Research

# Cross-Infectivity of Powdery Mildew Isolates Originating from Hemp (*Cannabis sativa*) and Japanese Hop (*Humulus japonicus*) in New York

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**Abstract**

In the recent decade, agricultural production of both hemp (*Cannabis sativa*) and hop (*Humulus lupulus*) has expanded throughout the Pacific Northwest, Midwest, and Eastern United States to support the growing industries for which these plants are key components. The significant and rapidly expanding overlap of production regions of these two Cannabaceae plant family members creates a potential dispersal route for organisms that are pathogenic to both hosts. Powdery mildew is a disease of high economic impact in both hemp and hop production systems, yet it was largely unknown whether the powdery mildew fungi commonly associated with hemp could also be pathogenic on hop, and vice versa. We isolated *Golovinomyces spadiceus* growing upon hemp in New York production greenhouses and *Podosphaera macularis* from feral hop (*H. japonicus*) plantings also in New York. Herein, we report the pathogenicity of *P. macularis* associated with hop to *C. sativa* cultivars 'Anka' and 'Wild Horse' and pathogenicity of *G. spadiceus* toward hop. The potential for *P. macularis* to establish, produce viable, infectious conidia, and undergo sexual recombination on hemp could complicate efforts to exclude the MAT1-2 mating type of *P. macularis* from western North America and could facilitate the spread of races pathogenic toward 'Cascade' hop, and hop cultivars with R6-based resistance to *P. macularis*, including 'Nugget'. Further assessment of the pathogenicity of diverse *P. macularis* isolates, in both geographic origin and the range of hop species, is necessary to better understand the dispersal risk of *P. macularis* on hemp.

*Keywords*: *Cannabis sativa*, *Humulus lupulus*, powdery mildew, host resistance, *Podosphaera macularis*, *Golovinomyces spadiceus*

## The Rise of Agricultural Hemp Production in the United States: New Avenues for Pathogen Dispersal

Production of hemp (*Cannabis sativa*) in the United States has steadily increased since reintroduction was allowed under the 2014 Farm Bill (Vote Hemp 2018a). Between 2017 and 2018, the acreage of hemp in production tripled from 25,713 to 78,176 acres (Vote Hemp 2018b). Following passage of the 2018 Farm Bill, hemp was removed from the U.S. Drug Enforcement Agency Controlled Substance List. Additionally, the definition of hemp was expanded to broadly cover all parts of *C. sativa* plants including seeds, derivatives, extracts, and cannabinoids with a tetrahydrocannabinol level of 0.3% or less (McConnell et al. 2018). Hemp acreage is expected to continue rapid growth (Empire State Development 2019; Vote Hemp 2018b), with 45 states having now enacted legislation to allow or encourage its production. Montana, the 2018 U.S. hemp acreage leader, alone reported 22,000 acres of harvested hemp in 2018 (Vote Hemp 2018b; Washington State Department of Agriculture 2019), and over 500,000 acres have been licensed for production in 2019 (Vote Hemp 2019).

Hemp is a member of the Cannabaceae plant family, along with the hop species *Humulus lupulus* (brewer's hop) and the broadly dispersed invasive hop species *H. japonicus* (Japanese hop) (USDA Natural Resources Conservation Service 2019). All are hosts of powdery mildew (PM) pathogens: *Golovinomyces spadiceus* in the case of hemp (Braun and Cook 2012) and *Podosphaera macularis* in the case of hop (Braun and Takamatsu 2000). Cross-infectivity of these pathogens on the foregoing hosts has not been previously reported. Although most PM pathogens are highly species-specific, cross-infectivity of PMs between host genera within the same botanic family is possible, in particular when they grow within a common range or their production is comingled with wild hosts. For example, within the Vitaceae, certain isolates of *Erysiphe necator* can move freely between *Vitis labrusca*, *V. vinifera*, *V. riparia*, *Ampelopsis glandulosa*, *A. brevipedunculata*, *Parthenocissus tricuspidata*, and *P. quinquefolia* (Gadoury and Pearson 1991).

The rapid expansion of hemp acreage, in particular within the Pacific Northwest (PNW), a region comprising >95% of U.S. hop production, should heighten concern with respect to the possibility

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of cross-infectivity within the Cannabaceae family. The expansion of hemp production in the Midwest and Eastern United States has been paralleled by a resurgence of local hop production due to growth of the craft brewing industry, thus creating the widespread and comingled production of hemp and hop (Hop Growers of America 2018; Vote Hemp 2018b). Quarantine of hop PM is already a national issue. *P. macularis* has been present in the PNW only since 1997 (Ocamb et al. 1999), caused great destruction to U.S. hop production upon its introduction (Gent 2008), and the pathogen populations established in the region contain only one of two possible mating type idiomorphs: MAT1-1 (Wolfenbarger et al. 2015). The primary emphasis of quarantine efforts has been to contain the other *P. macularis* mating type idiomorph (MAT1-2) to prevent its spread to western North America and the consequent increase in genotypic diversity of western populations that would inevitably result (Gent 2015). However, there is also a significant motivation to prevent certain PNW-derived *P. macularis* isolates from escaping the region and establishing in eastern U.S. hop production. More recently, *P. macularis* isolates virulent upon 'Cascade', a historically major U.S. cultivar, as well as isolates able to overcome R6-based resistance to *P. macularis* in hop cultivars such as 'Nugget', have emerged in the PNW.

Accordingly, we initiated a series of experiments to examine the potential for cross-infectivity of PM pathogens found naturally occurring on hemp and hop in New York. Herein we report the ability of *P. macularis* isolates to grow and reproduce on hemp, and *G. spadiceus* isolates to weakly grow and reproduce on hop, but to a degree unlikely to be commercially relevant.


**FIGURE 1**

*Golovinomyces spadiceus* colonies growing on *Cannabis sativa* **a,** leaves and **b,** female flowers, cultivar 17GH-F1.

## Acquisition of Original Infection Material and Maintenance of Isolates in Culture

Two hemp PM isolates identified growing on potted hemp plants in greenhouse production systems were used in this study. Hemp PM isolate 19001 was collected from the hemp cultivar 'Lifter' from a hemp greenhouse operation in Orange County, NY. Hemp PM isolate 19002 was collected from the hemp cultivar line '17GH-F1' from a greenhouse operation in Erie County, NY (Fig. 1). No chasmothecia were observed on PM-infected hemp plant material in either greenhouse. Infected leaves were incubated in growth chambers for 2 days to allow for production of new, viable conidia. Both isolates were then single-spore transferred from the original source leaves to young hemp leaves, cultivar 'TJ's CBD', in a double Petri dish detached leaf culture design (Pearson and Gadoury 1987). Isolates were subjected to two additional rounds of single-spore transfer, after which each culture was considered pure and maintained via a paint brush conidial inoculation thereafter (Gadoury and Pearson 1991).

Hemp cultivars used in this study include 'Anka' (seed from UniSeeds, Cobden, ON, Canada), 'Wild Horse' (seed from Winterfox Farms Eagle Point, OR), and TJ's CBD (clones from Stem Holdings Agri Inc., Eugene, OR).

Two hop PM isolates, hereafter referred to as NY002 and NY005, from our culture collection were used for the cross-infection assays. Both isolates were collected from feral *H. japonicus* hop in Geneva, NY. Both were reduced to clonal isolates from transfers of single conidial chains on detached leaves of the *H. lupulus* cultivar 'Symphony', using the same methods as described above for the hemp PM isolates, and maintained on *H. lupulus* Symphony detached leaves via paint brush conidia inoculations thereafter. Both the hemp PM and hop PM isolates were transferred to new leaves of their respective host every 11 to 15 days. Isolates were kept in growth chambers at 19C, 50% relative humidity, and 14-h day lengths.

## Taxonomic Classification of the Hemp PM and Hop PM Isolates

In order to confirm the identify of hemp PM isolates 19001 and 19002, the 28S and internal transcribed spacer (ITS) regions were polymerase chain reaction (PCR) amplified using the primers PM5G/NLP2 for the 3′ half of the ITS and 28S, and ITS5/PM6G for the 5′ half of the ITS (Bradshaw et al. 2017). Primer sequences are relisted

**TABLE 1**
**Primers used in this study, with space between primer pairings**

| Primer name | Sequence (5′ – 3′) | Citation |
|---|---|---|
| ITS_5 | GGAAGTAAAAGTCGTAACAAGG | White et al. (1990) |
| PM6G | CGAGCCCCAACACCAA | Scholler et al. (2016) |
| PM5G | GACCCTCCACCCGTGT | Scholler et al. (2016) |
| NLP2 | GGTCCCAACAGCTATGCTCT | Mori et al. (2000) |
| PmMAT1-1-1_BW_F | AGCGCCGATCGTTACATTTC | This study |
| PmMAT1-1-1_BW_R | CCGTCTCATCAGTGTAGCTAGT | This study |
| EnαF2 | AAAGATGCACCTCTCGATGAA | Brewer et al. (2011) |
| EnαR3 | AAGTTATAGAAGACATCGCAGTCA | Brewer et al. (2011) |
| PmMAT1-2-1_BW_F | CAACCCTGGTCTTAGCAATAATC | This study |
| PmMAT1-2-1_BW_R | GCAAGATCCTTGTAGGCATTTC | This study |
| G_o_MAT1-2_F | ACATGCCGAAACTGTTTTGC | This study |
| G_o_MAT1-2_R | GCCTTGTATGAATTCCGTCCT | This study |

here for future convenience (Table 1). PCR products were Sanger sequenced in both the forward and reverse directions, and consensus sequences for both isolates were deposited in GenBank (accessions MN365027 and MN381107). An NCBI BLAST search indicated the sequenced ITS and 28S regions of isolates 19001 and 19002 were identical and returned a 98% query coverage and 99.92 to 100% identity with *G. spadiceus*. All other significant *Golovinomyces* sp. alignments were less than 96.5% identity. These BLAST alignments agree with species classifications in previous reports of hemp PM in North America (Pépin et al. 2018; Szarka et al. 2019).

The hop PM isolates NY002 and NY005 were identical and confirmed as *P. macularis* through sequencing of the PM5G/ NLP2 region of the ITS. Both isolates returned a single high-yield amplification product (GenBank accessions MN381106 and MN381108) with 100% query coverage and 100% identity to *P. macularis*. The *Golovinomyces*-specific primer pairing PM5G (F)/ NLP2 (R) yielded multiple PCR products in hop PM isolates NY002 and NY005, indicating potential off-target amplification. As such, these amplicons were not used to further confirm species.

To further visualize the phylogenetic relationship between the hemp- and hop-associated PM isolates, a consensus neighbor-joining tree of the 28S and ITS region was constructed using the Tamura–Nei genetic distance model (Tamura and Nei 1993) with a bootstrap resampling method and 1,000 replications (Geneious Prime 2019.2.3). The *G. spadiceus* and *P. macularis* isolates closely grouped with other deposited GenBank isolates of their respective species, on distinct branches from other related mildew species including *G. tabaci*, *G. macrocarpus*, *P. xanthii*, *P. aphanis*, and *Blumeria graminis* (Fig. 2).

We paired the ITS classification of the hemp PM and hop PM isolates with an analysis of PM colony morphology. Hemp PM isolate 19001 and hop PM isolate NY005 were surveyed for conidia size, conidiophore dimensions, and morphological production of conidial chains (Zeiss Axiophot Trinocular Compound Light Microscope). Under a dissecting microscope (Wild Makroskop M420), both hemp PM isolate 19001 and hop PM isolate NY005 were also surveyed on each isolate's respective original host for latency period and daily conidial production numbers. Each inoculation set contained three biological replicates, and the entire inoculation protocol was repeated in triplicate.

Similar to previous reports (Braun 2002; Pépin et al. 2018; Szarka et al. 2019), mycelia on the original infected plant material from which both the hemp PM and hop PM isolates were obtained were amphigenous and caulicolous. In rare occasions, mildew growth extended onto short succulent stems. Conidiophores of hemp PM isolate 19001 were singular, hyaline structures that measured 131.46 µm in length ($n$ = 15, SD = 11.07). Conidia were ellipsoid to ovoid and measured 34.13 µm ($n$ = 20, SD = 2.69) × 19.36 µm ($n$ = 20, SD = 2.17). Once conidiophores were produced, multiple (six to 10) mature conidia were produced over a 24-h period in single chains. Conidiophores of hop PM isolate NY005 were similarly singular, hyaline, erect structures that measured 59.32 µm in length ($n$ = 15, SD = 11.51). Conidia were ellipsoid to ovoid and measured 30.25 µm ($n$ = 20, SD = 1.23) × 21.01 µm ($n$ = 20, SD = 1.99). Hop PM isolate NY005 also produced multiple mature conidia in single chains over each course of a 24-h sporulation period. Morphological characteristics for hemp PM isolate 19001 were consistent with descriptions of *G. spadiceus* (Pépin et al. 2018; Szarka et al. 2019), and isolate morphology of hop PM isolate NY005 was consistent with descriptions of *P. macularis* (Braun 2002). The convergence of the molecular and morphological data confirmed that hemp PM isolates 19001 and 19002 are *G. spadiceus* and hop PM isolates NY002 and NY005 are *P. macularis*.

## Cross-Infectivity Assays of the *G. spadiceus* and *P. macularis* Isolates

All hemp PM and hop PM isolates were surveyed for evidence of pathogenicity on hemp and hop leaf tissue. For the hemp infection assays, inoculations were executed on the adaxial surface of detached leaves in double Petri dish culture, on 10- to 14-day-old seedlings, and on 3- to 4-week-old potted hemp plants. The hemp cultivars TJ's CBD, Anka, and Wild Horse were collectively

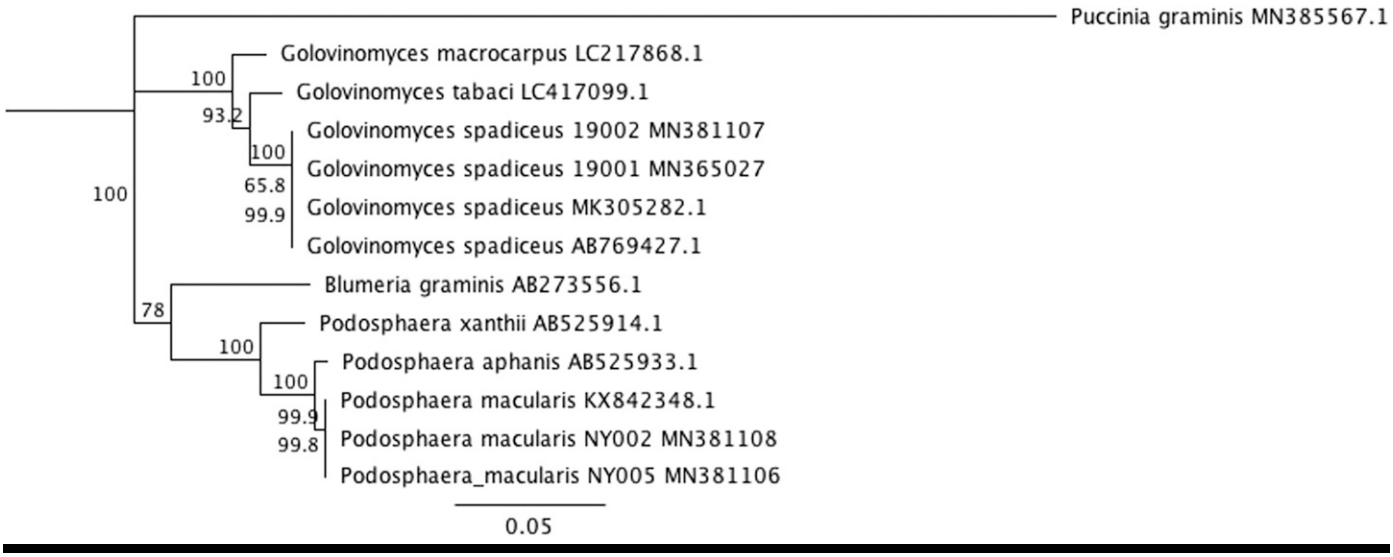


**FIGURE 2**

Neighbor-joining consensus tree of the 28S and internal transcribed spacer regions of various powdery mildew species, including the two *Golovinomyces spadiceus* and two *Podosphaera macularis* isolates of interest in this study. Samples are labeled by species, isolate name (if relevant), and their associated GenBank accession number. *Puccinia graminis* (GenBank accession MN385567.1) was used as an outgroup to root the tree. Branches are labeled with the consensus support percentage based on 1,000 replications of the Tamura–Nei model.

surveyed at various growth stages, depending on plant tissue availability. All inoculations were "dry" inoculations using a fine-tipped paint brush and 12- to 14-day-old mildew colonies from detached leaves in double Petri dishes as the inoculum source. For hop infection assays, inoculations were done both on detached hop leaves and potted plants of Symphony and 'Zeus' (often also marketed as 'CTZ', a complex of the identical cultivars 'Columbus', 'Tomahawk', and Zeus). Both hop cultivars are known to be highly susceptible to *P. macularis*. For all assays, the adaxial leaf surface was dry inoculated with conidia from 10- to 12-day-old mildew colonies from detached leaves in double Petri dishes using a fine-tipped paint brush. A compatible host–pathogen interaction was defined as colony formation through hyphal growth and completion of the latency period through production of infectious conidia. All cross-infectivity assays contained three biological replicates. Inoculation assays on detached leaves and seedlings were repeated in triplicate, whereas potted-plant inoculations were repeated twice.

No difference in pathogenicity was observed between *G. spadiceus* isolates 19001 and 19002. On detached leaves of both hop cultivars Symphony and Zeus, *G. spadiceus* was capable of initial infection, vegetative growth, and production of viable conidia that could be returned to hemp and be pathogenic (Table 2). However, when compared with the level of colonization and sporulation on hemp over the same 14-day timeframe, *G. spadiceus* was a significantly slower developing and macroscopically less prolific pathogen on hop tissue (Fig. 3). The latency period of *G. spadiceus* was delayed by 1 to 3 days on hop in comparison with control inoculations on hemp leaf tissue. When *G. spadiceus* was inoculated onto second and third node leaves of potted hop Symphony and Zeus plants, we were unable to produce a compatible infection event that resulted in macroscopically visible PM colonies, and as such considered *G. spadiceus* to be nonpathogenic in a practical sense. When young leaf tissue is collected for use in a detached leaf culture system, the leaf remains consistent at its given growth stage. Alternatively, when that same aged leaf is inoculated while it is still attached to the rest of a potted plant, the leaf continues to grow, increasing in both size and cuticle thickness (Ficke et al. 2002; Twomey et al. 2015). As such, we hypothesize that as part of a potted plant, the leaf acquires a degree of ontogenic resistance in the days following inoculation, which outcompetes the *G. spadiceus* hyphal growth rate, preventing the fungus from growing well enough to complete its latency and produce conidia, as reported in other PM pathosystems (Asalf et al. 2014; Ficke et al. 2002; Twomey et al. 2015). Based on these results, it is unlikely that the *G. spadiceus* isolates tested would become significant pathogens of hop.

*P. macularis* isolates NY002 and NY005 inoculated onto TJ's CBD induced a nonhost resistance type response, in which *P. macularis* conidia germinated at a low rate, and those that germinated did not grow past production of an initial germination tube and primary appressorium (Fig. 4). In some instances, a localized hypersensitive response could be observed in the plant host epidermal cell immediately below the *P. macularis* appressorium (Fig. 4b). *P. macularis* isolates were pathogenic on hemp Anka and Wild Horse (Table 2). Ten- to 14-day-old seedling and detached leaf inoculations of both varieties yielded compatible infection events, with a cumulative *P. macularis* infection success rate of 53% ($n = 36$) across the two varieties. In many of the compatible infections, a notably dense hyphal mat developed prior to production of conidiophores (Fig. 5a). *P. macularis* isolate NY005 averaged a latency period 1 day shorter than isolate NY002 across all infection events, including the control infections on hop cultivar Symphony. Controlled inoculations onto potted hemp plants of cultivars Wild Horse and Anka also produced macroscopically visible *P. macularis* colonies (Fig. 5b and c).

**TABLE 2**
**Compatibility of *Golovinomyces spadiceus* and *Podosphaera macularis* isolates on hemp and hop[a]**

| | | *Cannabis sativa* | | | *Humulus lupulus* | |
|---|---|---|---|---|---|---|
| **Species** | **Isolate** | **Anka** | **Wild Horse** | **TJ's CBD** | **Symphony** | **Zeus** |
| *Golovinomyces spadiceus* | 19001 | + (9) | + (9) | + (9) | +/– (10) | +/– (11) |
| | 19002 | + (8) | + (9) | + (9) | +/– (10) | NA |
| *Podosphaera macularis* | NY002 | + (9) | + (8) | – | + (7) | + (9) |
| | NY005 | + (8) | + (7) | – | + (6) | + (7) |

[a] + = compatible pathogen interaction; – = a noncompatible pathogen interaction; and +/– = a compatible interaction in detached leaf culture, but not so on a potted plant of the given cultivar. The mean latency period rounded to the nearest whole day is in parentheses.

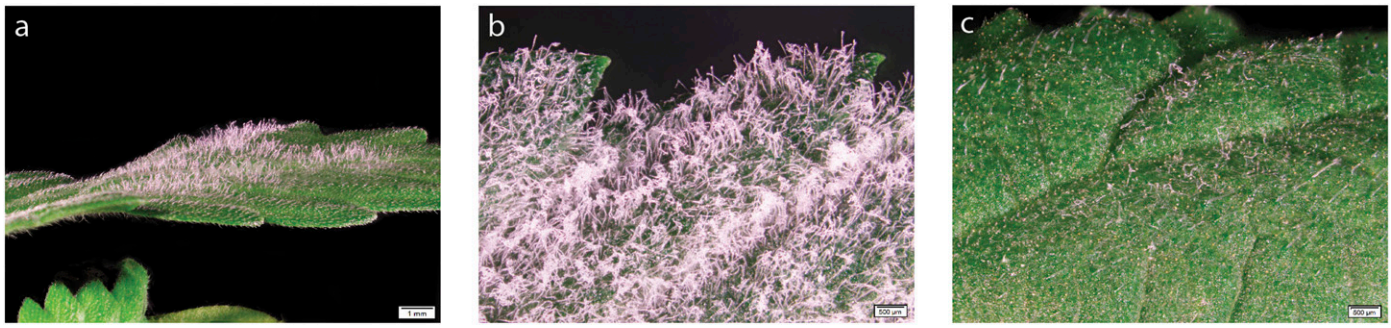


**FIGURE 3**

*Golovinomyces spadiceus* growing on **a,** the first leaf node of the hemp cultivar Anka seedling; **b,** the hemp cultivar TJ's CBD 14 days postinoculation (dpi); and **c,** the hop cultivar Symphony 14 dpi.

## Sexual Mating Compatibility Assays

The mating types of the two hop PM isolates (NY002 and NY005) were confirmed through PCR amplification of the MAT1-1 and MAT1-2 loci using primers based on deposited NCBI sequences of each (Wolfenbarger et al. 2015) and confirmation of sequence accuracy through Sanger sequencing of PCR products. To further confirm sexual compatibility, the two hop PM isolates were crossed with one another on detached hop leaves (Symphony) and surveyed for production of chasmothecia.

The hemp PM isolates (19001 and 19002) were surveyed for mating type through attempted PCR amplification of the MAT1-1-1 and MAT1-2-1 mating type loci. Due to limited sequence availability of the mating type regions in the genus *Golovinomyces*, the MAT1-1 locus was targeted using one set of primers designed from the *E. necator* MAT1-1 locus (Brewer et al. 2011) and a second set of primers designed from the *P. macularis* MAT1-1 locus (Wolfenbarger et al. 2015). Two MAT1-2 HMG domain locus primer sets were designed based on a *G. orontii* isolate (GenBank KJ909541, *unpublished*) and a *P. macularis* isolate (Wolfenbarger et al. 2015). The sexual compatibility of the two respective hemp PM isolates was checked by inoculating both isolates onto the same detached hemp leaf, varieties TJ's CBD and Anka. No coinoculation of hemp PM isolates 19001 and 19002 resulted in the production of chasmothecia, providing evidence that they are likely the same mating type idiomorph (Table 3). Prior publications have reported the formation of viable *G. spadiceus* chasmothecia in hemp production systems of the eastern and southeastern United States, documenting structures that contain five to 15 asci that each bear two ovoid ascospores (Szarka et al. 2019). Because the existence of sexual reproduction in *G. spadiceus* is not in question, nor is there any reported evidence across PM systems to suggest mating type as a driving factor of cross-host infection capability, additional *G. spadiceus* isolates of the alternate mating type were not considered necessary for the context of this study.

When coinoculated onto the same detached hop leaf (Symphony), *P. macularis* isolates NY002 and NY005 transitioned from asexual growth to production of sexually reproduced chasmothecia at any point where mycelial growth of the two isolates intersected, confirming that the two isolates are of opposite mating type and are sexually compatible. Targeted amplification of the MAT1-1-1 and MAT1-2-1 mating type loci confirmed that *P. macularis* isolate NY002 is mating type MAT1-2, whereas *P. macularis* isolate NY005 is mating type MAT1-1. None of the PCR primers targeting the MAT1-1 and MAT1-2 loci yielded amplified DNA products in either *G. spadiceus* isolate. Ultimately, whole-genome sequencing-based approaches will allow for targeted design of primers capable of differentiating *G. spadiceus* mating type, as well as many other important gene regions of PM organisms, such as the demethylase inhibitor fungicide gene target, *CYP51*, for example (Jones et al. 2014). Although undoubtedly a worthwhile endeavor in improving our downstream ability to manage *G. spadiceus*, the timeline required to produce a high-quality whole genome of an obligately biotrophic fungus such as *G. spadiceus* is significant and fell outside the scope of this specific cross-infection study.

The ability of *P. macularis* to undergo sexual reproduction on hemp plant tissue was investigated on 10- to 14-day-old Anka seedlings. *P. macularis* isolates NY002 and NY005 were coinoculated onto Anka seedlings, and chasmothecia initials could be observed under a dissecting microscope as early as 18 days postinoculation (Fig. 6a). Over the next 14 days the chasmothecia initials matured from ascocarps appearing externally clear, to yellow, to brown, to black. Once the ascocarp was a brown-black color, the ascocarps were transferred to a glass slide, where pressure was applied to the coverslip to reveal the inner ascus. A single ascus was observed, which is consistent with *P. macularis* morphology (Braun 2002) (Fig. 6b). Staining with Sudan Black B revealed the expected granularity and lipid droplets within a *P. macularis* ascus (Wolfenbarger et al. 2015) (Fig. 6c).

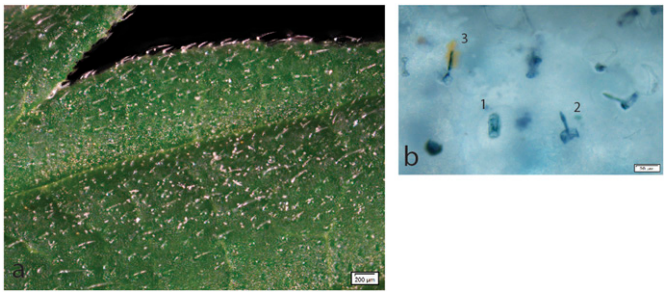


**FIGURE 4**

An incompatible host-resistance interaction between the hemp cultivar TJ's CBD and *Podosphaera macularis*. **a,** An incompatible infection result at 10 days postinoculation. **b,** *P. macularis* spore germination and growth at 48 h postinoculation: 1 indicates an example of a nongerminated conidium; 2 indicates an example of a conidium that has germinated and terminated growth at the formation of an initial appressorium; and 3 indicates a necrosed epidermal cell, part of a localized host hypersensitive response to presence of the powdery mildew conidium.


**FIGURE 5**

*Podosphaera macularis* growing on **a,** a first leaf node of the hemp cultivar Anka seedling; **b,** a young leaf from the secondary bud of a potted hemp plant cultivar Wild Horse; and **c,** a maturing leaf from the primary shoot of a potted hemp cultivar Wild Horse.

Coinoculations of *G. spadiceus* isolate 19001 with each respective *P. macularis* isolate were made to investigate potential sexual compatibility between the two species (Table 3). Crosses were made on detached hop leaves (Symphony) and detached hemp leaves (Anka). In the case of the inoculations on the detached hop leaves, to accommodate differing growth rates, the hemp PM isolates were inoculated and allowed to grow for 3 days before inoculating the same leaf with the respective hop PM isolate. No chasmothecia were formed in any of the coinoculations between *G. spadiceus* and the *P. macularis* isolates of either mating type.

## Larger Implications on the Management of Hop and Hemp PMs

Total planted hemp acreage is expected to continue to increase dramatically, and production regions now frequently overlap with regions of hop production (Vote Hemp 2018b). With the recent reports of hemp PM in Canada (Pépin et al. 2018) and the United States (Szarka et al. 2019) and the overlapping of production regions, understanding the cross-infectivity of hemp-associated and hop-associated PM is highly relevant to future successful disease management of both pathogens.

Using *G. spadiceus* originally cultured on detached hemp leaves and then inoculated onto detached leaves of hop cultivars Symphony and Zeus, we were able to demonstrate pathogenicity and confirm cross-infection capability on hop. The inability to induce *G. spadiceus* infection on potted hop plants of either cultivar suggests this PM species is unlikely to be a significant pathogen in hop production systems from a disease management perspective. However, a broader survey of additional hop cultivars is warranted.

Using *P. macularis* originally cultured on detached hop leaves and then inoculated onto a suite of hemp varieties, we were able to confirm the cross-infection capability of *P. macularis* on hemp cultivars Anka and Wild Horse. Inoculation events on hemp cultivar TJ's CBD yielded an incompatible reaction highly similar to the poor germination and hypersensitive response associated with host resistance. Additionally, when compatible mating types of *P. macularis* were paired on Anka seedlings, chasmothecia formation occurred at growth points where the two isolates overlapped, demonstrating the pathogen's ability to reproduce both sexually and

**TABLE 3**
**Sexual reproductive compatibility between isolates of *Podosphaera macularis* and *Golovinomyces spadiceus*[a]**

| | | *Cannabis sativa* | | |
|---|---|---|---|---|
| **Species** | **Isolate pair** | **Anka** | **TJ's CBD** | ***Humulus lupulus* (Symphony)** |
| *Golovinomyces spadiceus* | 19001 + 19002 | – | – | – |
| *Podosphaera macularis* | NY002 + NY005 | + | NA | + |
| *Golovinomyces spadiceus* + *Podosphaera macularis* | 19001 + NY002 | – | NA | – |
| | 19002 + NY005 | – | NA | – |

[a] + = sexual reproductive compatibility and formation of chasmothecia fruiting bodies; – = an incompatible sexual reproductive pairing of isolates; and NA = the cultivar was not used for that isolate pairing because one or both isolates could not successfully colonize the given host.

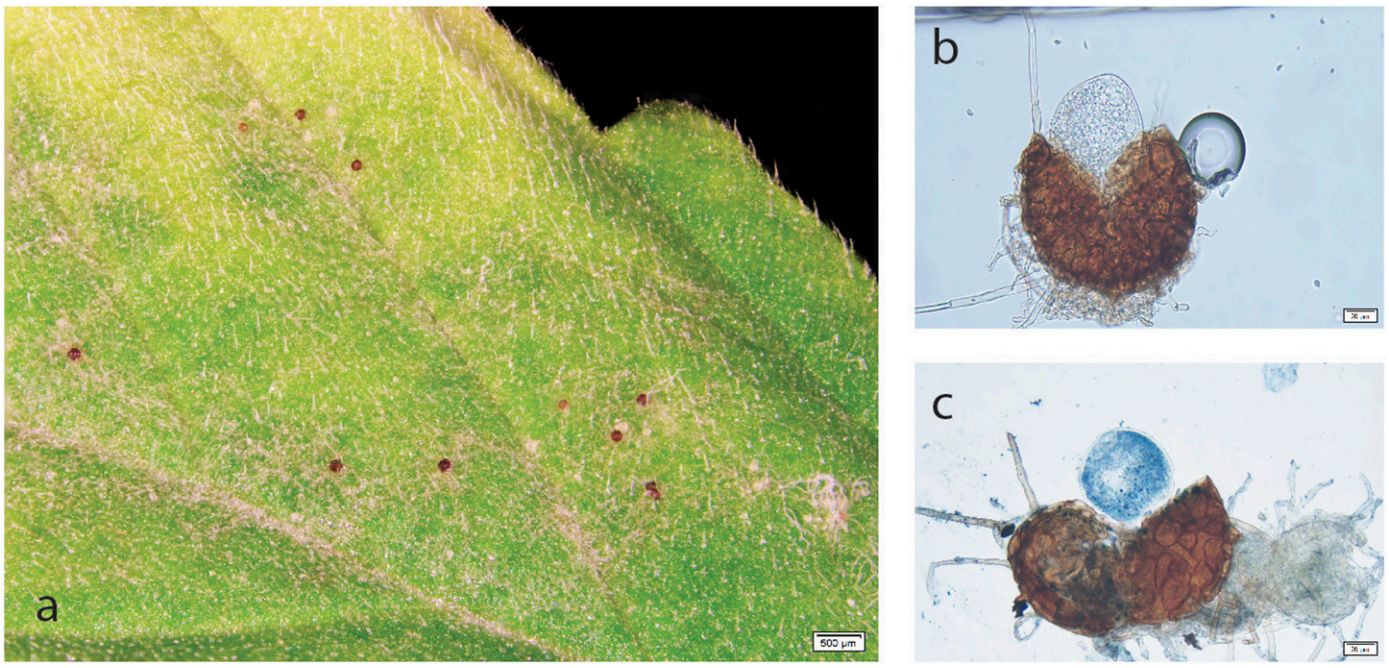


**FIGURE 6**
**a,** Formation of *Podosphaera macularis* chasmothecia on the hemp cultivar Wild Horse. **b,** *P. macularis* ascocarp, isolated from a colony originating on hemp, that has dehisced and is releasing a single ascus. **c,** Ascus contents are stained with 1% Sudan black B to confirm presence of the expected lipid droplets within a maturing ascus.

asexually on hemp. Future research will survey additional hemp cultivars to determine if resistance to *P. macularis* is unique to TJ's CBD.

Currently, the MAT1-2 mating type idiomorph of *P. macularis* has yet to be documented within the PNW hop growing region, whereas both mating types are present in approximately a 1:1 ratio in hop production regions of the Midwestern and Eastern United States and Europe (Wolfenbarger et al. 2015). The introduction of the second mating type would likely mean enhanced overwintering capability of the pathogen through sexual reproduction and formation of chasmothecia, as well as enhanced distribution and variation of virulent strains of *P. macularis* as a genetic result of sexual recombination. Because of these implications, a quarantine on the import of hop plant material from anywhere outside of the PNW was put in place in 2015 (Gent 2015). However, if *P. macularis* can also infect hemp to some degree, a possible alternative route for the MAT1-2 mating type to enter the region exists. Methods to monitor and prevent introduction of the *P. macularis* MAT1-2 mating type idiomorph through this route should be considered. Additional work investigating the extent to which *P. macularis* isolates derived from hop of various geographic origins and hop species can infect hemp would provide a clearer picture into the risks of *P. macularis* being an economically relevant pathogen of *C. sativa*.

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