HEMP NEWSLETTER

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Aspergillus Ecology and Mitigation Strategies in Hemp Production

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Aspergillus fungus is an increasing concern to Oregon hemp industry. This fungus belongs to *Aspergillus* genus, contains over 300 species, and occurs across the globe. *Aspergillus* is frequently found in various natural habitats and agricultural settings, is especially common in soil, considering this fungus reservoir. Only certain *Aspergillus* species produce mycotoxins known as aflatoxins, including *A. flavus* and *A. parasiticus*. Aflatoxins are poisonous to humans and animals including birds, cats, cattle, dogs, sheep, and swine. While some *Aspergillus* species, such as *A. niger*, are sources of beneficial metabolites (antibiotics and enzymes), they contain pathogenic strains that incite human, animal and plant diseases. *Aspergillus* infection in plants can ruin harvested crop portions and result in mycotoxin contamination.

Aspergillus affects a wide range of crop species, and important crops impacted by this fungus include corn, peanuts, cottonseed, and tree nuts. Recently, this fungus has been found to infect industrial hemp in the US. Species of *Aspergillus* commonly associated with hemp are *A. flavus, A. fumigatus, A. niger,* and *A. terreus*. Environmental conditions that promote plant diseases incited by this fungus are variable and depend on the crop grown. Plant-pathogenic *Aspergillus* strains may be host-specific (Sweany et al. 2011) as well as saprophytic (able to grow and reproduce on dead or dying organic matter), and this versatility helps to ensure the perpetuation of the fungus.

Aspergillus Biology

Aspergillus mainly produce asexual spores known as conidia and sclerotia (Fig. 1) and sometimes produce sexual spores (ascospores) as well (Horn et al. 2014). Sclerotia are small (< 0.04 inch in diameter), durable, and have compact fungal mass mycelium (Fig. 1) with a darkly pigmented outer layer. This layer protects the sclerotia from the detrimental effects of ultraviolet energy in sunlight. Sclerotia are formed in/on infected plant material or in association with saprophytic growth on dead organic matter. Sclerotia eventually end up in the soil as infected plant material decomposes in the field. Under conducive environmental conditions, sclerotia can activate and produce conidia or ascospores, and result in pathogen spread.

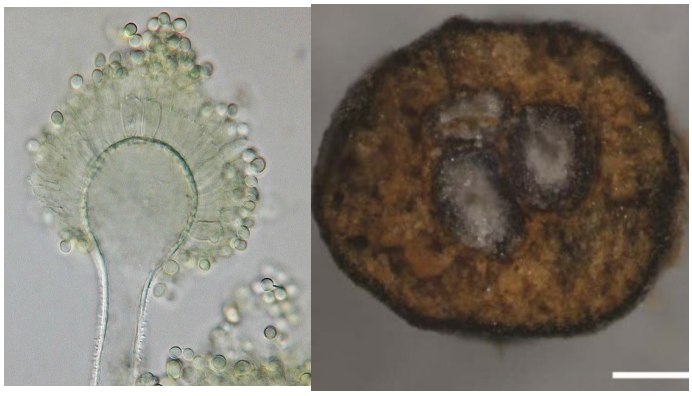

Figure 1. *Aspergillus flavus* showing the microscopic round conidia (**Left**) (Photo Credit: Schooch et al. 2020); A sclerotium cut in half showing darkened rind and hyaline ascospore-bearing structures towards the center (**Right**) (Horn et al. 2014)

In Canada, 34 different fungal species were recovered from harvested cannabis buds with discoloration, including *A. flavus*, *A. niger*, and *A. ochraceus*, which were recovered at relatively low levels (≤ 10%). In Oregon, Ocamb and Thomas

(unpublished) found *Aspergillus* occurring at low levels in hemp seeds of some varieties when 100-seed samples were plated onto a *Fusarium* semi-selective medium (Fig. 2; Table 1). When a larger prevalence of *Aspergillus* is found in association with harvested cannabis portions, it is likely that the greater presence developed post-harvest.

Figure 2. A yellowish *Aspergillus* colony growing from an infested hemp seed. Photo by Cynthia M. Ocamb, Oregon State University

Aflatoxins in Aspergillus

Four aflatoxin types produced by *Aspergillus* species are the most important: B1, B2, G1, and G2 (Shabeer et al. 2022). Among them, aflatoxin B1 has the greatest toxicity and is the most common aflatoxin type found in food. We can be exposed to aflatoxins by eating contaminated plants, meat, or dairy products. However, agricultural workers may be exposed to both aflatoxins and spores by inhaling dust generated during the handling and processing of contaminated crops (https://www.cancer.gov/). Aflatoxins can contaminate crops before or after harvest, with disease continuing to spread in storage if environmental conditions are not well controlled during storage. The US regulates corn, peanut, and other crops for mycotoxins; the standard for aflatoxins is 20 ppb for human food and animal feed.

Table 1. Detection of fungi associated with seeds of 5 hemp varieties on amended Nash-Snyder medium

	% seeds				
Hemp Variety	No microbial growth (clean seed)	Confirmed *Fusarium*	*Cladosporium* and/or *Penicillium*	*Aspergillus* species	Other fungi
A	98	0	1	0	1
B	18	0	29	1	52
C	80	0	6	0	14
D	36	4	10	4	46
E	11	1	7	1	80

John Hughes Bennett first described *Aspergillus* growing in the lung tissue of humans in 1842 (Bueid 2012). Around 20 *Aspergillus* species have been confirmed to cause human infections, although around 95% of *Aspergillus* diseases in humans are caused *A. flavus*, *A. niger*, and *A. fumigatus*. Infection routes include inhalation of airborne spores, exposure to contaminated water when bathing, and exposure in hospital settings to contaminated hospital fabrics and plastics. The incubation period after exposure before symptoms develop is 2 days to 3 months, but not everyone exposed to *Aspergillus* spores will develop the disease. Individuals with compromised or weakened immune systems are more prone to developing lung infections. However, humans and other animals are generally susceptible to the toxic effects of aflatoxin exposure, and intestinal bleeding, liver damage, death, and/or cancer may result after exposure. In the 1960s, more than 100,000 young turkeys died in England from 'Turkey-X disease', which was eventually understood to be due to peanut meal contaminated with aflatoxins that was fed to the young birds. In 2004, 125 people died of aflatoxin exposure in Kenya and more than half of the maize samples from markets in the region had aflatoxin levels greater than 20 ppb (Lewis et al. 2005). In 2005, more than 75 dogs in the US died due to pet food contaminated with aflatoxins, and hundreds more experienced severe liver problems. Again in 2020, another outbreak of aflatoxin-contaminated pet food occurred with more than 110 dogs dying after consuming affected pet food (https://www.avma.org). Both times, there were

national dog food recalls after batches of pet food were found to contain fatal levels of aflatoxins.

Potential mitigation strategies

- *Aspergillus* are ubiquitous fungi and can thrive in all types of cannabis cultivation systems, whether indoors or outdoors.
- Specifically, during the harvesting and storing processes, 1) discard insect- and pathogen-infested materials because these materials may contain *Aspergillus* and may promote pathogen growth, 2) choose the appropriate drying methods such as a hot air drying method that provides uniform drying, 3) trim the flowers after the harvest (vs wet trimming) to reduce mold and pathogen risks, 4) don't store unprocessed samples in an uncontrolled environment, and 5) avoid using plastic materials for storing and packaging to reduce pathogen growth.
- The introduction of *Aspergillus* spores into facilities can happen through various channels including contaminated clones or seeds, inadequate hygiene and sanitation practices among employees, and failures in heating, ventilation, air conditioning, and dehumidification (HVACD) systems. Effective mitigation largely hinges on adopting good agricultural and cleaning practices. Being acutely aware of the specifics of your growing environment enables the identification of potential risks and the implementation of appropriate strategies.
- Employing air purification techniques lowers the risk of mold proliferation. Incorporating HEPA filtration and UV lighting in HVAC systems can be helpful. Regular maintenance and monitoring of air quality are important.
- Prompt and appropriate pruning improves under-canopy airflow and discourages the creation of moisture-rich microenvironments that nurture mold development.
- Implement appropriate sanitation practices - utilize chemical sanitizers or UV light for facility and equipment sanitation, handle cannabis on clean surfaces, and ensure the use of personal protective equipment (PPE) such as masks and gloves to lessen contamination risks.
- During the postharvest phase, monitoring of temperature and humidity levels is essential.
- From the limited research findings so far, *Y*-ray can effectively eliminate *Aspergillus*. Other promising decontamination alternatives include Electron Beam (E-beam) irradiation and X-rays, which function similarly to Y-rays but require further validation.
- Cold plasma is a novel nonthermal decontamination approach in the food industry, which demonstrates not only a reduction in *Aspergillus* counts but also in aflatoxin levels. However, its impact on the chemical composition or texture of cannabis products necessitates further study.
- Extensive research is required to assess the efficacy of these methods and their effects on the desired active chemical compounds portfolio. corn.

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Late-season Foliar Diseases in Hemp: Sclerotinia and Botrytis Diseases

By Cynthia Ocamb, Oregon State University

As the 2023 field season draws to a close for Oregon hemp growers, the recent change to moist, cooler weather could bring on disease problems caused by *Sclerotinia sclerotiorum* (hemp canker) and *Botrytis cinerea* [gray mold (Botrytis bud blight and stem canker)]. Both of these fungal diseases result in stem cankers and bud infections in hemp. The diseases begin with the appearance of water-soaked lesions on leaves and/or buds. Weak tissues, such as flower buds and senescing leaves are susceptible to infection. After successful infection, the fungi grow into healthy plant portions and may invade the stem portion near infected buds, thereby causing death of the upper shoot portion, but cankers can potentially develop lower on plant stems as well. Eventually, infected buds turn brown as tissues die. Stem cankers are typically lighter in color than healthy stem portions. *Sclerotinia*-infected stems and buds are whitish in appearance due to the production of fluffy, white-colored mycelium (Fig. 1), while *Botrytis*-infected hemp buds have grayish colored mycelium on infected bud or stem portions (Fig. 2). Also, *Sclerotinia*-infected buds frequently have sclerotia (singular: sclerotium) embedded in them, both

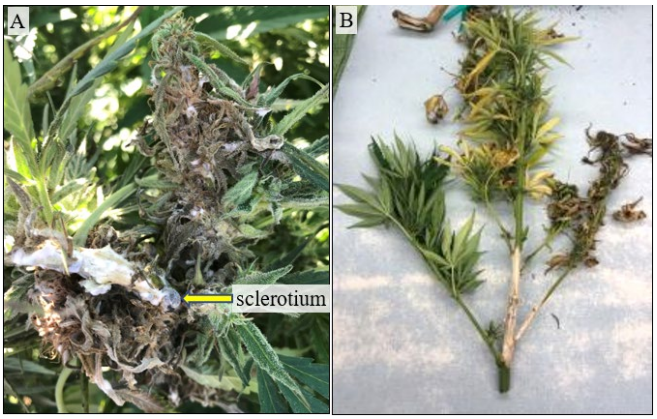

Figure 1. Hemp plants with *Sclerotinia*-infected portions. Infected buds (A) showing the fluffy white mycelium and black-colored sclerotia; stem portions (B) infected by *Sclerotinia*. Photos by C. M. Ocamb (L) and H. Rivedal (R), Oregon State University.

internally and on external surfaces. Newly forming sclerotia are white in coloration and darken (Fig.1) as they mature.

In Oregon, both hemp canker and gray mold fungi are found in field-grown hemp plants. These pathogens have a broad host range. Other susceptible host plants include beans, potatoes, and other vegetables as well as certain fruit crops, seed crops, herbs, and a large number of ornamentals both woody and herbaceous. Weeds are also hosts for *Sclerotinia sclerotium*. For example, dandelion (*Taraxacum officinale*) is a very susceptible weed host.

Figure 2. Hemp bud in early stage of gray mold development on a cola, denoted by the brown-colored tissues (A), a hemp plant with bud blight due to gray mold with multiple affected colas (B), a hemp bud infected with *Botrytis* that is exhibiting gray-brown sporulation (C), and a portion of a hemp stem with a *Botrytis* canker and profuse gray-colored sporulation (D) characteristic of this disease. Photos by C.M. Ocamb, Oregon State University.

Sclerotinia sclerotiorum survives as sclerotia, which may be associated with infected plant residues, contained in seed lots (external or internal to seed), or persist in the soil. Sclerotia are small, compact, hardened masses of hyphae, and can survive three years or longer in the soil, depending on environmental conditions and cropping practices. Sclerotia germinate when within a half inch of the soil surface, and produce a small, stalked, cup-shaped fruiting structure known as an apothecium. This fruiting structure forcibly ejects millions of ascospores into the air that can then move on wind currents. For infection, ascospores require free moisture or a relative humidity close to 100% with a temperature between 50°F and 86°F. *Botrytis* requires similar moist conditions for infection and also may produce sclerotia. But sclerotia are not necessary for *Botrytis* to survive. *Botrytis* occurs on a large number of plant hosts throughout the year, producing asexual spores (conidia) that can be wind-blown to susceptible plants during most months. Moist conditions within the plant canopy favor infection by either of these fungi, as do rain, dew, and/or irrigation practices that keep foliage wet for long periods. Rainy periods during bud maturation can bring on large disease outbreaks in hemp.

Recommended management for hemp canker and gray mold includes cultural control practices to improve air circulation and minimize leaf wetness, such as plant spacing, pruning of the plants, and irrigation timing. If *Sclerotinia* is detected, it is recommended that growers remove and destroy infected plant portions when possible. Rotate with non-hosts for 8 years to achieve the best control of *Sclerotinia*, but for at least two years to reduce the population of sclerotia; grasses and cereals are not affected by *Sclerotinia*.

Contans WG, which has an OMRI certification for organic production, can be used as a preplant or postharvest soil application and will help to reduce the survival of sclerotia produced by *Sclerotinia*. For the management of *Botrytis*, LifeGard WG, Regalia, and Stargus are possible options. A field trial conducted by OSU in 2020 showed that Stargus at 2 quarts/A on a weekly interval during bud development reduced *Botrytis* incidence and severity on hemp compared to nontreated plants (Table 1). Additional fungicide and biocontrol options are listed at the Oregon Department of Agriculture hemp pesticide guide list at https://www.oregon.gov/oda/shared/Documents/Publications/PesticidesPARC/GuidelistPesticideCannabis.pdf.

Table 1. Results of OSU evaluation of bio-fungicides for management of *Botrytis* in hemp

	% of plants with *Botrytis*[z]				% of *Botrytis*-infected buds present in 1-ft portions[z]	
Treatment (rate)[y]	**25 Sep**	**2 Oct**	**9 Oct**	**Treatment average[x]**	**9 Oct[w]**	
Nontreated control	15.5	38.0	83.0	45.1 A	14.9	A
Botrystop (3 lb/a)	7.5	22.5	59.0	24.5 B	6.7	B
Stargus (2 qt/a)	8.0	30.0	58.0	27.1 B	6.5	B
Stargus (4 qt/a)	10.0	18.0	55.5	23.8 B	7.1	B
Average date[x]	9.9 a	26.5 b	65.1 c			

From: Bates, T. A., M. Dietrich, and C. M. Ocamb. 2021. Evaluation of biofungicides for gray mold on hemp in Oregon, 2020. Plant Dis. Manage. Rep.: Rep. No. 15:V048.

[z] Visual evaluations of disease incidence in the uppermost 1-ft portion of eight individual inflorescences on each of five randomly chosen plants in each plot were made on 25 Sep, 2 Oct, and 9 Oct 2020.

[y] Applications were made on 25 Aug, 1 Sep, 16 Sep, 22 Sep, 29 Sep, and 6 Oct 2020.

[x] Means within a column or row followed by the same letter are not significantly different based on a generalized linear mixed effects model and pairwise t-test at $P \leq 0.05$.

[w] Means within a column followed by the same letter are not significantly different based on a linear mixed effects model and pairwise t-test at $P \leq 0.05$.

Research Update on Flowering Time Effects on Insecticide Efficacy on Corn Earworm Larval Mortality

By Alison Merkle and Govinda Shrestha, Oregon State University

Damage to floral and cannabinoid hemp from corn earworm pest (*Helicoverpa zea*) is a major concern to hemp growers potentially resulting in economic losses. The corn earworm is native to the temperate regions of North America. The larvae of this moth are destructive in the agricultural industry, as this species is a generalist feeder with several host plants. Crops susceptible to corn earworm larva include but are not limited to tomato, cotton, peppers, corn, and hemp. Particular to hemp, moths are attractive to floral inflorescences (also called colas), making flowers highly vulnerable to corn earworm damage. During the growing season, female moths lay eggs on flower buds, and eggs hatch into larvae within 2-3 days. Emerged larvae feed on flower buds that cause bud rots, thereby impacting marketable hemp flower products.

Figure 1. Outdoor potted hemp plants for insecticide efficacy experiment

At this time, Oregon hemp growers primarily rely on biological insecticides to manage corn earworm,

and there are no other solutions available. A commonly used biological insecticide for corn earworm is known as Gemstar LC. This insecticide contains polyhedrosis occlusion bodies of nuclear polyhedrosis virus of *Helicoverpa zea*. When corn earworm larvae feed on a treated plant, the virus enters the stomach, and kills the larva. This viral bioinsecticide efficacy may depend on the application timing with respect to hemp flowering stages.

Figure 2. Alison inoculating corn earworm larvae on hemp flowers after insecticide spray and caging larvae with muslin cloth (L); hemp flowers (mid flowering stage) with inoculated larvae (R)

For example, hemp flowers at an early stage (flower initiation) provide less space for corn earworm larvae to burrow, whereas flowers at the mid-stage (mid-flowering) are more intricate and allow more space for larvae to hide. Corn earworm larvae found at mid-flowering plants are nearly inconspicuous to the human eye. This can prevent a thorough insecticide application, hindering the product's efficiency and decreasing the overall mortality of the target species.

Therefore, to understand the flowering time effects, we conducted outdoor potted experiment at Southern Oregon Research and Extension Center in summer 2023 (Fig. 1). We grew hemp plants (CV. Photo CBD) on gardening soil in 5-gallon pots and sprayed plants with Gemstar LC (4fl oz/a) at early and mid-flowering stages. Immediately after spraying at each flowering stage, we inoculated 1-2 days old larvae (40/plant) on flowers and caged flowers with muslin cloth (Fig. 2), and checked live and dead ones after 1 week. In our study, we did not observe a difference in survival corn earworm population with respect to flowering stages. However, we observed clear differences in survival populations between Gemstar LC treated (1-2 live caterpillars/plant) and control (7-14 live caterpillars/plant) plant groups. Our study also concluded that Gemstar has a potential to control corn earworm larvae on hemp.

NEWS and UPDATES

Aspergillus Workshop

OSU Extension hosted an Aspergillus Workshop in August 2023. Here is the link to the recordings.