2019

# Hemp Flower Fungicide Evaluation Trial

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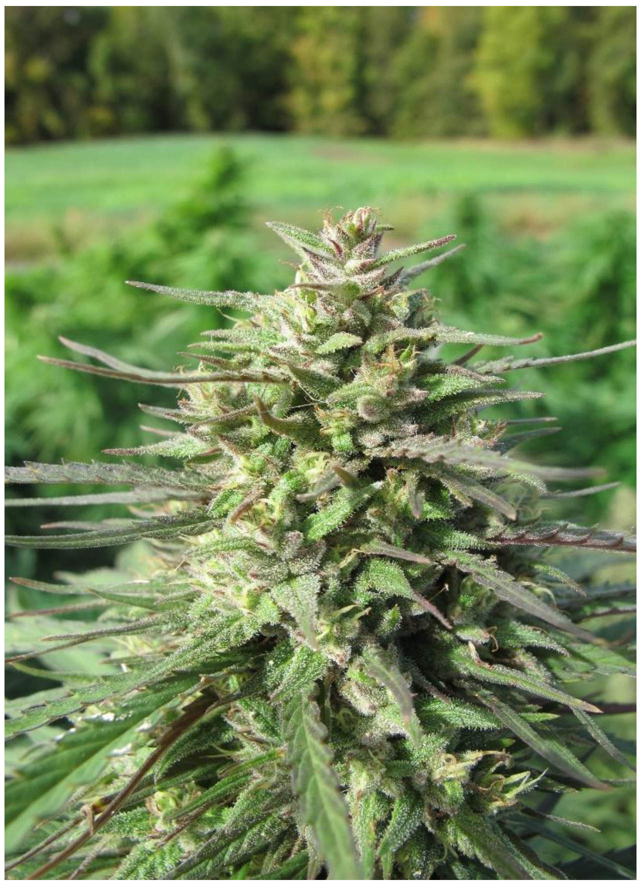

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THE UNIVERSITY OF VERMONT
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**2019 HEMP FLOWER FUNGICIDE EVALUATION TRIAL**

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Hemp is a non-psychoactive variety of *Cannabis sativa L*. The crop is one of historical importance in the U.S. and re-emerging worldwide importance as medical providers and manufacturers seek hemp as a renewable and sustainable resource for a wide variety of consumer and industrial products. Hemp grown for all types of end-use (health supplement, fiber, and seed) contains less than 0.3% tetrahydrocannabinol (THC). Hemp varieties intended to produce a health supplement contain relatively high concentrations of a compound called cannabidiol (CBD), potentially 10-15%. CBD has purported benefits such as relief from inflammation, pain, anxiety, seizures, spasms, and other conditions. The CBD is the most concentrated in the female flower buds of the plant; however, it is also in the leaves and other plant parts as well.

To produce hemp for flower, the plant is generally grown intensively as a specialty crop and the flowers are cultivated for maximum growth. A number of diseases are known to impact the quality and marketability of flower material, and may result in the inability to sell the crop.

*Sclerotinia sclerotiorum (*white mold) can infect stem material and greatly inhibit or completely stop flower development, and result in toppling branches depending on severity. In humid conditions, browning stems and formation of a white mold can be seed on the surface of the stem. As the disease progresses, reproductive structures known as sclerotia develop within stems as hard, black, oblong bodies which can allow the disease to overwinter in detritus or soil.

*Botrytis cinerea* (gray mold) has the potential to impact all types of *Cannabis* and thrives under cool, damp conditions often prevalent in our Northeast climate. Hemp flowers are most susceptible as they are born in dense clusters, creating ideal conditions for this disease. Most common symptoms include browning of flower material and the formation of gray fuzz on the plant material under humid conditions, whereas drier conditions will just result in brown and withered plant flowers. Leaf and stalks may also turn chlorotic and cankers may form further weakening stalks. Reproducing bodies such as the conidia, formed in the gray fuzz, can be blown or splashed from plant to plant or the disease can overwinter on infected seeds or plant debris in the form of sclerotia.

*Glovinomyces spp.* (powdery mildew) is most commonly seen starting as white spots on leaves or stems and can spread covering the entirety of a plant. This disease may be more prevalent with poor air circulation and drier conditions with spores carried by wind. Severe cases of powdery mildew may result in premature yellowing, browning, or dieback, and may impact overall flower quality.

To help farmers succeed, agronomic research on hemp is needed in the United States. University of Vermont, in partnership with the CASE Institute (https://www.caseinstitute.org/), evaluated the efficacy of four products intended for control of various pathogens including gray mold *(Botrytis cinerea)*, white mold (*Sclerotinia sclerotiorum*), and powdery mildew (*Glovinomyces spp.).* This preliminary study evaluated the efficacy of Cease, Actinovate, Regalia, and Trifecta on control of these three major hemp diseases. Efficacy of these four products was evaluated under two growing conditions: 1.) “indoors” under a plastic hoop house and 2.) “outdoors” without cover. Plastic hoop houses or “caterpillar tunnels” can be an inexpensive alternative to typical high tunnels and can provide greater control over environmental conditions for a variety of crops, yet may provide conditions ideal for various diseases without control.

A list of currently approved active ingredients and example commercial products for use in industrial hemp cultivation can be found here:

https://agriculture.vermont.gov/sites/agriculture/files/documents/PHARM/hemp/Hemp%20products.pdf

As stated by Vermont Agency of Agriculture, Food and Markets: currently approved active ingredients (as of May 14, 2019) for industrial hemp cultivation must be exempt from a tolerance by US EPA, be intended for unspecified food crops, be registered in Vermont (section 3, or minimum risk), and have an agricultural use label for hemp intended for commercial sale. Products must also be used in accordance with the label and applicators must be Vermont certified if applying product on someone else's hemp crop. Organic and OMRI listings do not automatically make products approved for use on industrial hemp.

## MATERIALS AND METHODS

The experimental design was a randomized complete block with split plots and 4 replicates. The main plots were fungicides and the split plots were growing condition (Indoor and Outdoor). Plots consisted of single plants spaced 5' apart in the row and between rows with one buffer plant in between treatments to avoid overspray of products between plants (Table 1). On 19-Jun, clones (var Boax) were transplanted into black plastic mulch with drip tape. Fertility amendments were based on soil test results received from the University of Vermont Agricultural and Environmental Testing Laboratory (Burlington, VT). The field was fertilized with 120 lbs N ac$^{-1}$ over the course of six weeks via fertigation. Nitrogen was applied in the form of ammonium nitrate plus sulfur (28-0-0) distributed evenly through 1000 gallons of water using a Dosatron unit. In addition, potassium chloride (0-0-62) was applied at a rate of 100 lbs ac$^{-1}$ just following planting. Based on soil test results, no further nutrients were required for production of hemp. Irrigation was applied on a weekly basis at a rate of 8000 gallons of water per acre delivered via drip tape. Irrigation duration and amount was modified based on weekly rainfall.

**Table 1. Agronomic information for the hemp variety trial 2019, Alburgh, VT.**

| **Location** | **Borderview Research Farm Alburgh, VT** |
|---|---|
| **Soil type** | Benson rocky silt loam, 3-5% slope |
| **Previous crop** | Organic corn |
| **Hemp variety** | Boax |
| **Plant spacing (ft)** | 5 x 5 |
| **Planting date** | 19-Jun |
| **Harvest date** | 25-Oct |
| **Fertilization** | 120 lbs N ac$^{-1}$, 60 lbs K ac$^{-1}$ |

From 13-Sep through 15-Oct, fungicide product treatments were applied every 7 to 10 days to treatment plants. Recommended rates of Cease, Actinovate, Regalia, Trifecta, and a Control (water) were mixed in water to fill a 3.6-gallon backpack sprayer (Table 2). Products were applied via a 3.6 gallon Invatech Italia 868 backpack sprayer (Baltimore, MD) with each plant receiving an equal application of fungicide solution applied thoroughly to the entire plant.

**Table 2. Fungicide application rates.**

| Product | Application rate $ac^{-1}$ |
|---|---|
| **Actinovate** | 12 oz |
| **Cease** | 2 qt |
| **Regalia** | 2 qt |
| **Trifecta** | 25 oz |
| **Control (water)** | N/A |

### Actinovate AG

Actinovate (Novozymes BioAg Inc., EPA Reg. No. 73314-1) contains a strain of *Streptomyces lydicus* and can be used to suppress or control foliar fungal, root rot, and damping off pathogens. This product is labeled for use against downy mildew and other pathogens. Actinovate AG contains live spores of the *Streptomyces* microbe and works best if it is used prior to disease onset.

http://www.monsantobioag.com/global/us/Products/Documents/actinovateag_biofungi_18oz_case_calif_4_13114.pdf

### Cease

Cease (Bioworks Inc., EPA Reg. No. 264-1155-68539) contains a strain of *Bacillus subtilis* and uses multi-site modes of action to avoid the development of resistance. This product can be used to control a variety of fungal pathogens and bacterial diseases and is intended for use as both a foliar spray and a soil drench. Cease is to be used at the onset of favorable disease conditions prior to the onset of symptoms.

http://www.bioworksinc.com/products/cease.php

### Regalia

Regalia (Marrone Bio Innovations, EPA Reg. No. 84059-3) is a broad-spectrum bio-fungicide that is active against soil borne and foliar pathogens. The active ingredient is extracted from giant knotweed (*Fallopia sachalinensis*). Regalia works by stimulating the plant's natural defenses and has antifungal and antibacterial properties.

http://marronebioinnovations.com/ag-products/brand/regalia/

### Trifecta

Trifecta Crop Control (Trifecta LLC, EPA/FIFRA exempt (25b) insecticide/fungicide) is a nano-emulsified essential oil based multi-purpose mold, mildew and pest control. The active ingredients include soap, isopropyl alcohol, thyme oil, clove oil, garlic oil, vinegar, peppermint oil, corn oil, geraniol, citric acid and rosemary oil. Trifecta uses the various essential oils to act as a repellent, suffocant, reproductive inhibitor, and fungicide and can be applied directly to foliage or as a soil drench depending on target pest.

https://www.trifecta.com.bz/

On a weekly basis, scouting took place from 13-Sep until 15-Oct. One plant per plot was scouted for major diseases. Entire plant assessments were made for disease with total number of infected buds, leaves, or stems counted and severity rated for gray mold *(Botrytis cinerea)* (Image 1), white mold (*Sclerotinia sclerotiorum*) (Image 2), and powdery mildew (*Glovinomyces spp.)* (Image 3). Severity was rated on a 1-5 scale, with a rating of 1 being least severe and a rating of 5 being most severe. Least severe cases were noted as single flower clusters showing degradation or infection, most severe cases would be indicative of entire stems or colas showing disease infection.

**Image 1.** ***Botrytis cinerea on hemp flower, Alburgh, VT.***

On 25-Oct, two pounds of plant material was harvested from flowering branches distributed across the plant. This subsample was used to determine the percentage of unmarketable, diseased flower material from each treatment. Smaller branched sections and larger "fan" or "sun" leaves were removed by hand, while smaller leaves were left attached since they subtend from the flower bract.

**Image 2. *Sclerotinia sclerotiorum* on hemp stem. Alburgh, VT.**

The data were analyzed using mixed model analysis using the mixed procedure of SAS (SAS Institute, 1999). Mean comparisons were made using the Least Significant Difference (LSD) procedure when the F-test was considered significant ($p<0.10$). Data was analyzed using the PROC MIXED procedure in SAS with the Tukey-Kramer adjustment, which means that each variable was analyzed with a pairwise comparison (i.e. 'variety 1' statistically outperformed 'variety 2', 'variety 2' statistically outperformed 'variety 3', etc.). Relationships between variables were analyzed using the GLM procedure.

**Image 3. *Glovinomyces spp.* on hemp plant. Alburgh, VT.**

Variations in yield and quality can occur because of variations in genetics, soil, weather, and other growing conditions. Statistical analysis makes it possible to determine whether a difference among treatments is real or whether it might have occurred due to other variations in the field. At the bottom of each table a p-value is presented for each variable that showed statistical significance (p-value ≤ 0.10). In this case, the difference between two treatments within a column is equal to or greater than the least significant difference (LSD) value and you can be sure that for 9 out of 10 times, there is a real difference between the two treatments. In this example, hybrid 3 is significantly different from hybrid 1 but not from hybrid 2. Hybrid 2 and hybrid 3 have share the same letter 'a' next to their yield value, to indicate that these results are statistically similar. The difference between hybrid 3 and hybrid 2 is equal to 1.5, which is less than the LSD value of 2.0. This means that these hybrids did not differ in yield. The difference between hybrid 3 and hybrid 1 is equal to 3.0, which is greater than the LSD value of 2.0. This means that the yields of these hybrids were significantly different from one another. The letter 'b' next to hybrid 1's yield value shows that this value is significantly different from hybrid 2 and hybrid 3, which have the letter 'a' next to their value.

| Treatment | Yield |
|---|---|
| Hybrid 1 | 6.0 b |
| Hybrid 2 | 7.5a |
| Hybrid 3 | **9.0**a |
| | |
| LSD (p-value ≤ 0.10) | 2.0 |

Participants of State Hemp Programs intending to grow are required to follow state and federal regulations regarding hemp production and registration. Growers must register within their intended state for production and must adhere to most current or active rules and regulations for production within a grower's given state. Regulations are subject to change from year to year with the development and approval of proposed program rules and it is important to note that regulations may vary across state lines and may be impacted by pending federal regulations. Please refer to this https://agriculture.vermont.gov/sites/agriculture/files/documents/PHARM/hemp/Industrial_Hemp_Rule_%20SOS_05172019.pdf for a detailed outline of proposed rules in Vermont. Additional information regarding the Vermont Agency of Agriculture, Food and Markets (VAAFM) Hemp Program can be found on the VAAFM website here:

https://agriculture.vermont.gov/public-health-agricultural-resource-management-division/hemp-program.

# RESULTS

Seasonal precipitation and temperature were recorded with a Davis Instrument Vantage Pro2 weather station, equipped with a WeatherLink data logger at Borderview Research Farm in Alburgh, VT (Table 3). The month of July was hot and dry when compared to the 30-year average, followed by a slightly cooler than normal August and September. Fortunately, October saw higher than normal temperatures allowing the hemp plants to develop fully. July and August were below average precipitation amounts but this was made up or during the month of October. Overall, there were an accumulated 2211 Growing Degree Days (GDDs) this season, approximately 197 more than the historical average, with much of the heat coming mid-season. Hemp plants received supplemental irrigation to account for precipitation deficits throughout the growing season, as needed.

**Table 3. Seasonal weather data collected in Alburgh, VT, 2019.**

| **Alburgh, VT** | June | July | August | September | October |
|---|---|---|---|---|---|
| Average temperature (°F) | 69.2 | 73.5 | 68.3 | 60.0 | 50.8 |
| Departure from normal | 0.84 | 2.84 | -0.53 | -0.62 | 0.14 |
| | | | | | |
| Precipitation (inches) | 1.71 | 2.34 | 3.50 | 3.87 | 3.85 |
| Departure from normal | 0.33 | -1.81 | -0.41 | 0.23 | 1.88 |
| | | | | | |
| Growing Degree Days | 446 | 716 | 568 | 335 | 146 |
| Departure from normal | -29 | 76 | -13 | 17 | 146 |

Based on weather data from a Davis Instruments Vantage Pro2 with WeatherLink data logger. Historical averages are for 30 years of NOAA data (1981-2010) from Burlington, VT.

### *Fungicide x location interactions*

There were statistically significant fungicide x location interactions for the seasonal average of gray mold severity and white mold incidence within this trial. These interactions indicate that the fungicide treatments performed differently depending on growing conditions. Applying Cease in the indoor location may be slightly more effective than when applying in the outdoor location for white mold control (Figure 1). Conversely, Trifecta may be more effective when applied in an outdoor location compared to indoors.

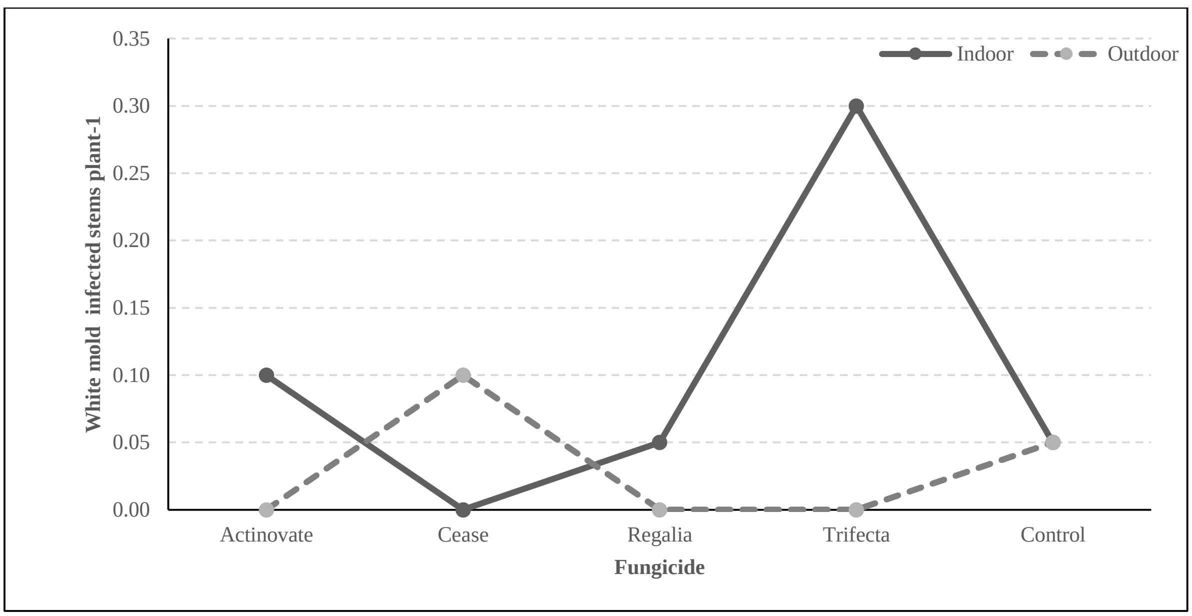


**Figure 1. Interaction of fungicide applications and location for average white mold infected stems plant$^{-1}$,Alburgh, VT, 2019.**

In the case of gray mold severity, Actinovate, Cease, and Trifecta appeared to be more effective in reducing severity in the indoor location compared to outdoors, whereas Regalia appeared to be more effective in reducing severity in the outdoor location (Figure 2). As a number of these products were derived from biological sources, they may respond to locational growing conditions differently, potentially impacting their efficacy under varied conditions.

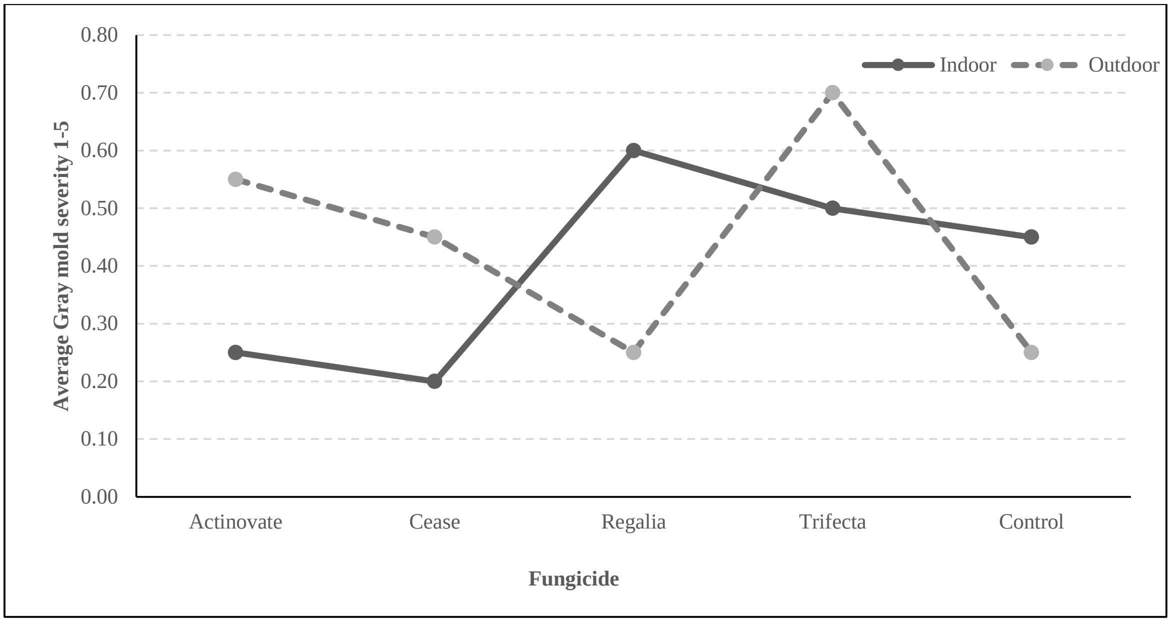


**Figure 2. Interaction of fungicide applications and location for average gray mold severity, Alburgh, VT, 2019.**

***Impact of fungicide***

Scouting took place from 13-Sep through 15-Oct and results were recorded for disease incidence and severity at each date. Table 4 shows the incidence and severity of disease averaged across all scouting events. Severity was rated on a 1-5 scale, with a rating of 1 being least severe and a rating of 5 being most severe. Least severe cases for gray mold were noted as single flower clusters showing degradation or infection, most severe cases would be indicative of entire stems or colas showing disease infection. Least severe cases of white mold were noted as having single point infections on stems with most severe cases including branches entirely molded or browned. Least severe cases of powdery mildew were noted as small white spots on leaves with severe cases having leaves entirely covered with white powdery symptoms.

There were low levels of disease incidence recorded throughout the scouting period. When averaged over the entire scouting period, the incidence and severity of disease in the control treatment was not significantly different from the top performing fungicide treatment. In a few cases, the fungicide treatment had more disease than the control. In this study, the fungicide treatments appeared to provide little control of the target diseases (Table 4).

**Table 4. Average disease incidence and severity for powdery mildew, white mold, and gray mold for applied fungicides, Alburgh, VT, 2019.**

| Treatment | Powdery mildew | | White mold | | Gray mold | |
|---|---|---|---|---|---|---|
| | # infected leaves plant$^{-1}$ | severity 1-5‡ | # infected stems plant$^{-1}$ | severity 1-5 | # infected colas plant$^{-1}$ | severity 1-5 |
| **Actinovate** | 0.05 ab† | 0.05 | 0.05 ab | **0.00 a** | 0.55 | 0.40 ab |
| **Cease** | 0.23 b | 0.05 | 0.05 ab | 0.10 ab | 0.43 | **0.33 a** |
| **Regalia** | 0.08 ab | 0.03 | **0.03 a** | 0.13 ab | 0.45 | 0.43 ab |
| **Trifecta** | **0.00 a** | 0.00 | 0.15 b | 0.20 b | 0.70 | 0.60 b |
| **Control** | 0.00 a | 0.00 | 0.05 ab | 0.08 ab | 0.68 | 0.35 a |
| **LSD (0.10)** | 0.19 | NS ¥ | 0.11 | 0.19 | NS | 0.24 |
| **Trial mean** | 0.07 | 0.03 | 0.07 | 0.10 | 0.56 | 0.42 |

†Within a column treatments marked with the same letter were statistically similar (p=0.10). Top performers are in **bold.**
‡Rating of 1-5 where 1= least severe and 5=most severe.
¥ NS – There was no statistical difference between treatments in a particular column (p=0.10).

While none of these diseases appeared to be in great presence over the course of the scouting period, it is worth noting that disease was most prevalent and severe in the last week of scouting (Table 5). While there was no significant difference in powdery mildew and white mold incidence or severity, there were significant differences amongst treatments for the severity of gray mold on flowers. Gray mold appeared to be most severe in the Trifecta treatment and was only significantly different from the Cease treatment. Cease performed similarly to all other treatments.

**Table 5. 15-Oct disease incidence and severity for powdery mildew, white mold, and gray mold for applied fungicides, Alburgh, VT, 2019.**

| Treatment | Powdery mildew | | White mold | | Gray mold | |
|---|---|---|---|---|---|---|
| | # infected leaves plant$^{-1}$ | severity 1-5‡ | # infected stems plant$^{-1}$ | severity 1-5 | # infected colas plant$^{-1}$ | severity 1-5 |
| **Actinovate** | 0.25 | 0.25 | 0.25 | 0.00 | 2.25 | 1.63 ab |
| **Cease** | 0.50 | 0.13 | 0.25 | 0.50 | 1.38 | **1.25 a** |
| **Regalia** | 0.00 | 0.00 | 0.13 | 0.63 | 1.88 | 1.88 ab |
| **Trifecta** | 0.00 | 0.00 | 0.50 | 0.75 | 3.00 | 2.50 b |
| **Control** | 0.00 | 0.00 | 0.13 | 0.25 | 3.13 | 1.50 ab |
| **LSD (0.10)** | NS | NS | NS | NS | NS | 1.13 |
| **Trial mean** | 0.15 | 0.08 | 0.25 | 0.43 | 2.33 | 1.75 |

†Within a column treatments marked with the same letter were statistically similar (p=0.10). Top performers are in **bold.**
‡Rating of 1-5 where 1= least severe and 5=most severe.
¥ NS – There was no statistical difference between treatments in a particular column (p=0.10).

On 25-Oct, two pounds of hemp flower was harvested at random from various flowering branches distributed across the plant. This was used to determine the percentage of unmarketable flower caused by disease (Table 6). The quantity of diseased flower was low across all fungicide treatments with a trial average of 1.87%. Cease had a significantly lower amount of diseased flower (0.800%) compared to all other treatments. Actinovate and Trifecta had significantly more diseased material than all other treatments.

**Table 6. Percentage of diseased flower material at harvest for applied fungicides, Alburgh, VT, 2019.**

| Treatment | Diseased flower<br>% |
|---|---|
| **Actinovate** | 2.71 b |
| **Cease** | **0.800a** |
| **Regalia** | 1.64 ab |
| **Trifecta** | 2.27 ab |
| **Control** | 1.91 ab |
| **LSD (0.10)** | 0.156 |
| **Trial mean** | 1.87 |

†Within a column treatments marked with the same letter were statistically similar (p=0.10). Top performers are in **bold.**

### *Impact of location*

Overall, location appeared to have no impact on the incidence or severity of any disease throughout the entirety of the scouting period, or while looking at the percentage of diseased harvested material (Tables 7 and 9).

**Table 7. Average disease incidence and severity for powdery mildew, white mold, and gray mold for location, Alburgh, VT 2019.**

| Treatment | Powdery mildew | | White mold | | Gray mold | |
|---|---|---|---|---|---|---|
| | # infected leaves plant$^{-1}$ | severity 1-5‡ | # infected stems plant$^{-1}$ | severity 1-5 | # infected colas plant$^{-1}$ | severity 1-5 |
| **Indoor** | 0.08 | 0.02 | 0.10 | 0.15 | 0.44 | 0.40 |
| **Outdoor** | 0.06 | 0.03 | 0.03 | 0.05 | 0.68 | 0.44 |
| **LSD (0.10)** | NS ¥ | NS | NS | NS | NS | NS |
| **Trial mean** | 0.07 | 0.03 | 0.07 | 0.10 | 0.56 | 0.42 |

†Within a column treatments marked with the same letter were statistically similar (p=0.10). Top performers are in **bold.**
‡Rating of 1-5 where 1= least severe and 5=most severe.
**¥** NS – There was no statistical difference between treatments in a particular column (p=0.10).

During the last scouting date, white mold incidence was significantly higher outdoors (Table 8). While not significantly different, gray mold and powdery mildew incidence and severity was highest in the outdoor location.

**Table 8. 15-Oct disease incidence and severity for powdery mildew, white mold, and gray mold for location, Alburgh, VT, 2019.**

| Treatment | Powdery mildew | | White mold | | Gray mold | |
|---|---|---|---|---|---|---|
| | # infected leaves plant$^{-1}$ | severity 1-5‡ | # infected stems plant$^{-1}$ | severity 1-5 | # infected colas plant$^{-1}$ | severity 1-5 |
| **Indoor** | 0.00 | 0.00 | 0.40 | 0.65 | 1.75 | 1.60 |
| **Outdoor** | 0.30 | 0.15 | **0.10** | 0.20 | 2.90 | 1.90 |
| **LSD (0.10)** | NS ¥ | NS | 0.29 | NS | NS | NS |
| **Trial mean** | 0.15 | 0.08 | 0.25 | 0.43 | 2.33 | 1.75 |

†Within a column treatments marked with the same letter were statistically similar (p=0.10). Top performers are in **bold.**
‡Rating of 1-5 where 1= least severe and 5=most severe.
**¥** NS – There was no statistical difference between treatments in a particular column (p=0.10).

**Table 9. Percentage of diseased flower material at harvest for location, Alburgh, VT, 2019.**

| Treatment | Diseased flower |
|---|---|
| | % |
| **Indoor** | 1.43 |
| **Outdoor** | 2.30 |
| **LSD (0.10)** | NS ¥ |
| **Trial mean** | 1.87 |

¥ NS – There was no statistical difference between treatments in a particular column (p=0.10).

# DISCUSSION

During the scouting period, the vast majority of plants were not impacted by disease until the last two weeks of scouting. At this point, powdery mildew, gray mold, and white mold became much more prevalent on plants within the trial area. Throughout this trial, we observed a large amount of branch splitting as terminal bud mass increased, which may have provided opportunity for some infection points for disease during these later periods of scouting. Large plant size, later maturation and harvest dates, and dense growth structure of Boax variety may have been contributing factors that could increase chance of disease. Overall, the disease incidence and severity of this variety was low and may indicate some resistance to disease. During weekly scouting, the control treatment performed as good or better than all fungicide treatments in the trial. However, when measuring percentage of flower material at harvest with disease, it appeared that treatment with the fungicide Cease was most effective in controlling disease. Due to the influence of yearly weather on disease incidence and severity, this study should be repeated to further determine efficacy of these products in hemp.

Overall, there were few differences between plants in the indoor location indicating that this particular covered structure may not have much of an impact on the disease incidence and severity regardless of fungicide applications. The use of a caterpillar structure for hemp production could potentially provide the added benefits of season extension and environmental control without increasing the chance of disease, however this represents only one year of data.

As stated by Vermont Agency of Agriculture, Food and Markets: currently approved active ingredients (https://agriculture.vermont.gov/sites/agriculture/files/documents/PHARM/hemp/Hemp%20products.pdf) as of May 14, 2019, for industrial hemp cultivation must be exempt from a tolerance by US EPA, be intended for unspecified food crops, be registered in Vermont (section 3, or minimum risk), and have an agricultural use label for hemp intended for commercial sale. Products must also be used in accordance with the label and applicators must be Vermont certified if applying product on someone else's hemp crop. Organic and OMRI listings do not automatically make products approved for use on industrial hemp. The list provided by the VAAFM includes products designed to be used as pesticides, including those specific as fungicides. The products tested within this trial were applied for experimental purposes and use within this trial does not indicate their allowance for use in industrial hemp cultivation.

# ACKNOWLEDGEMENTS

The UVM Extension Northwest Crops and Soils Program would like to thank the Nutraceuticals Science Laboratory for their collaboration on this project. Special thanks to Roger Rainville and the staff at Borderview Research Farm for their generous help with the trials. We would also like to thank Catherine Davidson, Hillary Emick, Haley Jean, Ivy Luke, Rory Malone, and Sara Ziegler for their assistance with data collection and entry. We would also like to acknowledge Taylor Readyhough for his assistance with cultivar selection and propagation. The information is presented with the understanding that no product discrimination is intended and no endorsement of any product mentioned or criticism of unnamed products is implied.

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