbs/A outperformed all other treatments
+ Better than Captan (chemical standard) and Serenade (biological standard)
+ BotryStop WP at 3 lbs/A performed as well as the standard treatments
+ All treatments yielded statistically equal marketable yield and were significantly higher than the untreated control (UTC)
+ Numerically, BotryStop WP at 3.0 lb/A gave the highest marketable yield

The trial was carried out on 8-year-old "Berkeley" blueberry bushes, Burlington WA; AgroSci Agriculture Consulting.

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III. INTEGRATION OF BOTRYSTOP WP WITH SYNTHETIC FUNGICIDES VS. *BOTRYTIS* FRUIT ROT IN FIELD-GROWN STRAWBERRIES, FLORIDA

FUNGICIDE TREATMENT	TOTAL YIELD (POUNDS/A)[1]	INCIDENCE OF *BOTRYTIS* FRUIT ROT
Switch 62.5WG 14 oz Captan Gold 80WDG 1.9 lb	26,439 a	4.2 hij
Switch 62.5WG 14 oz BotryStop WP 3 lb/A + Kinetic 0.1%	23,729 abcd	4.4 hij

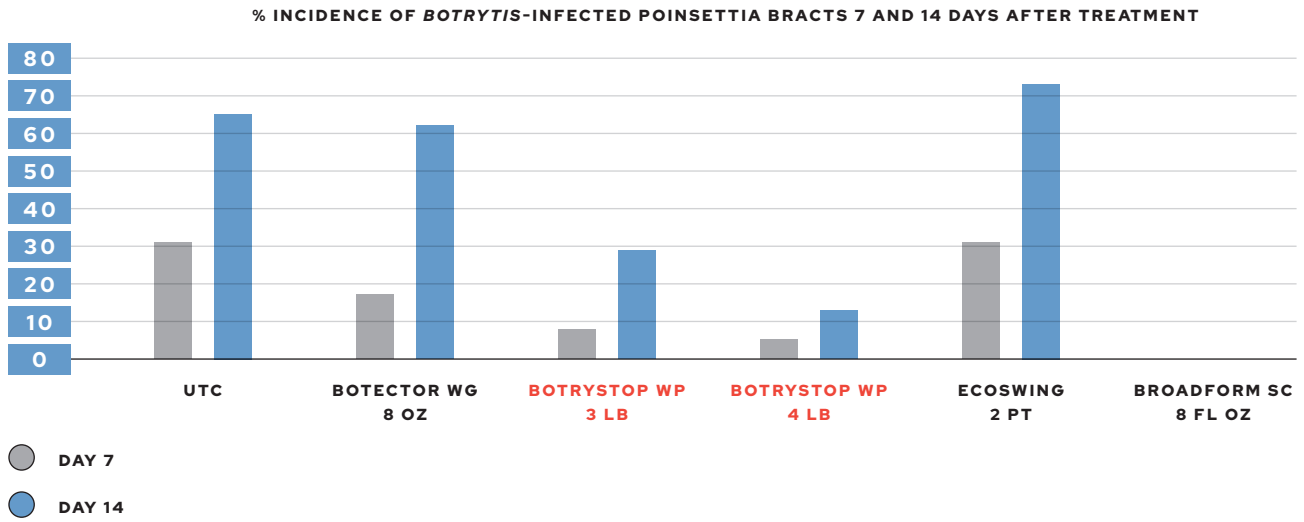
[1] Marketable yield; harvest data from 1 Dec 2020 to 26 Mar 2021 (21 harvests total).
Values in a column followed by the same letter are not significantly different by Fisher's Protected LSD test (α = 0.05)

Key takeaways:

- Captan/Switch is a standard chemical rotation program used in outdoor strawberry production in Florida
- Captan sprays were replaced with BotryStop WP in the integrated program
- Numerically, marketable fruit yields were slightly lower in the biological rotation, but were not statistically different from the chemical standard
- The incidence of *Botrytis* was no different between the two treatment regimes
- The efficacy of the program was maintained while reducing chemical inputs and resistance risks

Evaluation of biorational products to control *Botrytis* and *Pestalotia* fruit rot on annual strawberry, 2020-21. Zuniga, Baggio, Peres. University of Florida-GCREC

IV. RELATIVE EFFICACY OF BIOFUNGICIDES VS *BOTRYTIS CINEREA* IN POINSETTIA (*EUPHORBIA PULCHERRIMA* 'PRESTIGE RED')



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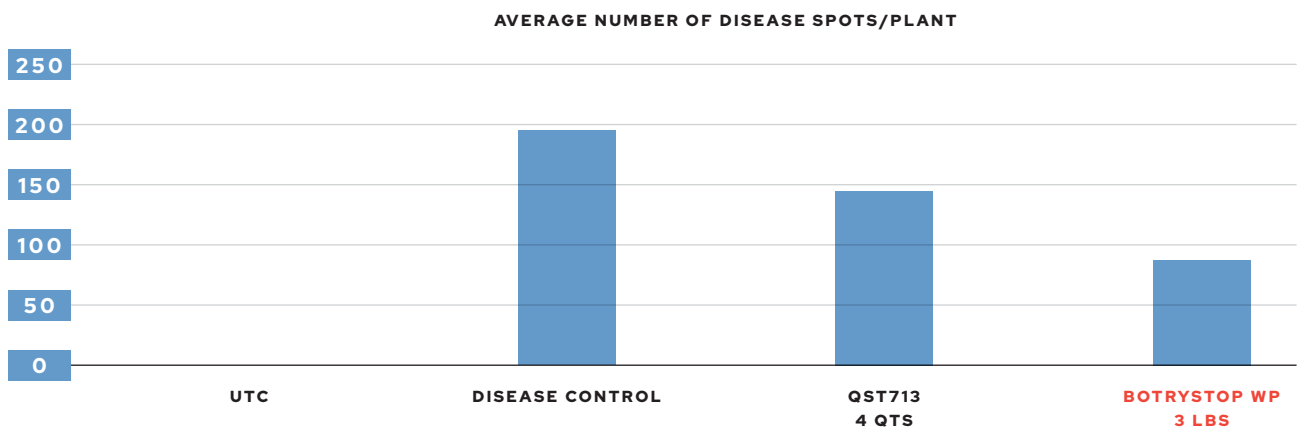
Key takeaways:

- Of three biofungicides tested, BotryStop WP performed the best under the "heavy disease pressures" experienced in the trial
- BotryStop WP at 4 lbs/100 gals was the best treatment tested, providing control that was significantly better than competing biopesticide treatments, and provided control that was statistically the same as that provided by the chemical standard, Broadform
- *Botrytis* infection levels were significantly lower in the BotryStop WP treatments at 3 and 4 lbs/100 gals and were superior to the other biofungicides tested (Botector, EcoSwing)

Research trials carried out by M.K. Hausbeck and B.R. Harlan, Michigan State University.

V. BOTRYSTOP WP VS *XANTHOMONAS* (BLS) ON GERANIUM



Key takeaways:

- BotryStop WP provided superior control of BLS on geranium (results taken 14 days after inoculation)
- Out-performed *B. subtilis* QST-713 CEASE®, the biological standard
- BotryStop WP is an effective biopesticide for some bacterial leaf spot diseases

Research trial carried out by Dr. Dave Norman, University of Florida.

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USE OF BOTRYSTOP WP IN AN INTEGRATED PLANT HEALTH STRATEGY

Earlier, we reviewed different cultural practices that will reduce *Botrytis* inoculum levels, and avoiding environmental conditions that facilitate infection, sporulation and spread of the disease within a susceptible crop. These should always be the first line of defense; BotryStop WP then provides another layer of protection for the crop. Additional supportive measures, which are fully compatible with the use of BotryStop WP, include use of nutritional supplements, other biopesticides (e.g., MilStop) and, if necessary, synthetic fungicides. Several commonly used fungicides are safe to use together with BotryStop WP, either in a tank mix or spray rotation (see BioWorks compatibility guide for more up to date information **https://bioworksinc.com/**).

Calcium is an important component of plant cell walls and reinforces the structure of plant tissue. But it is one of the hardest nutrients to translocate efficiently throughout the plant, particularly to leaf margins or flowers, which often leads to necrosis. Consequently, anything we can do to help calcium uptake helps plants build stronger cells. This reduces the likelihood of necrotic leaf margins developing, and hence the prevalence of infection sites, and produces healthy cells that are more resistant to infection. That's why calcium sprays are applied in susceptible crops (e.g., poinsettia, petunia) at times of peak nutrient demand; but uptake efficiency is still poor.

Calcium uptake efficiency can be significantly improved if applied with sprayable protein hydrolysates, especially in hard-to-reach parts of the plant like leaf margins, bracts and flowers. ON-Gard® Calcium is a pre-formulated product containing both calcium and protein hydrolysate. Dr. Jim Faust, Clemson University, carried out trials on petunia, whose flowers are very susceptible to grey mold; susceptibility is linked to poor movement of calcium into the flowers resulting in deficiencies, weaker cell walls, and increased susceptibility to *Botrytis*. Spray application of ON-Gard® Calcium alone significantly reduced disease severity in petunia, reducing incidence to levels that were on par with the untreated control plants, and lower than those observed in the standard chemical fungicide treatment. In the same trial, a drench application of RootShield® WP also significantly reduced the severity of *Botrytis* infections in the petunia flowers, either because of its ability to enhance plant's access to soil nutrients, and/or its ability to activate plant defense pathways.

These trials clearly demonstrate the benefits of using an integrated approach, and how actions not directly targeting foliar pathogens can help plants resist infection by *Botrytis*. Actions that decrease the prevalence of susceptible plant tissues lead to fewer infection sites and lower disease pressures, a much better starting point for biofungicides like BotryStop WP.

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Further reading

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GOING ABOVE AND BEYOND

At BioWorks, we not only help eradicate harmful diseases and pests that threaten your crops, but we design and support integrated plant health management programs. And because we know how quickly some diseases can work, we respond to any questions you might have in just four hours. So you can get back to the business of successful growing.

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BIOWORKS
100 Rawson Road • Suite 205
Victor, NY 14564
expert@bioworksinc.com
bioworksinc.com • 800.877.9443