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After an unusually high incidence of Fusarium canker was observed in commercial hop fields of the Pacific Northwest, field surveys were conducted and revealed that canker incidence ranged from 20 to 60% of bines sampled in six commercial fields, as well as wide-spread *Hop stunt viroid* infection in these six fields. A variety of inoculation techniques and incubation conditions were evaluated in laboratory and greenhouse studies to determine whether *Fusarium sambucinum* incites girdling symptoms on hop bines, which is characteristic of later stage Fusarium canker infection in commercial hop fields. Koch's postulates were fulfilled, confirming that *F. sambucinum* incites Fusarium canker and produces girdling, killing the bine. Colonization of detached hop stems with green fluorescent protein-labeled *F. sambucinum* or *F. verticillioides* were observed microscopically, but *F. sambucinum* colonized more aggressively and to a greater extent. Investigation into the effect of relative humidity on colonization of hop stems demonstrated that relative humidities greater than 88% are required *for F. sambucinum* to colonize green hop stems. Hilling of commercial hop plants was

explored as a management strategy to ameliorate canker symptoms or improve yields in commercial fields with wide-spread *Hop stunt viroid* infection and results indicate that hilling can improve cone yields in commercial hop plantings co-infected with HpSVd and *F. sambucinum*.

Examinations of *Fusarium sambucinum* on *Humulus lupulus* and Co-infection with *Hop stunt viroid* in Commercial Hop Fields

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Natasha R. Cerruti, Author

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Introduction

The Pacific Northwest is the leading producer of hops in the United States,harvesting 43 x 10^6 kg of dried cones with a net worth of 356,375,000 US dollars (USDA-NASS) during 2009. During 2004, *Hop stunt viroid* (HpSVd) (Genus *Hostviroid*; family *Pospiviroidae*) was discovered in commercial hop in Washington State (12), and soon after HpSVd was found in Oregon. This viroid poses a threat to hop production due to the reduction it causes in cone numbers, cone size, and brewing quality. An increase in incidence of Fusarium canker, caused by the soil inhabitant, *Fusarium sambucinum*, has been observed in hop fields with high incidence of HpSVd. Fusarium canker symptoms are typically sporadic in a hop field, but when HpSVd-infected rootstock is planted, an widespread incidence of cankered bines has been observed.

This thesis reports on studies into *F. sambucinum* pathogenicity on hop, green fluorescent protein-labeled *F. sambucinum* and *F. verticillioides* colonization of detached hop stems, the effect of relative humidity on *F. sambucinum* colonization of hop stems, Fusarium canker incidence in commercial hop fields co-infected with HpSVd, and hilling of plants for management of co-infected commercial fields.

Literature Review

Hop belongs to the family, Cannabaceae, and the genus, *Humulus,* which contains three dioecious species, *H. lupulus* L., *H. japonicus* Siebold & Zucc., and *H. yunnanensis* Hu; all are native in China (26). *Humulus japonicus* and *H. yunnanensis* have cones structurally similar to *H. lupulus,* but cones of the first two species lack the quantity of lupulin glands, if they are present at all. *Humulus japonicus* are annual plants, while *H. yunnanensis* and *H. lupulus* are perennial (29). *Humulus lupulus*, the commercially-important species of the genus, was first cultivated in eastern Europe somewhere between the 9 and 12th century and spread throughout Europe, making its way to the United States in the mid-17th century (29). *Humulus lupulus* consists of perennial rhizomes that produce annual bines. The bines, which are similar to vines, climb clockwise with hooked hairs along supporting strings, instead of with tendrils. The main bines produce lateral branches (sidearms) which give rise to inflorescences that develop into cones. Female cones contain lupulin glands and these can be found throughout the inflorescence on the bracteoles, bracts, and strig. Lupulin glands produce resins, which were originally used as a preservative against bacteria in beer production, but now also are a staple ingredient for imparting bitterness and aroma to beer (29).

Fusarium sambucinum

The fungal genus, *Fusarium,* is characterized by macroconidia that have a fusiform shape. *Fusarium* is a diverse genus, infecting a broad range of hosts and causing an array of symptoms such as wilt, root rot, and canker. *Fusarium*

sambucinum Fuckel (teleomorph: *Gibberella pulicaris* (Fr.) Sacc.) belongs to the order Hypocreales, which produces macroconidia and microconidia on monophyletic conidiogenous cells. The characteristic macroconidia are curved, short, and stout, with a well-developed foot cell. On potato dextrose agar, *F. sambucinum* appears pink to tan in color. Aerial mycelium is white, floccose, and can be tinged with pink. Chlamydospores can develop as single cells, in chains, or clumps (28) and can be found as stroma with perethecia on woody hosts (8). Under the taxonomic system of Nelson et al., *F. bactridioides, F. trichothecioides*, *F. sambucinum* var. *trichothecioides, F. roseum* var. *sambucinum* and *F. sulphureum* are indistinguishable from *F. sambucinum*; all are considered the same species (28).

Fusarium sambucinum can cause a variety of diseases including cankers on *Sambucus* and *H. lupulus*, hop cone tip blight, seedling rot and root rot in cereals and *Amaranthus*, storage rot of potatoes, and fruit blotch on strawberry (7-9,17). *Fusarium sambucinum* has been recovered from hops expressing symptoms of cone tip blight and canker (5,11). *Fusarium sambucinum* and *F. avenacium* have been found to be pathogenic on 'Nugget' and 'Willamette' cones in the Pacific Northwest causing necrosis of hop cones(5). *Fusarium crookwellense* has exhibited similar cone symptoms on cultivars 'Nugget', 'Willamette' and 'Agate' in Tasmania (33). In addition to affecting plant yield, the mycelium of *F. sambucinum* can produce mycotoxins which can be present in harvested plant parts used for food. Certain *F. sambucinum* isolates from potato and corn, strains 8.3 and 8.4, were found to be toxic to brine shrimp. Isolates recovered from potatoes in Iran, strains 8.1 and 8.2,

were associated with areas of esophageal cancer in humans, found to be lethal to ducklings, and caused lesions and toxicoses in rats (23). Some isolates recovered from potato, wheat, and corn were found to produce trichothecenes, a mycotoxin which inhibits protein synthesis in eukaryotes (8,23). It is unknown whether *F. sambucinum* produces mycotoxins in hop cones.

Fusarium canker

Fusarium canker was first reported in England on hop in the early 1900's (11,41). Cankered plants are characterized by swelling and frequently girdling near the base of bines at the soil line. The accumulation of photosynthetic products due to vascular involvement causes swelling at the base of the bine (41). Leaves can turn chlorotic on infected bines, wilting may occur, and infected bines may die from girdling anytime before harvest (41,49,52). A physical force on an affected bine, such as from wind, machinery, or workers, can easily sever a girdled stem from the rhizome. *Fusarium sambucinum* can also infect rhizomes, causing degradation and discoloration in the cortex of the underground stems (10,11,36,41). Symptoms caused by *Verticillium dahliae* and *V. albo-atrum* appear similar to Fusarium canker, due to swelling of the base of the bine, wilting, and chlorotic/necrotic leaves. While *Verticillium*-infected bines can be swollen at the base, bines do not exhibit a girdling typical of *Fusarium*-infected bines, and the vascular tissues are discolored when *Verticillium* is present.

Fusarium canker in hop is reported to be associated with wet winters, areas of the field with poor drainage, or damage of bines/rhizomes by wind or during

routine cultural practices (22,41,43). The lower main bine leaves are often removed during a process called 'stripping', which is performed mechanically or chemically. Stripping is done on the bottom meter of stem to increase air-flow for downy and powdery mildew management (29), and herbicide-treated bines appear to be more susceptible to *F. sambucinum* (C. M. Ocamb; personnel communication). Because areas with poor drainage or damaged plants tend to a have a higher incidence of canker, field experiments have been carried out previously to mimic various field conditions and observe the development of Fusarium canker. Field experiments showed that wounding and wet conditions favored development of canker by *F. sambucinum* (37,52). Pathogenicity tests have been performed to determine the casual agent of the canker but have yet to reproduce the classic Fusarium canker symptoms of stem swelling and girdling. Cuttings exposed to infested soil developed discoloration of stem and rhizomes and *F. sambucinum* was later recovered from affected stems and rhizomes (10,11,37).

Although no immune cultivars have been found, severity of Fusarium canker is variable, ranging from swollen to completely girdled bines. 'Fuggle', 'Cobb', and 'Old Golding' are reported to have lower incidences of Fusarium canker, while 'Rodmersham Golding', 'Bramling', and 'Tolhurst' appear to be highly susceptible (41,49). Some high alpha cultivars appear to be more susceptible to *F. sambucinum* (37). Experimental trials for Fusarium canker control examined various chemicals including thiabendazole, Benlate, Brestan 60, metalaxyl, and mercury, were mostly unsuccessful in significantly reducing canker levels. Some success was found in

reducing canker with thiabendazole and Brestan 60, but control varied among years, due to the incidence and severity of the canker in the nontreated hills (10,36).

Viroids

Viroids consist of a covalently closed circular single-stranded RNA with a high amount of secondary structure. The 30 known viroids that infect plants are 246 to 401 nucleotides in size. Unlike viruses, viroids are not encapsulated with a protein coat and do not encode for specific proteins (13,46). Viroids replicate autonomously, using a rolling circle mechanism which differs between the two families, Avsunviroidae and Pospiviroidae. The Pospiviroidae family replicates and accumulates in the nucleus following the "asymmetric pathway", which uses one rolling circle, while members of the Avsunviroidae family replicate and accumulate in the chloroplast and use two rolling circles adhering to a "symmetric pathway". The asymmetric mechanism begins with the (+) viroid strand, which is replicated by RNA polymerase II, producing a (–) strand, which is replicated continuously. Popsidiviroidae relies on RNase activity from host enzymes to cleave the (+) strand, while Avsunviroidae possess self-cleaving "hammerhead" ribozymes. Asymmetric replication concludes with host enzymatic ligation (13,46).

Intracellular movement of viroids is believed to be facilitated by host proteins. Viroids move cell to cell via the plasmodesmata, the natural openings connecting plant cells, and enter into the vascular system and spread systemically with plant sap (13). Most viroids are transmitted mechanically plant to plant, although certain viroids can be transmitted via seeds, pollen, or insect vectors (13).

Hop latent viroid (HpLVd) was first identified as an unknown viroid simultaneously occurring with HpSVd in hop plants in 1987 (31) and was characterized in 1988 (34). Although HpLVd is distributed worldwide in the hop germplasm and shares 45% sequence homology of its 256 nucleotides with HpSVd, it apparently does not have the same detrimental effect on hop as HpSVd (34). One hop cultivar, Omega, is known to be severely affected by HpLVd, showing a reduction in vigor that can cause approximately a 37% loss in fresh cone weight and a 30% reduction in alpha acid content (4). 'Wye Northdown' and 'Wye Challenger' are affected to a lesser extent than 'Omega', with an 8% loss in fresh cone weight and 15% loss in alpha acid content (4). These two cultivars also showed a slight increase in beta acid content when infected, suggesting early cone maturation (1,4)

Early experiments examining the distribution and spread of HpLVd infection in UK hop plantings suggested a possible vector due to the randomly scattered infections in a 'Wye Challenger' planting (3). *Phorodon humuli,* the damson hop aphid, did not transmit HpLVd, but a high frequency of transmission did occur with mechanical tools (2).

During 1952, a stunting of plants was noticed in Japanese hop fields, along with abnormal plant growth and a significant decrease in cone yields. This stunting disease was described as hop stunt in 1973 and was believed to be of viral origin (51). The causal agent was determined to be a viroid rather than a virus (42), 297 nucleotides in length (30), and named *Hop stunt viroid*. Prior to its detection in

commercial hop fields in the Pacific Northwest, HpSVd had only been reported in Japan and Korea (12).

HpSVd can result in an overall reduction in growth, causing shorter internodes and a visible stunting of the bine. The bines appear less vigorous and thinner, with poorly developed hooked hairs on the main bine (43,51). Hop plants infected with this viroid may come into bloom 8 to 14 days earlier than HpSVd-free plants (25) and the fresh cone weight and cone numbers may be reduced up to 40% (51). 'Kirin II' plants infected with HpSVd produced cones which were smaller in diameter and length, resulting in cones that appear more elongated (51). Although the distribution of lupulin glands in HpSVd-infected cones is similar to that of HpSVd-free cones, the density of lupulin glands in HpSVd-infected cones is 40% less than that of HpSVd-free plants (25). The glands of infected plants appear shriveled and produce half to one-third of the alpha acids found in healthy plants, while the beta acid levels appear to be less affected (25,51).

Foliar symptoms of HpSVd can vary among hop cultivars as well as growing regions, and may take up to five year to be fully expressed (15). Leaves of infected 'Kirin II' in Japan appear smaller and chlorotic, and leaves near the top of the plant may curl downwards (43,51). 'Glacier' in Washington State exhibits similar stunting symptoms and juvenile leaves tend to be lime-green while mature leaves develop veinal chlorosis, yellow speckling, and epinasty (12). *Humulus japanicus* exhibits stunting symptoms three months after inoculation with HpSVd (43). *Cucumis sativus* L. cv. 'Suuyou' develops distinct rugose leaves and shortened

internodes when injected with HpSVd, and thus, has been used as an indicator plant for detection of HpSVd (42,43). *Hop stunt viroid* has been detected in other hosts exhibiting no visible symptoms, including grape and certain tree fruits, like apricot, almond and peach (15).

HpSVd is transmitted mechanically via sap movement from infected plants, as was demonstrated by dipping hands or tools in sap from infected plant material and rubbing the buds of HpSVd-free plants, or using contaminated tools for plant maintenance (51). Contaminated razorblades that were not dipped in chemicals transferred HpSVd with or without water rinses while a 10 minute soak in 0.1% formaldehyde, 0.1% NaOH, 0.5% NaOCl, 0.1% CaOCl, or 1% Na_3PO_4 x 12 H_20 was effective in preventing transmission of HpSVd (47). The use of tools in an infected planting (field or greenhouse) without proper cleaning between plants can spread HpSVd, as well as vegetative propagation using infected mother plants. Although HpSVd has not yet been transmitted experimentally by pollen or seed in hop (50), transmission is reported to occur via seed in grape (48).

There are currently no methods for curing plants of HpSVd, thus eradication has been used extensively for controlling HpSVd in Japan. Hop growers for Kirin Brewery Co. Ltd. were advised to perform yearly inspections of hop fields and test suspect plants for HpSVd (44). When a HpSVd infection was detected, 10 plants on either side along the same row and three plants in adjacent rows on either side of the infected plant were sprayed with herbicide and/or cut down and burned. Urea was then used to kill the remaining rootstock, and HpSVd-free rootstock would be

planted after decomposition of the infected plant residues. Sterilization of tools, HpSVd-free root stock, and routine inspection when used together were successful in controlling HpSVd in Japan (44).

HpSVd spread throughout Japanese hops before it was detected, and similarly HpLVd spread throughout hop germplasm worldwide before being detected. So it is expected that HpSVd has the potential to spread throughout the U.S. hop industry and have serious impacts on yield. Preliminary observations in Willamette Valley hop plantings infected with HpSVd suggest that U.S. growers may face the additional problem of wide-spread Fusarium canker. This thesis explores *Fusarium* spp. as pathogens on hop, with an emphasis on *F. sambucinum*, and examines the prevalence of plants co-infected with HpSVd in commercial fields.

Methods and Materials

Fusarium isolation and inoculum production

To obtain *Fusarium* isolates, cankered bines were severed from the rhizome, and then rinsed with water to remove soil and debris. Small pieces of tissue were excised from the tapered or girdled end of the bine. The tissue was dipped in a 0.05% NaOCl solution and embedded in Nash-Synder medium (27) amended with 120 µg/ml chlorotetracycline HCl (18), a medium selective for *Fusarium* species. Plates were then incubated at 24 C, away from direct light, for up to 21 days. Suspect *Fusarium* colonies were transferred to potato dextrose agar (PDA) (Difco, Sparks, MD) and carnation leaf agar (CLA), and then incubated under fluorescent

lamps (three General Electric or Sylvania 40W tubes, Danvers, MA) supplemented with black light (one Sylvania 40W tube, BLB series) with a 12-hour photoperiod at 24 C (28). *Fusarium sambucinum* isolates were identified to species according to the system of Nelson, Toussoun and Marasas (28). Isolates were purified by single-sporing and stored on CLA at 4 C.

For increasing inoculum, agar pieces (0.5 x 0.5 cm) from stock cultures of *Fusarium* isolates (Table 1) were transferred to CLA and incubated for 7 to 14 days at 24 C. Liquid cultures were made by transferring isolates from 14- to 21-days-old CLA cultures to 75-ml aliquots of potato dextrose broth (Difco, Sparks, MD) and then liquid cultures were incubated on an Innova 2300 platform shaker (New Brunswick Scientific, Edison, NJ) at 90 rpm for 10 to 14 days under ambient light. Conidia and hyphal fragments from potato dextrose broth cultures were collected by vacuum filtration using a Buchner funnel and Whatman # 1 filter paper (Whatman International Ltd, Maidstone, England). Conidia and hyphal fragments were washed three times with sterile, reverse osmosis water (RO water). The conidia and hyphal fragments were re-suspended in 100 ml of sterile, RO water, and then homogenized with a hand-held Tissue-Tearor (985-370 Biospec, Bartlesville, OK) for 40 seconds at 5,000 rpm. Conidia were collected by filtering the slurries through 100 µm pore size Nytex cloth (Tetko, Briarcliff Manor, NY). Spores and hyphal fragments were counted with a hemocytometer and were then diluted to a final concentration of 10^5 conidia and hyphal fragments/ml using sterile, RO water.

Table 1. *Fusarium* isolates used in hop pathogenicity studies

Isolate Name	***Fusarium* spp.**	**Source**
Fsam 1	*F. sambucinum*	Fusarium canker on hop
Fsam 3	*F. sambucinum*	Fusarium canker on hop
Fsam 5	*F. sambucinum*	Fusarium canker on hop
Fsam 6	*F. sambucinum*	Fusarium canker on hop
Fsam 7	*F. sambucinum*	Fusarium canker on hop
Fsam 8	*F. sambucinum*	Fusarium canker on hop
gF131	*F. sambucinum**	Cone tip blight on hop
gF234	*F. verticillioides**	Corn seed

*****Isolates were transformed with the green fluorescent protein gene (20).

Hop plant propagation

Five to 15 cm lengths were cut from the growing tip of bines, and included three nodes and the apical meristem. Leaves were removed from the bottom two nodes while the upper leaf pair and meristem remained intact. The bottom two nodes were planted in Sunshine SB40 potting mix (Sun Gro Horticulture, Bellevue WA) contained inside a 1-quart plastic zip-sealed bag. Bags were watered until potting mix was saturated, and then sealed until cuttings were rooted (Figure 1). Cuttings were incubated in a greenhouse under ambient light; temperatures were maintained at approximately 24/18 C (day/night). Plastic pots (8.5 x 8.5 cm) were dipped in 0.05% NaOCl, allowed to air dry, and then filled with SB40 potting mix, after which one rooted-cutting was planted per pot. Potted plants were kept on a single bench in the greenhouse under conditions as previously stated, and used for pathogenicity assays 30 to 60 days later.

Figure 1. A hop cutting with new root growth.

***Fusarium sambucinum* pathogenicity trials with potted hop plants**

Study 1- Greenhouse pathogenicity trial using *Fusarium*-colonized agar plugs. Plants received a 1-cm scratch on the epidermis from a sterile scalpel and a 1-cm diameter piece of CLA or *F. sambucinum*-colonized CLA (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) was attached to the wound with 1.27-cm transparent tape (3M, St. Paul, MN). Thirty plants were placed in a random arrangement on a single bench in the greenhouse and watered every two days. For eight weeks, plants were visually examined weekly for bine swelling or decay.

Study 2- Greenhouse pathogenicity trial using *Fusarium*-infested toothpick pieces. The ends of round, wooden toothpicks (Penly, West Paris, ME) were cut to 1-cm lengths. Cut pieces were placed in 75-ml aliquots of potato dextrose broth (PDB) and then inoculated with one agar plug of each of the *F. sambucinum* isolates: Fsam 1, 3, 5, 6, 7, and 8 (Table 1). Inoculated PDB-toothpicks were incubated on an Innova 2300 platform shaker at 90 rpm for 10 to 14 days under

ambient light. To ensure the toothpicks were successfully infested, toothpicks were embedded in CLA for *Fusarium* identification. Subsequently, plants were scratched with a *F. sambucinum*-infested or non-infested toothpick piece, just below the soil line, and the toothpick piece was left next to the wounded area of the plant. Twenty plants (10 plants/treatment) were placed in a complete random design on a single bench in the greenhouse.

Trays (15 cm diameter) were placed under the pots (8.5 x 8.5 cm) and filled with water every other day for four weeks. Then the trays were removed from under the pots and plants were watered every seven days for an additional four weeks. Then the plants were moved to a humidity chamber for another eight weeks. The humidity chamber consisted of a wooden bench and frame wrapped in poly plastic with a cool-mist humidifier (V400 Kaz, Southborough, MA) placed inside. Trays were placed under plants in the humidity chamber and were filled with water every two days. During the final eight weeks of the 16-week study, plants were visually examined weekly for swelling or decay near the base of bine.

Study 3- Greenhouse pathogenicity trial using *Fusarium* inoculum drenches.

Plants were divided into five treatment groups (Table 2) with 10 plants/treatment. Plants were injured with a sterile scalpel, causing a 1-cm scratch to the epidermis just above the soil line. Ten ml aliquots of liquid *F. sambucinum* (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) inoculum (10^5 conidia and hyphal fragments/ml) were poured over the stem region into the soil of each inoculated plant. Plants were placed in a complete randomized design on a single bench in the greenhouse. Plants

were examined for lesion development, bine swelling or decay over 16 weeks. Every four weeks, one plant from each treatment was arbitrarily selected for destructive examination of the rhizome and roots for observation of canker symptoms.

Table 2. Study 3- Greenhouse *Fusarium* pathogenicity trial treatments

Treatment #	**Injured**[1]	***F. sambucinum* inoculated**[2]
1	No	No
2	Yes	No
3	No	Yes
4	Yes	Yes
5	Yes; 5 days after inoculation	Yes

[1] Stems of "injured" plants received a 1-cm scratch from a sterile scalpel.
[2] Each inoculated plant received 10 ml of *F. sambucinum* (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) at 10^5 conidia and hyphal fragments per ml.

Study 4- Cold frame pathogenicity trial using *Fusarium* inoculum drenches.

Twenty hop rhizomes were each planted individually in black, 1.8 L plastic pots filled halfway with Sunshine SB40 potting mix. Plants were placed outside in a cold frame in September. Four weeks later, 10 plants each received 100 ml of *F. sambucinum* (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) inoculum (10^5 conidia and hyphal fragments/ml) while 10 plants remained non-inoculated, and then all pots were filled with SB40 potting mix to about 3-5 cm from the top. Plants were placed in a random arrangement in the cold frame and kept saturated throughout the winter months by natural rainfall or hand watering. Four months later, the study was repeated. Eight months after inoculation, plants were examined for symptoms of Fusarium canker.

Study 5- Pathogenicity trials in glass jars with *Fusarium* inoculum drenches.

Glass canning jars (1.9L) were sterilized for 30 minutes at 121 C. Sunshine SB40 potting mix was pasteurized in 61 x 91 cm autoclavable bags (sealed with 2.54-cm labeling tape wrapped around a 11-cm foam square) at 90 C for one hour, and again 24 hours later. Jars were filled half-way with the pasteurized SB40 mix and one hop rhizome of either 'Sterling' or 'Nugget' was planted in each jar. Jars were watered with RO water until just before the water pooled on the surface of the potting mix. The jars were sealed with a Whatman #3 filter paper inserted into the canning jar ring. One week later, 10 ml aliquots of liquid *F. sambucinum* (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) inoculum (10^5 conidia and hyphal fragments/ml) or RO water were added each jar and then one week after that, 5 ml aliquots of *F. sambucinum* or RO water were added to the respective jars. Three weeks later, plants were examined for swelling or decay of the bines or rhizomes. Three to five jars per treatment were placed in a complete randomized design on a single shelf supplemented with two fluorescent lamps (General Electric or Sylvania 40W tubes) for a 16-hour photoperiod. The study was repeated twice with 'Nugget' plants (Study 5b and 5c).

Hop stem inoculations with GFP-labeled *F. sambucinum* and *F. verticillioides*

'Nugget' bines, approximately 2 cm in diameter, were collected from a single hill at the OSU Botany Field Laboratory for each run, and were cut into 8 cm lengths, dipped in 0.05% NaOCl solution, and allowed to air dry. Plastic moist chambers and galvanized steel wire mesh were soaked in 0.05 % NaOCl for ten

minutes and rinsed with RO water. Germination paper (Anchor Paper, St. Paul, MN) was sterilized for 30 minutes at 121 C. Six pieces of hop stem were placed in each plastic moist chamber (20.5 x 13 x 5 cm) on top of wire mesh (5 x 5 mm) above two pieces of wet germination paper (Figure 2). Two plastic boxes were used for each treatment in each run of the study (12 stems/*Fusarium* spp.).

Figure 2. Plastic moist chambers with two pieces of germination paper and folded wire mesh used for GFP-*Fusarium* inoculations on detached hop bine pieces.

A small piece of CLA or GFP-*Fusarium*-colonized CLA (isolates Fsam 1, 3, 5, 6, 7, and 8 in Table 1) was placed on the stem surface towards the middle of each bine. Stems were sealed in the plastic moist chamber and incubated at 25 C with a 16-hour photoperiod provided by one fluorescent light (General Electric or Sylvania 40W tube). Stems were examined at 10 and 20 days using a Leica MZ FLIII stereo-fluorescence microscope (Leica, Heerburg, Switzerland) with a GFP2 filter. From each treatment, a subset of stems with mycelial growth was destructively sampled on 10 day to determine whether the GFP-*Fusarium* colonized the internal tissue of the hop stem. The study was repeated two times. Stems were collected in September 2009 for the first run; stems were collected during April 2010 for the second and third run.

Effect of relative humidity on hop stem colonization by *F. sambucinum*

Controlled relative humidity chambers were made by an agar dish isopiestic equilibrium method described by Harris et al. (16). Water agar (2%) was amended with sodium chloride (NaCl) in order to obtain relative humilities ranging from 87.7 to 99.6% (Table 3) (35). Twenty ml aliquots of each NaCl agar (Table 3) was cooled in plastic Petri dishes (100 x 15 mm). Fiberglass window screen (100 mm diameter) was sterilized in an autoclave for 30 minutes at 121 C. 'Nugget' stems from greenhouse-grown plants were cut to about 5 cm lengths. One end of the stem piece was dipped for one minute in a 10-ml beaker containing 2 ml of GFP-*Fusarium* (isolates gF131 and gF234 in Table 1) inoculum (10^5 conidia and hyphal fragments/ml). Inoculated or non-inoculated stem pieces were placed in each Petri dish on a screen above the agar (Figure 3). A second piece of fiberglass window screen was placed on top of stem pieces followed by a slab of the respective NaCl agar. Twelve Petri dishes (4 plates per treatment), containing five to eight stems each, were wrapped with parafilm and randomly arranged in an incubator at 25 C with a 16-hour photoperiod provided by one fluorescent light (General Electric or Sylvania 40W tubes). After 12 days, stems were evaluated microscopically for incidence of mycelial growth. Stems were examined microscopically using a Leica MZ FLIII stereo-fluorescence microscope with a GFP2 filter. Colonized stems were summed by % relative humidity and were analyzed with PROC REG in SAS 9.1. The study was repeated twice with gFsam (gF131) using 15 stems per treatment.

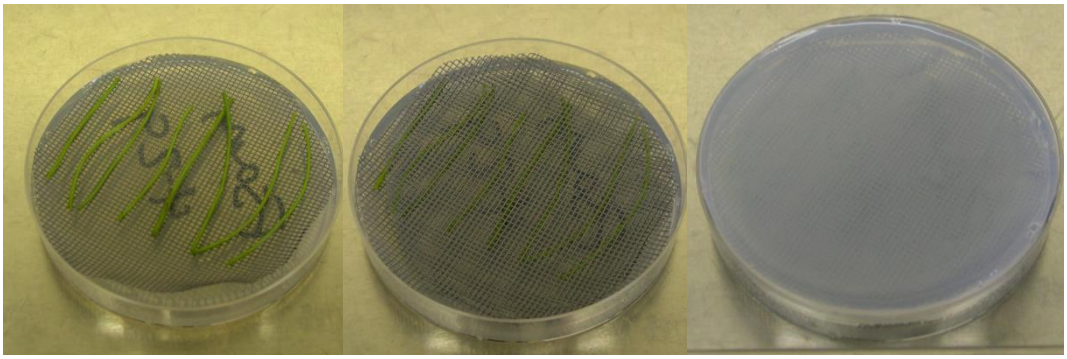

Figure 3. Petri dish (100 x 15 mm) humidity chambers.

Table 3. Relative humidity and concentration of NaCl tested in Study 7

Relative Humidity (%) at 25 C	Concentration of NaCl (molality)
99.6	0.1
97.7	0.8
94.6	1.6
87.7	3.4

***Hop stunt viroid* and Fusarium canker survey in commercial fields**

During 2008, HpSVd and canker surveys were conducted in six hop fields with suspected widespread HpSVd presence; two were 'Tettnanger' plantings and four were 'Sterling'. A seventh hop planting, located on the Oregon State University Botany Field Laboratory, which was virtually free of HpSVd but had received *F. sambucinum* inoculations during previous years was also surveyed. The number of rows were counted in each field and divided by 20 to determine the number of rows to skip between transects. Poles, situated parallel with the rows, were counted along the edge row and the sum was divided in half to determine the number of poles to skip between transects. The number of transects in a field varied, depending on field shape and size. Each transect contained 10 consecutive plants in a row, except for transects at the Botany Field Laboratory which contained five consecutive plants.

Transects were arranged in a 'V' or 'W' shape and spanned the entirety of the field (Figure 4).

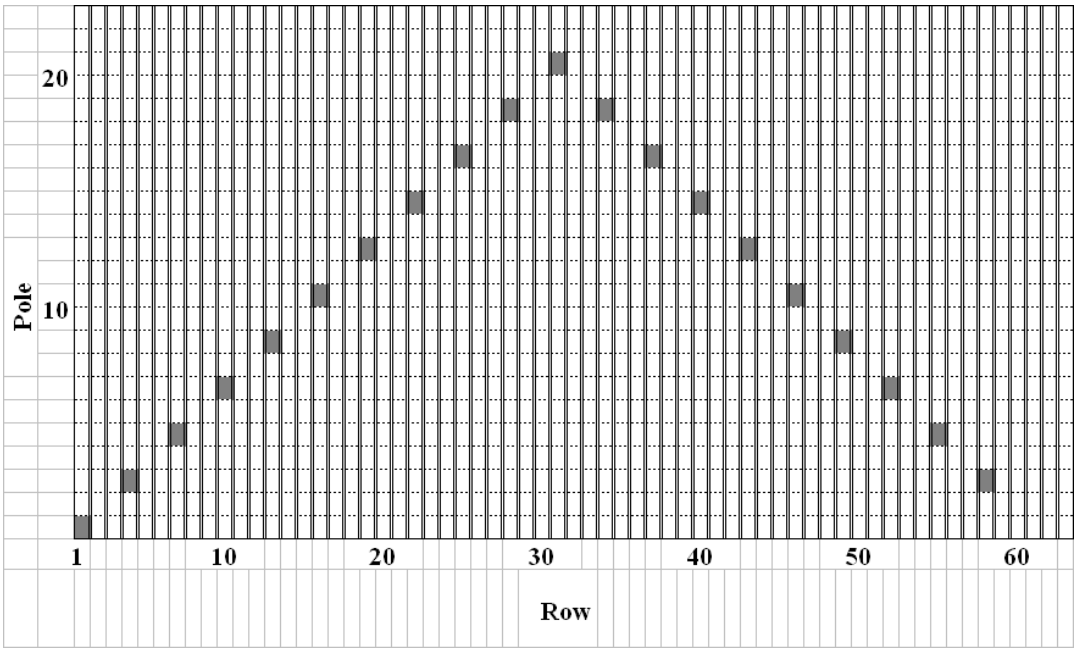

Figure 4. Example of the sampling pattern used in commercial hop fields for HpSVd and Fusarium canker surveys. Horizontal dotted lines represent 'poles' along the length of a field. Vertical spaces between the solid black lines represent 'rows' along the width of a field. Gray squares are transects of 10 consecutive plants.

From each plant (hill), a single leaf was sampled. Leaves presumptively symptomatic for HpSVd (Figure 5) were preferentially chosen and if a plant did not appear symptomatic, a mature leaf was sampled arbitrarily. Using a hand-held hole-puncher, 5 to 10 leaf disks, each 6 mm in diameter, were removed from each leaf in the field. Between each leaf, hole-punchers were dipped in 0.05% NaOCl for at least one minute, dipped in 95% ethanol, and then flamed. Leaf discs were stored in microcentrifuge tubes containing 0.5 ml of RNALater (Ambion, Austin, TX) in the

field and transported on ice, stored at 4 C for 24 hours, and then stored in a -20 C freezer.

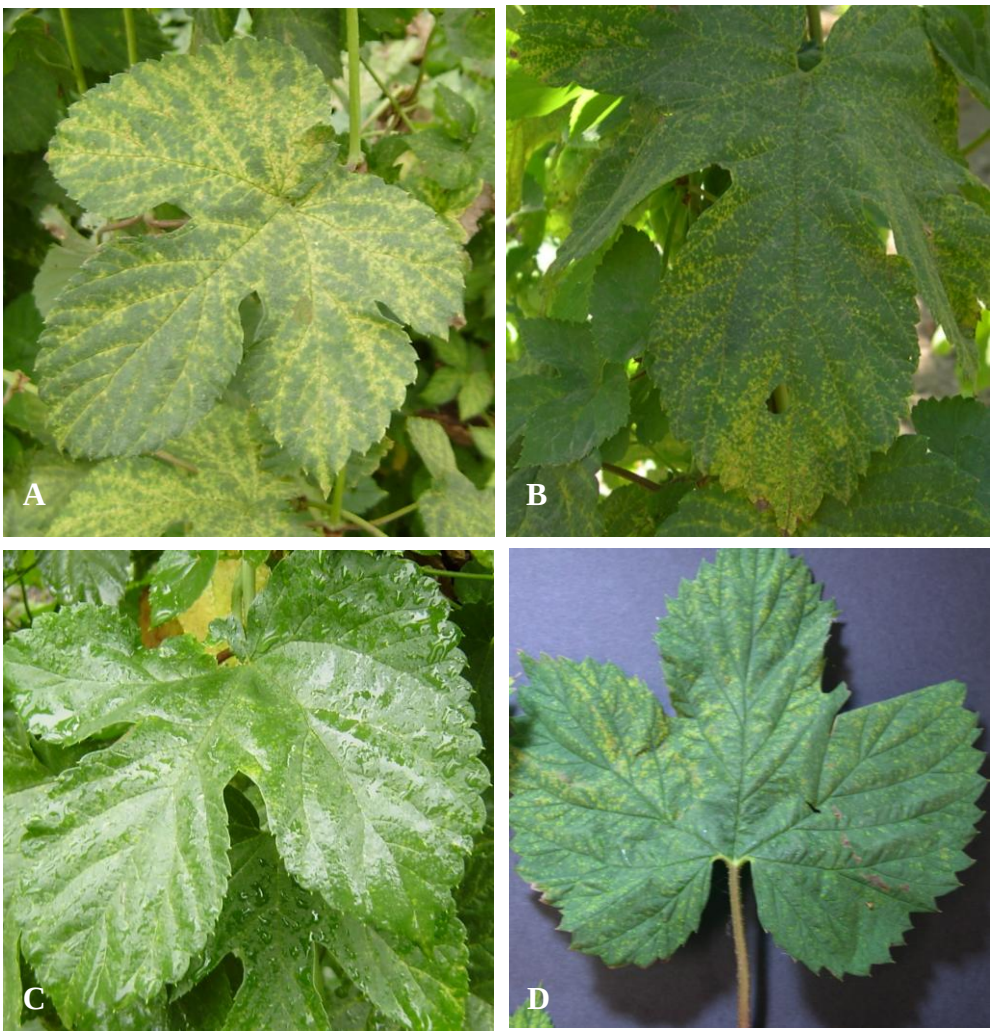

Figure 5. 'Sterling' plants with A, veinal chlorosis and B, chlorotic speckling characteristic of HpSVd-infected plants. C, 'Sterling' leaf with subtle chlorotic speckling. D, chlorotic speckling on 'Tettnanger' leaf.

After commercial cone harvest, hills within each transect were evaluated for Fusarium canker. Every bine was tugged on each hill sampled. Girdled bines could be severed from the rhizome and were categorized as such. Bines that could not be severed from the rhizome but had swelling near their base were categorized as

'swollen'. The remaining bines were categorized as healthy. Three to five cankered bines from each transect were embedded in amended Nash-Snyder medium as previously described. Percentage of cankered bines and plants with symptoms of HpSVd were analyzed with PROC GLM using SAS 9.1.

Leaf disk samples were removed from tubes of RNAlater stored at -20 C, patted dry on paper wipes, and any visible salt crystals that were removed. Leaf disk samples were placed in Lysing Matrix D tubes (MP Biomedicals, Solon, OH,) containing 742.5 µl of RLT buffer (Qiagen Rneasy Mini Kit, Valencia, CA), 0.015 g of polyvinylpolypyrrolidone, and 7.5 µl of beta-mercaptoethanol. Samples were lysed in a FastPrep Instrument (MP Biomedicals, Solon, OH) on setting '6' for 40 seconds. The lysed leaf disks were transferred to QIAshredder spin columns and centrifuged for five minutes at 14,000 rpm. The supernatent was transferred to a new microcentrifuge tube without disturbing the pellet and centrifuged for 3 minutes at 14,000 rpm. The lysate was transferred to a new microcentrifuge tube containing 50% volume of 100% EtOH and mixed by pipetting for 5 seconds. The lysate and EtOH was transferred to the RNeasy spin column and centrifuged for 15 seconds at 14,000 rpm. The flow-through was discarded, and 700 µl of RW1 buffer was added to the spin column. The spin column was removed from the collection tube, inverted and rolled several times to remove any residuals before returning the spin column to the collection tube, and then centrifuged for 15 seconds at 14,000 rpm. The flow-through was discarded, and 500 µl of RPE buffer was added to the spin column. The spin column was removed from the collection tube, inverted and rolled

several times before returning to the collection tube, and then centrifuged for 15 seconds at 14,000 rpm. The flow-through was discarded, 500 µl of RPE buffer was added to each spin column. The spin columns were removed from the collection tube, inverted and rolled several times, returned to the collection tube, and then tubes were centrifuged for two minutes at 14,000 rpm. The flow-through and collection tubes were discarded. The spin column was placed in a new 2-ml collection tube and centrifuged for 2 minutes at 14,000 rpm to remove any residual buffer. The spin column was placed in a new microcentrifuge tube, 40 µl of RNase-free water was added directly on the membrane of the spin column, and the tubes were centrifuged for one minute at 14,000 rpm. The spin columns were discarded and the eluted RNA was transferred to a new microcentrifuge tube and stored at -20 C for l week or less, or at -80 C for long term storage.

RNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR). Five µl of extracted RNA was added to 2.5 µl of 10x ThermoPol Buffer (M02367, New England BioLabs, Ipswich, MA), 0.5 µl of 10 mM dNTPs, 0.25 µl of each primer (5'-GCCCCGGGGCTCCTTTCTAGGTAAG-3', 5'-GGCAACTCT TCTCAGAATCC-3'(12)) at a concentration of 20 nm (Integrated DNA Technologies, San Diego, California), 0.5 µl of *Taq* DNA polymerase (M02367, New England BioLabs, Ipswich, MA), 0.5 µl of SuperScript III/RNaseOUT Enzyme Mix (18080-400, Invitrogen, Carlsbad, California), 0.5 µl of 50% acetonitrile, 0.5 µl purified bovine serum albumin, 0.9 µl 10% polyvinylpyrrolidone, and 13.6 µl of RNase-free water for a final PCR reaction volume of 25 µl. cDNA was synthesized

in a thermocycler (Eppendorf, Hauppauge, NY) at 55 C for 60 minutes. Then immediately after in the same reaction tube, PCR was initiated at 94 C for 4 minutes, followed by 35 cycles of denaturing at 94 C for 1 minute, annealing at 50 C for 2 minutes and extension at 72 C for 3 minutes. After the 35 cycles, tubes were held at 72 C for 7 minutes for a final extension.

Ten µl of PCR product was mixed with 2 ul of loading dye and processed by electrophoresis on a 1.6% agarose gel made with TAE and ethidium bromide (0.5 $\mu g\ ml^{-1}$) at 75 volts for 30 minutes. A 100 base pair molecular size marker (N04675, New England BioLabs, Ipswich, MA) was used for reference. The gels were visualized and photographed in a BioDoc-it imaging system (UVP, Upland, CA).

Evaluation of hilling practice as a management strategy for Fusarium canker

'Hilling' treatments were applied during the summer 2009 in three commercial hop fields. In the 'hilled' treatment, additional soil was piled on top of each hop hill by the grower, using either plows or shovels, for approximately 50 meters along each row (Figure 6). The height of soil added to each hill ranged between 100 and 200 cm. The nontreated group did not receive any additional soil (Figure 7). The study design at sites 'Sterling' II and IV was a complete block design with three replicates (Table 4). The design at site 'Tettnanger' II consisted of three consecutive rows hilled and three consecutive rows of nontreated. Plants in both treatments were subjected to the same grower practices prior to and after hillling.

Table 4. Timing and cultivars used in study on hilling for Fusarium canker management

Site name	Date hilling was done	Sidearm harvest date	Number of replicates
Tettnanger I	3rd week of July	10-Aug	1
Sterling II	4th week of June	18-Aug	3
Sterling IV	5th week of June	19-Aug	3

Figure 6. Hop plants pre-harvest in A, hilled plant and B, nontreated plant.

Figure 7. Hop plants post-harvest in A, hilled and B, nontreated.

During the week of commercial harvest, sidearms were collected from the same side of each of 10 consecutive plants in each plot. Sidearms were arbitrarily

sampled at approximately 2 m above the ground. All sidearms selected had active terminal growth. One sidearm was sampled from each plant in sites ‘Sterling’ II; two sidearms were sampled from each plant in site IV and were averaged per plant. Within 24 hours of collection, cone number and fresh weight were determined for each sidearm sampled. The vegetative sidearm weight was also recorded.

After harvest, ten hills (plants) in each row, for a total of 60 plants in each field, were assessed for canker as previously described. The bine diameter was recorded for all bines showing symptoms of Fusarium canker; the diameter of the swollen portion and normal portion was determined. The extent of root development along the length of girdled bines was measured and these bines were also rated for relative root abundance using a scale of 0 (none) to 4 (greatest), as illustrated in Figure 8. The hilling studies were analyzed using a PRCO GLM in SAS 9.1, and included only fields ‘Sterling’ II and IV. Field ‘Tettnanger’ II was excluded from the analysis because treatments were applied much later (4 weeks before harvest), compared to the other two fields (Table 4).

Figure 8. Rooting classes of hop bines with girdling from Fusarium canker. A, class '0' bines had no root development. C, class '1', '2', and '3' (left to right, respectively) had increasing amounts of root growth. B, class '4' bines with relatively abundant root development above the point of girdling.

Results

Fusarium sambucinum pathogenicity trials with potted hop plants

When plants were inoculated with *F. sambucinum*- colonized agar plugs (Study 1), grown in the greenhouse, and examined eight weeks later, no plants developed canker symptoms. Some plants developed a slight swelling around the scalpel wound. Plants inoculated with *Fusarium*-infested toothpicks (Study 2) did not develop canker symptoms in the greenhouse when examined four and eight weeks after inoculation. Greenhouse plants inoculated by conidial drenches, with and without injury (Study 3), did not develop canker symptoms by 16 weeks after inoculation. None of the plants developed canker after being drenched with *F. sambucinum* and incubated in outdoor cold frames (Study 4) for four months.

Canker symptoms did appear on inoculated 'Nugget' and 'Sterling' plants when grown for five weeks inside glass jar (Study 5). Some plants developed girdled or partially girdled bines, and some rhizomes and roots were rotted (Figure 9). Swelling of the bine above the point of girdling was not obvious on bines growing in glass jars, but girdled bines were easily severed from the rhizome. Although the number of bines that developed in each jar varied between one and six, at least one bine in each jar treated with *F. sambucinum* developed canker symptoms (Table 5). Thirty of the 50 total bines (60%) inoculated with *F. sambucinum* in jars developed girdled bines (Table 5). *Fusarium sambucinum* was recovered from 71% of the girdled bines. One bine from non-treated 'Nugget' jars in Study 5a and one from Study 5c also developed canker symptoms.

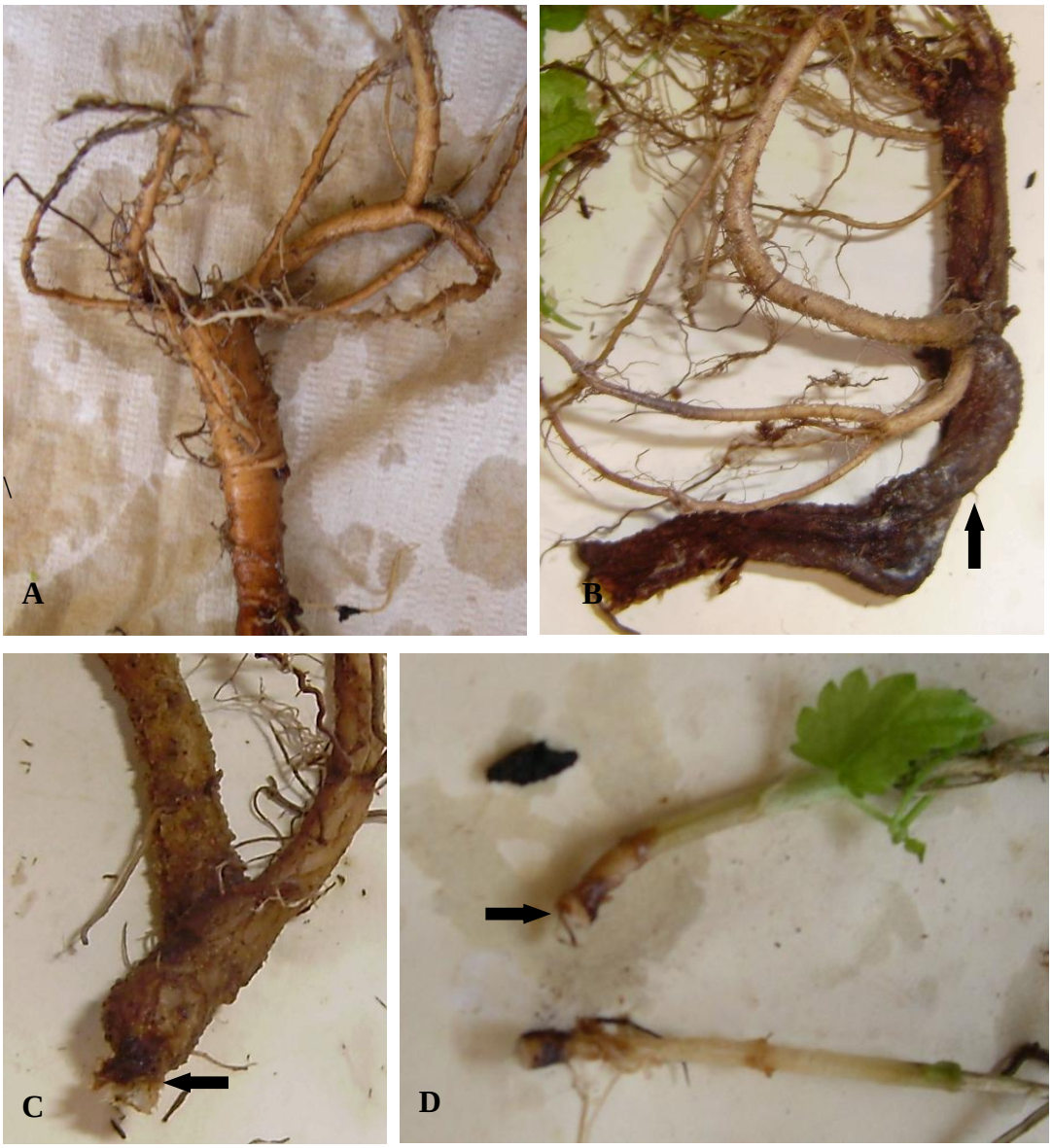

Figure 9. Symptoms of *F. sambucinum* infection of hop rhizomes and bines growing in glass jars. A, a rhizome with no obvious Fusarium canker symptoms from a nontreated jar. B, rotted rhizome from a jar inoculated with *F.sambucinum*. C and D, examples of girdled bines growing in jars inoculated with *F. sambucinum*.

Table 5. Development of Fusarium canker on hop 'Nugget' plants planted in glass jars

		***F. sambucinum* inoculated[1]**				**Nontreated**			
Study	**Cultivar**	**Jars[2]**	**Bines[3]**	**Canker[4]**	**%[5]**	**Jars[2]**	**Bines[3]**	**Canker[4]**	**%[5]**
5a	Sterling	4	7	7	85	4	9	0	0
5a	Nugget	5	14	12	67	4	11	0	0
5b	Nugget	3	16	7	71	3	11	1	0
5c	Nugget	5	13	4	50	4	8	1	0
	Total	17	50	30	68	15	39	2	0

[1]Jars were inoculated with *F. sambucinum* at 10^5 conidial and hyphal fragments/ml twice, one week apart.
[2] Number of jars in each study. Each jar contained one rhizome.
[3] Total number of bines in each jar.
[4] Cankered bines were girdled and could easily be removed the rhizome.
[5] % recovery of *F. sambucinum* from cankered bines.

Hop stem inoculations using GFP-labeled *F. sambucinum* and *F. verticillioides*

Under the GFP2 filter, living hop cells containing chloroplasts usually appeared red (Figure 10a), and epidermal cells sometimes had a slight yellow-greenish auto-fluorescence (Figure 10b). GFP-*Fusarium* could be easily be distinguished from the hop plant's auto-florescence because the *Fusarium* mycelium was brighter and the mycelium could be visualized.

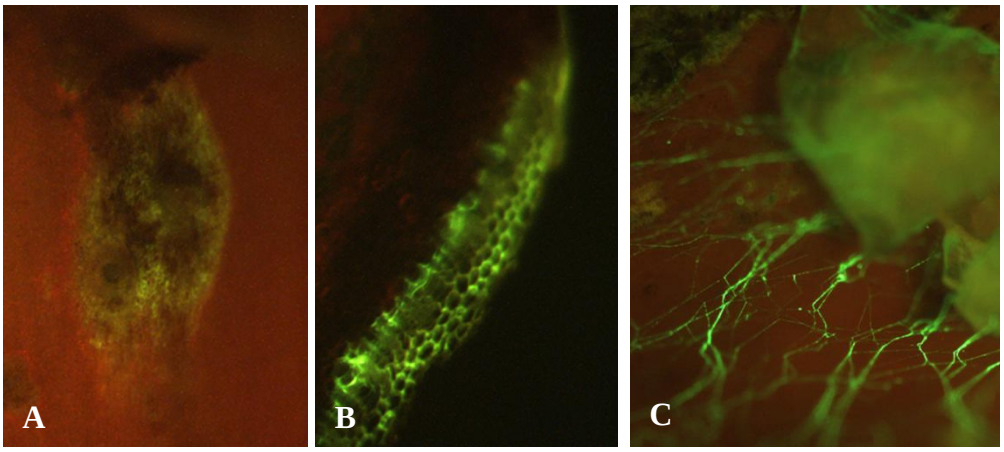

Figure 10. Hop bines viewed through a GFP2 filter. A and B, epidermal cells can auto-fluoresce similar in appearance to GFP-*Fusarium*. Living cells with chloroplasts appear red. C, florescence of GFP-*Fusarium* is brighter and *Fusarium* florescent mycelium is visible.

Ten days after inoculation, most inoculated stems had GFP-labeled *F. sambucinum* (gFsam) mycelium growing along the surface of stems regions with necrotic epidermal cells (Figure 11, Table 6). Some stems in two runs of this study (6a and 6c) had gFsam colonization on the surface of green stem areas (Table 6). Mycelium of this fungus was found growing in the pith, cortex, xylem and phloem (Figure 11) in at least one stem inoculated with gFsam in study 6b. Ten days after stems were inoculated with the GFP-labeled *F. verticillioides* (gFvert), the fungus was observed only on necrotic portions of the stem surface. Mycelium was not observed internally in the stem. Stems inoculated with water agar did not have any fluorescent mycelial growth on the surface or in internal stem tissues.

Table 6a. Study 6a- Presence of *F. sambucinum* and *F. verticillioides* in 'Nugget' hop stems collected in September 2009

	10 days[1]			20 days[1]		
Tissue[2]	**gFsam[3]**	**gFvert[3]**	**WA[3]**	**gFsam**	**gFvert**	**WA**
Green[4]	8	0	0	0	0	0
Necrotic[4]	69	23	0	85	46	0
Cortex[5]	-	-	-	+	+	-
Pith[5]	-	-	-	+	+	-
Xylem[5]	-	-	-	+	-	-
Phloem[5]	-	-	-	+	-	-

Table 6b. Study 6b- Presence of *F. sambucinum* and *F. verticillioides* in 'Nugget' hop stems collected in April 2010

	10 days			20 days		
Tissue	**gFsam**	**gFvert**	**WA**	**gFsam**	**gFvert**	**WA**
Green	0	0	0	0	0	0
Necrotic	50	8	0	92	84	0
Cortex	+	-	-	+	+	-
Pith	+	-	-	+	+	-
Xylem	+	-	-	+	-	-
Phloem	+	-	-	+	-	-

Table 6c. Study 6c- Presence of *F. sambucinum* and *F. verticillioides* in 'Nugget' hop stems collected in April 2010

	10 days			20 days		
Tissue	**gFsam**	**gFvert**	**WA**	**gFsam**	**gFvert**	**WA**
Green	25	0	0	0	0	0
Necrotic	42	0	0	75	20	0
Cortex	+	-	-	+	+	-
Pith	-	-	-	+	+	-
Xylem	-	-	-	+	-	-
Phloem	-	-	-	+	-	-

[1]Days after inoculation.
[2]Type of hop stem tissue examined.
[3]gFsam= GFP-*F. sambucinum* (gF131), gFvert= GFP-*F. verticillioides* (gF234), and WA= 2% water agar.
[4]Percentage of hop stems with colonization by resepctive *Fusarium* spp. on green or necrotic epidermis regions.
[5] (+) indicates that GFP-*Fusarium* mycelium was found in at least one stem in the tissues indicated. (-) indicated no mycelium was observed on any stems.

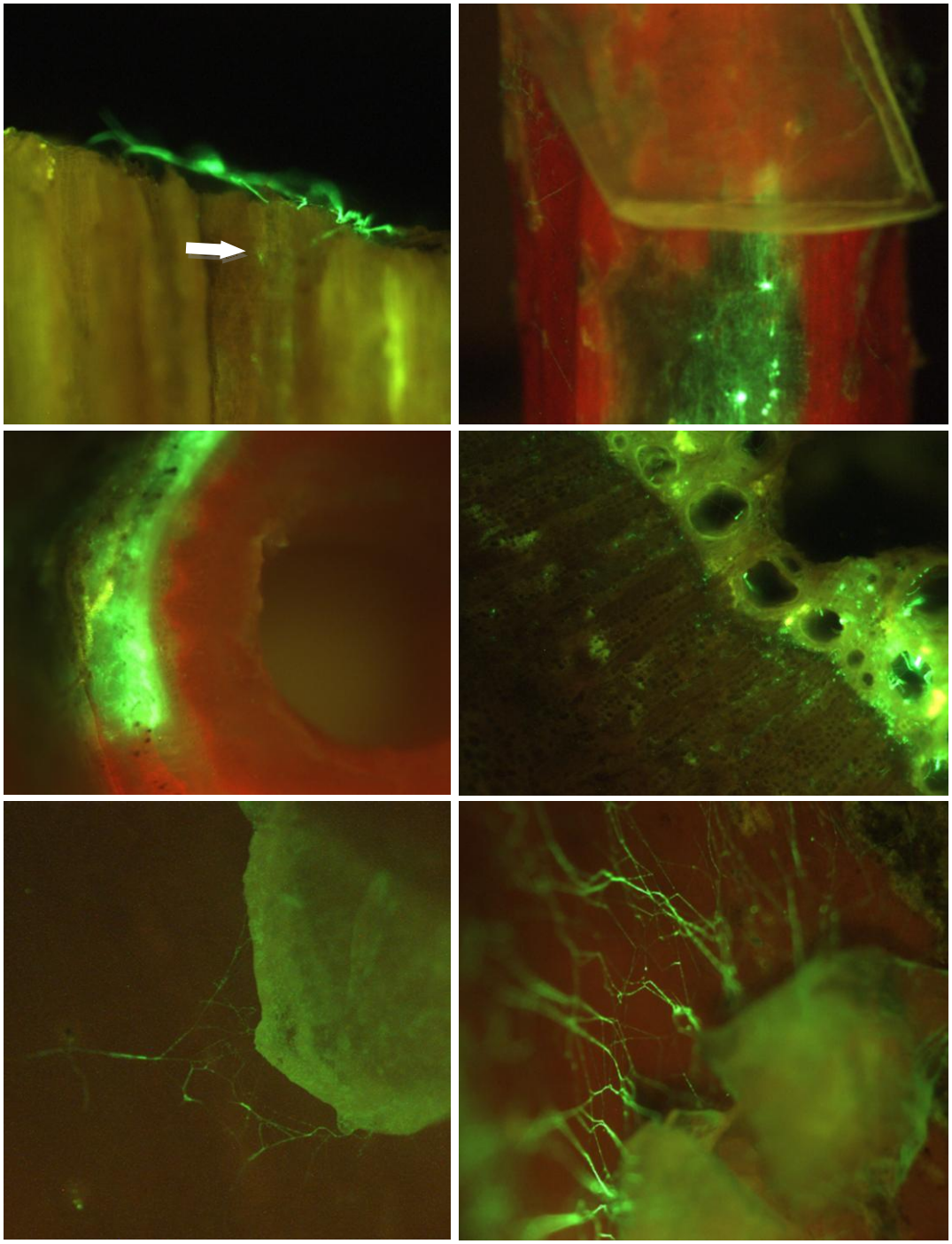

Figure 11. Mycelial growth of GFP-*Fusarium* on 'Nugget' hop stems. A, growing along the surface to the ends of stems and entering vascular tissue. B, growing as densely-colonized areas on the stem surface. C, growing in the cortex (Cx) under areas of dense mycelial growth, D, growing in xylem (X) and phloem (Ph). E, hyphae growing from *Fusarium*-agar plug. F, mycelium colonizing stem region with green surface tissue.

When stems inoculated with gFsam were examined 20 days after inoculation, most stems were colonized and at least one stem in all replicates of this study had mycelium growing internally (Table 6). Twenty days after inoculation with gFvert, between 20 to 84% of the stems were colonized, but only where the tissue was necrotic. When stems collected during September 2009 were inoculated with gFvert, mycelium grew along the surface of the stems to the cut end(s) and entered the pith and then grew into the cortex (Figure 12) of at least one stem. Mycelium from gFvert did not grow to the ends of stems collected during April 2010, but gFvert was found in the cortex and pith.

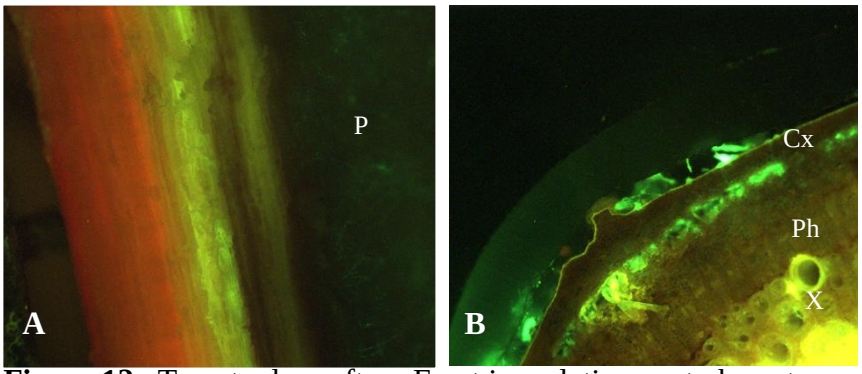

Figure 12. Twenty days after gFvert inoculations onto hop stems. A, longitudinal section of a stem where gFvert mycelium was present only in the pith (P). B, cross section of a stem showing a slight penetration by gFvert into the cortex (Cx) but not the phloem (Ph) or xylem (X).

Effects of relative humidity chambers on stem colonization by *F. sambucinum*

When cut hop stems were dipped in *F. sambucinum* and incubated in controlled humidity chambers, the proportion of stems colonized by *F. sambucinum* increased as relative humidity levels increased. The mean percentage of infected stems (93%) leveled out around 97% relative humidity (Figure 13). The percentage of stems infected had a significant positive relationship with relative humidity (R^2=

0.825, $P \leq 0.0001$). The mean percentage of colonized stems did not vary among study runs, except for stems incubated at 87.7% relative humidity; a single stem was colonized in Study 1 (Table 7). Stems dipped in water were not colonized with fluorescent mycelium.

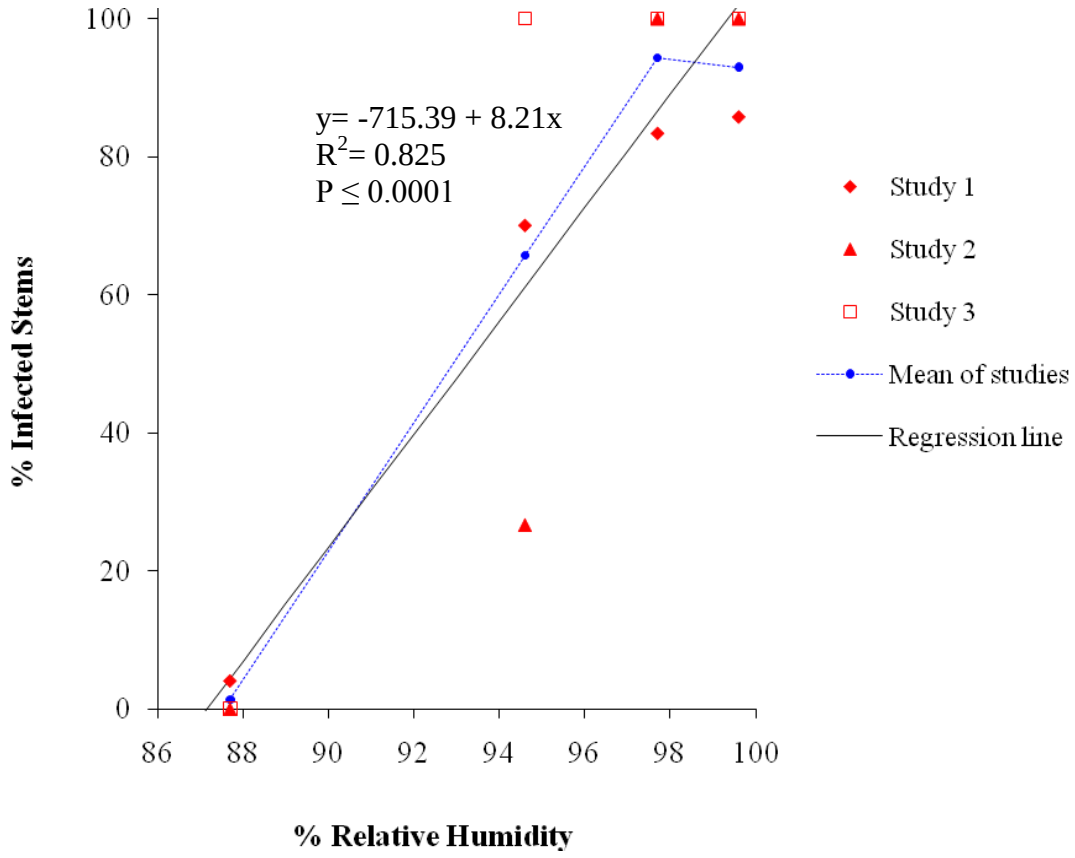

Figure 13. Percentage of stems colonized with *F. sambucinum* after 12 days at different relative humidities.

Table 7. Incidence of detached hop stems colonized by *F. sambucinum* at various % relative humidities in Petri dish humidity chambers

RH[1]	Mean[2]
87.7	1
94.6	66
97.7	94
99.6	93

[1]% relative humidity.
[2]Mean incidence (%) of hop stems colonized, summed across three replicates using 60 stems per RH.

Hop stunt viroid and Fusarium canker survey in commercial fields

Nearly all hop plants examined in grower fields were symptomatic for HpSVd (Figure 5) and at least one leaf sample from each field was verified to be infected with HpSVd by RT-PCR. All leaves sampled in the 'Sterling' fields (Table 8) had chlorotic speckling or venial chlorosis, which is characteristic of HpSVd infection on this cultivar. Ninety-eight ('Tettnanger' I) and 92% ('Tettnanger' II) of the leaves examined in 'Tettnanger' plantings were symptomatic for HpSVd. Plants sampled in Field VII, the experimental planting of 'Perle' on the OSU Botany Field Laboratory were not symptomatic for HpSVd, and had significantly lower levels of cankered bines, relative to the other six fields. On average, 35 and 38% of bines per transect were cankered in 'Tettnanger' fields, while the mean percentage of cankered bines (per transect) in the 'Sterling' fields varied from 20 to 62%. A portion of a 'Sterling' planting with notable bine mortality as well as leaf chlorosis and necrosis due to Fusarium canker is shown in Figure 14, while a range of Fusarium canker symptoms of bines' bottoms is illustrated in Figure 15.

Table 8. Percent of leaves found to have HpSVd symptoms and bines found to have Fusarium canker in commercial hop fields

		Plants with HpSVd[1]		Cankered bines[2]		
Field	**Transects[3]**	**Mean[4]**	**SE[5]**	**Mean[6]**	**SE[5]**	**%[7]**
'Tettnanger' I	20	92	3.94	38 *	3.94	81
'Tettnanger' II	23	98	0.87	35 *	3.10	50
'Sterling' I	18	100	0	20 *	2.40	38
'Sterling' II	18	100	0	38 *	2.41	65
'Sterling' III	20	100	0	22 *	2.28	64
'Sterling' IV	19	100	0	62 *	2.65	63
'Perle/Nugget' VII	5	0	0	7	3.02	60

[1]HpSVd infection was based on visual examination of leaves. Symptomatic leaves had either chlorotic speckling or veinal chlorosis (Figure 15).
[2]Cankered bines in the field include girdled and swollen bines (Figure 16).
[3]Total number of transects evaluated. 10 plants/transect were examined.
[4] Mean % of plants with foliar symptoms of HpSVd.
[5]Standard error by LS means in a general linear model.
[6] Mean % of cankered bines.
[7]% recovery of *F. sambucinum* from cankered bines.
*Indicates means are significantly different ($P \leq 0.05$) when compared to HpSVd-free planting (Field VII).

Figure 14. 'Sterling' planting with dead and dying bines due to Fusarium canker.

Figure 15. Hop bines affected by Fusarium canker. A, completely girdled and swollen bines with some sporodochia on the surface. B, cankered bines are often connected by only a few vascular strands. C, swollen bine with slight girdling and which could not be severed from the crown.

Effects of hilling in HpSVd-and Fusarium canker-affected fields

Overall, bines produced an average of 18 cm of new root growth along the stem above the point of girdling when hilled, significantly more than affected bines in non-hilled rows (Table 9). There was no significant difference between hilled and non-hilled plants in the extent of bine base swelling. Cankered bines in 'Sterling' IV had a greater diameter of swelling than affected bines in 'Sterling' II. The hilled plants in both fields developed a more extensive root system than plants that were nontreated. There was no significant difference in sidearm vegetative weight or the incidence of cankered bines between hilled and non-hilled plants, but hilled plants produced a greater cone weight per plant than plants that were not hilled (approaching significance at P=0.056) (Table 10). Average cone yield was significantly greater in plants that were hilled when compared to nontreated plants in 'Sterling' IV.

Table 9. New root growth and bine swelling of hilled and non-hilled 'Sterling' hop plants

	Rooted Length (cm)[1]			Cankered Bine Swelling (mm) [2]		
Variable	**n[3]**	**Mean[4]**	**SE[5]**	**n[3]**	**Mean[4]**	**SE[5]**
'Sterling' IV Hilled	36	21.6 *	2.2	65	7.2	0.3
'Sterling' IV nontreated	47	2.9	1.9	61	7.2	0.3
'Sterling' II Hilled	74	19.1 *	2.1	101	4.6	0.3
'Sterling' II nontreated	38	1.9	1.5	82	4.6	0.3
Hilled	110	20.2 **	1.6	143	5.6	0.2
nontreated	85	2.2	0.3	166	5.7	0.2
'Sterling' IV	83	11.8	1.4	83	7.2 **	0.2
'Sterling' II	112	10.4	4.3	112	4.6	0.2

[1]New root growth along the length of the bine above the point of girdling.
[2]Difference in bine size on cankered bines measured at the largest area near the base of the bine and the normal stem above the diseased area.
[3]Number of bines sampled.
[4]Data were analyzed using the general linear model with a LS means.
[5]Standard error from LS means statement.
[6]Data were log-transformed to correct abnormalities in the variance.
*Indicates that means of hilled treatment were significantly different from non-hilled treatment within that same field using a T-test (P=0.05).
**Indicates that means of hilled treatment were significantly different from non-hilled treatment when fields were combined or Field V was significantly different than Field VI using a T-test (P=0.05).

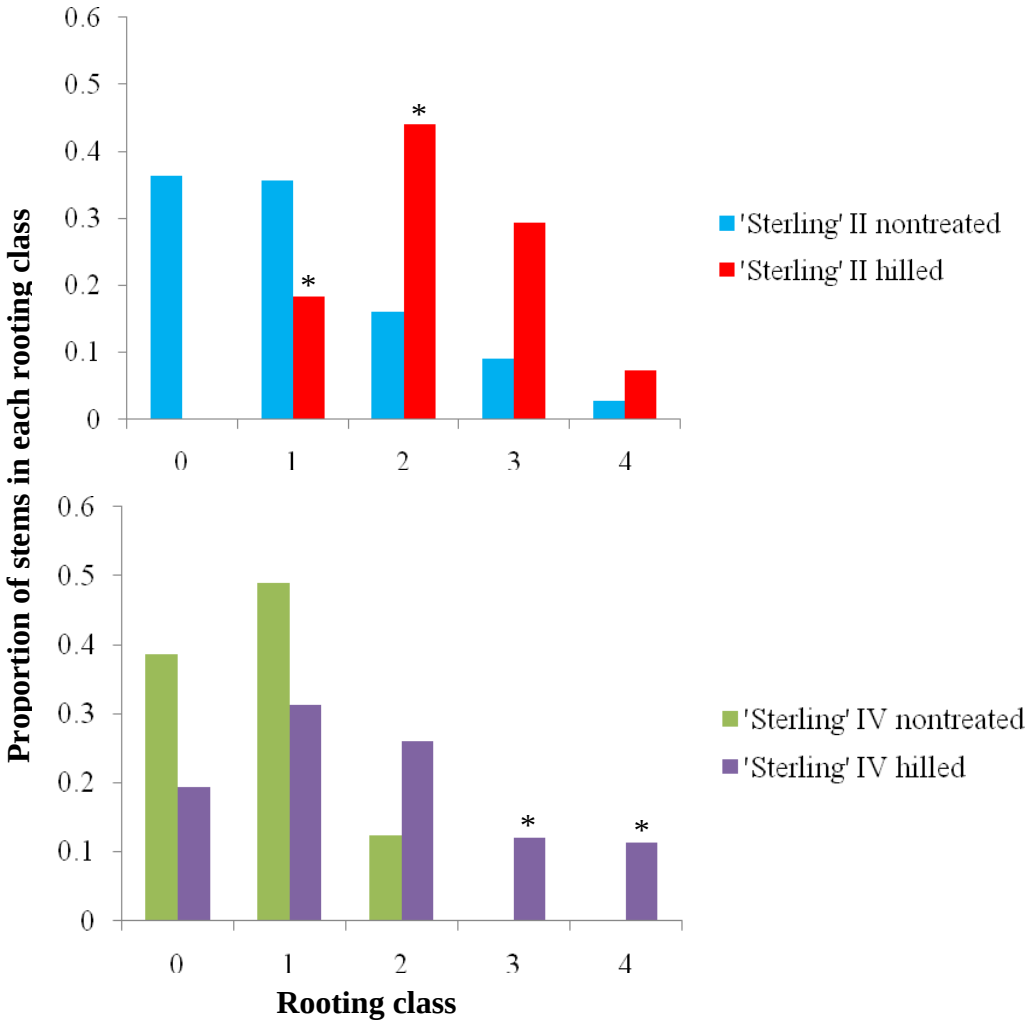

Figure 16. Proportion of stems in each rooting class (class 0=no rooting to 4=relatively abundant rooting; Figure 8) by hilled and nontreated for A, 'Sterling' II and B, 'Sterling' IV. * indicates there was significant difference between hilled and nontreated as tested by LS means (P=0.05).

Table 10. Fusarium canker incidence, average cone yield, and sidearm weight of hilled and non-treated 'Sterling' hop plants

	Canker Incidence[1]		Sidearm Weight (g)[2]		Cone Yield (g)[3]	
Variable	**Mean[4]**	**SE[5]**	**Mean[4]**	**SE[5]**	**Mean[4]**	**SE[5]**
'Sterling' IV Hilled	81	5.1	195	18	4.45	0.21
'Sterling' IV nontreated	83	5.1	228	18	4.36	0.21
'Sterling' II Hilled	85	3.5	224	23	4.65 *	0.22
'Sterling' II nontreated	84	3.5	217	23	3.79	0.22
Hilled	83	2.9	209	14	4.55 +	0.15
nontreated	84	2.9	222	14	4.07	0.15
'Sterling' IV	82	2.9	212	14	4.40	0.15
'Sterling' II	85	2.9	220	14	4.20	0.15

[1]Mean % cankered bines per replicate; cankered bines include girdled and swollen bines.
[2]Average vegetative weight of sidearm per plant by replicate.
[3]Average cone yield (green weight) per plant by transect.
[4]Each variable was summed across six replicates and data were analyzed using the general linear model with a LS means in SAS 9.1.
[5]Standard error from LS means statement.
*Indicates that means of hilled treatment were significantly different from non-hilled treatment at P=0.05.
+ Indicates that means of hilled treatment were significantly different from non-hilled treatment at P= 0.056.

Discussion

Studies presented in this thesis reproduce disease in small 'Nugget' and 'Sterling' hop plants, showing conclusively that *F. sambucinum* incites canker symptoms on hop bines. When hop plants were inoculated with *F. sambucinum* and incubated in sealed glass jars, 60% of the bines were girdled, which is typical of the end-stage Fusarium canker. However, pathogenicity tests for this thesis that were carried out in a greenhouse humidity chamber and a cold frame using various methods of inoculation and incubation were unsuccessful in reproducing Fusarium canker on hop plants incubated under the conditions of testing. Previous pathogenicity testing by others for Fusarium canker reported difficulty in reproducing symptoms typical of Fusarium canker on hop. In one study (37), 1-node, 2-node, and 1-node-split-cuttings from a healthy field were planted in *F. sambucinum*-infested compost and misted for 2 weeks. One-node-split-cuttings had the highest mortality, with 6 to 7 of the 10 cuttings dead. The mortality of the other cuttings was between 2 and 6 out of 10 cuttings for the 1-node and 2-node cuttings. Whether these cuttings died from *F. sambucinum* is unknown as recovery of pathogens was not reported. However, the plants that rooted and survived were planted outside and re-evaluated 6 months later, at which time *F. sambucinum* was recovered from lesions that showed "slight discoloration" on the rhizome. Additional rhizomes were planted outside in *Fusarium*-infested compost, but no disease was reported 4 months after planting.

In another study (11), *F. sambucinum* mycelium and ascospores from *Gibberalla pulicaris* (teleomorph of *F. sambucinum*) were placed on the cut surface of woody cuttings. Plants developed necrosis, starting at the inoculation site, then wilted and died; incidence was not reported but *F. sambucinum* was recovered from the diseased tissue. *Fusarium sambucinum* has been shown to cause necrosis in larger hop plants and wilting or death of cuttings, but girdling by *F. sambucinum* was never reported in these previous pathogenicity studies. Rhizomes inoculated in glass jars developed girdled bines that were easily severed from the rhizome, but these bines did not swell. Swelling probably did not occur on hop bines growing in jars because plants were small and were grown for only 5 weeks after inoculation. In contrast to commercial fields, plant are can produce more developed, bigger bines and can be diseased over a much longer period of time and crown material on infected plants may survive for years with *F. sambucinum* infections, which could contribute to the swelling. As Fusarium canker develops, a continuum of symptoms can be observed at any time. In commercial hop fields *F. sambucinum* colonizes the lower bines near the soil line, and slight stem swelling occurs at the stem base due to the accumulation of photosynthetic materials as the fungus slowly invades and begins to girdle the bine at the soil line. The girdling, and perhaps due to the mycelial infiltration of vascular elements, can both impede water and mineral transport to the developing bine and sidearms, can result in the bine wilting and possible death of the bine.

The inoculum was probably not the cause for the lack of canker symptoms in my unsuccessful greenhouse and cold frame trials as the same *Fusarium* isolates and conidial/mycelial fragment concentration were used in all trials. Differences between plants incubated in the humidity chamber in a greenhouse and plants incubated in glass jars was the frequency of *Fusarium* inoculation, as well as growing conditions post-inoculation. Plants received the same amount of inoculum per volume of soil, but plants in jars received inoculum twice, one week apart, while plants in other studies were inoculated once with the full equivalent amount of *F. sambucinum*. Plants in the greenhouse humidity chamber were incubated for 8 weeks post-inoculation, three weeks longer than the plants in glass jars so time post-inoculation should not have been a limiting factor, unless the pathogen invaded much more slowly in the greenhouse study. The plants in the greenhouse humidity chamber remained non-cankered probably due to the difference in environmental conditions between the two studies.

Detached stems were inoculated near the middle of the hop stem with GFP-*Fusarium* to observe the process of infection by *F. sambucinum* and to contrast *F. sambucinum*, now shown conclusively to incite canker and cone tip blight of hop, with *F. verticillioides* - not known as a pathogen of hop. Stems were incubated in moist chambers to achieve a constant high humidity, similar to the conditions in sealed glass jars. *Fusarium sambucinum* readily colonized necrotic and a portion of healthy stem surfaces by 10 days after inoculation. Under necrotic stem tissue, where *F. sambucinum* mycelium was dense on the stem surface, *Fusarium* growth

could be observed internally in the xylem and phloem, showing the potential of *Fusarium sambucinum* to invade the vascular system and impede fluid movement.

Colonization by *F. sambucinum* on detached hop stems was more frequent when stems were incubated at increasing higher relative humidities. Although a relatively broad range of colonization incidence was observed when stems were incubated at 94.6% relative humidity, stems incubated at 88% relative humidity had nearly a zero incidence of colonization in all studies, while at least 80% of stems were colonized when relative humidity was greater than 97.7%. *Fusarium sambucinum* appears to colonize detached hop stems much more readily when incubated close to or above 90% relative humidity. Disease is often positively associated with high relative humidity and surface wetness (21). In addition to or in spite of high relative humidity, free water on the surface of bine could be also affect Fusarium canker infection. Experiments have shown that fungal pathogens such as *Marssonina juglandis* which causes Walnut anthracnose, actually cause more disease when relative humidity is near or above 98%, although previously it was thought that infection by *M. juglandis* was optimal at or above 70% relative humidity (6). Additional investigations on relative humidity at and above 90% would give more precise knowledge of the optimal relative humidity range for *F. sambucinum* colonization of hop stems, especially since the highest relative humidity evaluated in this thesis seemed to slightly inhibit colonization.

Recovery of other *Fusarium* species from diseased hop bines has been reported. Fusarium canker research in Yugoslavia and Poland detected a number of

Fusarium species, including *F. oxysporum*, in infected bine tissue from symptomatic fields, but no pathogenicity testing have been reported for other than *F. sambucinum* (40,45). Though not associated with Fusarium canker, *Fusarium verticillioides* and *F. oxysporum* (Appendix C), as shown in studies reported in this thesis, can colonize hop stems but mycelial growth was only evident where the stem had turned necrotic, and occurred at a lower incidence than found with *F. sambucinum*. Twenty days after inoculation, stems in all treatments including the control treatment were mostly necrotic, at which time, *F. verticillioides* was observed beneath the epidermis in the cortex and the pith while *F. oxysporum* was present in the xylem and pith, but mycelium of both species grew along the stem surface and entered the pith from the cut end (Study 6a and Appendix C). Although *F. verticillioides* was observed in the pith and cortex in young hop stems (6b and 6c), and did not appear to grow along the stem surface. But apparently *F. verticillioides* grew from the necrotic surface regions on young stems, escaping detection in the vascular region perhaps by limited hyphal expansion between cells, and once in the pith it proceeded to amass for mycelium. Although a variety of *Fusarium* species may colonize hop stems under certain conditions that may include high relative humidity, *F. oxysporum* and *F. verticillioides* were less aggressive in my studies, and probably do not incite Fusarium canker, but this was not investigated with intact plant in this thesis. Because most *Fusarium* species are general soil inhabitants that can casually colonize decaying tissue, further studies should be carried out to evaluate the pathogenicity of frequently isolated *Fusarium* species, such as *F. oxysporum*.

Fusarium sambucinum was recovered from 60% of the cankered bines sampled from commercial fields studied in this thesis. The remaining organisms were mostly non-*Fusarium* fungi, although a small percentage (less than 5%) were non-identified *Fusarium* spp. *Fusarium* spp. can be masked by other fungi when isolations are made as the amended Nash-Snyder medium used allows other fungi like *Trichoderma* and *Penicillium* to grow and suppress growth of *Fusarium* so that it is macroscopically overlooked and only detected with laborious dilution plating (C. M. Ocamb, personal communication). Two cankered bines developed in non-inoculated glass jars, this was probably due to *F. sambucinum* already being present in the rhizome cuttings. *Fusarium sambucinum* is commonly found in western Oregon soils (C. M. Ocamb, unpublished) and may be a rhizome inhabitant, propagated during vegetative reproduction, and is not readily apparent unless the plant is over-watered or maintained at too high of relative humidity, and perhaps if HpSVd is present.

When Fusarium canker is present in a commercial hop field, canker incidence is typically sporadic and at low levels in a field (C. M. Ocamb, unpublished). Surveys in Oregon of six commercial hop fields suspected of being propagated from HpSVd-infected rootstock revealed an unusually high incidence of Fusarium canker as well as widespread HpSVd symptomology in leaves. On average, fields with wide-spread HpSVd infection had 20 to 62% cankered bines per transect while Field VII on the Botany Field Lab which visually has no HpSVd infection had less than 7% incidence of Fusarium canker. The following year, two

of the commercial hop fields were evaluated again for canker symptoms in the hilling studies, and these two fields still had a high incidence of canker (83 and 84%), suggesting that perhaps HpSVd-infected plants are more susceptible to Fusarium canker. Czech Republic and Great Britain, both currently free of known HpSVd-infection, reported no economic loss from Fusarium canker (32) and that Fusarium canker is "rarely troublesome" (14). Additional surveys should be conducted in Oregon with a larger number of hop cultivars and field sites, including hop plantings that are HpSVd-free to better understand the relationship between HpSVd-infected hop fields and Fusarium canker. Evaluations of canker incidence over sequential years within same fields would be invaluable for tracking canker incidence in fields that are infected with HpSVd. Full expression of HpSVd can take up to five years to develop (15), and it is possible that soilborne disease incidence may increase over the early years of a new planting of HpSVd-infected stock.

HpSVd can reduce the root volume (50), creating a smaller root system which could make the plant more susceptible to water stress or other factors, and perhaps more susceptible to Fusarium canker. Certain winter wheat cultivars infected with *Barley yellow dwarf virus* have an increased susceptibility to Fusarium head blight of wheat (19). Florist's chrysanthemum inoculated with *Chrysanthemum stunt viroid* and symptomless *Chrysanthemum chlorotic mottle viroid*, plants were consistently more susceptible to *Erwinia chrysanthemi* (24). Experiments have shown that certain cultivars of sugar beet, when infected with

Beet mild yellowing virus, are susceptible to infection by *Peronospora farinose* (39) or *Alternaria* spp. (38).

It is unknown whether the presence of HpSVd increases a hop plant's susceptibility to Fusarium canker, but this appears probable, based on the results of the field survey reported in this thesis. Further research should directly test the susceptibility of hop, when infected with HpSVd, to Fusarium canker, and compare HpSVd-infected to HpSVd-free plants in the presence of *F. sambucinum*.

Currently there is no cure for HpSVd infection in hop, cleaning-up infected stock has not been reported for hop. Growers with infected fields are advised to eradicate infected plants from the field, or eventually remove the entire planting and replant with clean root stock (C. M. Ocamb, unpublished). As HpSVd spreads throughout fields on farms with infected new plantings, and if infected rootstock continues to populate the reproductive stream, eradication of all infected fields in a short period of a time is unlikely, and thus management of Fusarium canker could become pertinent to ameliorate symptoms and maintain cone yields, before replanting with HpSVd-free rootstock can occur.

There are currently no specific control measures for Fusarium canker. Lesions associated with Fusarium canker on bines have been significantly reduced when certain demethylation-inhibiting fungicides have been applied prior to herbicide application (C. M. Ocamb, unpublished). Past experiments have investigated chemical sprays for management of Fusarium canker but have been unsuccessful (10,36). Hop plants can be hilled to promote growth for rhizome

propagation, which may help Fusarium-infected plants develop new roots and tolerate girdling. So hop plants were 'hilled' in two fields to investigate hilling as a management strategy. The addition of soil was thought to promote root growth above the point of girdling, and to continue to allow water and mineral uptake. Although hilling of plants did not prevent swelling or girdling near the base of bine, hilled plants grew significantly more roots along the length of the buried bine, and root production was significantly denser above the point of bine girdling in hilled plants. Not only was hilling successful in promoting root growth, hilled plants may produce greater cone weights. The increased presence of roots probably maintained water and mineral transport for continued growth and cone development, while the non-hilled plants were stressed from a lack of water/mineral due to erosion of vascular elements. Further research should be done to determine optimal timing of hilling and soil level among other factors.

Conclusion

Under conditions of controlled high humidity, *Fusarium sambucinum* causes girdling of hop bines. Similarly, detached hop stems are more readily colonized by *F. sambucinum* above 90% relative humidity detached hop stems. Other *Fusarium* species can also colonize detached hop stems but to a lesser extent than *F. sambucinum*. Further research should examine whether other *Fusarium* spp., such as *F. oxysporum,* can cause girdling of hop bines in controlled studies.

A higher incidence of Fusarium canker was detected in field with wide-spread HpSVd-infection, but it has yet to be established that HpSVd is the direct cause for the increased incidence of Fusarium canker in commercial fields. Additional fields which are planted with HpSVd-free and HpSVd-infected rootstock, as well as fields where HpSVd has been introduced should be examined to understand the extent that Fusarium canker can develop. Further research should directly examine whether plants infected with HpSVd are more susceptible to Fusarium canker under controlled, experimental conditions.

If HpSVd continues to spread through-out commercial hop production in the Pacific Northwest, management of Fusarium canker and other soilborne pathogens such as *Verticillium* spp. may become necessary. Hilling of hop plants slightly improved average cone weight through promotion of root development along the buried bine. But further optimization of hilling to alleviate Fusarium canker symptoms should be examined as well as other strategies for management of soilborne pathogens inHpSVd-infected hop plantings.

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Appendices

Appendix A- Effect of temperature on growing HpSVd-infected cuttings

Materials and Methods

A large rhizome from Field III was cut into 6-8 cm length pieces with a single bud on each piece and the pieces were planted as previously stated. Twenty-four potted rhizomes were placed in incubators (model 2015, VWR International, West Chester, PA) at temperatures 18 C and 28 C. Plants were exposed to a 16-hour photoperiod and watered twice each week. Four weeks after planting, the height of each plant was recorded. Five weeks later, plant height and symptoms were recorded. The study was repeated. Plants were arranged in a complete randomized design and analyzed using the GLM procedure in SAS 9.1.

Results

HpSVd-infected hop plants growing in incubators at 18 C were 5.58 cm shorter in height than plants growing at 28 C (Table A1). When the same plants were measured 5 weeks later, the mean heights of plants growing at 18 C was 3.29 cm shorter than that of plants incubated at 28C, but the difference was insignificant. The mean height of plants at nine weeks was greater than mean height of plants at the four weeks, however when the difference between height measurements was recorded by plant, the overall mean difference in height decreased. Plants grown at 28 C were 1.17 cm shorter on average at the second measurement than at the first measurement where plants grown at 18 C averaged 0.79 shorter between the two measurements. When difference in height between the second and the first reading was compared between pairs of plants between incubators, 16 plants still had pairs

and were used in the analysis. The mean of visual HpSVd symptoms in each treatment were not significantly different when analyzed over the studies.

Table A1. Means of heights of hop plants incubated at 18 and 28 C

	18 C[1]		**28 C[1]**	
	Mean[5]	SE[6]	Mean[5]	SE[6]
Height 1[2]	12.25 *	1.98	17.58	1.69
Height 2[3]	14.29	2.22	18.21	1.71
H2-H1[4]	-0.79	0.84	-1.17	0.65

[1]Plants were grown in incubators at 18 and 28 C.
[2]Heights of plants were measured (cm) 4 weeks after planting.
[3]Heights of plants were measured (cm) 9 weeks after planting.
[4]Height 2 –Height 1. Difference in height between first and second measurements.
[5]Means are analyzed over 2 studies with 24 plants in each treatment using LS means in a general linear model.
[6]Standard error from LS means in general linear model.
*indicates that plants grown at 18C were significantly different than grown at the 28C.

Plants incubated at 18 C have a higher percentage of leaves developing epinasty symptoms, and plants grown at 28 C developed more rugose leaves (Table A2). Both treatments had a high incidence of meristem death at the time of the second measurement. Chlorotic speckling or chlorosis of leaves ranged from 19 to 33% in plants at 18 C and 24 to 50% in plants at 28 C between studies.

Table A2. Percentage of 'Sterling' plants developing certain HpSVd symptoms incubated at 18 C and 28 C for 9 weeks

Study	Temp[1]	n[2]	Epinasty[3]	Chlorotic[4]	Rugose[5]	M. death[6]
1	18	16	69	19	31	69
1	28	21	38	24	67	57
2	18	3	67	33	33	0
2	28	14	29	50	50	50

[1]Temperature treatment at which plants were incubated.
[2]The number of plants that survived out of the original 24, and were examined for symptoms.
[3]Curling downwards of leaves.
[4]Lime colored leaves or chlorotic speckling.
[5]Irregular appearance leaf surface.
[6]Death of the meristem between the first and second time of measurement.

Appendix B- Hop stem inoculations using labeled-*F. oxysporum*

Materials and Methods

'Nugget' bines, approximately 2 cm in diameter, were collected from the same hill at the OSU Botany Field Laboratory, and were cut into 8 cm lengths, dipped in 0.05% NaOCl solution, and allowed to air dry. Stems were collected in Septmenber 2009. Plastic moist chambers and welded wire mesh were soaked in 0.05 % NaOCl for ten minutes and rinsed with RO water. Germination paper (Anchor Paper, St. Paul, MN) was sterilized for 30 minutes at 121 C. Six pieces of hop stem were placed in each plastic moist chamber (20.5 x 13 x 5 cm) on top of wire mesh (5 x 5 mm) above two pieces of wet germination paper (Figure 2). Two plastic boxes were used for each treatment.

A small piece of plain or GFP-*Fusarium*-colonized CLA (Table 1) was placed on the stem surface towards the middle of each bine. Stems were sealed in plastic moist chamber and incubated at 25 C with a 16-hour photoperiod provided by

one fluorescent light (General Electric or Sylvania 40W tube). Stems were examined at 10 and 20 days using a Leica MZ FLIII stereo-fluorescence microscope (Leica, Heerburg, Switzerland) with a GFP2 filter. A subset of stems with mycelial growth from each treatment were destructively sampled on 10 days to determine whether there was GFP-Fusarium colonization of the internal stem tissue.

Results

At ten days, *F. oxysporum* transformed with the green fluorescent protein (gFoxy*)* colonized necrotic surface tissues on 39% of stem samples, but was not observed in the internal tissues. When stems were observed 20 days after inoculation, no additional stem samples were colonized. On colonized stems, mycelia had grown to the end of the stem segments, over the cut edge, and into the pith. From the pith, mycelium grew into the xylem. Mycelium was also evident beneath the epidermal cells growing partially into the cortex.

Table B1. Incidence and presence of labeled-*F. oxysporum* in hop stem tissues

	10 days[1]		**20 days**	
Tissue[2]	**gF219**[3]	**WA**[3]	**gF219**	**WA**
Green[4]	0	0	0	0
Necrotic[4]	39	0	39	0
Cortex[5]	-	-	+	-
Pith[5]	-	-	+	-
Xylem[5]	-	-	+	-
Phloem[5]	-	-	-	-

[1]Days after inoculation.
[2]Type of stem tissue examined for gFoxy colonization.
[3]Inoculations with a plug of *F. oxysporum*= gFoxy (gF219)*;* WA=2% water agar.
[4]Percentage of hop stems with of gFoxy growth.
[5] (+) indicates that gFoxy was found in the cortex, pith, xylem, or phloem of at least one hop stem. (-) indicates no gFoxy was observed.

Appendix C- Effect of relative humidity on hop stem colonization by *F. verticillioides*

Materials and Methods

Controlled relative humidity chambers were made by an agar dish isopiestic equilibrium method described by Harris et al. (16). Water agar (2%) was amended with sodium chloride (NaCl) in order to obtain relative humilities ranging from 87.7 to 99.6% (Table C1) (35). Twenty ml of respective NaCl agar was cooled in plastic Petri dishes (100 x 15 mm). Fiberglass window screen (100 mm in diameter) was sterilized in an autoclave for 30 minutes at 121 C. 'Nugget' stems from greenhouse-grown plants were cut to about 5 cm lengths. One end of the stem piece was dipped for one minute in a 10-ml beaker containing 2 ml of GFP-*Fusarium* inoculum (10^5 conidia and hyphal fragments/ml). Inoculated or non-inoculated stem pieces were placed in each Petri dish on a screen above the agar. A second piece of window screen was placed on top of stem pieces followed by a slab of the respective NaCl agar. Twelve Petri dishes (3 plates per treatment), containing five stems each, were wrapped with parafilm and randomly arranged in an incubator at 25 C with a 16-hour photoperiod provided by one fluorescent light (General Electric or Sylvania 40W tubes). After 12 days, stems were evaluated microscopically with for mycelial growth. Stems were examined microscopically using a Leica MZ FLIII stereo-fluorescence microscope with a GFP2 filter. Colonized stems were summed by % relative humidity and were analyzed with a regression model in SAS 9.1.

Table C1. Relative humidity and concentration of NaCl tested in hop stem colonization of *F. verticillioides*

Relative Humidity (%) at 25 C	Concentration of NaCl (molality)
99.6	0.1
97.7	0.8
94.6	1.6
87.7	3.4

Results

When the cut end of detached hop stems were dipped in *F. verticillioides* and incubated in NaCl-agar plate humidity chambers, the proportion of stems colonized by *F. verticillioides* increased as relative humidity increased. Incidence of colonized stems (90%) peaked at 97.7% relative humidity and at 99.8 % relative humidity dropped to 76.5 % incidence (Figure C1). Relative humidity and the percentage of stems infected had a significant correlation with relative humidity (R^2 of 0.6469, P = 0.0161). The mean percentage of infected stems by relative humidity was insignificant among studies, except for stems incubated at 94.6% (Table C2). Stems dipped in water were not colonized with *F. verticillioides*.

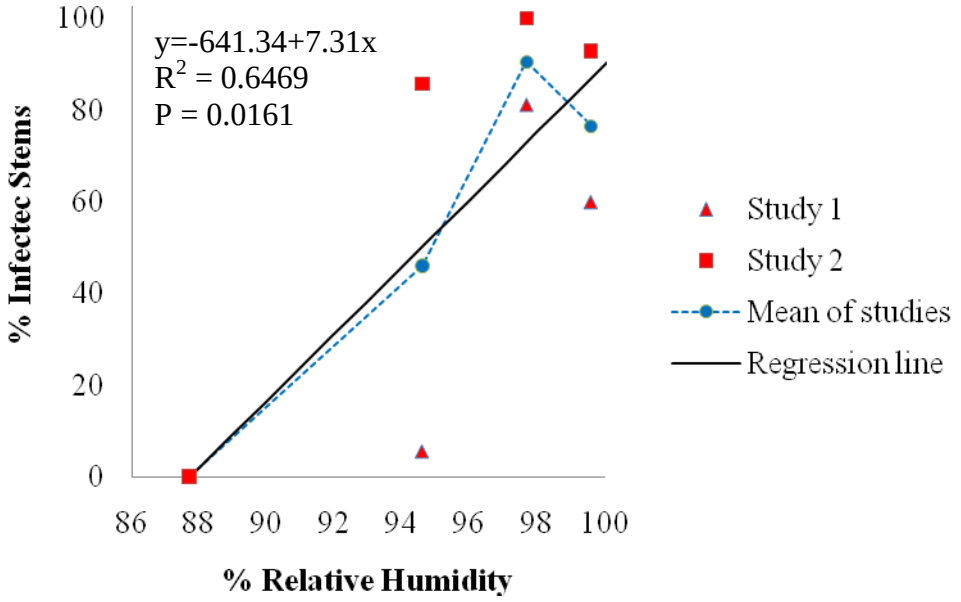

Figure C1. Percentage of stems infected with *F. vertcillioides* different relative humidities after 12 days.

Table C2. Incidence of hop stems colonized with *F. verticillioides* at various relative humidities

RH[1]	Mean[2]
87.7	0
94.6	46
97.7	90.5
99.6	76.5

[1]% relative humidity.
[2]Mean incidence (%) of hop stems colonized.