Identification and resistance to thiophanate-methyl of *Botrytis* species on Kansas greenhouse crops and a specialty crops grower survey to assess extension IPM resource needs

by

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Abstract

Gray mold, caused by the fungus *Botrytis* spp., is a disease that occurs worldwide and infects over 170 plant families, including 200 horticultural commodities such as geraniums, lavender, ornamental peppers, petunias, and other greenhouse commodities. *Botrytis cinerea*, a necrotrophic generalist, is the major cause of gray mold. However, in recent years, other *Botrytis* species have been identified on an array of crops using molecular diagnostic techniques. The objective of this study was to identify *Botrytis* species associated with horticultural crops in Kansas and determine sensitivity to methyl benzimidazole carbamate (MBC) fungicides in Kansas strains. To do this, 80 strains were collected from symptomatic plant parts from greenhouses from 19 sites off 43 different hosts. To determine fungicide sensitivity levels to MBC fungicides, strains were single-spored and tested for sensitivity to 100µg/ml of thiophanate-methyl using fungicide-amended media and control plates. Relative mycelial growth (RMG) was calculated as the average diameter on fungicide-amended media divided by the average diameter on the control × 100. The entire experiment was conducted three times. Of the 80 strains: 63 were highly resistant, RMG ≥ 75%; 9 strains had moderate resistance, RMG ≤50%, but ≥75%; 4 strains had low resistance, RMG between 0 and 50%; and 4 strains were sensitive, RMG=0%. Since the use of morphology has proven to be unreliable for species identification, three nuclear protein-coding genes (RPB2, G3PDH, and HSP60) were used in a phylogenetic analysis that included comparison to known species sequences. 75 strains were identified as *Botrytis cinerea* and 5 strains were inconclusive. Understanding the species of *Botrytis* and fungicide sensitivity levels to different active ingredients in Kansas provides growers with science-based information to improve pre- and post-harvest management practices.

Many fruit and vegetable producers grow a wide range of crops with a diverse range of pest problems. To understand and prioritize research and extension needs, 107 fruit and vegetable growers were surveyed to gather information about farmer backgrounds, farm systems, quantify top pest problems, current practices, and resource needs. Surveys were distributed at 6 conferences and workshops, as well as online. Nearly half (46%) of farms were less than 5 acres and 33% were novice growers, with farms operated for less than 5 years. Half (51%) of growers said they could identify diseases “usually” or “always” as opposed to “never” or “sometimes”, while 48% never use a disease diagnostic lab. Currently, 73%, 59%, and 46% frequently use online materials, conferences/ workshops, and printed resources, but the most preferred resources were conference/ workshops (23%), online written publications (20%), and online videos (18%). Many growers were smaller-scale and less experienced, and they seek information in diverse formats. Both organic and conventional farmers’ main diagnostic challenge was disease identification, yet many growers did not report using the plant disease diagnostic lab, indicating a need for further training and resources. Our results form a baseline to develop and optimize research and extension projects to better serve growers.

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Dedication

I dedicate this thesis to Dr. Dave Appel and Sheila McBride for sharing the world of extension plant pathology and diagnostics. The mentorship I receive from you both is the driving force behind my success.

Chapter 1 - Identification and sensitivity to thiophanate-methyl of Botrytis isolates from Kansas greenhouse crops

Introduction

The fungal genus *Botrytis* was established in 1729 by Mecheli and linked to the genus *Botryotinia*, teleomorph, in 1940s and 1950s (Elad, 2004). *Botrytis* species, causal agents of gray mold and similar diseases, are necrotrophic plant pathogens in the Sclerotiniaceae family and infect both monocotyledon and eudicotyledon hosts that include many horticultural commodities (Fillinger and Elad, 2015). Infections occur on a variety of plant parts including seeds, leaves, stems, petioles, flowers, and fruit, and *Botrytis* is both a pre- and post-harvest problem. This fungus causes infection through several methods including penetration of natural openings (stomata, trichomes, and micro-fissures), latent infections in specific plant structures (styles, sepals, carpels, and flowers), infection of intact host tissue, and colonization of host tissue (Elad, 2004). *Botrytis* species cause plant diseases in the field in diverse climates worldwide ranging from temperate, tropical to subtropical which include all continents except for Antarctica (Farr et al., 2019). Along with field infections, *Botrytis* is a problem in greenhouse production worldwide (Fillinger and Elad, 2015; Moorman and Lease, 1992; Moyano et al., 2003).

Economic Impacts

Botrytis cinerea is the most studied species in the genus. In 2012, *B. cinerea* was listed as the 2nd most important fungal pathogen in molecular plant pathology partly due to its wide host range (Dean et al., 2012). Although it is challenging to determine crop losses and economic impacts that *Botrytis cinerea* causes, Steiger (2007) stated that average cost of controlling *Botrytis* on all crops is around €40/ha. Steiger (2007) also stated that wine and table grapes make

up 50% of the total global market for botryticides, fungicides to control *Botrytis* and other fungi, while specialty crops and ornamentals only make up to 5-9% of the total market.

As of 2004, botryticides had a $15-25 million global market size, showing the large need from growers to control gray mold (Elad, 2004). More specifically, Dutch rose growers experience post-harvest revenue losses around € 1.3 million during annual auction trading (Vrind, 2005), while grape growers in Australia experience profit losses around $ 52 million/year (Scholefield and Morison, 2010).

A horticulture survey conducted in 2006 by the Kansas Department of Agriculture (KDA) found that the nursery/greenhouse industry occupies 4,780 acres in Kansas and provides $157 million dollars of gross sales to the Kansas economy (KDA, 2007). The Netherlands and the United States are the top two floral producers worldwide, followed by China and Japan (Nelson, 2012). Due to the widespread production through the world, greenhouse products, including cut flowers, foliage plants, propagative materials, flowering potted plants and bedding plants, are often shipped, in high humidity, and at temperature averaging 16°C. These conditions create a conducive environment for gray mold infections and contribute to annual losses.

Host Range

A few decades ago it was thought that *Botrytis* species infected over 200 different plant species (Jarvis, 1977), but in recent years over 1,400 host species in 170 different families have been reported. (Fillinger and Elad, 2015). The genus *Botrytis* infects 596 genera of vascular plants with 580 belonging to the Spermatophyta (seed-bearing plants), one in the Lycopodiophyta (spore-bearing plants) and 15 in the Pteridophyta (flowerless plants) (Fillinger and Elad, 2015). Most species within the genus *Botrytis* have a narrow host range, with the exception of *B. cinerea* and *B. pseudocinerea* (Hyde et al., 2014). *B. cinerea* infects a variety of

plants parts including seeds, leaves, petioles, stems, flowers, and fruit (Jarvis, 1977). *B. pseudocinerea* was discovered in 2011 and the host range continues to be studied (Walker et al., 2011). *B. pseudocinerea* has been reported on five unrelated genera: *Rubus/Fragaria* (family Rosaceae), *Solanum* (family Solanaceae), *Vaccinium* (family Ericaceae), *Brassica* (family Brassicaceae), and *Vitis* (family Vitaceae) (Farr, D.F., and Rossman, A.Y., 2019). The U.S. Fungus-Host Database has 1928 reports of *Botrytis* spp. in North America. Of these 1928 reports, 251 are listed as *Botrytis* sp., 277 are listed under a specific *Botrytis* species, and 1400 are *Botrytis cinerea*. Within the 1400 *B. cinerea* reports, there are 319 different host genera and 595 different host species (Farr, D.F., and Rossman, A.Y., 2019).

Epidemiology and Ecology of *Botrytis*

Most of the epidemiological studies related to the genus *Botrytis* have been done with *B. cinerea*. Environmental conditions affect growth and development of *B. cinerea*. Temperature, light, relative humidity, and wetness duration on plant surfaces all play a key role. The optimal growing conditions of *Botrytis* and the ideal temperatures for growing ornamentals and vegetables within greenhouses are often the same, which pose great challenges for controlling *Botrytis* infections (Jarvis, 1992). For example, the optimum temperature for growing geraniums (*Pelargonium* spp.), African violets (*Saintpaulia* spp.), and chrysanthemums (*Chrysanthemum* spp.) are 10°-22°C, 21°-22°C, and 17°-18°C, respectively (Nelson, 2012; White, 1993). The optimum temperature for this fungus is between 18° and 20°C (Fillinger and Elad, 2015). Earlier studies have shown that *Botrytis cinerea* conidia will germinate at temperatures as low as 0°C and as high as 35°C, aligning with a broad range of temperatures for greenhouse production (Brooks and Cooley, 1917; Shiraishi et al., 1970). Optimum temperatures for sclerotia production occur between 11-13°C (can be produced between 2 and 27°C), for sporulation

temperatures between 12-22°C, and for appressorium production temperatures between 27-28°C (Martínez et al., 2009). Kerssies et al. (1995) conducted a study where spore traps were randomly scattered throughout greenhouses of two crops, high- and low-density gerbera daisies (*Gerbera* spp.) and open and high rose (*Rosa* spp.) crops. Under all conditions and in both crops, conidial dispersal was uniform and there were no significant differences between locations and number of flower lesions. The authors concluded that conidial dispersal is rapid regardless of crop density once gray mold is introduced into greenhouse production of gerbera and roses (Kerssies et al., 1995).

Along with temperature and moisture, light also affects fungal growth and development. *B. cinerea* has been shown to have a "two-receptor-model" where near-UV/blue and red/far-red-reversible photoreceptors regulate asexual reproduction (Fillinger and Elad, 2015; Schumacher, 1996). Near-UV (nUV) light has a greater effect on asexual reproduction when combined with white light and increased *B. cinerea* sporulation by 54-fold when compared to white light only (West et al., 2000). Two different types of polyethylene that filter out nUV or far-red regions of the visible spectrum have been studied as a method of controlling *Botrytis* sporulation in greenhouses. A study conducted with polyethylene tunnels revealed that the use of polyethylene sheets that filtered out nUV and blue/ultraviolet light reduced conidial production by 50% (Mueller et al., 2013; Reuven and Raviv, 1992). Elad (1997) concluded that a green-pigmented polyethylene film that filtered out light in the 560-800 nm range also decreased *Botrytis* conidial loads in commercial greenhouses by 35-75%. Although polyethylene filters prove to be a good method for controlling *Botrytis* in organic and conventional greenhouses, Elad (1997) also found that some field isolates were able to sporulate in the dark leading to light filtration being ineffective.

Relative humidity (RH) and leaf wetness are two factors, as well as temperature, that impact conidial germination and disease incidence. In terms of RH for *Botrytis* species, Rippel (1930) found that all conidia germinated in 95% RH and at temperatures at 5°, 15°, and 25°C while 80-85% germinated at 90% RH, but no germination occurred below 85% RH. In the field, *Botrytis cinerea* infections on grape (*Vitis* spp.) berries is favored at 36 hours of wetness duration and is rare at 3 hours of wetness duration (Ciliberti et al., 2015).

Disease Management

Knowledge of the biology and ecology of *Botrytis* spp. has contributed to the development of disease management strategies. For example, understanding the optimal growing temperatures and leaf wetness periods for *Botrytis* is also useful for determining fungicide timing. Bulger et al. (1987) established equations that predicted disease incidence on strawberry (*Fragaria* spp.) flowers and bunches, which MacKensize & Peres (2012) used for a fungicide field trial experiment. Using known average temperature and leaf wetness optima for *Botrytis* to time applications, total fungicide applications could be cut in half without reducing yields (Bulger, 1987; MacKenzie and Peres, 2012). This prediction model is usable by strawberry growers, is web-based, and uses the Florida Automated Weather Network system (MacKenzie and Peres, 2012).

Gray mold is one of the most abundant diseases in greenhouses and integrated management strategies are used for control (Daughtrey, 1995; Elad, 2004; Fillinger and Elad, 2015; Nelson, 2012). Common practices within greenhouses include multiple cultural control strategies to reduce leaf wetness including reduction of relative humidity through proper ventilation (HAF, horizontal airflow fans), proper plant spacing, use of raised mesh or screen bench tops, and drip irrigation (Dik and Wubben, 2007; Nelson, 2012). Another common

cultural practice is sanitation, which includes removing diseased plant material from the growing area, keeping plant debris swept up, and trash cans covered (Dik and Wubben, 2007).

When cultural practices are insufficient, fungicides are used to control *Botrytis*. On average fungicide treatments are applied between one and twenty times throughout the growing season depending on crops grown, field or greenhouse conditions, and weather (Elad, 2004). When fungicides are applied frequently, especially single-site inhibitors, the risk of developing resistance increases. Greenhouse growers use routine fungicide applications as part of gray mold management, and resistance to multiple fungicide classes has been reported (Fan et al.,2014; Kanetis et al., 2017; Saito and Xiao, 2018; Samarakoon et al., 2017). To reduce fungicide resistance, the Fungicide Resistance Action Committee (FRAC) classifies different fungicides based on their active ingredients (a.i.) and Mode of Action (MOA) to assess risks of resistance.

Botryticides, fungicides used to control *Botrytis* and also widely used to control other fungi, include five single-site fungicide groups that target distinct cellular functions including the cytoskeleton (MBC, methyl benzimidazole carbamates), mitochondrial respiration and ATP-synthesis (SDHI, succinate-dehydrogenase inhibitors; QoI, quinone outside inhibitors), ergosterol biosynthesis/cell membrane (DMI-fungicides, demethylation Inhibitors), biosynthesis of proteins or amino acids (AP, anilnopyrimidines), and signal transduction/ osmoregulation (dicarboximides and PP-fungicides, phenylpyrroles) (Elad, 2004; Fillinger and Elad, 2015; Mueller et al., 2013). Fungicide resistance in *Botrytis cinerea* populations is widespread in field and greenhouse conditions and is problematic in both pre- and post-harvest crops. In California, strains of *B. cinerea* from mandarin fruit were resistant to azoxystrobin (QoI), pyrimethanil (AP) and thiabendazole (MBC) in both in vitro and fruit inoculation assays (Saito and Xiao, 2018). In greenhouse crops (cucumber (*Cucumis sativus*), green bean (*Phaseolus vulgaris*), strawberry

(*Fragaria* spp.), eggplant (*Solanum melongena*), and tomato (*Solanum lycopersicum*)), an in vitro study in Cyprus showed widespread resistance in *B. cinerea* strains to thiophanate-methyl (MBC), pyraclostrobin (QoI), boscalid (SDHI), cyprodinil (AP), fenhexamid (DMI), and iprodione (dicarboximides) (Kanetis et al., 2017). Strains of *B. cinerea* recovered from petunias grown in greenhouses in Florida showed in vitro and in vivo resistance to multiple fungicides including boscalid (SDHI), fenhexamid (DMI), fludioxonil (PP), iprodione (dicarboximides), pyraclostrobin (QoI), and thiophanate-methyl (MBC) (Samarakoon et al., 2017).

Alternative control products exist such as plant extracts, biological control, micro-organisms, and mineral oils. Examples include *Melaleuca alternifolia*, tea tree extract; and *Reynoutria sachalinensis*, giant knotweed extract (plant extracts); *Aureobasidium pullulans*, bacterium, *Bacillus subtilis*, actinomycetes, *Streptomyces*, and other fungi such as *Trichoderma* (living microorganisms); and Paraffinnic oil and neem oil (mineral oils and organic acids) (Fillinger and Elad, 2015). These products are commercially available and have different modes of action including antifungal (*Melaleuca alternifolia*), antimicrobial (*Bacillus subtilis*), induced systemic resistance, (ISR) (*Reynoutria sachalinensis* and *Bacillus subtilis*), competition/competitive exclusion (*Aureobasidium pullulans*, *Trichoderma*, and *Streptomyces*), and fungicidal/fungistatic, growth inhibitors, (paraffinic and neem oils (Fillinger and Elad, 2015). You et al. (2016) tested 72 different strains of 11 different species of *Trichoderma* for control of *Botrytis cinerea*. In a tomato (*Solanum lycopersicum*) assay, two strains of *T. koningiopsis* and two strains of *T. harzianum* induced significant systemic resistance against *B. cinerea*. *Bacillus subtilis* (Serenade MAX) reduced *Botrytis* blight on geranium compared to untreated controls similar to levels of suppression by some conventional fungicides (Elmhirst et

al, 2011). As for plant extracts Shao et al. (2013) demonstrated that tea tree oil vapor (0.9 g/L) reduced post-harvest fruit decay in strawberries (Shao, Wang, Xu, & Cheng, 2013).

Identification of *Botrytis* species

The genus *Botrytis* was named after 'botryose', which refers to how macroconidia are arranged on their conidiophores in a shape that resembles a cluster of grapes (Hyde et al., 2014). Botryose is formally defined as "arranged like a cluster of grapes; racemose, racemiform" (Ulloa, 2012). The asexual states of species in this genus produce mycelia, sclerotia, chlamydospores, microconidia, and macroconidia. The sclerotia are irregular shaped black survival structures that are resilient in harsh environments and are between 1 and 10 mm (Whetzel, 1945). All species of *Botrytis* create sclerotia, although they vary in shape and size, while only a few species create chlamydospores (Elad, 2004). Sclerotia are important to the life cycle of *Botrytis* as they can give rise to apothecia after sexual reproduction and produce large amounts of conidia after asexual reproduction (Elad, 2004). In 1939, Groves and Drayton were the first to successfully produce apothecia of the *B. cinerea* type but were uncertain of its identity compared to *Botryotinia fuckeliana*. In 1953 the connection between *B. cinerea* and *B. fuckeliana* was established from single ascospore isolates from apple (*Malus* spp.), potato (*Solanum tuberosum*), celery (*Apium graveolens*), and grape (*Vitis* spp.) (Groves and Loveland, 1953).

The morphology within the genus *Botrytis* makes identification to the species level challenging because of diversity within a single species and overlapping characteristics among different species (Jarvis, 1977). For example, *B. pseudocinerea* and *B. cinerea* do not have significantly different sized conidia, *B. pseudocinerea* with 12.04 ± 1.55 µm and *B. cinerea* with 11.86 ± 1.45 µm (Walker et al., 2011). *B. pseudocinerea* and *B. cinerea* have overlapping

morphological characteristics, but can be distinguished molecularly using three genes: glyceraldehyde 3-phosphate dehydrogenase (G3PDH), heat stock protein 60 (HSP60), and the ATP-dependent RNA helicase DBP7, from the reference gene in *B. cinerea* B0510 genome: BC1G_03202.1 (MS547) (Walker et al., 2011). In that study, the authors estimated that the two species diverged over a million years ago.

As morphology has continued to be challenging for identifying to the species level, the use of DNA-based molecular sequencing, specifically the protein-coding G3PDH, HSP60, and DNA-dependent RNA polymerase subunit II (RPB2) provide more clarity when identifying *Botrytis* species. (Hyde et al., 2014; Staats et al., 2005) RPB2 is the second largest subunit of RNA polymerase II and is a large single-copy gene that has moderate rates of evolutionary changes (Lui et al., 1999). G3PDH has been shown to be a single-copy gene and with a relatively high level of variability therefore making it a good gene for phylogenetic studies (Smith, 1989; Taylor et al., 2000). G3PDH is involved in basic cellular carbohydrate metabolism. HSP60 is one of the heat-shock proteins that are also known as stress proteins. They are highly conserved genes and are cytoprotective proteins involved in protein folding (Mothay and Ramesh, 2019) All the genes used to determine evolutionary divergence within the genus *Botrytis* are single-copy nuclear genes that encode enzymes that are used in basic cellular processes. When RPB2, G3PDH and HSP60 are combined they provide higher resolution to species determination and species divergence (Staats et al., 2005). Similar strategies are used for other fungi the are difficult to distinguish morphologically. For example, species of *Colletotrichum* have similar morphological characteristics, broad host ranges, and minimal resolution in the ITS region (Cannon et al., 2012; Hyde et al., 2014). Therefore, several genes including GPDH (Glyceraldehyde-3-phosphate dehydrogenase), β-tubulin (Beta-tubulin), ApMat

(Intergenic region of apn2) and MAT1-2, GS (glutamine synthetase), HIS3 (Histone3) and ACT (Actin), have been used distinguish among species within the genus *Colletotrichum* (Hyde et al., 2014).

In recent years several new species of *Botrytis* have been discovered using RPB2, G3PDH, and HSP60 sequences including *B. sinoallii*, *B. fabiopsis*, and *B. caroliniana* (Li et al., 2012; J. Zhang et al., 2010; L. Zhang et al., 2010)*B. sinoalli* was identified on green onion, *Allium fistulosum*, and garlic chives, *Allium tuberosum*, and formed a unique lineage that was closely related to *B. squamosa*, a common pathogen of *Allium*, but distantly related to *B. cinerea* (L. Zhang et al., 2010). In contrast, *B. sinoalli* was not found on broad bean (*Vicia faba* L.), pea (*Pisium sativum* L.), oilseed rape (*Brassica napus* L.), or wheat (*Triticum aestivum* L.), indicating that this species of *Botrytis* is not of concern in these cropping systems (L. Zhang et al., 2010). The new species found on broad bean in central China, *B. fabiopsis*, is distantly related to *B. fabae* and *B. cinerea*, the two previously known casual agents of gray mold on broad bean, and closely related to *B. galanthina*, the causal agent of gray mold on *Galanthus* spp. (J. Zhang et al., 2010). In South Carolina, *B. caroliniana* was discovered on blackberry and is also closely related to *B. fabiopsis* and *B. galanthina* (Li et al., 2012). The discovery of *B. caroliniana* has led to more questions about different *Botrytis* species and their host range. While many *Botrytis* species have a narrow host range (Jarvis, 1977), *B. caroliniana* was shown to infect broad bean and blackberries, plants in two different families (Li et al., 2012).

Molecular methods for phylogenetic analysis

Along with identification, molecular methods have been used to relationships among *Botrytis* species and within subgroups of *B. cinerea*. The genus *Botrytis* is monophyletic and has two distinct clades (Andrew et al., 2012; Staats et al., 2005). Holst-Jensen et al. (1998) were the

first to study the genus *Botrytis* in relation to other members of the Sclerotiniaceae using nuclear ribosomal ITS sequencing. They found that there is a lack of variability in the ITS region that made it challenging to determine relationships within the genus. Andrews et al. (2012) used G3PDH, HSP60, and a 500 bp segment of the calmodulin gene (cal) to further determine the phylogeny of the family Sclerotiniaceae, which provided further confirmation of the usefulness of the G3PDH and HSP60 genes.

Staats et al. (2005) studied the evolutionary relationships among species to examine trends among host ranges. To do this, they examined sequences of G3PDH, HSP60 and RPB2 for 22 species and one hybrid (*Botrytis allii*). Two different clades were classified based on host range and a phylogenetic analysis. Clade 1 was found to have four species that infect only eudicot hosts and clade 2 has eighteen species that infect both eudicots, three species of *Botrytis*, or monocots, fifteen species of *Botrytis*. The authors conducted a follow-up study to determine higher evolutionary rates within the genus using two genes encoding phytotoxic necrosis and ethylene-inducing proteins 1 and 2 (NEP1 and NEP2) (Staats et al., 2007). The use of the NEP1 and NEP2 genes as neutral markers has been criticized because they were shown to have evolved under positive selection suggesting that these genes are involved in the infection process (Hyde et al., 2014; Staats et al., 2007).

Research Objectives

As morphology continues to be challenging for species identification in *Botrytis*, the use of molecular identification tools is more accurate for identification to the species level. The goal of the study was to identify what species of *Botrytis* occur in Kansas greenhouses and begin to understand the fungicide resistance profile to improve disease management. The specific research objectives were: 1) determine the species of *Botrytis* occurring on Kansas greenhouse crops; 2) assess the sensitivity of Kansas *Botrytis* isolates to thiophanate-methyl.

Methods

Collection, isolation, and storage of strains

During spring 2018 and 2019, 80 strains of *Botrytis* were collected from plant material from greenhouse crops across 19 sites in Kansas. The Kansas Department of Agriculture and Dan McGinnis (Hummert International) assisted with the collection process. Strains were isolated by scraping conidia off plant material using a needle and plated on 1% Malt Extract Agar (MEA) (Oxoid, United Kingdom) for slower growth, full strength MEA amended with streptomycin and chloramphenicol antibiotics for faster/bacteria-free growth, and water agar for sporulation. Strains were sub-cultured to obtain pure cultures. Once in pure culture, strains were single-spored by transferring spores onto 3% water agar slides (22×50 mm). A single conidium was separated with a micromanipulator under a compound microscope and left to grow overnight. The next day, slides were checked for conidium germination and then transferred to MEA. Once strains were single-spored, they were sub-cultured for storage and DNA extraction.

Eighty strains were stored using two techniques. Filter disks (5 mm punches from filter paper) were autoclaved and placed on MEA culture plates until the strain grew over the disks. Once filter disks were covered, they were removed using sterile forceps and placed in labeled sterile bags and stored at -20°C. Second, cultures were stored in a glycerol-milk solution at -80°C. To make the 7% milk solution, reconstituted dehydrated non-fat milk powder was added to water and autoclaved, being careful not to curdle the milk. Graduated transfer pipettes were used to spread sterile milk solution across the surface of mycelium, scraped to dislodge conidia and mycelium into the milk suspension, and ~1 ml transferred into a 2 ml microcentrifuge tube. One ml of sterile 50% glycerol was added to the same 2 ml microcentrifuge tube. The tubes were mixed through inversion, placed in 4°C overnight, and then moved to -80°C. Two

microcentrifuge storage tubes were made for every strain. Although the filter disk storage method worked, after a year many strains became contaminated. Therefore, the glycerol milk solution storage method was selected for subsequent characterization.

DNA Extraction and Amplification

Mycelia were harvested by adding 0.1 M magnesium chloride to pure *Botrytis* sp. cultures. One and a half ml of the solution was centrifuged and rinsed with sterile water three times. DNA was harvested from cleaned mycelia using the MasterPure Yeast DNA Purification Kit (Lucigen, Madison, Wisconsin) according to the manufacturer's instructions. DNA pellets were dissolved in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Amplification of three protein-coding genes regions, G3PDH, RPB2 and HSP60, was performed using Go Taq Gel Green Master Mix (Promega, Madison, Wisconsin). Reaction mixtures (50 µl) included 50 ng/µl DNA template, Go Taq Gel Green Master mix, and 10 µM forward and reverse primers. Forward and reverse primer sequences from Staats et al. (2005), excluding the M13 forward/reverse primers used to extend these regions for batch sequencing, were used to amplify the desired sequences. PCR was performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Foster City, California) at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s; and 72°C for 10 min for RPB2 and HSP60. PCR was performed on G3PDH under the same conditions except an annealing temperature of 64°C was used instead of 55°C. PCR products (RPB2, HSP60 and G3PDH) were separated on a 1.5% agarose gel at 80 volts for 60 min. Bands were visualized with ethidium bromide under UV light and compared to GeneRuler 100-bp PLUS Ladder (Fisher Scientific, Pittsburgh, Pennsylvania).

After the appropriate product size was confirmed, PCR products were shipped overnight to McLab (San Francisco, CA) for sequencing.

Phylogenetic Analysis

Contigs were constructed from chromatographs of forward and reverse sequences in Geneious 7.1.9. Consensus sequences from G3PDH, HSP60 and RPB2 regions were concatenated for alignment. Sequences from the 80 Kansas strains and reference strains from Staats et al. (2005) (42 strains), Walker et al., (2011) (two strains), Leroch et al. (2013) (1 strain) and Saito et al. (2016) (six strains) (exported from NCBI GenBank and imported into Geneious 7.1.9) were aligned for each gene region and the concatenated sequences (G3PDH, HSP60, and RPB2: sequence order) using the MUSCLE algorithm (Edgar, 2004). Table 1.1 shows the GenBank accession numbers for the chosen reference sequences, where bolded accession numbers are ex-type specimens. Once aligned, sequences were analyzed by visual inspection for errors and manually adjusted.

Table 1.1 Phylogenetic Reference List*

Clade I		**Genes and GenBank Accession Numbers**		
Source	*Botrytis* species	G3PDH	HSP60	RPB2
(Staats et al., 2005)	*B. calthae*	AJ704999	AJ716060	AJ745671
	B. calthae	AJ705000	AJ716061	AJ745672
	B. calthae	AJ705001	AJ716062	AJ745673
	B. cinerea	AJ705002	AJ716063	AJ745674
	B. cinerea	AJ705003	AJ716064	AJ745675
	B. cinerea	**AJ705004**	**AJ716065**	**AJ745676**
	B. cinerea	AJ705005	AJ716066	AJ745677
	B. cinerea	AJ705006	AJ716067	AJ745678
	B. fabae	AJ705013	AJ716074	AJ745685
	B. fabae	**AJ705014**	**AJ716075**	**AJ745686**
	B. pelargonii	AJ705029	AJ716090	AJ745701
	B. pelargonii	**AJ704990**	**AJ716046**	**AJ745662**
Saito et al. (2016)	*B. pseudocinerea*	KJ796651	KJ796655	KJ796647
Saito et al. (2016)	*B. pseudocinerea*	KJ796652	KJ796656	KJ796648
Leroch et al. (2013)	*B. pseudocinerea*	NA	JX266722	NA
Walker et al. (2011)	*B. pseudocinerea*	JF421574	JF421576	NA
Clade II		**GenBank Accession Numbers**		
(Staats et al., 2005)	*B. aclada*	AJ704991	AJ716049	AJ745663
	B. aclada	AJ704992	AJ716050	AJ745664
	B. aclada	AJ704993	AJ716051	AJ745665
	B. allii	AJ704996	AJ716055	AJ745666
	B. allii	AJ704997	AJ716056	AJ745667
	B. allii	AJ704996	AJ716052	AJ745668

	B. allii	AJ704995	AJ716053	AJ745669
	B. byssoidea	AJ704998	AJ716059	AJ745670
	B. convoluta	AJ705007	AJ716068	AJ745679
	B. convoluta	AJ705008	AJ716069	AJ745680
	B. croci	AJ705009	AJ716070	AJ745681
	B. elliptica	AJ705010	AJ716071	AJ745682
	B. elliptica	AJ705011	AJ716072	AJ745683
	B. elliptica	AJ705012	AJ716073	AJ745684
	B. ficariarum	AJ705015	AJ716076	AJ745687
	B. ficariarum	AJ705016	AJ716077	AJ745688
	B. galanthina	AJ705017	AJ716078	AJ745690
	B. galathina	AJ705018	AJ716079	AJ745689
	B. gladiolorum	AJ705019	AJ716080	AJ745691
	B. gladiolorum	AJ705020	AJ716081	AJ745692
	B.	AJ705022	AJ716083	AJ745693
	B. hyacinthi	AJ705023	AJ716084	AJ745695
	B. hyacinthi	AJ705024	AJ716085	AJ745696
	B. narcissicola	AJ705025	AJ716086	AJ745698
	B. narcissicola	AJ705026	AJ716087	AJ745697
	B. paeoniae	AJ705028	AJ716089	AJ745700
	B. paeoniae	AJ705027	AJ716088	AJ745699
	B. polyblastis	AJ705030	AJ716091	AJ745702
	B. polyblastis	AJ705031	AJ716092	AJ745703
	B. porri	AJ705032	AJ716093	AJ745704
	B. porri	AJ705033	AJ716094	AJ745705
	B. ranunculi	AJ705034	AJ716095	AJ7456706
	B. sphaerosperma	AJ705035	AJ716096	AJ745708
	B. sphaerosperma	AJ705036	AJ716097	AJ745709
	B. squamosa	AJ705039	AJ716100	AJ745707

	B. squamosa	AJ705037	AJ716098	AJ745710
	B. squamosa	AJ705038	AJ716099	AJ745711
	B. tulipae	AJ705040	AJ716101	AJ745712
	B. tulipae	AJ705041	AJ716102	AJ745713
	B. tulipae	AJ705042	AJ716103	AJ745714
Outgroups		GenBank Accession Numbers		
(Staats et al., 2005)	*Monilinia fructigena*	AJ705043	AJ716047	AJ745715
	Sclerotinia sclerotiorum	AJ705044	AJ716048	AJ745716

*References, accession numbers (bold: ex-type strain), and *Botrytis* species are the reference strains compiled.

Once aligned, Geneious Tree Builder was used for phylogenetic analysis of the 80 unknown Kansas strains. The phylogenetic analysis was conducted on the three different protein-coding gene regions (HSP60, G3PDH, RPB2) and on the concatenated sequences using *Monolinia fructigena and Sclerotinia sclerotiorum* as the outgroups (Staats et al., 2005). Jukes-Cantor was used as the genetic distance model with neighbor-joining (NJ) as the tree building method. To ensure tree outcomes were accurate, 1000 bootstrap replications were used which denotes branch node as a confident percentage. If a branch node does not have a percentage, that means there is less confidence in that branch.

Fungicide sensitivity in mycelial growth assay

In preliminary tests, five strains were initially screened on amended media with three different concentrations, 0.1 μg/ml, 1 μg/ml, 10 μg/ml, and 100 μg/ml of thiophanate-methyl. All isolates exhibited resistance at every concentration, except for one strain that was unable to grow on media amended with 100 μg/ml of thiophanate-methyl. Therefore, 100 μg/ml of thiophanate-methyl was chosen to screen of all 80 strains. Strains were removed from storage and grown for

7 days on 1% MEA in the dark at 20°C. These strains were screened on 9 cm diameter 1% MEA plates amended with Cleary 3336F (Cleary Chemical, Alsip, Illinois) (active ingredient: thiophanate-methyl). Each strain was transferred onto three fungicide-amended (100 μg/ml of thiophanate-methyl) and three unamended control plates. The plates were incubated for 5 days in the dark at 20° C. Two colony diameters per plate were measured, excluding the 5mm starter plug and recorded. Relative mycelial growth (RMG) was calculated as the average diameter on fungicide-amended media divided by the average diameter on the control × 100. The entire experiment was repeated three times. The RMG ratios were averaged and categorized into one of four categories modified from (Fernández-Ortuño et al., 2014). These included high resistance strains (HR; > 75% RMG), moderate resistance strains (50-75% RMG), low resistance strains (1-50% RMG), and sensitive strains (0% RMG).

Results

Sequencing

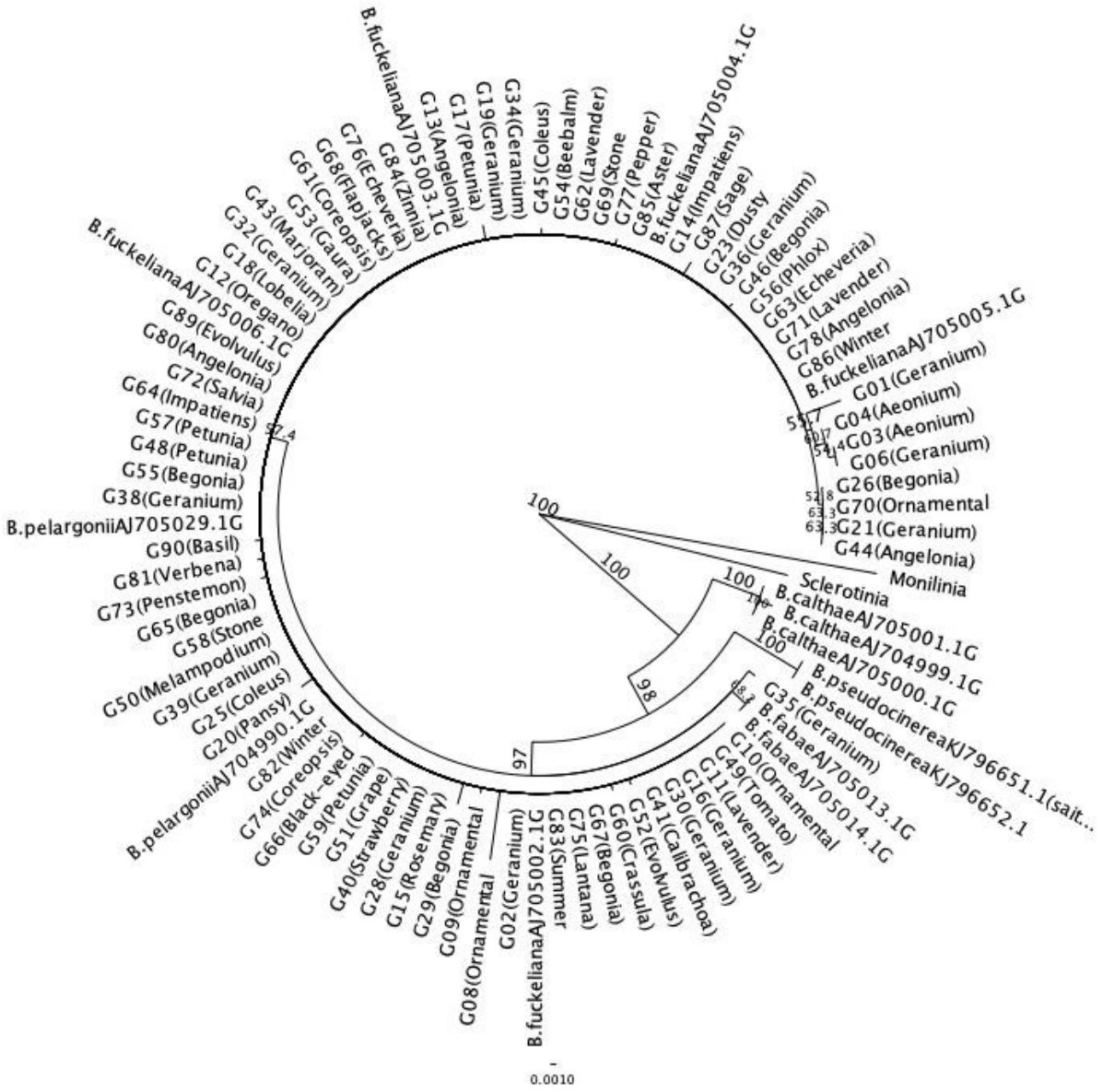
Sequences were obtained for all three gene regions for 78 of the 80 Kansas *Botrytis* strains. Strain B42 had amplification in all three gene regions, but after multiple sequencing attempts sequences in the RPB2 and G3PDH region, the forward and reverse chromatographs did not align and therefore were inconclusive. As for strain B47, amplification was shown in HSP60 and RPB2, but not in G3PDH. Multiple attempts were made to amplify the G3PDH region of strain B47, but they all failed. Strains B42 and B47 were removed from the concatenated phylogenetic analysis due to inconclusive sequences or lack of amplification. Strain B47 was included in phylogenetic analyses of the RPB2 and HSP60 regions and strain B42 was included only in the HSP60 analysis.

Phylogenetic identification

Seventy-eight Kansas strains grouped into clade I for every gene region. Clade 1 includes 5 different species *(B. cinerea*, *B. fabae*, *B. calthae*, *B. pseudocinerea* and *B. pelargonii*) of *Botrytis.* Strains B42 and B47 lacked information for the G3PDH region but did group with clade 1 for the other two regions.

Using the G3PDH region, all Kansas strains, except G35, grouped with *B. cinerea* and *B. pelargonii* (node confidence of 57.4%), while *B. pseudocinerea* (node confidence of 100%), *B. calthae* (node confidence of 100%) and *B. fabae* (68.2%) each had its own distinct branch (Figure 1.1). Strain G35 did not cluster with (node confidence of less than 50%) any known reference strains or any Kansas strains.

Figure 1.1 Botrytis Phylogenetic Tree (G3PDH)

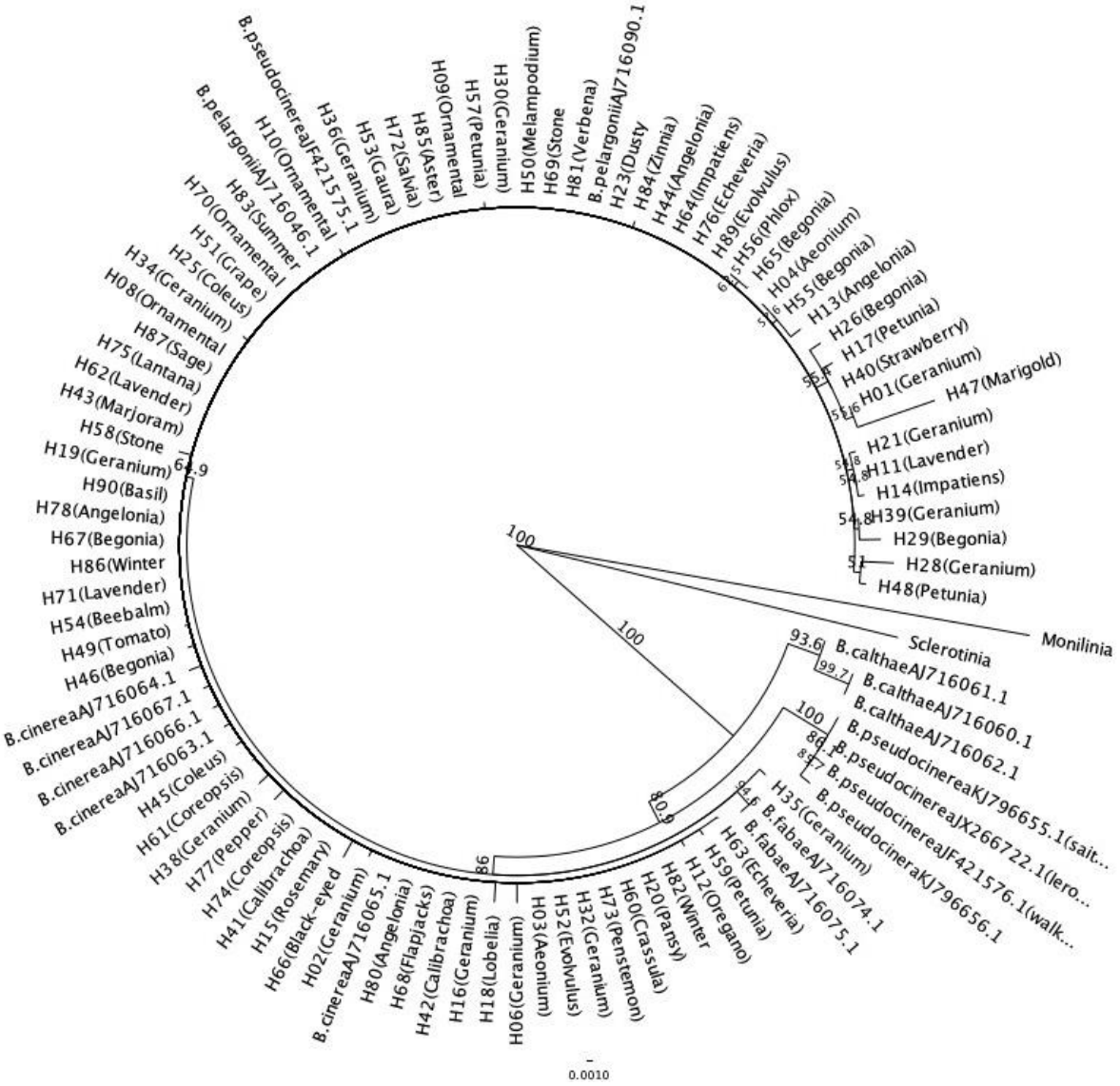


A phylogenetic tree of G3PDH region from 78 Kansas strains. In the figure, Kansas strains are labeled as GXX, where the XX refers to the strain number and the original host is listed in parentheses. For example, G82 indicates strain B82. Strains B42 and B47 are not included in this analysis as strains G42 and G47 did not produce a product and the sequence for strain B42 and B47 was inconclusive. Reference sequences are taken from *Botrytis cinerea*, *Botrytis pelargonii*, *Botrytis pseudocinerea*, *Botrytis calthae* and *Botrytis fabae*; and two outgroups *Monilinia fructigena* and *Sclerotinia sclerotiorum*. Jukes-Cantor was used as the genetic distance model with neighbor-joining (NJ) as the tree building method.

Similarly, for the HSP60 region tree (Figure 1.2), all Kansas strains, except B35 (sequence H35), clustered with *B. cinerea*, *B. pelargonii* (node confidence of 64.9%) strains, and

one *B. pseudocinerea* strain, while other strains of *B. pseudocinerea* (node confidence of 100%) and all *B. calthae* (node confidence of 93.6%) and *B. fabae* (node confidence of 94.6%) reference strains were branched separately from the Kansas strains. Again, strain H35 (less than 50% confidence) did not cluster with any Kansas strains or reference strains.

Figure 1.2 Botrytis Phylogenetic Tree (HSP60)

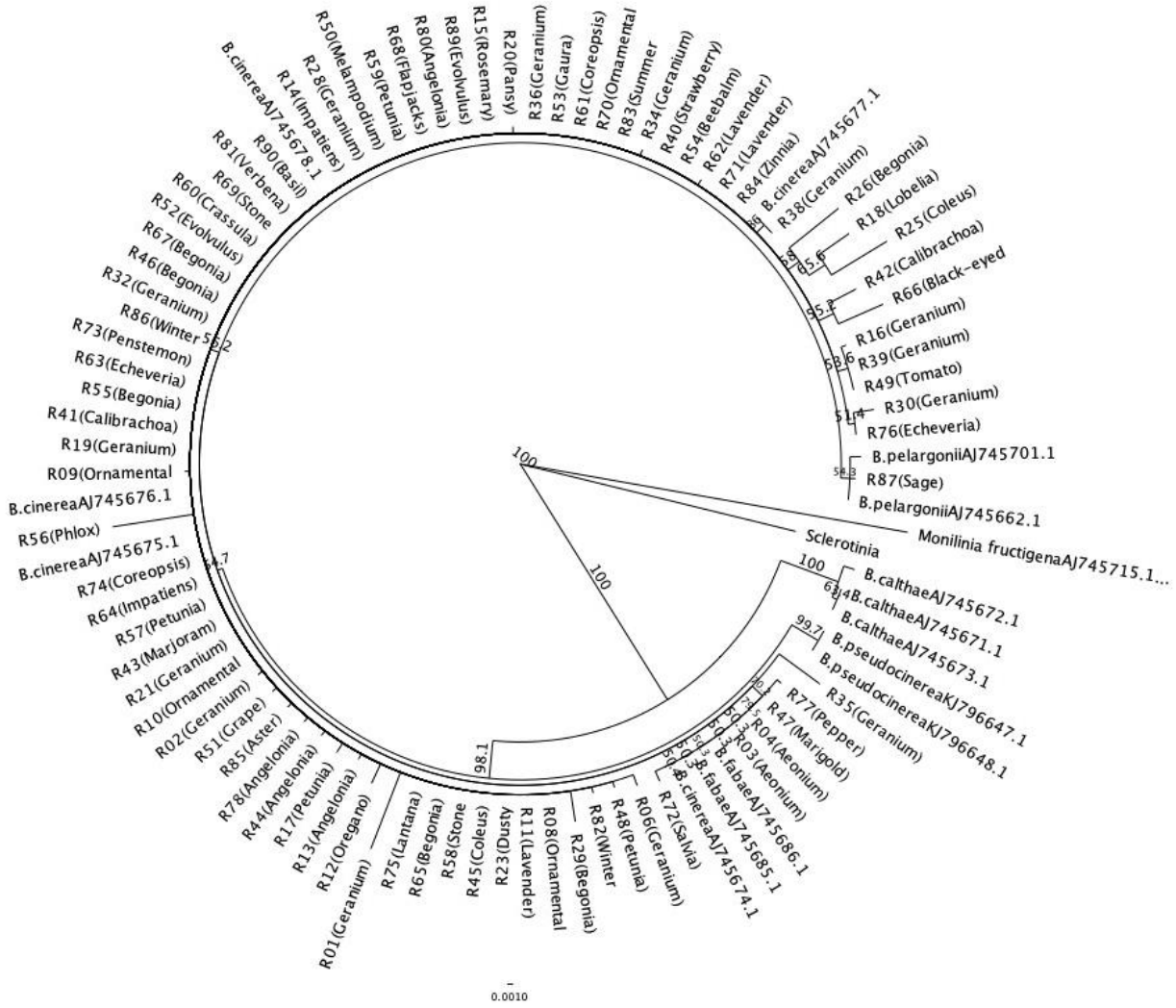


Phylogenetic tree of HSP60 region from 78 Kansas strains. In the figure, Kansas strains are labeled as HXX, where the XX refers to their strain number original host is listed in parentheses. For example, H82 indicates strain B82. Reference sequences are taken from *Botrytis cinerea*, *Botrytis pelargonii*, *Botrytis pseudocinerea*, *Botrytis calthae* and *Botrytis fabae*; and two outgroups *Monilinia fructigena* and *Sclerotinia sclerotiorum*. Jukes-Cantor was used as the genetic distance model with neighbor-joining (NJ) as the tree building method.

The phylogenetic analysis of the RPB2 region tree (Figure 1.3) placed the majority of Kansas strains with *B. cinerea* (node confidence of 55.2%). Strain B35 (sequence R35) did not cluster with any Kansas strains or reference strains. As for Kansas strains B77, B03, B04, B47, B72 (sequences R77, R03, R04, R47, R72), they clustered with *B. fabae* (node confidence of

70.2%). The two known *B. pelargonii* strains and B87 (node confidence of 54.3%) strain clustered together.

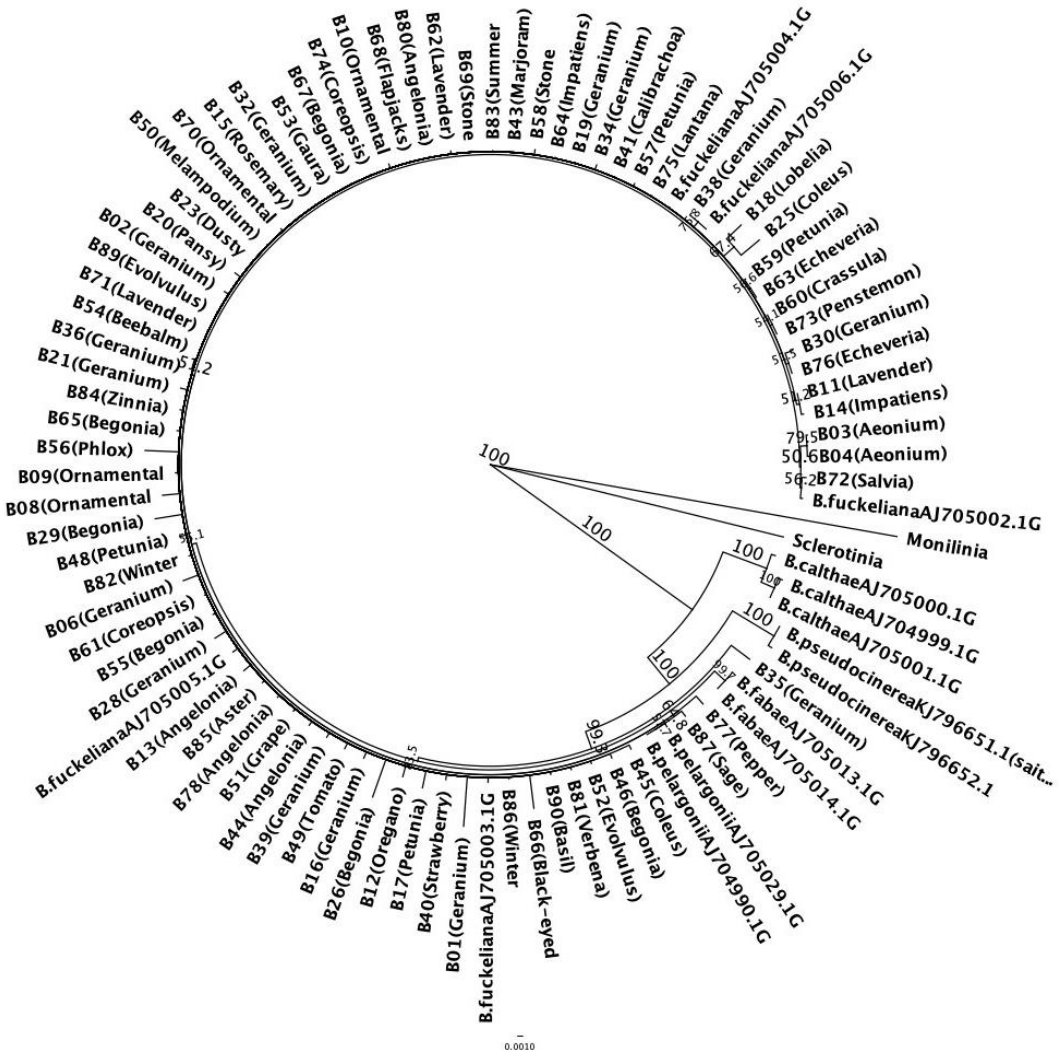
Figure 1.3 Botrytis Phylogenetic Tree (RPB2)



Phylogenetic tree of RPB2 region from 78 Kansas strains. In the figure, Kansas strains are labeled as RXX, where the XX refers to their strain number and the original host is listed inside the parentheses. For example, H82 indicates strain B82. Reference sequences are taken from *Botrytis cinerea*, *Botrytis pelargonii*, *Botrytis pseudocinerea*, *Botrytis calthae* and *Botrytis fabae*; and two outgroups *Monilinia fructigena* and *Sclerotinia sclerotiorum*. Jukes-Cantor was used as the genetic distance model with neighbor-joining (NJ) as the tree building method.

As for the concatenated phylogenetic tree, G3DPH, HSP60, and RPB2 (Figure 1.4), all but three Kansas strains clustered with all *B. cinerea* (node confidence of 55.1%) reference strains. Strain B87 grouped with both *B. pelargonii* (node confidence of 64.8%) reference strains, similarly to the RPB2 region analysis. B35 and B77 (less than 50% node confidence) both did not group with any other Kansas strain or any of the reference strains.

Figure 1.4 Botrytis Phylogenetic Tree (Concatenated)



Phylogenetic tree of G3PDH, HSP60, and RPB2 regions concatenated from 78 Kansas strains. In the figure, Kansas strains are labeled as BXX, where the XX refers to their strain number and the original host is listed inside the parentheses. Strains B42 and B47 are not included in this analysis as strains G42 and G47 did not produce a product and the sequence for strain B42 and B47 was inconclusive. Reference sequences are taken from *Botrytis cinerea, Botrytis pelargonii*, *Botrytis pseudocinerea*, *Botrytis calthae* and *Botrytis fabae*; and two outgroups *Monilinia fructigena* and *Sclerotinia sclerotiorum*. Jukes-Cantor was used as the genetic distance model with neighbor-joining (NJ) as the tree building method.

Fungicide sensitivity

There were 62 strains (78% of the total) that showed high resistance to thiophanate-methyl with an average of 90% RMG, nine strains (11% of the total) had moderate resistance with an average of 66% RMG, four strains (5% of the total) had low resistance with an average of 1% RMG, and four strains (5% of the total) that were sensitive with no growth or 0% RMG. A summary table (Table 1.2) of the results is shown below. Table 1.3 displays the average RMG for each isolate.

All strains grew consistently except for strain B44. Growth from storage of strain B44 was normal and covered the petri dish, but when sub-cultured this strain did not grow consistently or at all on the control and amended plates. This was true across all three replications and therefore this strain was not included in any RMG categories.

Table 1.2 Summary of Fungicide Sensitivity and Species Identification Results*

Kansas County	Number of Strains Collected	Number of Hosts	RMG Category Average %RMG, and number of strains in the category				Species Found
			Sensitive	Low	Moderate	High	
Brown	2	1	-	-	-	89% (2)	*B. cinerea* & *B. fabae* or *B. pelargonii*
Butler	1	1	0% (1)	-	-	-	*B. fabae* or *B. pelargonii*
Coffey	1	1	-	-	-	88% (1)	*B. cinerea*
Crawford	1	1	-	-	-	95% (1)	*B. cinerea*
Harper	1	1	-	-	-	81% (1)	*B. cinerea*
Johnson	2	1	-	-	-	92% (2)	*B. cinerea*
Leavenworth	2	1	-	2% (1)	69% (1)	-	*B. cinerea* & *B. fabae* or *B. pelargonii*
Montgomery 1	1	1	-	-	-	90% (1)	*B. cinerea*
Montgomery 2	2	2	-	-	-	90% (2)	*B. cinerea*
Morris	11	9	-	-	71% (2)	90% (9)	*B. cinerea*
Nemaha	3	3	0% (1)	-	-	87% (1)	*B. cinerea*
Pottawatomie 1	1	1	-	-	-	100% (1)	*B. cinerea*
Pottawatomie 2	9	9	0% (1)	3% (1)	54% (1)	89% (6)	*B. cinerea* & *B. pelargonii*
Reno 1	2	1	-	-	-	85% (2)	*B. cinerea*
Reno 2	2	1	-	-	-	91% (2)	*B. cinerea*
Riley 1	36	23	0% (1)	1% (1)	64% (4)	91% (30)	*B. cinerea*
Riley 2	2	2	-	1% (1)	73% (1)	-	*B. cinerea* & *B. fabae* or *B. pelargonii*
Rooks	1	1	-	-	-	82% (1)	*B. cinerea*

*Kansas strain collection location shown with number of strains and hosts from each location, resistance level to thiophanate-methyl, and species identification. Relative mycelial growth (RMG) was calculated as the average diameter on fungicide-amended media divided by the average diameter on the control × 100. These included high resistance strains (HR; > 75% RMG), moderate resistance strains (50-75% RMG), low resistance strains (1-50% RMG), and sensitive strains (0% RMG). The % in each category indicates the average %RMG for all the strains in that group. The number in parentheses indicates the number of strains. Most species (76 strains) were identified by using neighbor-joining phylogenetic analysis from concatenated sequences of the G3PDH, HSP60, and RPB2 regions. The other 4 species determinations were made based on NCBI BLAST matches against ex-type specimens.

Table 1.3 Kansas *Botrytis* species Identification and Fungicide Resistance for Each Strain*

Strain	Kansas County	Species	Relative Mycelial Growth (RMG)				Level of Resistance	Host
			Trial 1	Trial 2	Trial 3	Average		
B01	Riley 1	*B. cinerea*	131%	83%	106%	107%	High	Rocky Mountain Red Zonal Geranium (*Pelargonium zonale*)
B02		*B. cinerea*	76%	77%	74%	75%	High	Rocky Mountain Red Zonal Geranium (*Pelargonium zonale*)
B03		*B. cinerea*	90%	74%	76%	80%	High	Aeonium 'kiwi' (*Aeonium haworthii*)
B04		*B. cinerea*	92%	93%	79%	88%	High	Aeonium 'kiwi' (*Aeonium haworthii*)
B06		*B. cinerea*	104%	79%	90%	91%	High	Classic Salmon Geranium (*Pelargonium* sp.)
B08		*B. cinerea*	111%	69%	77%	86%	High	Ornamental Pepper 'Sedona sun' (*Capsicum annuum*)
B09		*B. cinerea*	80%	78%	87%	82%	High	Ornamental Pepper 'Sedona sun' (*Capsicum annuum*)
B10		*B. cinerea*	117%	64%	90%	90%	High	Ornamental Pepper 'Sedona sun' (*Capsicum annuum*)
B11		*B. cinerea*	81%	74%	76%	77%	High	Lavender 'Munstead' (*Lavandula angustifolia*)
B12		*B. cinerea*	72%	91%	82%	82%	High	Greek Mountain Oregano (*Origanum vulgare*)
B13		*B. cinerea*	101%	111%	97%	103%	High	Angelonia white (*Angelonia angustifolia*)

B14		*B. cinerea*	68%	84%	66%	73%	Moderate	Impatiens (*Impatiens walleriana*)
B15	Riley 1	*B. cinerea*	65%	39%	55%	53%	Moderate	Rosemary (*Rosmarinus officinalis*)
B16	Montgomery 1	*B. cinerea*	106%	82%	82%	90%	High	Calliope Geranium large salmon (*Pelargonium* sp.)
B17	Montgomery 2	*B. cinerea*	109%	78%	91%	93%	High	Petunia Glow Cappuccino (*Petunia* sp.)
B18		*B. cinerea*	100%	74%	88%	87%	High	Lobelia techno heat dark blue (*Lobelia erinus*)
B19	Crawford	*B. cinerea*	99%	97%	90%	95%	High	Geranium True Red (*Pelargonium* sp.)
B20	Morris	*B. cinerea*	90%	75%	73%	79%	High	Pansy (*Viola tricolor*)
B21		*B. cinerea*	84%	82%	86%	84%	High	Geranium survivor neon violet (*Pelargonium* sp.)
B23		*B. cinerea*	105%	82%	72%	86%	High	Dusty Miller (*Jacobaea maritima*)
B25		*B. cinerea*	136%	74%	79%	96%	High	Coleus 'Sunset boulevard' (*Plectranthus scutellarioides*)
B26		*B. cinerea*	88%	87%	85%	87%	High	Rieger Begonia 'Amstel Batik' (*Begonia* sp.)
B28		*B. cinerea*	83%	79%	86%	83%	High	Geranium (*Pelargonium* sp.)
B29		*B. cinerea*	128%	101%	78%	102%	High	Rieger Begonia 'Amstel Blitz' (*Begonia* sp.)
B30	Reno 1	*B. cinerea*	91%	83%	87%	87%	High	Geranium Americana Rose Mega Splash (*Pelargonium zonale*)
B32		*B. cinerea*	90%	87%	69%	82%	High	Geranium Rocky Mountain Dark Red (*Pelargonium* sp.)

B34	Leavenworth	*B. cinerea*	75%	72%	60%	69%	Moderate	Geranium (*Pelargonium* sp.)
B35		*B. fabae/ B. pelargonii**	1%	2%	2%	2%	Low	Geranium (Pelargonium hirsutum)
B36	Harper	*B. cinerea*	88%	87%	68%	81%	High	Dynamo Geranium Hot Pink (*Pelargonium* sp.)
B38	Reno 2	*B. cinerea*	104%	65%	86%	85%	High	Geranium (*Pelargonium zonale*)
B39		*B. cinerea*	121%	77%	94%	97%	High	Geranium (*Pelargonium zonale*)
B40	Rooks	*B. cinerea*	88%	89%	69%	82%	High	Strawberry (*Fragaria ananassa*)
B41	Brown	*B. cinerea*	99%	69%	95%	88%	High	Calibrachoa ‘aloha nani red cartwheel’ (*Calibrachoa parviflora*)
B42		*B. fabae/ B. pelargonii**	84%	87%	99%	90%	High	Bloomtastic rose quartz' Calibrachoa (*Calibrachoa parviflora*)
B43	Nemaha	*B. cinerea*	91%	83%	87%	87%	High	Compacta' Marjoram (*Origanum majorana*)
B44		*B. cinerea*	-	-	-	-	-	Angelonia ’Angelface white' (*Angelonia angustifolia*)
B45		*B. cinerea*	0%	0%	0%	0%	Sensitive	Coleus (*Plectranthus scutellarioides*)
B46	Coffey	*B. cinerea*	0%	192%	71%	87%	High	Hielmlis Begonia Eva (*Begonia* sp.)
B47	Riley 2	*B. fabae/ B. pelargonii**	1%	0%	1%	1%	Low	Marigold (*Tagetes* sp.)
B48		*B. cinerea*	79%	75%	65%	73%	Moderate	Petunia (*Petunia* sp.)

B49	Riley 1	*B. cinerea*	86%	87%	89%	100%	High	Tomato ‘jetsetter’ (*Solanum lycopersicum*)
B50	Morris	*B. cinerea*	68%	79%	72%	73%	Moderate	Melampodium (*Melampodium* sp.)
B51	Pottawatomie 1	*B. cinerea*	120%	80%	100%	98%	High	Grape ‘Vignoles’ (*Vitus vinifera*)
B52	Riley 1	*B. cinerea*	108%	91%	95%	78%	High	Evolvulus 'blue my mind' (*Evolvulus* sp.)
B53		*B. cinerea*	74%	78%	81%	89%	High	Gaura 'pink foundation' (*Gaura lindheimeri*)
B54		*B. cinerea*	100%	92%	76%	113%	High	Bee balm ' pink lace' (*Monarda didyma*)
B55		*B. cinerea*	147%	129%	62%	82%	High	Begonia 'upright fire' (*Begonia* sp.)
B56		*B. cinerea*	88%	79%	80%	88%	High	Creeping Phlox 'white delight' (*Phlox stolonifera*)
B57		*B. cinerea*	65%	77%	68%	70%	Moderate	Petunia (*Petunia* sp.)
B58		*B. cinerea*	88%	83%	94%	92%	High	Stonecrop ' class act' (*Sedum telephium*)
B59		*B. cinerea*	108%	65%	102%	93%	High	Petunia 'plus pinkalicious' (*Petunia* sp.)
B60		*B. cinerea*	102%	88%	89%	123%	High	Crassula ' princess pine' (*Crassula ovata*)
B61		*B. cinerea*	142%	123%	104%	88%	High	Coreopsis ' leading lady sophia' (*Coreopsis lanceolata*)
B62		*B. cinerea*	113%	66%	84%	106%	High	Lavender 'phenomenal' (*Lavandula intermedia*)

B63		*B. cinerea*	1%	0%	1%	1%	Low	Echeveria (*Echeveria* sp.)
B64		*B. cinerea*	136%	82%	100%	75%	High	Impatiens 'rose aurora' (*Impatiens walleriana*)
B65	Riley 1	*B. cinerea*	0%	0%	0%	0%	Sensitive	Begonia (*Begonia* sp.)
B66		*B. cinerea*	112%	58%	54%	103%	High	Black-eyed Susan ' indian summer' (*Rudbeckia hirta*)
B67		*B. cinerea*	46%	71%	62%	60%	Moderate	Begonia (*Begonia* sp.)
B68		*B. cinerea*	124%	101%	82%	93%	High	Flapjacks (*Kalanchoe thyrsiflora*)
B69		*B. cinerea*	119%	94%	67%	107%	High	Stonecrop ' dark magic' (*Sedum telephium*)
B70		*B. cinerea*	113%	141%	66%	110%	High	Ornamental pepper 'midnight fire' (*Capsicum annuum*)
B71		*B. cinerea*	156%	101%	73%	78%	High	Lavender (Darwin) ' otto quast' (*Lavandula stoechas*)
B72		*B. cinerea*	81%	83%	69%	82%	High	Salvia 'lyrical blues' (*Salvia nemorosa*)
B73		*B. cinerea*	88%	79%	78%	82%	High	Penstemon 'rocky mountain' (*Penstemon strictus*)
B74	Morris	*B. cinerea*	95%	64%	89%	108%	High	Coreopsis ' double the sky' (*Coreopsis lanceolata*)
B75		*B. cinerea*	173%	64%	89%	84%	High	Lantana 'havanna red sky' (*Lantana camara*)
B76		*B. cinerea*	79%	73%	57%	70%	Moderate	Echeveria (*Echeveria* sp.)

B77	Butler	*B. fabae/ B. pelargonii**	0%	0%	0%	0%	Sensitive	Pepper (*Capsicum annuum*)
B78	Johnson	*B. cinerea*	96%	85%	69%	101%	High	Angelonia ' archangel dark purple' (*Angelonia angustifolia*)
B80		*B. cinerea*	115%	87%	101%	121%	High	Angelonia 'archangel blue bicolor' (*Angelonia angustifolia*)
B81	Pottawatomie 2	*B. cinerea*	140%	114%	110%	76%	High	Verbena 'lascar white' (*Verbena* sp.)
B82		*B. cinerea*	0%	0%	0%	0%	Sensitive	Winter Squash (*Cucurbita maxima*)
B83		*B. cinerea*	65%	87%	75%	78%	High	Summer Squash ' gold crookneck' (*Cucurbita pepo*)
B84		*B. cinerea*	85%	77%	72%	84%	High	Zinnia 'Zahara yellow' (*Zinnia elegans*)
B85		*B. cinerea*	1%	3%	4%	3%	Low	Aster (*Aster dumosus*)
B86		*B. cinerea*	90%	73%	88%	89%	High	Winter Savory (*Satureja montana*)
B87		*B. pelargonii*	100%	81%	87%	89%	High	Sage (*Salvia officinalis*)
B89		*B. cinerea*	63%	43%	56%	54%	Moderate	Evolvulus (*Evolvulus* sp.)
B90		*B. cinerea*	118%	72%	76%	77%	High	Basil ' Italian large leaf' (*Ocimum basilicum*)

*****Kansas strains shown with Kansas county location, species identification, resistance level to thiophanate-methyl, and host. Most species (76 strains) were determined by using neighbor-joining phylogenetic analysis from concatenated sequences of the G3PDH, HSP60, and RPB2 regions. *B35, B42, B47, and B77 species determinations were determined based on NCBI BLAST matches against ex-type specimens. Relative mycelial growth (RMG) was calculated as the average diameter on fungicide-amended media divided by the average diameter on the control × 100. These included high resistance strains (HR; < 75% RMG), moderate resistance strains (50-75% RMG), low resistance strains (1-50% RMG), and sensitive strains (0% RMG). Strain B44 did not produce consistent growth and therefore was removed from the fungicide sensitivity analysis.

Discussion and future work

Identification

Out of eighty strains, seventy-five strains were confirmed as *Botrytis cinerea*, one strain (B87) clustered with *Botrytis pelargonii* type specimens, and the other four (B35, B42, B47, and B77) were inconclusive between *Botrytis pelargonii* and *Botrytis fabae*. Although the *Botrytis pelargonii* reference sequences did show separation from *B. cinerea* for the concatenated sequences in our study, other studies have not consistently separated *B. pelargonii* from *B. cinerea* (Fillinger and Elad, 2015; Staats et al., 2005; Staats et al., 2007; Walker et al., 2011). *B. pelargonii* was originally described on geranium species (Røed, 1949) (*Pelargonium* spp.), but has been recently reported on ginseng root (*Panax ginseng)* (Lu et al., 2019). Our strain B87 was isolated from sage (*Salvia officinalis*). Therefore, additional work is needed to clarify the identity of strain B87.

In terms of conidia size, B35, B77, and B87 all had slightly smaller average (n=20) conidia, 8.1 µm, 8.4 µm, and 8.9 µm respectively compared to a subset (7 other KS strains) of the Kansas strains, which ranged from 9.1µm to 10.8 µm. All three region sequences from strains B35 and B77 were analyzed individually through National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) in comparison to sequence information from ex-type specimens. Interestingly, both strains aligned with the same two species, *B. pelargonii* and *B. fabae*, and the same 2 specimens for all three gene regions (Table 1.4). Strains B35 and B77 also both showed some level of sensitivity to thiophanate-methyl (low resistance and sensitive, respectively). For strains B42 and B47, similar results were found where the HSP60 and RPB2 regions sequences aligned with the same 2 specimens as B35 and B77. Sequences from the NEP1 and NEP2 gene regions and other genes that distinguish *B. cinerea*

from *B. pseudocinerea*, fungicide sensitivity trials with different active ingredients are needed to further understand and analyze the five strains that show inconclusive results.

Table 1.4 Accession Numbers of BLAST matches*

			NCBI BLAST Matches				
Strain	Host	Gene Region	Species Identified	Percent Identity	Base Pair Ratio	Accession Number	Reference Strain
B35	Geranium (*Pelargonium hirsutum*)	G3PDH	*B. pelargonii*	99.66%	883/886	AJ704990	CBS 497.50
			B. fabae	99.32%	880/886	AJ705014	MUCL98
		HSP60	*B. pelargonii*	99.69%	950/953	AJ716046	CBS 497.50
			B. fabae	99.48%	948/953	AJ716075	MUCL98
		RPB2	*B. pelargonii*	99.09%	1083/1093	AJ745662	CBS 497.50
			B. fabae	98.99%	1082/1093	AJ745686	MUCL98
B77	Pepper (*Capsicum annuum*)	G3PDH	*B. pelargonii*	99.89%	885/886	AJ704990	CBS 497.50
			B. fabae	99.32%	880/886	AJ705014	MUCL98
		HSP60	*B. pelargonii*	99.80%	974/976	AJ716046	CBS 497.50
			B. fabae	99.39%	970/976	AJ716075	MUCL98
		RPB2	*B. fabae*	99.91%	1092/1093	AJ745686	MUCL98
			B. pelargonii	99.63%	1089/1093	AJ745662	CBS 497.50
B42	Calibrachoa ‘Bloomtastic rose quartz' (*Calibrachoa parviflora*)	HSP60	*B. pelargonii*	100%	976/976	AJ716046	CBS 497.50
			B. fabae	99.59%	972/976	AJ716075	MUCL98
		RPB2	*B. fabae*	98.99%	1082/1093	AJ745686	MUCL98
			B. pelargonii	98.90%	1081/1093	AJ745662	CBS 497.50
B47	Marigold (*Tagetes* sp.)	HSP60	*B. pelargonii*	98.36%	960/976	AJ716046	CBS 497.50
			B. fabae	97.95%	956/976	AJ716075	MUCL98
		RPB2	*B. fabae*	100%	1093/1093	AJ745686	MUCL98
			B. pelargonii	99.73%	1090/1093	AJ745662	CBS 497.50

*****Four Kansas strains matches to two ex-type specimens, CBS 497.50 and MUCL98, in NCBI BLAST database for three gene regions G3PDH, HSP60 and RPB2. Percent identity was calculated based on the base pair ratio.

The goal of this study was to identify the strains to the species level and not progress into population structure within any species. However, this is an area for future research that might

help to confirm the identification of the five inconclusive strains. Understanding the diversity within and between *B. cinerea* species complex has been challenging. The presence or absence of two transposable elements *Boty* (Diolez et al., 1995) and *Flipper* (Levis et al., 1997) have been used in the past to categorize *B. cinerea* strains into two subgroups (Group I: *vacuma* (neither transposon) and Group II: *Boty* only, *Flipper* only, and *transposa* (both transposons). However, the description of *B. pseudocinerea* complicated the story by identifying the *Flipper* element in group I and therefore makes the use of transposable elements obsolete (Fillinger and Elad, 2015; Kecskeméti et al., 2014; Walker et al., 2011) Currently, the vegetative incompatibility locus *Bc-hch*, developed from the vegetative incompatibility loci of *Neurospora crassa* (Nc-het-c) and *Podospora anserina* (Pa-hch), helps define group I as *B. pseudocinerea*, which is naturally resistant to the hydroxyamide fungicide fenhexamid and group II as *B. cinerea* (Fournier et al., 2003; Walker et al., 2011). Other diagnostic sequence polymorphisms include nine microsatellites (Fournier et al., 2002), a sterol 14-α demethylase gene, cyp51(Albertini et al., 2002), a 3-keto reductase gene (Albertini and Leroux, 2004) , G3PDH (Walker et al., 2011), and HSP60 (Walker et al., 2011), and MS547 (Walker et al., 2011).

Fungicide Sensitivity

In previous studies, strains of *B. cinerea* that were resistant to methyl benzimidazole carbamate (MBC) fungicides have shown mutations in the β-tubulin gene (Baggio et al., 2018; Leroux et al., 2002; Rupp et al., 2017; Yarden and Katan, 1993). Specifically, the amino acid at position 198 can change from glutamic acid to alanine, glycine, lysine, or valine, or at position 200 researchers have observed a change from phenylalanine to tyrosine (Baggio et al., 2018; Banno et al., 2008; Leroux et al., 2002; Yarden and Katan, 1993). Similar mutations at amino

acid positions 198 and 200 have been reported in other filamentous fungi such as *Aspergillus nidulans* (May et al., 1987), *Collectotrichum graminicola*, and *Colletotrichum gloeosporioides* (Buhr and Dickman, 1994). In future work, our strains can be characterized to determine which mutation(s), E198A, E198G, E198K, E198V, or F200Y, is/are present. Strains highly resistant to benzimidazole fungicides have mutations in codon 198, while moderately resistant strains have mutations in codon 200. Negative cross-resistance has been found for benzimidazoles and N-phenylcarbamates, a fungicide group that disrupts β-tubulin assembly in mitosis (diethofencarb and zoxamide) (https://www.frac.info/expert-fora/benzimidazoles/soa-and-mechanism(s)-of-resistance). This means that strains with the E198A mutation are resistant to benzimidazoles and are then sensitive to diethofencarb and zoxamide. In contrast, the F200Y mutation corresponds to resistance to both diethofencarb and benzimidazole fungicides (Banno et al., 2008; Leroux et al., 2002; Yarden and Katan, 1993). In terms of fitness costs, strains resistant to six or seven different chemical classes grow more slowly and are hypersensitive to osmotic stress compared to sensitive strains (Chen et al., 2016). Although there are some fitness costs, these resistant and sensitive strains do not differ when it comes to oxidative sensitivity, aggressiveness and in vivo spore production and sclerotia production and variability. Specifically, for benzimidazole resistance, mutations in the β-tubulin gene have been shown reduce fitness of organisms, but there have been cases where resistance persisted long after use was stopped. However, there have been exceptions where resistance was not stable (https://www.frac.info/expert-fora/benzimidazoles/soa-and-mechanism(s)-of-resistance).

Greenhouse growers have multiple fungicide groups available to use, and many already uses a variety of products, but some still use thiophanate-methyl in their production systems. Future work also includes screening the 80 strains for resistance to other fungicide groups. In

addition to in vitro testing, molecular tests have been developed to aid in resistance detection including a primer set Quinone outside Inhibitors (cytB), Dicarboximides (bos1), phenylpyrroles (mrr1), and MBCs (β-tub) (Plesken et al., 2015; Rupp et al., 2017). Understanding which mutations are selected by fungicide resistance and to which group within *B. cinerea* would help growers to know which fungicides have lower risk for fungicide resistance.

Overall, most *Botrytis* isolates were clearly delineated as *B. cinerea*. Phylogenetic analyses that used three concatenated gene regions were useful for identifying *Botrytis* to the species level compared to using BLAST for a single gene region. Resistance to thiophanate-methyl was widespread within and across Kansas greenhouses. Growers should stop using this active ingredient and rotate to other fungicides for gray mold.

References

Albertini, C., Thebaud, G., Fournier, E., & Leroux, P. (2002). Eburicol 14 alpha-demethylase gene (CYP51) polymorphism and speciation in *Botrytis cinerea*. *Mycological Research; Mycol.Res., 106*(10), 1171-1178. doi:10.1017/S0953756202006561

Albertini, C., & Leroux, P. (2004). A *Botrytis cinerea* putative 3-keto reductase gene (ERG27) that is homologous to the mammalian 17β-hydroxysteroid dehydrogenase type 7 gene (17β-HSD7). *European Journal of Plant Pathology; Published in Cooperation with the European Foundation for Plant Pathology, 110*(7), 723-733. doi:10.1023/B:EJPP.0000041567.94140.05

Baggio, J. S., Peres, N. A., & Amorim, L. (2018). Sensitivity of *Botrytis cinerea* isolates from conventional and organic strawberry fields in Brazil to azoxystrobin, iprodione, pyrimethanil, and thiophanate-methyl. *Plant Disease,* 102(9), 1803-1810. doi:10.1094/PDIS-08-17-1221-RE

Banno, S., Fukumori, F., Ichiishi, A., Okada, K., Uekusa, H., Kimura, M., & Fujimura, M. (2008). Genotyping of benzimidazole-resistant and dicarboximide-resistant mutations in *Botrytis cinerea* using real-time polymerase chain reaction assays. *Phytopathology, 98*(4), 397. doi:10.1094/PHYTO-98-4-0397

Brooks, C., & Cooley, J. S. (1917). *Temperature relations of apple-rot fungi* (8th ed.) J. Agric. Res.

Buhr, T. L., & Dickman, M. B. (1994). Isolation, characterization, and expression of a second {beta}-tubulin-encoding gene from *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. *Applied and Environmental Microbiology, 60*(11)

Bulger, M. A. (1987). Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. *Phytopathology, 77*(8), 1225. doi:10.1094/Phyto-77-1225

Cannon, P. F., Damm, U., Johnston, P. R., & Weir, B. S. (2012). *Colletotrichum* current status and future directions *Studies of Mycology,* 73(9), 181-213.

Chen, S. N., Luo, C. X., Hu, M. J., & Schnabel, G. (2016). Fitness and competitive ability of *Botrytis cinerea* isolates with resistance to multiple chemical classes of fungicides. *Phytopathology, 106*(9), 997. doi:10.1094/PHYTO-02-16-0061-R

Ciliberti, N., Fermaud, M., Roudet, J., & Rossi, V. (2015). Environmental conditions affect *Botrytis cinerea* infection of mature grape berries more than the strain or transposon genotype. *Phytopathology, 105*(8), 1090-1096. doi:10.1094/PHYTO-10-14-0264-R

Daughtrey, M. L. (1995). In Peterson J. L., Wick R. L. (Eds.), *Compendium of flowering potted plant diseases* St. Paul, Minn.: APS Press.

Dean, R., Van Kan, Jan A. L, Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Foster, G. D. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology, 13*(7), 804. doi:10.1111/j.1364-3703.2012.00822.x

Dik, A. J., & Wubben, J. P. (2007). Epidemiology of *Botrytis cinerea* diseases in greenhouses doi:10.1007/978-1-4020-2626-3_17

Diolez, A., Marches, F., Fortini, D., & Brygoo, Y. (1995). Boty, a long-terminal-repeat retroelement in the phytopathogenic fungus botrytis cinerea. *Applied and Environmental Microbiology, 61*(1), 103.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research, 32*(5), 1792-1797. doi:10.1093/nar/gkh340

Elad, Y. (2004). *Botrytis: Biology, pathology and control*. Dordrecht; Boston: Dordrecht; Boston: Kluwer Academic Publishers.

Fan, F., Hamada, M. S., Li, N., Li, G. Q., & Luo, C. X. (2017). Multiple fungicide resistance in *Botrytis cinerea* from greenhouse strawberries in Hubei province, China. *Plant Disease, 101*(4), 601. doi:10.1094/PDIS-09-16-1227-RE

Farr, D.F., & Rossman, A.Y. (2019). Fungal databases, U.S. national fungus collections, ARS, USDA; Retrieved from https://nt.ars-grin.gov/fungaldatabases/

Fernández-Ortuño, D., Grabke, A., Bryson, P. K., Amiri, A., Peres, N. A., & Schnabel, G. (2014). Fungicide resistance profiles in *Botrytis cinerea* from strawberry fields of seven southern U.S. states. *Plant Disease, 98*(6), 825-833. doi:10.1094/PDIS-09-13-0970-RE

Fillinger, S., & Elad, Y. (2015). *Botrytis - the fungus, the pathogen and its management in agricultural systems* Cham: Springer.

Fournier, E., Giraud, T., Loiseau, A., Vautrin, D., Estoup, A., Solignac, M., Brygoo, Y. (2002). Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (ascomycota). *Molecular Ecology Notes, 2*(3), 253-255. doi:10.1046/j.1471-8286.2002.00207.x

Fournier, E., Levis, C., Fortini, D., Leroux, P., Giraud, T., & Brygoo, Y. (2003). Characterization of bc-hch, the *Botrytis cinerea* homolog of the *Neurospora crassa* het-c vegetative incompatibility locus, and its use as a population marker. *Mycologia, 95*(2), 251-261. doi:10.1080/15572536.2004.11833110

Groves, J. W., & Loveland, C. A. (1953). The connection between *Botryotinia fuckeliana* and *Botrytis cinerea*. *Mycologia, 45*(3), 415-425. doi:10.1080/00275514.1953.12024279

Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. *Journal of Chemical Biology, 7*(4), 133-141. doi:10.1007/s12154-014-0113-1

Hyde, K. D., Nilsson, R. H., Alias, S. A., Ariyawansa, H. A., Blair, J. E., Cai, L., Zhou, N. (2014). One stop shop: Backbones trees for important phytopathogenic genera: I (2014). *Fungal Diversity, 67*(1), 21-125. doi:10.1007/s13225-014-0298-1

Jarvis, W. R. (1977). *Botryotinia and Botrytis species: Taxonomy, physiology, and pathogenicity: A guide to the literature* Ottawa: Research Branch, Canada Dept. of Agriculture: obtainable from Information Division, Canada Dept. of Agriculture.

Jarvis, W. R. (1992). *Managing diseases in greenhouse crops*. St. Paul, Minn., U.S.A.: St. Paul, Minn., U.S.A.: APS Press.

Kanetis, L., Christodoulou, S., & Iacovides, T. (2017). Fungicide resistance profile and genetic structure of *Botrytis cinerea* from greenhouse crops in Cyprus. *European Journal of Plant Pathology; Published in Cooperation with the European Foundation for Plant Pathology, 147*(3), 527-540. doi:10.1007/s10658-016-1020-9

Kecskeméti, E., Brathuhn, A., Kogel, K., Berkelmann-Löhnertz, B., & Reineke, A. (2014). Presence of transposons and mycoviruses in *Botrytis cinerea* isolates collected from a german grapevine growing region. *Journal of Phytopathology, 162*(9), 582-595. doi:10.1111/jph.12230

Kerssies, A., Zessen, B., & Frinking, H. D. (1995). Influence of environmental conditions in a glasshouse on conidia of *Botrytis cinerea* and on post harvest infection of rose flowers, *European Journal of Plant Pathology,* 101 (2).

Leroux, P., Fritz, R., Debieu, D., Albertini, C., Lanen, C., Bach, J., Chapeland, F. (2002). Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. *Pest Management Science, 58*(9), 876-888. doi:10.1002/ps.566

Levis, C., Fortini, D., & Brygoo, Y. (1997). Flipper, a mobile Fot1-like transposable element in *Botrytis cinerea*. *Molecular and General Genetics MGG, 254*(6), 674-680. doi:10.1007/s004380050465

Li, X., Kerrigan, J., Chai, W., & Schnabel, G. (2012). *Botrytis caroliniana*, a new species isolated from blackberry in South Carolina. *Mycologia, 104*(3), 650-658. doi:10.3852/11-218

Lu, B. H., Wang, X. H., Wang, R., Wang, X., Yang, L. N., Liu, L. P., Liu, X. N. (2019). First report of *Botrytis pelargonii* causing postharvest gray mold on fresh ginseng roots in China. *Plant Disease, 103*(1), 149. doi:10.1094/PDIS-01-17-0031-PDN

Lui, Y. J., Whelen, S., & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution; Mol.Biol.Evol., 16*(12), 1799-1808. doi:10.1093/oxfordjournals.molbev.a026092

MacKenzie, S. J., & Peres, N. A. (2012). Use of leaf wetness and temperature to time fungicide applications to control *Botrytis* fruit rot of strawberry in Florida. *Plant Disease, 96*(4), 529-536. doi:10.1094/PDIS-03-11-0182

Marion Andrew, Reeta Barua, Steven M Short, & Linda M Kohn. (2012). Evidence for a common toolbox based on necrotrophy in a fungal lineage spanning necrotrophs, biotrophs, endophytes, host generalists and specialists. *PLoS One, 7*(1), e29943. doi:10.1371/journal.pone.0029943

Martínez, J. A., Gómez-Bellot, M. J., & Bañón, S. (2009). Temperature-dependent growth of *Botrytis cinerea* isolates from potted plants. *Communications in Agricultural and Applied Biological Sciences, 74*(3), 729. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20222557

May, G. S., Tsang, M. L., Smith, H., Fidel, S., & Morris, N. R. (1987). *Aspergillus nidulans* beta-tubulin genes are unusually divergent. *Gene, 55*(2-3), 231.

Moorman, G., & Lease, R. (1992). Benzimidazole-resistant and dicarboximide-resistant *Botrytis cinerea* from Pennsylvania greenhouses. *Plant Disease; PLANT DIS., 76*(5), 477-480.

Mothay, D., & Ramesh, K. V. (2019). Evolutionary history and genetic diversity study of heat-shock protein 60 of *Rhizophagus irregularis*. *Journal of Genetics, 98*(2)

Moyano, C., Raposo, R., Gómez, V., & Melgarejo, P. (2003). Integrated *Botrytis cinerea* management in southeastern spanish greenhouses. *Journal of Phytopathology, 151*(2), 80-85. doi:10.1046/j.1439-0434.2003.00684.x

Mueller, D. S., Wise, K. A., Dufault, N. S., Bradley, C. A., & Chilvers, M. I. (Eds.). (2013). *Fungicides for field crops* APS Press.

Nelson, P. V. (2012). *Greenhouse operation and management* (7th edition. ed.). Englewood Cliffs, N.J.: Englewood Cliffs, N.J.: Prentice Hall.

Panaccione, D. G., & Hanau, R. M. (1990). Characterization of two divergent beta-tubulin genes from *Colletotrichum graminicola*. *Gene, 86*(2), 163. doi:10.1016/0378-1119(90)90275-V

Plesken, C., Weber, R. W. S., Rupp, S., Leroch, M., & Hahn, M. (2015). *Botrytis pseudocinerea* is a significant pathogen of several crop plants but susceptible to displacement by fungicide-resistant *B. cinerea strains*. *Applied and Environmental Microbiology, 81*(20), 7048-7056. doi:10.1128/AEM.01719-15

Reuveni, R., & Raviv, M. (1992). The effect of spectrally-modified polyethylene films on the development of *Botrytis cinerea* in greenhouse-grown tomato plants. *Biological Agriculture & Horticulture, 9*(1), 77-86. doi:10.1080/01448765.1992.9754618

Røed, H. (1949). *Botryotinia pelargonii* n. sp., the perfect stage of a *Botrytis* of the *cinerea* type on *Pelargonium*. *Blyttia, 7*, 65-79.

Rupp, S., Plesken, C., Rumsey, S., Dowling, M., Schnabel, G., Weber, R. W. S., & Hahn, M. (2017). *Botrytis fragariae*, a new species causing gray mold on strawberries, shows high frequencies of specific and efflux-based fungicide resistance. *Applied and Environmental Microbiology, 83*(9), E00269. doi:10.1128/AEM.00269-17

Saito, S., & Xiao, C. L. (2018). Fungicide resistance in *Botrytis cinerea* populations in California and its influence on control of gray mold on stored mandarin fruit. *Plant Disease, 102*(12), 2545-2549. doi:10.1094/PDIS-05-18-0766-RE

Samarakoon, U. C., Schnabel, G., Faust, J. E., Bennett, K., Jent, J., Hu, M. J., Williamson, M. (2017). First report of resistance to multiple chemical classes of fungicides in *Botrytis cinerea*, the causal agent of gray mold from greenhouse-grown petunia in Florida. *Plant Disease, 101*(6), 1052. doi:10.1094/PDIS-12-16-1778-PDN

Scholefield, P., & Morison, J. (2010). *Assessment of economic cost of endemic pests & diseases on the australian grape & wine industry.*

Schumacher, J. (2017). How light affects the life of *Botrytis*. *Fungal Genetics and Biology, 106 (9)* 26-41.

Shao, X., Wang, H., Xu, F., & Cheng, S. (2013). Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit. *Postharvest Biology and Technology, 77*, 94-101.

Shiraishi, M., Fukutomi, M., & Akai, S. (1970). On the mycelial growth and sporulation of *Botrytis cinerea* pers. the conidium germination and appressorium formation as affected by conidial age. *Annals of the Phytopathological society of Japan, 36 (4).*

Smith, T. L. (1989). Disparate evolution of yeasts and filamentous fungi indicated by phylogenetic analysis of glyceraldehyde-3-phosphate dehydrogenase genes. *Proceedings of the National Academy of Sciences of the United States of America, 86*(18), 7063-7066. doi:10.1073/pnas.86.18.7063

Staats, M., Baarlen, v., P, & Kan, v., J.A.L. (2005). Molecular phylogeny of the plant pathogenic genus botrytis and the evolution of host specificity. *Molecular Biology and Evolution, 22*(2), 333-346. doi:10.1093/molbev/msi020

Staats, M., van Baarlen, P., Schouten, A., Van Kan, J. A. L., & Bakker, F. T. (2007). Positive selection in phytotoxic protein-encoding genes of *Botrytis* species. *Fungal Genetics and Biology, 44*(1), 52-63. doi:10.1016/j.fgb.2006.07.003

Suvedi, M., Jeong, E., & Coombs, J. (2010). Education needs of michigan farmers.*48*(3)

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., & Fisher, M. C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology, 31*(1), 21-32. doi:10.1006/fgbi.2000.1228

Ulloa, M. (2012). In Hanlin R. T. (Ed.), *Illustrated dictionary of mycology* (Second ed.). St. Paul, Minn.: St. Paul, Minn.: APS Press: American Phytopathological Society.

Vrind, T. A. (2005). The Botrytis problem in figures. *Acta Horticulturae,* (669), 99-102. doi:10.17660/ActaHortic.2005.669.11

Walker, A., Gautier, A. L., Confais, J., Martinho, D., Viaud, M., Le P Cheur, P., Fournier, E. (2011). *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. *Phytopathology, 101*(12), 1433-1445. doi:10.1094/PHYTO-04-11-0104

West, J. S., Pearson, S., Hadley, P., Wheldon, A. E., Davis, F. J., Gilbert, A., & Henbest, R. G. C. (2000). Spectral filters for the control of *Botrytis cinerea. Annuals of Applied Biology,* 136 (2).

Whetzel, H. H. (1945). A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia, 37*(6), 648-714. doi:10.2307/3755132

White, J. W. (1993). *Geraniums IV: The grower's manual* Geneva, Ill., USA: Ball Pub.

Yarden, O., & Katan, T. (1993). Mutations leading to substitutions at amino acids 198 and 200 of beta-tubulin that correlate with benomyl-resistance phenotypes of field strains of *Botrytis cinerea*. *Phytopathology, 83*(12), 1478-1483. doi:10.1094/Phyto-83-1478

Zhang, J., Wu, M., Li, G., Yang, L., Yu, L., Jiang, D., Zhuang, W. (2010). *Botrytis fabiopsis*, a new species causing chocolate spot of broad bean in central China. *Mycologia, 102*(5), 1114-1126. doi:10.3852/09-217

Zhang, L., Li, G., Yang, L., Jiang, D., Zhuang, W., & Huang, H. (2010). *Botrytis sinoallii*: A new species of the grey mould pathogen on allium crops in China. *Journal Civic Hukum, 51*(6), 1. doi:10.1007/s10267-010-0057-4

Chapter 2 - Assessing Specialty Crop Growers' Extension Needs for Pest and Disease Management Information

Introduction

The United States Department of Agriculture (USDA) defines specialty crops as "fruits and vegetables, tree nuts, dried fruits and horticulture and nursery crops, including floriculture." The USDA Economic Research Services (ERS) vegetables and pulses yearbook tables show that U.S. fresh market vegetables have grown from 17,887 million pounds in 1970 to 35,890 million pounds in 2018 (USDA ERS, 2018). From 1997 to 2003 U.S. specialty crop value increased from $39.70 to $50.10 billion, representing a rise of 22.60% (Holm et al., 2007).

In Kansas, specialty crop production has increased in recent decades, both in acreage and sales. The Kansas Department of Agriculture (KDA) conducted a 2006 survey of the economic impact of the horticultural industry in Kansas (https://www.k-state.edu/turf/resources/docs/horticulture2007.pdf). In this survey production area and sales of specialty crops (fruits, berries, nuts, vegetables and melons, grapes/wine, nurseries/greenhouses, and florists) were compared from 2000 to 2006. In this six-year time period, increases in sales were 17% for fruit, 24% for berries, 30% for nuts, 36% for vegetables and melons, 94% for wine/grapes, 8% for Christmas trees, 81% for nurseries/greenhouses, and 5% for florists. As for acreage, from 2000 to 2006 acres grew in all sectors: nurseries/greenhouses (increased from 1,666 to 4,780 acres), grapes/wine (increased from 147 to 320 acres), vegetables and melons (increased from 3,299 to 7,800 acres), nuts (increased from 7,446 to 8,500 acres), berries (increased from 111 to 190 acres), and fruit (increased from 1,051 to 1,080 acres). The total acreage of specialty crops reported in 2000 was 13,720 acres and grew to 22,670 acres in 2006.

In terms of economic impact in 2006, florists contributed $66.5 million, farmers' markets contributed $1 million, and nurseries and greenhouses contributed $156.7 million of gross sales for the Kansas horticulture industry.

The KDA conducted another economic impact survey of specialty crops in 2017 and reported that Kansas specialty crop growers are diverse in experience, farm size, and scope of crops. The survey stated that specialty crop farms in Kansas range from less than 1 to more than 51 acres (KDA, 2017). The major specialty crops reported were tomato, pepper, beans, salad mix, herbs/spices, berries, and others. This report stated that 63% of farms are 1-3 acres and 14% are 4-6 acres. Not only are many of these farms small, many growers are relatively new to the industry with 78% of farms being founded after 2001. The increasing numbers of new produce farms has likely created a knowledge gap in various aspects of production such as horticultural practices and pest and disease management. Although the 2017 KDA report described the demographics and economics of specialty crop producers, the survey did not assess disease and pest problems, current pest and disease management solutions, and resource preferences. Information on these topics is needed to optimize research and extension programs.

In other states and within other commodity groups, surveys have provided important insights into grower practices and current needs. For example, 827 row crop and specialty crop growers from North America responded to questions about changes in agriculture and best management practices (Sulecki, 2018). Both types of growers responded that they actively seek better sources of information (62%). Specialty crop growers predicted most precision changes occurring around pest management and water, whereas row crop growers predicted more precision changes around NPK fertilizers and seed. Defining future challenges and what sources

of information growers are seeking helps enable extension professionals to create the specialized references that address growers' concerns.

An IPM survey of specialty crop growers in Missouri revealed useful information about specialty crop growers and their resource preferences (Piñero and Keay, 2018). Comparisons of organic and conventional farms, as well as fruit growers and vegetable growers, were used to separate and evaluate knowledge and use of IPM strategies, resource preferences, years farming, significant pests for each group, and size of farm. Missouri growers were specifically asked what types of challenges they face on their farm and the top two responses were pests (43%) and weather (21%) (Piñero and Keay, 2018). Understanding the specific challenges growers face is a necessary part of developing and implementing extension resources and more information on this topic is needed from Kansas specialty crop growers.

Researchers have gathered information about grower knowledge and pest management strategies in agronomic cropping systems. For example, Vommi et al. (2013) assessed grower perceptions and adoption of IPM practices in corn in West Virginia and used the results to make policy recommendations to enhance adoption, such as programs to share costs to help growers transition to new methods. A survey was conducted in six different states over weed management and glyphosate resistant weed challenges for cotton, corn, and soybean producers (Shaw et al., 2009). Producers were asked about their management practices including the use of irrigation, crop rotation, and tillage. Shaw et al. (2009) determined that crop rotation was more common in the Midwestern states compared to Southern states and that the most common cropping system that used glyphosate was glyphosate resistant soybean/non-glyphosate resistant crop. In Virginia, row crop producers (corn, soybeans, and small grains) were surveyed on their usage of integrated pest management strategies and different types of pest problems including

diseases, weeds, insects, and vertebrates (Malone et al., 2004). In the Vommi et al. (2013), Shaw et al. (2009), and Malone et al. (2004) studies, the researchers determined which IPM strategies were being used, specific pest problems, and different types of cropping systems.

Research Objectives

Although there is anecdotal evidence for growers' needs related to pest and disease management, formal quantitative data is lacking for Kansas specialty crops. More specifically, information is needed concerning specialty crops growers' pest problems, diagnostic abilities, and resource preferences as the specialty crop industry continues to grow. To address this gap, our goal was to gather baseline data related to grower practices and resource needs in order to prioritize future research and extension efforts. Specifically, the objectives of this study were to: 1) quantify farm demographics and top crops grown, 2) quantify use of current cultural management strategies, 3) assess use of laboratory-based resources of plant health problems, 4) identify current and future resource preferences, 5) determine growers' self-reported ability to identify plant health problems (insect pests, diseases, weeds, and abiotic stresses) and 6) quantify pest problems. Within those broad questions, we had an additional goal to assess differences in grower knowledge and years of experience as defined by three grower categories: novice (< 5 years), intermediate (6-20 years), and experienced (> 20 years).

Methods

Survey Development, Distribution, and Analysis

Previous surveys were examined (Burrows, 2008; Gelernter et al.2017; KDA, 2017; Mack et al., 2017; Sellmer et al., 2003; Vommi et al., 2013) that covered IPM and grower perceptions. In addition, discussions with the K-State IPM Coordinator, Franny Miller, helped to develop the survey questions. The survey received an Institutional Review Board (IRB) wavier for this research survey under proposal number 9084. The full survey can be found below as Figure 2.1. It was designed to be easy to complete in less than 5 minutes. The survey consisted of 16 questions. Of these, nine were open-ended and three of the main questions had multiple parts. Questions 1 through 4 addressed broad demographics, questions 5 through 10 addressed what crops were grown and how comfortable growers were at identifying pests. Questions 11 and 12 addressed current management strategies growers were using and questions 13 and 14 addressed where growers currently get information and how they would prefer to receive information in the future. The last two questions addressed viewing farming information on smartphones and provided the opportunity for growers to give feedback.

Figure 2.1 Specialty Crop Grower Survey

Thank you for participating in our survey! This survey is a research project to help us improve research, education, and resources for specialty crops, we would like to gather your responses to the questions below. Your participation is voluntary, and you do not have to respond to any questions you do not want to answer. You will not be identified in any way by the information you provide.

General background

1. In what state do you farm?

2. Approximately how many years have you been farming?

3. Please choose one of the following to describe your farm (Circle one)
   - USDA certified organic
   - Organic, but not certified
   - Strictly conventional
   - Both conventional and organic
   - Does not apply

4. What is the approximate size of your farm, in acres? (Circle one)
   - Less than 1 acre
   - 1-5 acres
   - 6-10 acres
   - 11-20 acres
   - 21-50 acres
   - >50
   - Does not apply

5. What are the top crops you grow on your farm? Please list up to FIVE.

6. If you wish, please list any additional crops you grow on your farm beyond your top five.

Scouting, Identification, and Diagnosis

7. Please indicate your response to the following questions (check one box)

Strategy	Never	Sometimes	Usually	Very frequently/Almost Always	Does not apply to my cropping system
I am able to identify most weeds on my farm					
I am able to identify insect and mite pests on my farm					
I am able to identify beneficial insects on my farm					
I am able to identify diseases on my farm					
I am able to identify environmental stresses on my farm (ex: nutrient deficiencies, drought, etc)					

8. Please list your top 3-5 weed problems, and the crops they are associated with:

9. Please list your top 3-5 insect problems, and the crops they are associated with:

10. Please list your top 3-5 disease problems, and the crops they are associated with:

Management Strategies

11. Which of the following plant health strategies do you use to reduce diseases, insects, and/or weeds?

Strategy	Never	Sometimes	Usually	Very frequently/Almost Always	Does not apply to my cropping system
Plant varieties resistant to diseases					
Crop rotation					
Mulch (plant-based)					
Mulch (plastic)					
Cover crops					
Biological controls (such as release of beneficial insects, nematodes, etc)					
Organically-labeled products					
Conventional products					
Managing moisture and humidity (such as improving drainage, using an irrigation system that avoids over-watering, promoting airflow, etc)					
Other? (open ended)					

12. Optional open-ended question: If you indicated use of biologicals, organic products, or conventional products, please list the ones you tend to use:

- Biologicals:
- Organically-labeled products:
- Conventional products:

Information Sources

13. Where do you currently receive information about crop production, including disease, pest, and weed management? Please check one response for each resource.

Resource	Never	Sometimes	Frequently	Does not apply
Other farmers				
Extension – Local County/Region/District Office				
Extension - University main or branch campus faculty				
Soil testing laboratory				
Plant disease diagnostic lab/clinic				
Insect identification laboratory				
Weed identification laboratory/herbarium service				
Companies (seed suppliers, chemical suppliers, etc				
State Department of Agriculture				
Printed brochures/fliers/fact sheets				
Written online material on websites				
Online videos				
Conferences and workshops				
Social media (Twitter, Facebook, etc)				
Books/Trade magazines				
Other (open-ended)				

14. How would you prefer to receive information about pest and disease management? (circle all that apply)

- Printed brochures/fliers/fact sheets
- Online written publications
- Online videos
- Conferences and workshops
- Social media (Twitter, Facebook)
- Books/magazines
- Other *(open-ended)*

15. Do you like to view farming information on a smartphone?

- Yes
- Maybe
- No
- Does not apply (do not have a smart phone)

16. Do you have any additional comments you would like to share about crop/pest/disease management? (open ended)

Thank you for submitting your answers. Your feedback is important to us!

*This survey was distributed in person by K-State county extension agents and at region conferences and workshops and online using Qualtrics Survey Tool.

The targeted population for the survey (Figure 2.1) was specialty crop growers in and near Kansas, but data is weighted towards Kansas. The survey was distributed in person at the following conferences and workshops: 2018 Kansas Grape Growers and Wine Makers Conference (Lawrence, KS); 2018 Food Safety Modernization Act (FSMA) High Tunnel Bus Tour (Johnson/Douglas Counties, KS); 2018 and 2019 Great Plains Growers Conference (St. Joseph, MO); 2018 and 2019 Central Kansas Market Grower and Vendor Workshop (Wichita, KS). A link to the survey was also distributed in a social media campaign through the Kansas Specialty Crop Growers Association Facebook group and through local Kansas State Research and Extension (KSRE) agents as liaisons for communication with specialty crop growers.

Surveys were input into an online survey tool, Qualtrics XM (Qualtrics, Provo, Utah), and data was summarized using Qualtrics and Microsoft Excel 2016. Frequency tables were created for each question and percentages were developed from the total responses.

Results

Eighty-eight growers responded to paper surveys through a local county agent, conferences, or workshops listed in the methods and nineteen growers responded to an electronic link to the electronic survey, for a total of 107 respondents. Although biases were unintended, the data is slightly weighted towards Kansas grape growers because 23.3% of responses were obtained from the 2018 Kansas Grape Growers and Wine Makers Conference (Lawrence, KS). We recognize that our sampling was not a random selection of growers. Since the majority of surveys were collected at conferences, the data also over-represent growers interested in conferences as a source of information.

Farm Demographics

Growers were asked what state they farm in and responses revealed five states, with over half of respondents farming in Kansas (Table 2.1).

Table 2.1 State Farmed*

	Kansas	Missouri	Iowa	Nebraska	Michigan	Total
Count	69	25	7	4	1	106
Percentage	65%	24%	7%	4%	1%	100%

*Growers reported the state in which they farm in an open-ended question.

Nearly half of growers reported a farm size of 5 acres or less (46%), but more growers reported farms greater than 20 acres (32%) compared to mid-size farms with between 6 and 20 acres (21%) (Table 2.2).

Table 2.2 Farm Size*

	< 1 acre	1-5 acres	6-10 acres	11-20 acres	21-50 acres	>50 acres	Total
Count	17	32	15	7	10	24	106
Percentage	16%	30%	14%	7%	9%	23%	100%

*Growers reported their farms' size in a multiple-choice question.

About one third of growers, 33%, have been farming for 5 years or less, and were designated as "novice" (Table 2.3). Growers who have farmed for more than 20 years (33%) were designated as "experienced". Growers with 6-20 years of experience (32%) were designated as "intermediate." These categories provide a framework for understanding growers' needs and are used in the subsequent figures and tables.

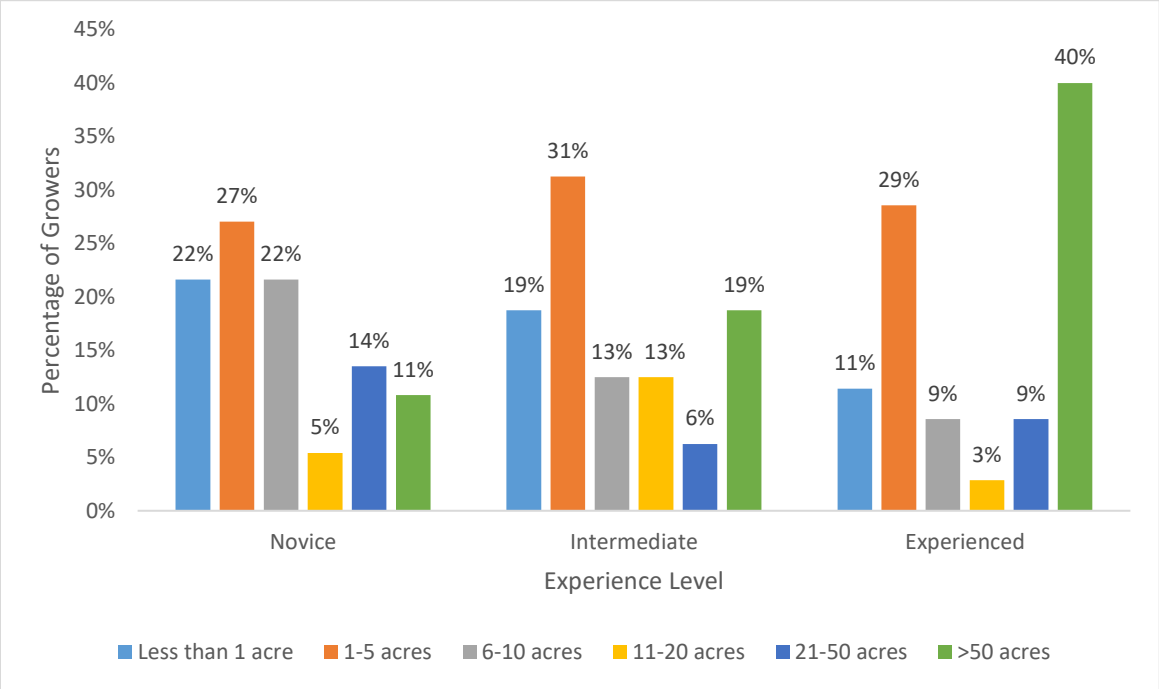
Table 2.3 Farm Experience Level*

	5 years or less	6 to 10 years	11 to 20 years	21 to 30 years	Over 30 years	Total
Category of experience	Novice	Intermediate		Experienced		
Count	29	14	16	11	17	87
Percentage	33%	16%	18%	13%	(20%)	(100%)

*****Growers reported the number of years they have been farming in an open-ended question, which was grouped into experience level categories; novice (5 years or less), intermediate (6 to 20 years), and experienced (over 20 years).

There is a clear trend that growers with larger farms (over 50 acres) are also experienced (farming over 20 years) growers (Figure 2.2). A large portion of growers that farm less than 5 acres are relatively new to growing (less than 5 years).

Figure 2.2 Grower Experience and Farm Size



*****Farm size is organized by experience level where percentages shown were calculated based on each experience category and each category totals 100%. (Novice: n = 37, Intermediate: n = 31, Experienced: n = 36).

Many respondents (40%) stated that they classify their farm as both conventional and organic. 34% of respondents classified their farm as strictly conventional, while 19% classified their farm as organic, but not certified. As for USDA certified organic farms, only 3% of respondents reported this as their classification and 4% chose "do not wish to respond" (Table 2.4).

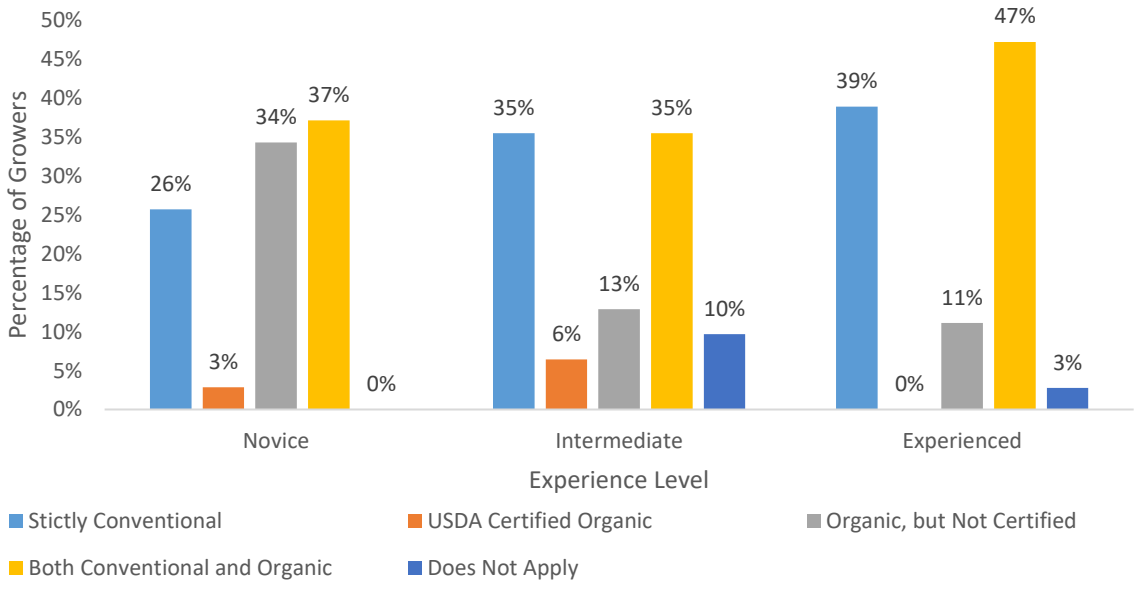
Table 2.4 Farm Type*

	USDA certified organic	Organic, but not certified	Strictly Conventional	Both Conventional and Organic	Do Not Wish to Respond	Total
Count	3	20	35	40	4	104
Percentage	3%	19%	34%	42%	4%	100%

*Growers were asked to select which farm type best described their farm.

In terms of farm types and how it relates to experience, over a third of growers classifying themselves as organic, either certified or not certified, (37%) have been farming for less than 5 years. As for growers that have been farming for more than 20 years, fewer (11%) reported being only organic growers, either certified or not certified organic. Instead, the largest number of growers farming more than 20 years classified their farms as both conventional and organic (47%) (Figure 2.3).

Figure 2.3 Grower Experience and Farm Type

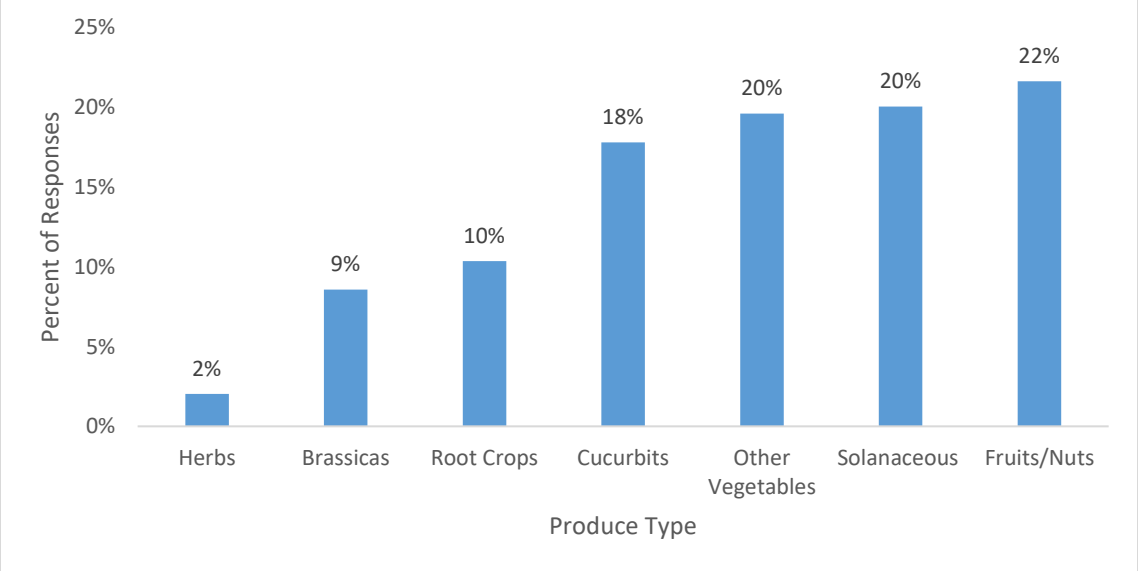


Farm type organized by experience level where percentages shown were calculated based on each farm type category and each farm type totals 100% (Novice: n = 29, Intermediate: n = 30, Experienced: n = 28).

Top Crops Grown

The ten top crops reported were tomatoes, peppers, grapes, pumpkins, cucumbers, potatoes, squash, apples, green beans, and sweet corn. Growers are producing a large variety of crops with a total of 92 different crops being reported as the top 5 primary crops grown. Figure 2.4 shows a summary by seven different produce categories. Fruits and nuts (22%) were reported as the most common produce category grown, followed by solanaceous (20%), other vegetables (20%), cucurbits (18%), root crops (10%), brassica crops (9%) and herbs (2%). Ninety-three different crops were reported out of the 444 total responses. Table 2.5 shows a list of the open-ended responses and some growers used generic terms such as "herbs or vegetables" while other responses are specific crops. We opted to show the raw responses to accurately represent the diversity of responses.

Figure 2.4 Top Crops Reported



Primary crops grown were grouped into different types of specialty crops (n = 444 responses from 106 growers).

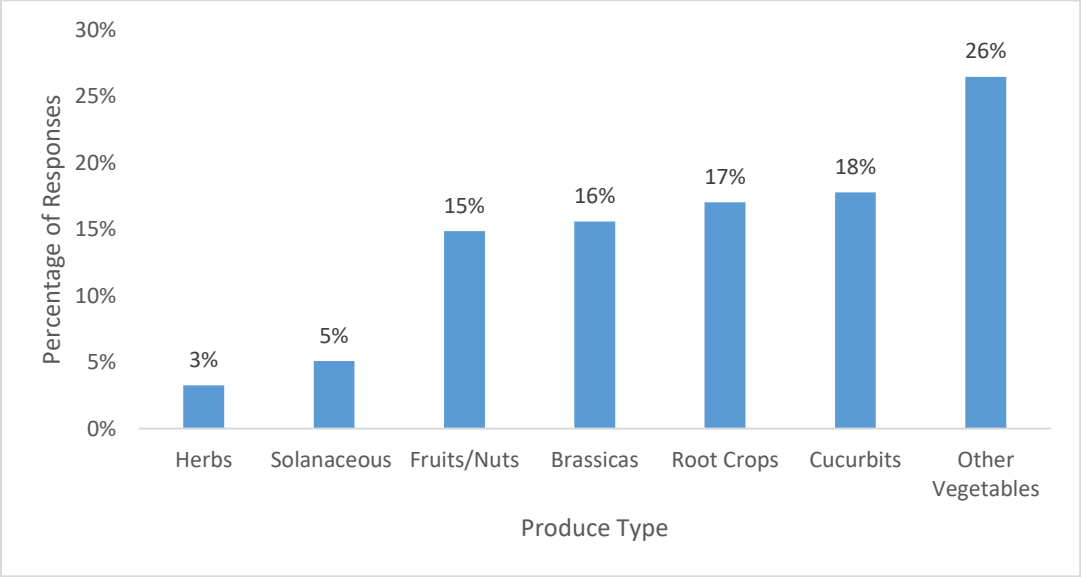
Table 2.5 Top Crops Reported*

Top Crops Grown	Count	Top Crops Grown	Count	Top Crops Grown	Count
Tomatoes	58	berries	4	escarole	1
Peppers	27	cantaloupe	4	fabaceae	1
Grape	25	melons	4	flintcorn	1
pumpkins	21	radishes	4	flowering plants	1
cucumbers	19	arugula	3	forage	1
potato	15	cucurbits	3	fruit	1
squash	15	microgreens	3	garlic	1
apples	13	peas	3	gourds	1
green beans	13	pecan	3	grass	1
sweet corn	10	blueberries	2	hard cider	1
blackberry	9	brassicas	2	honey	1
flowers	9	broccoli	2	koshihikari	1
peaches	9	collards	2	milo	1
watermelon	9	eggplant	2	mushrooms	1
lettuce	7	hops	2	mustard	1
onions	7	kiwi	2	natives fruit trees	1
strawberries	7	mizuna	2	perennial fruit	1
asparagus	6	salad greens	2	pickles	1
beets	6	spinach	2	roots	1
corn	6	zucchini	2	rhubarb	1
greens	6	alfalfa	1	salanova	1
okra	6	all variety trees	1	snow peas	1
sweet potatoes	6	alliaceae	1	solanaceae	1
vegetables	6	apricot	1	sorghum	1
carrots	5	beans	1	specialty greens	1
hay	5	brome	1	timber	1
herbs	5	brussel sprouts	1	tomatillos	1
kale	5	cherries	1	tree fruit	1
pears	5	citrus	1	wheat	1
raspberries	5	currant (white, red, and black)	1	future hazelnuts	1
soybeans	5	endive	1	Total responses	444

*****Growers reported their top five primary crops in an open-ended question.

After growers reported top crops, they were asked to list additional crops grown and these are shown as secondary crops. Overall, other vegetables (26%) were listed as the most common secondary crop being produced followed by cucurbits (18%), root crops (17%), brassica crops (16%), fruits/nuts (15%), solanaceous (5%), and herbs (3%) (Figure 2.5). Ninety-two different crops were reported out of 276 total responses. Vegetables, potatoes, asparagus, cucumber, lettuce, radishes, squash, broccoli, cabbage, and carrots were the most common secondary crops listed (Table 2.6). The responses in Table 2.6 are in a similar format as Table 2.5, where the table shows open-ended responses from growers that include generic terms and the data shown are the raw responses to accurately represent the diversity of responses.

Figure 2.5 Secondary Crops Reported



Secondary crops grown were grouped into different types of specialty crop categories (n=276 from 60 respondents).

Table 2.6 Secondary Crops Reported*

Other Crops Grown	Count	Other Crops Grown	Count	Other Crops Grown	Count
vegetables	10	grapes	3	brussel sprouts	1
potatoes	9	herbs	3	chard	1
asparagus	8	onion	3	cilantro	1
cucumbers	8	pears	3	collards	1
lettuce	8	peas	3	dill	1
radishes	8	rhubarb	3	dusty miller	1
squash	8	soybeans	3	field corn	1
broccoli	7	strawberries	3	fruit trees	1
cabbage	7	tomatoes (cherry)	3	gooseberries	1
carrots	7	artichoke	2	horseradish	1
okra	7	basil	2	hot peppers	1
cantaloupe	6	cherries	2	kabocha	1
melons	6	eggplant	2	leeks	1
peaches	6	elderberries	2	microgreens	1
peppers	6	garlic	2	mizuna	1
pumpkins	6	hay	2	nectarines	1
sweet corn	6	mushrooms	2	parsley	1
blackberries	5	plum	2	peanuts	1
green beans	5	raspberries	2	persimmon	1
greens of all types	5	root crops	2	pickles	1
kale	5	summer squash	2	red mustard	1
sweet potatoes	5	sunflowers	2	scallions	1
turnips	5	watermelon	2	small fruit	1
zucchini	5	wheat	2	snap peas	1
apples	4	apriaceae	1	spinach	1
beans	4	apricots	1	tomatoes (high tunnel)	1
Beets	4	arugula	1	winter squash	1
flowers (sunflowers, bedding plants, annual flowers)	4	barley	1	yellow squash	1
cauliflower	3	berries	1	fiber animals	1
corn	3	bell peppers	1	**Total**	276
cukes	3	blueberries	1		

*Growers reported their top five secondary crops in an open-ended question.

Disease and Pest Management Strategies

Growers reported using a variety of cultural methods to reduce pest and disease problems (Table 2.7). The top management strategies reported being used "usually" or "very frequently/almost always" were crop rotation (75%), plant varieties resistant to diseases (72%), and managing moisture and humidity (such as improving drainage, using an irrigation system that avoids over-watering, promoting airflow, etc.) (72%). In terms of crop rotation, 12% of growers also reported that crop rotation does not apply to their farming system, which likely means they produce perennial crops such as grapes or fruit trees. In contrast, a majority of growers reported they "never" or "sometimes" use biological control (such as release of beneficial insects, nematodes, etc.) (78%), cover crops (69%), and organically-labeled products (64%).

In an open-ended question about other plant health strategies, growers reported using a diverse array of practices. Methods used included companion cropping, biodynamic farming, no till/regenerative again, animal rotation, insolation, high tunnel production, torch blown killing, mulches with weeds, and pulling weeds by hand, each reported by one grower respondent.

Table 2.7 Plant Health Management Strategies Reported*

	Never		Sometimes		Usually		Very Frequently/ Almost Always		Doesn't Apply		Do Not Wish to Respond		Total	
	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Plant varieties resistant to diseases	3	3%	25	25%	39	39%	33	33%	1	1%	0	0%	101	100%
Crop rotation	5	5%	7	7%	24	24%	51	51%	12	12%	2	2%	101	100%
Mulch (plant-based)	17	27%	26	26%	20	20%	28	28%	6	6%	2	2%	99	100%
Mulch (plastic)	36	37%	16	16%	20	21%	17	18%	7	7%	1	1%	97	100%
Cover crops	21	22%	44	47%	9	10%	16	17%	4	4%	0	0%	94	100%
Biological controls	48	48%	30	30%	11	11%	6	6%	3	3%	1	1%	99	100%
Organically-labeled products	26	26%	38	38%	11	11%	22	22%	3	3%	1	1%	101	100%
Conventional products	25	26%	12	12%	30	31%	27	28%	2	2%	1	1%	97	100%
Managing moisture and humidity	3	3%	19	18%	32	31%	42	41%	6	6%	1	1%	103	100%

*Growers were asked to choose how often they use different cultural management strategies. Responds could select only one option and the number of respondents varies from 94 to 103.

Information on which biological controls, organic pesticides, and conventional pesticides growers were using was also of interest. Respondents were asked in an open-ended question to report what control products they use in these three categories. Not every grower answered these questions. For biological control products, the top three products reported were the release of biological insects such as ladybugs, Phytoline (*Phytoseiulus persimilis*, a predatory mite), and wasps (6 responses), Bt (*Bacillus thuringiensis*) (5 responses), and plants (4 responses) (Table 2.8). For organic control products, the top three products reported were Bt (*Bacillus thuringiensis*)/Dipel (9), Pyganic (9), and Neem Oil (7) (Table 2.9). For conventional products, the top three products reported were Sevin (14), Mancozeb (9) and Captan (9) (Table 2.10). In terms of pesticide category, 26 different insecticides, 19 different fungicides, and 9 different herbicides were reported across all three types of control products. Overall, growers reported more conventional products than organic or biological, with 90 responses for conventional products and only 57 and 24 for organic and biological, respectively.

Table 2.8 Biological Control Products Reported*

Biological products and methods listed	Active Ingredient	Control category	Number of growers
Release Beneficial (Ladybug, parasitic mites, parasitic wasps)	varies	Insecticide	6
Bacillus thuringiensis (Bt)	Bacillus thuringiensis (Bt)	Insecticide	5
Plants	-		4
Milky spore	Spore of Bacillus popillae	Insecticide	2
Nematodes	-	-	1
Guinea Hens	-	-	1
traps	-	Insect	1
Garlic	-	Insecticide	1
Spinosad	Spinosad	Insecticide	1
Probiotics	-	-	1
Diatomaceous Earth	-	-	1
Total	**24**		

***** Growers responded to an open-ended question about what biological control products they use. Biological control products organized by name reported, active ingredients, type of control product and the number of growers who responded to this open-ended question.

Table 2.9 Biological Control Products Reported*

Trade name/product listed	Active Ingredient	Control category	Number of growers
Dipel	*Bacillus thuringiensis* (Bt)	Insecticide	9
Pyganic	Pyrethrin	Insecticide	9
Neem Oil	Clarified Hydrophobic Neem Oil	Insecticide	7
Copper	Copper	Fungicide/ Bactericide	5
Soap Products	Potassium laurate	Insecticide	5
Pepper Spray/oil	Oils, Black Pepper	Insecticide	4
Sulfur	Sulfur	Fungicide	3
Surround	Kaolin Clay	Insecticide	3
Oil Extracts	Essential oil, cedar oil	Varying	2
Spinosad	Spinosad	Insecticide	2
Nicotine	Nicotine	Insecticide	1
Regalia	*Reynoutria sachalinensis*	Fungicide	1
Milky Spore	Spore of *Bacillus papillae*	Insecticide	1
OxiDate	Hydrogen peroxide	Fungicide	1
worm casting	-	-	1
Total	**57**		

*Growers responded to an open-ended question about what organic control products they use. Organic control products organized by trade name, active ingredients, type of control product and the number of growers who responded.

Table 2.10 Conventional Control Products Reported*

Product name listed	Active Ingredient	Control category	Number of growers
Sevin	Carbaryl	Insecticide	14
Mancozeb	Mancozeb	Fungicide	9
Captan	Captan	Fungicide	9
Round Up	Glyphosate	Herbicide	6
Rally	Myclobutanil	Fungicide	5
Mustang	Zeta-cypermethrin	Insecticide	5
Malathion	Malathion	Insecticide	4
Pyrethrin	Pyrethrin	Insecticide	3
Copper/ Copper Sulfate	Copper Sulfate	Fungicide	3
Warrior	Lambda-Cyhalothrin	Insecticide	2
Danitol	Fenpropathrin	Insecticide	2
Assail	Acetamiprid	Insecticide	2
Gramoxone	Paraquat dichloride	Herbicide	1
Elevate	Fenhexamid	Fungicide	1
Delegate	Spinetoram	Insecticide	1
Daconil	Chlorothalonil	Fungicide	1
Bravo	Chlorothalonil	Fungicide	1
Belt	Flubendiamide	Insecticide	1
Abound	Azoxystrobin	Fungicide	2
Weed B Gon	Dicamba	Herbicide	1
Treflan	Trifluralin	Herbicide	1
Topsin	Thiophanate-methyl	Fungicide	1
Switch	Fludioxonil/ Cyprodinil	Fungicide	1
Surflan	Oryzalin	Herbicide	1
Sulforix	Lime sulfur	Fungicide	1
Ridomil Gold	Metalaxyl-M	Fungicide	1
Pristine	Pyraclostrobin/ Boscalid	Fungicide	1
Preen	Trifluralin	Herbicide	1
Lifeline	Glufosinate	Herbicide	1
Lambda	Lambda-Cyhalothrin	Insecticide	1
Kocide 3000	Copper hydroxide	Fungicide	1
Baythroid	Cyfluthrin	Insecticide	1
Avian	Methyl anthranilate	Bird Repellent	1
Atrazine	Atrazine	Herbicide	1
Acuron	Bicyclopyrone	Herbicide	1
Total:	**90**		

* Growers responded to an open-ended question about what conventional control products they use. Conventional control products organized by trade name, active ingredients, type of control product and the number of growers who responded to this open-ended question.

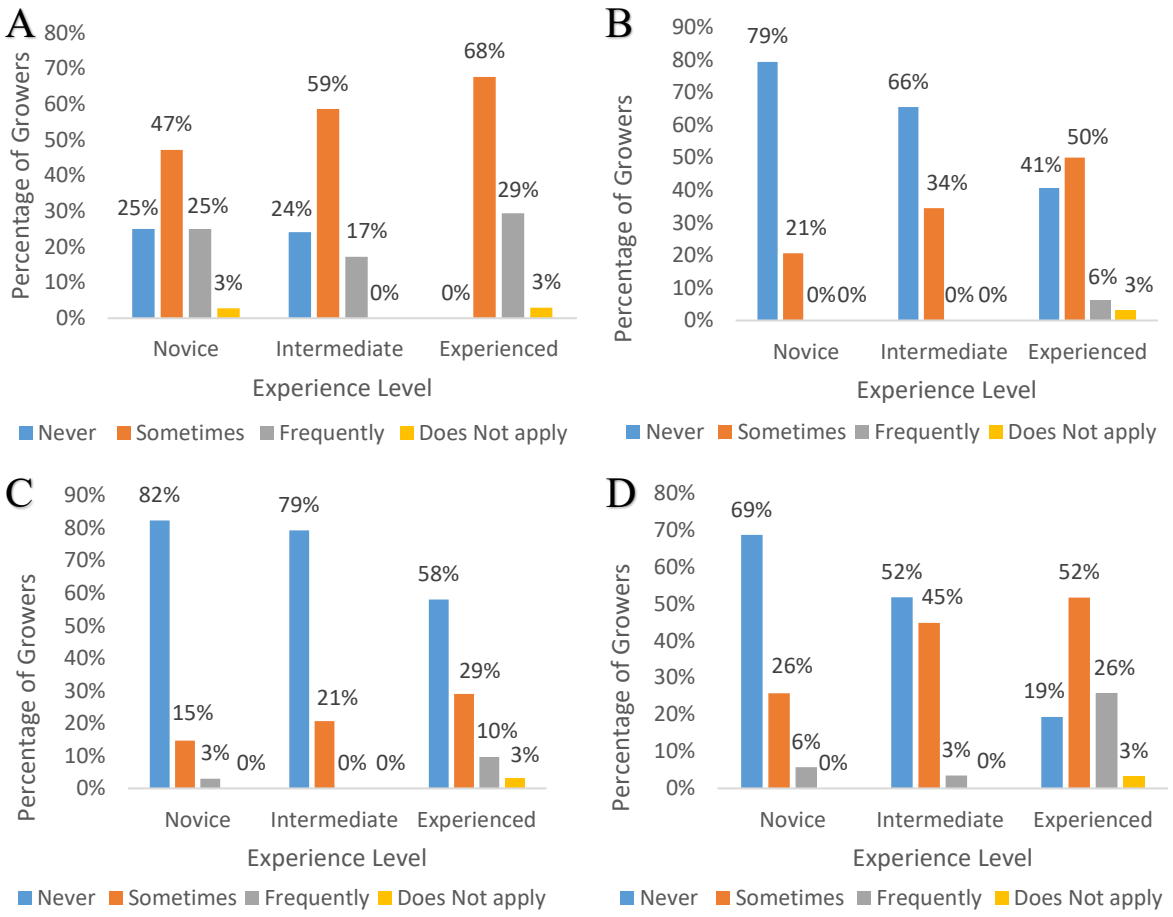
Current Use of Resources for Information

Growers were asked to categorize their current use of specified resources by choosing all the resources options that applied to them. Growers used some resources frequently and others very rarely (Table 2.11). The top five resources growers reported frequent use of were written online materials on websites (73%), conferences and workshops (59%), printed brochures/fliers/fact sheets (46%), books/trade magazines (46%), and other farmers (43%). In contrast, for the lowest used resources, only 8% reported frequent use of their state Department of Agriculture and only 17% reported frequent use of social media.

In an open-ended question to identify other resources, growers listed the following: “ebooks”, “spray guides”, “brother”, “own research”, “out of state producer conferences”, “hire out of state professional consultants”, and “agricultural specialists in Japan and other parts of the world”.

In regard to laboratory-based resources, averaged across all experience groups, growers reported more usage of soil testing laboratories compared to pest and disease identification services. Within pests/diseases, growers overall were more likely to use a plant disease diagnostic lab (sometime/frequently: 51% total), followed by insect identification (sometimes/frequently: 36% total), with weed identification (sometimes/frequently: 25% total) as the lowest (data not shown). Specifically, laboratory-based resources are utilized more by the experienced growers, while many novice growers reported never (soil testing; 25%, insect diagnostics; 79%, disease diagnostics; 69%, and weed identification; 82%) using laboratory-based resources (Figure 2.6). For insect identification laboratories (Figure 2.6B), 0% of novice and intermediate growers reported frequent usage and only 21% novice and 34% intermediate growers reported that they sometimes use insect identification laboratories.

Figure 2.6 Laboratory-based Resource Usage Reported



Laboratory-based resource usage by respondents' years of experience: Soil testing lab (A), insect diagnostic lab (B), weed identification lab (C), and disease diagnostic lab (D).

26% of experienced growers reported frequent use of a disease diagnostic lab compared to only 6% of novice and 3% of intermediate growers (Figure 2.6D). Around half of intermediate (45%) and experienced (52%) growers sometimes use a disease diagnostic laboratory. Overall, a weed identification laboratory (Figure 2.6C) was used the least across all experience categories, with 82% of novice, 79% of intermediate, and 58% of experienced growers stating that they never use a laboratory for weed identification. The soil testing lab was the most frequently used lab-based resource across all experience levels.

Table 2.11 Current Resource Usage Reported*

	Never		Sometimes		Frequently		Does not apply		Total	
	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Other farmers	2	2%	46	53%	37	43%	2	2%	87	100%
Extension - Local County/Region/District Office	11	11%	44	45%	40	41%	2	2%	97	100%
Extension - University main or branch campus faculty	11	11%	55	57%	30	31%	1	1%	97	100%
Soil testing laboratory	16	16%	57	57%	25	25%	2	2%	100	100%
Plant disease diagnostic lab/clinic	46	48%	38	40%	11	11%	1	1%	96	100%
Insect identification laboratory	60	63%	33	34%	2	2%	1	1%	96	100%
Weed identification laboratory/herbarium service	70	74%	20	21%	4	4%	1	1%	95	100%
Companies (seed suppliers, chemical suppliers, etc.)	23	24%	47	49%	24	25%	2	2%	96	100%
State Department of Agriculture	42	44%	44	46%	8	8%	1	1%	95	100%
Printed brochures/fliers/fact sheets	11	11%	41	43%	44	46%	0	0%	96	100%
Written online material on websites	2	2%	23	24%	69	73%	1	1%	95	100%
Online videos	11	12%	41	44%	39	42%	2	2%	93	100%
Conferences and workshops	2	2%	37	38%	58	59%	1	1%	98	100%
Social media (Twitter, Facebook, etc.)	40	44%	31	34%	17	19%	3	3%	91	100%
Books/Trade magazines	3	3%	48	49%	45	46%	1	1%	97	100%

*Current resource preferences reported by specialty crop growers. Not every grower responded to each resource preference.

Future resource preferences and smart phone usage

Growers were asked to indicate where they would prefer to receive information to prioritize future educational efforts. Growers could select more than one option. The responses showed a desire for both traditional face-to-face meetings and online resources (Table 2.12). The most preferred resources were conferences and workshops (23%), online written publications (20%), online videos (18%), and printed brochures/fliers/fact sheets (17%). The least preferred resources were social media (6%) and books/magazines (13%).

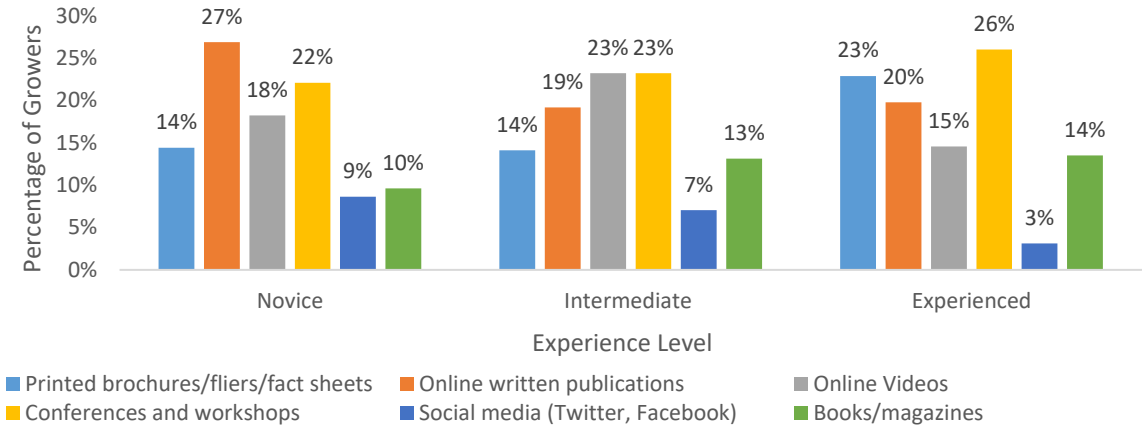
Table 2.12 Future Resource Preferences Reported*

	Count	Percent
Printed brochures/fliers/fact sheets	54	17%
Online written publications	66	20%
Online Videos	57	18%
Conferences and workshops	76	23%
Social media (Twitter, Facebook)	21	6%
Books/magazines	41	13%
Other (open-ended)	9	3%
Total	324	100%

*Growers selected all resources they would prefer to use in the future.

Online written publications (27%) were the most preferred resource for novice growers and social media (9%) was the least preferred resource (Figure 2.7). Intermediate growers preferred online videos (23%) and conferences and workshops (23%) the most, with social media as the lowest (7%). Experienced growers preferred conferences and workshops (26%) the most and social media (3%) the least. As for traditional face-to-face meetings, approximately a quarter of growers at all experience levels (novice; 22%, intermediate; 23% and experienced; 26%) preferred conferences and workshops.

Figure 2.7 Grower Experience and Future Resource Preferences



Growers future resource preferences is organized by experience level where percentages shown were calculated based on each experience category and each category totals 100% (Novice: n = 35, Intermediate: n = 31, Experienced: n = 34).

Growers were asked if they would like to view farming information on a smart phone. The majority (yes; 49% and maybe; 27%) of respondents were open to viewing farming information on a smart phone (Table 2.13).

Table 2.13 Smart Phone Preferences Reported*

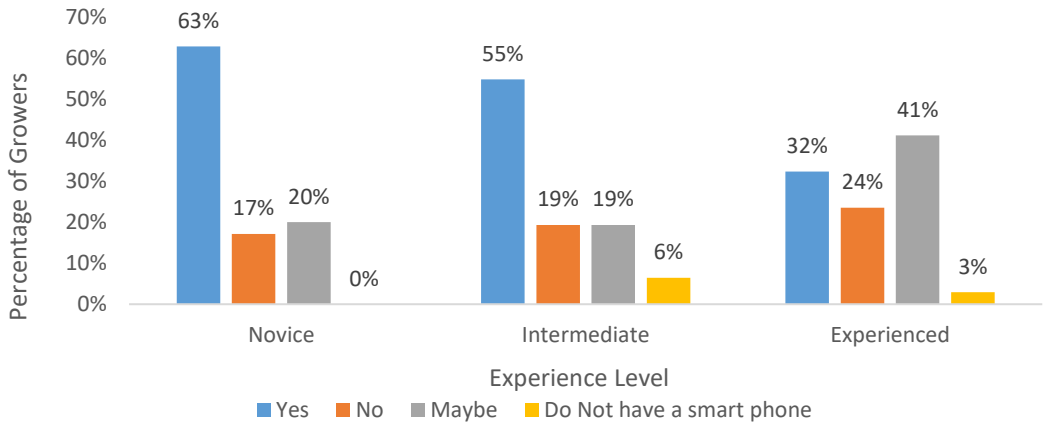
Smart phone Preferences	Yes	No	Maybe	Do not have a smart phone	Total
Count	50	21	28	3	102
Percentage	49%	21%	27%	3%	100%

*Growers were asked in a multiple-choice question on their preferences for view farming information on a smart phone.

More experienced growers reported “no” (24%) and “maybe” (41%) to viewing farming information on a smart phone, while the majority of novice (63%) and intermediate (55%) growers said “yes.” Only a few growers reported that they “do not have a smart phone” (3%).

Specifically, more intermediate growers reported they "do not have a smart phone" compared to the other experience levels (Figure 2.8).

Figure 2.8 Smart Phone Preferences and Grower Experience



Growers smart phone preferences is organized by experience level where percentages shown were calculated based on each experience category and each category totals 100% (Novice: n = 35, Intermediate: n = 31, Experienced: n = 34).

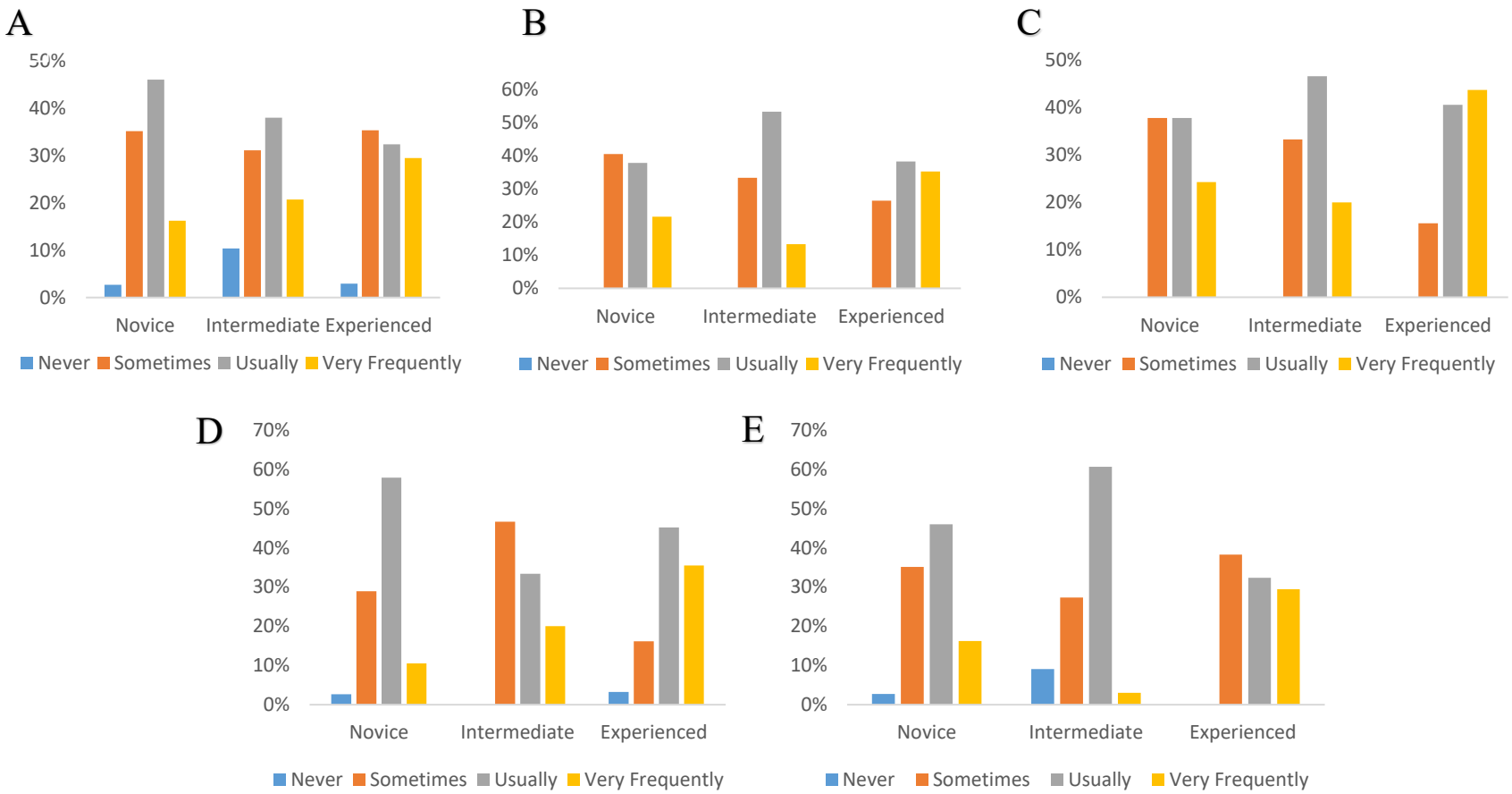
Ability to ID Pests and Top Pests Reported

Growers were asked to assess their ability to identify weeds, insects and mites, beneficial insects, diseases, and environmental stresses on their farm. The self-reported ability to identify weeds "usually" or "very frequently/almost always" was the highest, 73%, compared to other problems on their farm (Table 2.14). The self-reported ability to identify disease issues on their farm "usually" or "very frequently/almost always" had the lowest percentage, with only 51%. As for self-reported ability to identify insects and mites, beneficial insects, and environmental stresses, 67%, 62%, and 68% of growers said they were "usually" or "very frequently/almost always" able to identify these problems, respectively. Across all plant health categories, experienced growers more often stated that they "very frequently" (11%) can identify pest

problem compared to growers with less experience (novice: 6% responses; intermediate: 5%) (Data not shown).

There were trends between grower experience (years farming) and self-reported ability to identify beneficial insects, insect and mite pests, weeds, environmental stress, and diseases. Novice growers and experienced growers both reported that “usually” or “very frequently/almost always” able to identify environmental stress compared to intermediate growers. More novice growers reported they are “usually” able to identify beneficial insects compared to intermediate and experienced growers (Figure 2.9A).

Figure 2.9 Self-reported Ability to Identify Pests and Environmental Stresses



Growers responded to questions about their ability to identify (A) beneficial insects (Novice: n = 37, Intermediate: n = 29, Experienced: n = 34), (B) insects and mites (Novice: n = 37, Intermediate: n = 30, Experienced: n = 34), (C) weeds (Novice: n = 37, Intermediate: n = 30, Experienced: n = 32), (D) environmental stress (Novice: n = 38, Intermediate: n = 30, Experienced: n = 31), and (E) diseases (Novice: n = 37, Intermediate: n = 33, Experienced: n = 34). Data was then organized by growers' different levels of experience where percentages shown were calculated based on each experience category and each category totals 100%. The y-axis is based upon percentage of growers who responded, and the x-axis is experience level.

No growers stated that they are “never” able to identify insects and mites and weeds across all experience groups, showing growers may be more comfortable identifying these types of pests. For growers’ ability to identify diseases, more novice (46%) and intermediate (61%) growers stated that they are “usually” able to identify diseases compared to experienced (32%) growers. Interestingly, more experienced (29%) growers stated they are “very frequently” able to identify diseases, compared to novice (16%) and intermediate (3%) growers.

To gain insight on the top pest problems, growers were asked in an open-ended question to state their top pest problems in 3 categories (weeds, insects, and diseases). Tables 2.15-2.17 list the raw responses to illustrate the diversity of answers. The top ten weeds growers reported were pigweed (31 responses), bindweed (22 responses), crabgrass (18 responses), grasses (15 responses), johnsongrass (13 responses), marestail (11 responses), thistle (10 responses), morning glory (7 responses), bermudagrass (6 responses), henbit/deadnettle (6 responses) (Table 2.15). The top ten insect and mite pests reported were squash bugs (37 responses), cucumber beetles (34 responses), Japanese beetles (29 responses), aphids (16 responses), flea beetles (9 responses), spider mites (8 responses), grasshoppers (7 responses), white flies (6 responses), cabbage loopers (5 responses), and stink bugs (5 responses) (Table 2.16). The top ten diseases (or symptoms/nutrient deficiency) reported were powdery mildew (21 responses), black rot (17 responses), downy mildew (12 responses), blight (9 responses), anthracnose (8 responses), bacterial wilt (6 responses), blossom end rot (6 responses), fire blight (6 responses), rust (6 responses), and brown rot (5 responses) (Table 2.17).

Table 2.14 Self-reported Ability to Identify Pests and Environmental Stresses*

	Never		Sometimes		Usually		Always		Total	
	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
I am able to identify most weeds on my farm.	0	0%	28	27%	41	40%	33	32%	102	100%
I am able to identify insect and mite pests on my farm.	0	0%	34	33%	44	43%	25	24%	103	100%
I am able to identify beneficial insects on my farm.	5	5%	34	33%	40	39%	23	23%	102	100%
I am able to identify diseases on my farm.	4	4%	46	45%	35	34%	18	17%	103	100%
I am able to identify environmental stresses on my farm (nutrient deficiencies, drought, etc.).	2	2%	31	30%	45	44%	25	24%	103	100%

* Growers reported their ability to identify pest problems on their farm.

Table 2.15 Problematic Weeds Reported*

Weed Reported	Count	Weed Reported	Count
pig weed	31	queen ann's lace	2
bind weed	22	black eyed susan	1
crab grass	18	blue grass	1
grasses	15	buffalo bur	1
Johnson grass	13	button weed	1
mares tail	11	clover	1
thistle	10	curly dock	1
morning glory	7	doc weed	1
bermudagrass	6	fall panicum	1
henbit/ deadnettle	6	ground ivy	1
lambs quarters	4	hoary alyssum	1
purslane	4	iron weed	1
rag weed	4	lambs ear	1
bird weed	3	milk weed	1
cocklebur	3	mullins	1
foxtail	3	needle weed	1
lespedeza	3	night shade	1
perennial species	3	nut grass	1
velvetleaf	3	smart weed	1
vine weed	3	poison hemlock	1
water hemp	3	poke weed	1
chick weed	2	random grasses	1
dandelions	2	red thorny thing	1
hemp	2	sand bur	1
horse tail	2	scrabble	1
horsenettle	2	sedges	1
kochia	2	shattercane	1
not having any problems	2	smart weed	1
nutsedge	2	sunflower	1
plantain	2	terminated rye re-seed	1
quack grass	2	Texas sandbur	1
queen ann's lace	2	various weeds	1
round up resistant weeds	2	water grass	1
spurge	2	water plant	1
white clover	2	weeds	1
quack grass	2	Total:	238

*Growers reported their top five weeds they deal with in an open-ended question. Raw response data was used to illustrate the diversity of answers, with minimal editing.

Table 2.16 Problematic Insects Reported*

Insects Reported	Count	Insects Reported	Count
squash bugs	37	tomato worms	2
cucumber beetles	34	army worm	1
Japanese beetles	29	Asian beetles	1
aphids	16	caterpillar	1
flea beetles	9	colorado potato beetle	1
spider mites	8	corn borer	1
grasshoppers	7	drosophila	1
white flies	6	drosophila	1
cabbage loopers	5	europeans corn borer	1
grape berry moth	5	europeans corn borer	1
stink bug	5	grape leaf beetle	1
tomato horn worm	5	grape rootworm	1
beetle	4	green fruitworm	1
horn worm	4	green june beetle	1
mites	4	hoppers	1
Phylloxera	4	hummingbird moth	1
spotted wing drosophila	4	Insects	1
cabbage worm	3	june bugs (like)	1
codling moth	3	ladybugs	1
cut worms	3	leaf rollers	1
ear worms	3	multicolored asian lady beetle (MALB)	1
leaf hoppers	3	oriental fruit moth	1
plum curculio	3	possible nematodes	1
squash beetle	3	potato worm	1
worms	3	spotted squash bugs	1
blister beetles	2	squash borers	1
borers	2	striped army worm	1
cabbage moth	2	striped squash bugs	1
corn ear worm	2	tent caterpillar	1
corn worm	2	thrips	1
cucumber squash bug	2	tobacco horn worm	1
harlequin bugs	2	vertebrae	1
nematodes	2	vine borer	1
no major problems	2	webworm	1
peach borer	2	Total	267
potato beetle	2		

*Growers reported their top five insect/mite pests they deal with in an open-ended question. Raw response data was used to illustrate the diversity of answers, with minimal editing.

Table 2.17 Problematic Diseases Reported*

Diseases Reported	Count	Diseases Reported	Count
powdery mildew	21	black spot	1
black rot	17	black/white rots	1
downy mildew	12	cankers	1
blight	9	Cladosporium leaf spot	1
anthracnose	8	collar rot	1
bacterial wilt	6	cucumber wilt	1
blossom end rot	6	Diplodia	1
fire blight	6	environment?	1
rust	6	Eutypa dieback?	1
brown rot	5	fruit splitting	1
mildew	5	fungus	1
septoria	5	Fusarium wilt	1
bacterial canker	4	leaf issues	1
Botrytis	4	leaf mold	1
scab	4	mild mold	1
early blight	3	mold	1
late blight	3	nematode	1
leaf spot	3	Phyloxia	1
Phomopsis	3	red bloch	1
Phytophthora	3	rose rosette virus	1
viral	3	Scleriotinia	1
bacterial speck	2	smut	1
bunch rot	2	something on hops	1
cedar apple rust	2	sour rot	1
damping off	2	speck	1
grey mold	2	TMV	1
Phylloxera	2	Verticillium wilt	1
root rots	2	Zn deficiency	1
wilt	2	Total	181
bitter rot	1		

*Growers reported their top five diseases they deal with in an open-ended question. Raw response data was used to illustrate the diversity of answers, with minimal editing.

Discussion

Our survey found that Kansas specialty crops growers are diverse in farm size, experience, capacity, and needs. These results are similar to findings by other surveys in which many specialty crop growers surveyed had relatively small sized farms (< 5 acres), were less experienced (farming < 5 years), and have a variety of farm types (Baugher et al., 2017; KDA, 2017; Piñero and Keay, 2018; Pinero et al., 2015) Our survey found that 33% of growers have farmed for less than 5 years and 46% of growers farmed less than 5 acres, comparable to the Piñero (2015) results with 31% of growers farming for less than five years and 54% farming less than 5 acres. Similarly, in Pennsylvania, Baugher et al. (2017) reported 27% of growers had farmed for less than 5 years. The 2006 survey by Kansas Department of Agriculture also reported specialty crop growers farm on small acres with, 63% of farms between 1 and 3 acres.

In our study and the Piñero et al. (2018) survey, growers were asked to choose the production system that best described their farm. In our study, 42% classified their farm as both conventional and organic, while in Piñero et al. (2018) only 28% of growers classified their farm as both conventional and organic. In our study, 19% of growers classified their farm as organic but not certified, compared to 38% in the Piñero study (2018). Only 3% of growers from our survey classified their farm as USDA certified organic farms, while 23% of Piñero (2018) growers classified their farms as USDA certified organic farms. In our survey, growers were not asked if they were interested in becoming certified, but future studies could examine the questions: Do growers understand the criteria? Are there barriers to becoming certified? Since specialty crop growers are less experienced, there may be a need for more extension programs to support these growers.

In terms of top crops grown, 93 different crops were listed with tomatoes being the most common vegetable produced by survey participants, which is consistent with the 2017 KDA survey and the Missouri survey (KDA, 2017; Piñero and Keay, 2018). Understanding the diversity and top crops grown helps extension professionals develop targeted crop specific resources to address these growers needs.

For cultural management strategies, multiple surveys, including this survey, are in agreement that crop rotation is one of the most widely used management strategies for specialty crops and conventional row crops (corn, soybeans, and alfalfa) (Hammond et al., 2006; Piñero and Keay, 2018; Vommi et al., 2013). Moving forward, more research is needed to understand how profitable different crop rotation systems are on specialty crop farms. Quantifying the profitability of different rotation systems will help growers prioritize what cycles of crops should be planted.

Pest Problems

Understanding what types of pest problems specialty crop growers deal with are of high interest because it helps extension personnel to know where to focus educational resources. Pest problems were identified as one of the biggest challenges for growers (Piñero and Keay, 2018; Vommi et al., 2013). It is important to correctly identify crop insects and diseases so the appropriate management strategies can be applied. Interestingly, the conventional control products that were listed in our survey revealed that growers are using older, broader-spectrum products such as Sevin, Mancozeb, and Captan. Providing research-based pesticide evaluations and on farm field days might be good methods to demonstrate and raise awareness of newer materials that are available to growers.

Piñero et al. (2018) reported that 43% of specialty crop growers identified pests as their biggest challenge on their farm. More specifically, insects and diseases are the most problematic pest on farms, regardless of crops being grown (Jasinski and Haley, 2014; Piñero and Keay, 2018; Vommi et al., 2013). However, growers sometimes struggle with identifying those problems. In Vommi et al. (2013), sweet corn growers reported a higher ability to identify weeds compared to diseases or insects. However, in contrast to our study, sweet corn growers reported more difficulty identifying insects compared to this study where identifying diseases was reported as the most challenging.

Piñero et al. (2018) and our study both show that identifying and diagnosing diseases is a challenging aspect for specialty crop growers. Piñero et al. (2018) found that 43% of growers struggle with identifying diseases and our study found that 49% of growers reported that they never/sometimes are able to identify diseases on their farm. Interestingly, even though specialty crop growers reported low capacity with disease identification, 48% of growers stated they never utilize a plant disease diagnostic laboratory. The data was not separated to determine if the growers with low self-reported capacity to diagnose diseases are the same that rarely use diagnostic labs. Regardless, this knowledge gap highlights a strong need for awareness and educational materials about diseases and laboratory-based resources. Overall, these reports show a clear need for extension resources on common disease and insect pests on specialty crops. More information is needed to understand why growers are not utilizing the plant disease diagnostic laboratory. For example, are time, money, or lack of awareness of diagnostic services a barrier?

Current and Preferred Sources of Information

Understanding where growers are currently receiving information provides extension personnel the knowledge of the resources that are or are not being utilized. Our survey found that growers frequently use a wide range of resources including online materials (website publications and videos), face-to-face interactions (other farmers, extension personnel, conferences, and workshops), and printed resources (fact sheets, fliers/brochures, books, and trade magazines). Similarly, Piñero et al. (2015) and Jasinski et al. (2014) found that specialty crop growers are currently using diverse sources of information including other farmers, extension personnel/publications/presentations, and industry representatives. Growers prefer face-to-face interactions and online resources, which demonstrates a need for flexibility in regard to specialty crop information form and accessibility. The majority of survey responses were conducted at conferences, which may have biased results to increase the preference of face-to-face interactions compared to a random selection of growers.

Growers were queried about future resource preferences to identify what types of resources and delivery methods specialty crop growers desire in years to come. It is important that when resources are created and updated that they are in a format that growers will use. In this study and in Baughter et al. (2017) face-to-face (conferences and workshops, on farm demonstrations/tours) interactions were the most preferred followed by online materials (online publications, courses, and videos) and hard copy materials (fact sheets, brochures, newsletters, and production guides). This study and the Baughter et al. (2017) study collected many responses at conferences, which may have selected for a higher percentage of growers who report preference for face-to-face learning as compared to a completely random set of growers. More specifically, Church et al. (2012) found that North Carolina Extension Educators' stated bullet

points with photos and publications/fact sheets were the most effective formats, while one-on-one assistance, videos, and web-based materials were less effective. Overall, growers from this survey prefer a diversity of resources.

Similar to the social media results, Wright et al. (2018) found that 50% or more of grain growers in Australia infrequently used Facebook, Twitter, chat groups, blogs, and podcasts. Further survey work needs to be conducted to understand why growers do not prefer social media but do want information from other online sources. Such information would be of great use to our extension personnel to improve grower knowledge, educational resources, and community impact and engagement.

Questions about business practices such as who growers sell their produce to, marketing strategies, pesticide recordkeeping, and labor were not addressed in our survey. Suvedi et al. (2010) surveyed Michigan farmers (producers of dairy, livestock, swine, cash crops, fruit, vegetable and greenhouse/nursery), and found that specific educational topics such as bookkeeping/marketing skills, sustainable farming practices, and management/care of livestock were of interest. Producers were also asked about how the role of extension could be improved, and they identified farm management, business education, and overall improved knowledge among extension agents as top focus areas. Similar questions should be asked to specialty crops growers in Kansas for extension personnel to conduct targeted and relevant workshops and educational materials.

Our survey was designed to be short and to provide preliminary data for future efforts. Questions of why growers use certain resources and not others were not examined, but this can be an area of future work. Mack et al. (2017) asked ornamental nursery growers why they used best management practices for irrigation and fertilizer management, and respondents reported

saving money and water, efficiency/business/production, and environmental stewardship as reasons. Information such as this provides reasoning and incentives that should be included in resources and programs to help implement best management practices. To understand why growers are or are not adopting management strategies, more survey work must be done to understand the barriers for growers such as costs, needs for upgrades to be made first, not enough time, and lack of knowledge (Mack et al., 2017).

Conclusions

This survey work helped to create a starting point of research data on grower capacity and needs assessment for pest management for specialty crop production in Kansas and surrounding states. Further investigation is needed to better understand the needs of specialty crop growers. This includes but is not limited to more specific demographic questions about growers' age, gender, race, and how reliant growers are on farm income and how it relates to resource needs. Researchers with expertise in rural sociology can be consulted to provide guidance. Other questions include why laboratory-based resources are not highly utilized, why farming practices are or are not being adopted, and what obstacles/barriers are preventing organic growers from becoming certified if they desire to do so. A more comprehensive understanding of grower needs will help extension professionals provide practical and useful information to specialty crop growers.

References

Archibald, W. R., Bradshaw, J. D., Golick, D. A., Wright, R. J., & Peterson, J. A. (2018). Nebraska growers' and crop consultants' knowledge and implementation of integrated pest management of western bean cutworm. *Journal of Integrated Pest Management*, 9(1) doi:10.1093/jipm/pmx033

Baugher, T., Estrada, M. F., Lowery, K., & Contreras, H. N. (2017). Learning preferences of next generation hispanic/latino specialty crop growers. HortTechnology, 27(2), 263-268. doi:10.21273/HORTTECH03581-16

Burrows, M. E. (2008). Using local farmer's markets to promote extension programming. *Journal of Extension*, 46 (6).

Church, C. S., Buhler, W. G., Bradley, L. K., & Stinner, R. E. (2012). Assessing extension educators' needs for homeowner pesticide use and safety information. *Journal of Extension*, 50(5).

Gelernter, W. D., Stowell, L. J., Johnson, M. E., & Brown, C. D. (2017). Documenting trends in land-use characteristics and environmental stewardship programs on US golf courses. *Applied Turfgrass Science*, 3(1), doi:10.2134/cftm2016.10.0066

Hammond, C. M., Luschei, E. C., Boerboom, C. M., & Nowak, P. J. (2006). Adoption of integrated pest management tactics by Wisconsin farmers. *Weed Technology*, 20(3), 756-767. doi:10.1614/WT-05-095R1.1

Holm, R. E., Baron, J. J., & Kunkel, D. L. (2007). Challenges faced by the IR-4 programme and US specialty crop growers. EPPO Bulletin, 37(1), 204-208. doi:10.1111/j.1365-2338.2007.01103.x

Jasinski, J. R., & Haley, J. (2014). An integrated pest management adoption survey of sweet corn growers in the great lakes region. Journal of Integrated Pest Management, 5(2), 1-10. doi:10.1603/IPM13002

Kansas Department of Agriculture. (2017). Economic impact survey of specialty crops. Retrieved from https://agriculture.ks.gov/docs/default-source/ag-marketing/specialty-crop-flyer-2017.pdf?sfvrsn=f18685c1_4

Mack, R., Owen, J. S., Niemiera, A. X., & Latimer, J. (2017). Virginia nursery and greenhouse grower survey of best management practices. *HortTechnology*, 27(3), 386-392. doi:10.21273/HORTTECH03664-17

Malone, S., Herbert, D. A. J., & Pheasant, S. (2004). Determining adoption of integrated pest management practices by grains farmers in Virginia.42(4)

Piñero, J. C., Quinn, J., Byers, P., Miller, P., Baker, T., & Trinklein, D. (2015). Knowledge and use of integrated pest management by underserved producers in Missouri and the role of extension. *Journal of Extension*, 53(3).

Piñero, J. C., & Keay, J. (2018). Farming practices, knowledge, and use of integrated pest management by commercial fruit and vegetable growers in Missouri. Journal of Integrated Pest Management, 9(1) doi:10.1093/jipm/pmy011

Sellmer, J. C., Kelley, K. M., Suchanic, D. J., & Barton, S. (2003). An interactive survey to assess consumer knowledge about landscape plant health care and IPM practices. *Journal of Extension*, 41(2).

Shaw, David. R., Givens, Wade A., Farno, Luke A., Gerard, Patrick D., Jordan, David, Johnson, William G., Owen, Michael D. K. (2009). Using a grower survey to assess the benefits and challenges of glyphosate-resistant cropping systems for weed management in U.S. corn, cotton, and soybean. *Weed Technology*, 23(1), 134-149. doi:10.1614/WT-08-042.1

Sulecki, J. C. (2018). Specialty crop growers take unique path to precision. Growing Produce, Retrieved from https://www.growingproduce.com/fruits/specialty-crop-growers-take-unique-path-precision/

Suvedi, M., Jeong, E., & Coombs, J. (2010). Education needs of Michigan farmers. *Journal of Extension*, 48(3).

Vommi, H. K., LaVergne, D. D., & Gartin, S. A. (2013). Growers' perceptions and adoption practices of integrated pest management in West Virginia, *Journal of Extension*, 51(2).

Wright, D., Hammond, N., Thomas, G., MacLeod, B., & Abbott, L. K. (2017). The provision of pest and disease information using information communication tools (ICTs): An Australian example. *Crop Protection*, 103, 20-29.