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RESPONDING TO GROWERS' NEEDS: EVALUATION OF MANAGEMENT STRATEGIES FOR ONION CENTER ROT, CAUSED BY *PANTOEA ANANATIS* AND *PANTOEA AGGLOMERANS*

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Abstract

Onion growers in Pennsylvania are continually challenged by in-field and post-harvest yield losses due to bacterial pathogens. In 2013, losses due to bacterial disease reduced the number of marketable onion boxes over 40%, resulting in a total loss of $488,000. The primary bacterial pathogens in Pennsylvania include the onion center rot pathogens *Pantoea ananatis* and *Pantoea agglomerans* and are the focus of this research. Although, there are a number of cultural and in-season management practices currently used by growers to reduce the risk of center rot, unacceptable losses still frequently occur and there is a need to develop more targeted strategies that can be incorporated into an integrated pest management program. The three management strategies evaluated in this research were cultivar selection, augmented nitrogen fertigation programs and pre-plant onion transplant treatments. Currently, there are no known onion breeding programs targeting center rot, nor have there been many trials to evaluate the susceptibility of commercially available cultivars. To address this knowledge gap, thirteen onion cultivars were evaluated between 2015 and 2016 for center rot susceptibility, marketability and select horticultural characteristics. The only cultivar to have lower disease incidence and severity and comparable yields to grower standard cv. Candy was cv. Spanish Medallion. Preliminary data suggested that applying total crop nitrogen prior to onion bulbing increased total yield and reduced bacterial disease incidence. Also, it was observed that low lying areas in heavily manured fields used for onion production have had up to 83% bacterial bulb decay incidence at harvest. Based on this knowledge, we evaluated whether the timing and rate of nitrogen application could reduce center rot losses at harvest. A positive, quadratic relationship was found between foliar nitrogen levels at bulbing and center rot incidence at harvest. An interaction existed between rate and time but trends in this relationship were variable based on field trial location. Eliminating *P. ananatis* and *P. agglomerans* prior to planting through use of a transplant bactericide could reduce a potential source of inoculum and provide growers with another management tool. Hydrogen dioxide, hydrogen peroxide with mono- and di-potassium salts of phosphorus acid, copper sulfate pentahydrate and streptomycin sulfate were all effective at reducing *P. ananatis* and *P. agglomerans* as epiphytes on onion transplants and *in-vitro*. The results of this research would also be widely applicable to other onion production systems

and has been influential in the development of a horticultural production guide to aid professionals and growers in low-input horticulture and disease management decisions in Honduras. The results of this research have increased the collective knowledge on the potential use of cultivar selection, nitrogen application rate and timing and bactericide sensitivity to manage onion center rot and will be used to provide growers higher precision disease management options.

Table of Contents

List of Tables

List of Figures

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CHAPTER 1: LITERATURE REVIEW

Taxonomy and significance of onion throughout history

Onions (*Allium cepa* L.) are one of the most important *Allium* species, and are widely cultivated throughout the world. Onions were originally placed in the Liliaceae family, and later the Amaryllidaceae based on inflorescence. Takhtajan (1997) placed *Allium* into the family Alliaceae due to molecular data suggesting it was a distinct family (Rabinowitch and Currah, 2002). Classification of the genus *Allium* is still up for debate and currently can be referred to in any of the three families discussed. The word *Allium*, or *alium*, is the latin name for garlic and *caepa* or *cepa* for onion (Davies, 1992). To distinguish onion and garlic, it is thought that *unio* and later *union* were used to describe onion as one bulb (Davies, 1992). It was not until the Norman Conquest that *onion* became common usage (Davies, 1992).

The bulb onion is thought to have originated in central Asia (Griffiths et al., 2002). The first uses of onion are unclear, but it is estimated that they were one of the first cultivated plants. Onions have been found in Ancient Egyptian tombs suggesting that their cultivation began as early as 3200 B.C. (Schwartz et al., 2008). Radishes, onions, and leeks have been listed as a primary food source of the laborers building the pyramid of Khufu (Cheops) (Davies, 1992). Although onions were considered a food of the lower class, they also thought of onions as a representation of eternal life because of their circular structure. King Ramses IV, who died in 1160 B.C., was entombed with onions in his eye sockets (NOA, 2014). Before Olympic games, Greek athletes would consume pounds of onions, drink onion juice, and rub onions on their bodies (NOA, 2014). Onions were a staple crop by the Middle Ages (NOA, 2014). Pilgrims brought onions with them to the new world, but found that wild onions already grew throughout North America and were consumed by the Native Americans (NOA, 2014).

The first medicinal use of onions was by Charaka Sanhita as a diuretic for improved digestion, heart, eyes, and joints (NOA, 2014). In the middle ages, onions were

used to alleviate headaches, snakebites, and hair loss (NOA, 2014). Today onion is still recognized as a medicinal plant providing many health benefits. The chemical groups found in onions believed to be aids in human health are flavonoids such as anthocyanins in red onions, quercetin in yellow onions, and the alk(en)yl cysteine sulphoxides (ACSOs) which give onions their odor when cleaved by the enzyme alliinase (Griffiths et al., 2002). Some of the health benefits of onion include anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects (Griffiths et al., 2002). In the United States of America (U.S.A.), the National Onion Association (NOA) was established to promote and raise awareness of the health benefits of onions (Griffiths et al., 2002; NOA, 2014).

Relatives of onions include garlic, leek, chives, Welsh onion, and shallots. Onion is a biennial plant. It produces the characteristic bulb that the plant is famous for in the first year of growth. After undergoing a cold vernalization process, the foliage of onion returns along with the production of flowers producing an umbel inflorescence. Bulb formation is under genetic control via day-length response which would theoretically favor production in tropical areas to near the Arctic Circle although these are the regions that tend to favor vegetatively propagated shallot or multiplier onions (Griffiths et al., 2002; Rubatzky and Yamaguchi, 1997). Onions only keep up to 11 leaves at a time (Pfeufer, 2014). Onions are composed of approximately 80-90% water (Griffiths et al., 2002). The remaining dry matter consists primarily of non-structural carbohydrates such as fructon (Darbyshire and Steer, 1990; Griffiths et al., 2002). The most common size of onions sold in the U.S. is 5.0-9.5 cm (2.0-3.7 in.) in diameter (NOA, 2014).

Onion production worldwide

Approximately 3.7 million ha (9.2 million A) of onions are harvested annually (NOA, 2014). Asia encompasses the largest amount of dry onion production in the world at 62% and green onion production at 64.9% (FAO, 2014). The top five producers of dry onions across the world include China, India, U.S.A, and Turkey (FAO, 2014). China produces an average of 15 million tons (31 billion lb) of dry onions a year while the

U.S.A. produces an average of 3 million tons (6 billion lb) of dry onions per year (FAO, 2014). For green onions and shallots, China is still the top producer at an average of 586,084 tons (1 billion lb) per year. Since 1993, dry onion production in the U.S.A. has been on the rise with 2004 resulting in the highest production of 3 million tons (7 billion lb) (FAO, 2014). The U.S.A. produces approximately 4% of the world's annual supply of dry onions (NOA, 2014).

U.S.A. farmers produce approximately 50,585 ha (125,000 A) of onion annually for fresh market (NOA, 2014). It is estimated that less than 1,000 U.S.A. growers produce onions commercially, and of those growers, onion is usually only one of their agricultural crops (NOA, 2014). The U.S.A. exports about 11-14 million 22.6 kg (50 lb) bags of onion per year and imports about 12-17 million 22.6 kg (50 lb) bags per year (NOA, 2014). California, Idaho-Eastern Oregon, and Washington are the top three onion producing regions in the U.S.A. (NOA, 2014). Georgia follows close behind through its 'Sweet Vidalia' onion production. Pennsylvania (PA) onion acreage is among the minor onion production states in the U.S.A. but provides a niche market for diversified vegetable farms.

If dried properly along with modern refrigerated or controlled atmosphere storage, fresh bulbs can be stored up to nine months (Griffiths et al., 2002; MAFF- ADAS, 1982; Smittle, 1988). Onion production systems have a wide range of production practices depending on what is the final product of the system. Onions can be grown for fresh market green onions, fresh market dry onions, dehydrated for food processing, seed production, sets (small bulbs produced at high density), or transplants (Griffiths et al., 2002). Approximately 87% of the onions produced in the U.S.A. are yellow, Spanish cultivars, and of those, the best known is the sweet onion (NOA, 2014). Sweet, low pungency onions are growing in popularity globally, although the more pungent cultivars still dominate the market (Griffiths et al., 2002). The sweet, low pungency cultivars bring their own problems because they have a shorter storage and shelf life (Griffiths et al., 2002). The availability of U.S.A. grown sweet, yellow onions is between March and

September (NOA, 2014), and from October to February they are usually imported from Central and South America as Peruvian or Mayan Sweets™ (B. Gugino, pers. comm.)

Onion production in PA

Onion production can be a great asset to diversified vegetable growers, especially in PA. Transplants are generally planted earlier than many other agricultural crops, so planting does not interfere with other crop production. In PA, they are harvested in early to mid-July and therefore the field can be double cropped with a fall brassica or a summer cover crop. In addition, the storability of onions allows for a sufficient income even into the late fall. This is a huge benefit from a marketing standpoint. Most of the PA onion growers are located in Lancaster and Chester counties, and participate in an onion grower cooperative. In addition there is an increasing number of onion growers in central PA also growing for the grower cooperative (B. Gugino, pers. comm.). The majority of onions grown in PA are marketed through the PA Simply Sweet Onion Program, which was established in 2002 by the Pennsylvania Vegetable Growers Association (PVGA). Since then, acreage in the program has tripled and in 2013, approximately 44.1 ha (109 A) were planted with 5.5 million transplants resulting in an estimated crop value of $1.2 million (B. Gugino, pers. comm.). Onions are the state's only trademarked crop. In addition, there is an increasing number of non-program onions that are being grown for retail markets such as roadside stands, farmer's markets or community supported agriculture (CSAs).

Onion plants are typically either sourced from transplant producers in Arizona or Texas, or from local transplant growers who start their own seeds in December through January. Transplants are usually 10-12 weeks old when planted in the field in late March through early to mid-April. After May 1, growers will forgo planting onions and plant another crop because there are not enough days for the plant to develop sufficient foliage before bulbing at the end of June. Onions in the program are grown on black plastic mulch in four rows at 15 cm x 15 cm (6 in. x 6 in.) spacing on beds that are 0.91 m (3 ft) wide and 20-30 cm (8-12 in.) tall with a double row of drip irrigation. Fields typically

range in size between 0.2 and 0.6 ha (0.5 and 1.5 A). The currently accepted cultivars in the PA Simply Sweet Onion Program are cvs. Candy, Expression, and Enterprise. These onions are sweet, Spanish, yellow onions that are intermediate-long day summer onions. They are marketed as fresh-slicing onions rather than dry, storage onions. The bulbs in this program must meet a minimum size of 7.62 cm (3 in.) in diameter. The pyruvic acid concentration is used to evaluate pungency, and typically ranges between 1-4 μmol pyruvic acid/kg bulb, which is very mild. The soluble solids (sugars) of these onions must be greater than 6%. Bulbs are usually harvested in early to mid-July totaling a three-month field season.

At harvest, onion leaves can be removed (topped) immediately and the bulbs placed in shade or cool storage for curing. Alternatively, onions can be pulled, and laid on plastic mulch for 1-2 days before being topped, placed in bins and transferred to cool storage. While on the plastic mulch, the leaves of one plant typically cover the bulbs of the adjacent plant to prevent sunscald. Once in cool storage, forced air fans are used to promote neck drying, internal moisture sealing, and the formation of dry papery layers. Harvested bulbs that are greater than 10.16 cm (4 in.) in diameter are graded as 'colossal' and ones that are between 7.62 cm (3 in.) and 10.16 cm (4 in.) in diameter are 'jumbo'. If onions are smaller than 7.62 cm (3 in.) in diameter, they are sold in netted bags at much lower prices or sold through the retail market.

Bacterial rots of onion

Bacterial bulb rots of onion and their causal pathogens include: sour skin, caused by *Burkholderia cepacia* Burkholder; slippery skin, caused by *Burkholderia gladioli* pv. *allicola* Burkholder; leaf streak, caused by *Pseudomonas viridiflava* Burkholder; leaf blight, caused by *Xanthomonas axonopodis* Kadota; soft rot, caused by *Pseudomonas marginalis* pv. *marginalis* Brown and *Pectobacterium carotovorum* subsp. *carotovorum* Jones; and center rot, caused by *Pantoea ananatis* Serrano and *Pantoea agglomerans* Beijerinck (Bull et al., 2010). A bulb decay caused by *Enterobacter cloacae* can occur in storage, but is to our knowledge infrequent in PA, and more commonly found in New

York and the Pacific Northwest. Identification of the primary bacterial species causing these diseases may be difficult since multiple species of bacteria can be isolated from a symptomatic bulb and diagnostic symptoms can be similar. Of these pathogens, *P. carotovorum* subsp. *carotovorum*, *P. agglomerans*, and *P. marginalis* pv. *marginalis*, are the most common pathogens in the PA onion cropping system (Pfeufer, 2014). As *P. ananatis* and *P. agglomerans* incidence has recently increased, they are often considered emerging pathogens of onion and will be the focus of this research (Anderson et al., 2004).

Center rots of onion- *P. ananatis* and *P. agglomerans*

Center rot caused by *P. ananatis* was first reported on sweet onions in Georgia in 1997 (Gitaitis and Gay, 1997). It was confirmed in Colorado one year later in dry-bulb pungent onions (Schwartz and Otto, 1998, 2000). The first report of *P. agglomerans* (synonymous with *Erwinia herbicola*) in the U.S.A. was in Georgia in 2006. Since that time, pathologists in New York, Michigan and PA have also observed *P. agglomerans* on onion in their states. Although *P. ananatis* and *P. agglomerans* are closely related, at one point considered the same species, and cause indistinguishable symptoms on onion, they may individually cause center rot of onion (Dutta et al., 2014). Often, *P. ananatis* and *P. agglomerans* are found co-infecting symptomatic bulbs in PA (Pfeufer, 2014).

Center rot pathogens are thought to enter the leaf through stomata or wounds. As lesions enlarge, the tissue can become water-soaked, soft and bleached white. Infected leaves collapse and hang beside the onion neck. Severe symptoms may include complete wilting and bleaching of all leaves. Carr et al. (2013) described transmission of *P. ananatis* from the leaves to the bulb occurring as the infected leaf lodges. Center rot bulb symptoms typically include one or a few discolored scales with macerated tissue apparent when the bulb is cut in half. Infected bulbs are usually odorless unless another pathogen or secondary agent is infecting the bulb. Center rot symptoms often go unidentified at the time of harvest because the discolored ring in the onion neck can be difficult to distinguish from plants that have lodged or been dried in the field prior to harvest.

Different strains of *Pantoea* species may contribute to a wide range in virulence and possible preference for leaf or bulb infection. It has been suggested that the mechanisms of pathogenicity of *P. ananatis* and *P. agglomerans* is the type VI and type III secretion systems respectively (Barash and Manulis-Sasson, 2009; De Maayer et al., 2011). The type III secretion system is believed to pump effector proteins into onion cells (Barash and Manulis-Sasson, 2009). Although the type VI secretion system needs further exploration, it may be that it delivers lytic enzymes and secretes bacterially-synthesized antimicrobials (De Maayer et al., 2011; Pusey et al., 2008). Both *Pantoea* species can produce indole-3-acetic acid (IAA), which may aid in loosening cell walls (Barash and Manulis-Sasson, 2009; Enya et al., 2007).

Current management of bacterial rots of onion in PA

Onion growers in PA are continually experiencing reductions in yield due to bacterial rots, even though they actively trying to manage these diseases. In 2013, losses due to bacterial disease reduced the number of marketable boxes over 40%, resulting in a total loss of $488,000 (B. Gugino, pers. comm.). These losses tend to increase post-harvest as center rot often affects only one or two inner scales, leaving the outer scales firm, and making the disease difficult to detect at harvest. It is often bacterial disease epidemics that cause the growers to harvest early and sacrifice size (jumbo and colossal-sized bulbs) for a greater proportion of smaller asymptomatic bulbs. Although there are a number of cultural and chemical management practices currently used by growers to reduce the risk of center rot and other onion bulb rots, unacceptable losses still frequently occur and there is a need to develop more targeted strategies that can be integrated into an IPM program.

Most commonly, growers use copper-based fungicides to reduce secondary spread of the bacterial pathogens. However, these products cannot protect plants if the plant tissue is already infected. Also, copper-tolerant strains of *P. ananatis* have been identified in Georgia onion fields (Nischwitz et al., 2007). Warm temperatures can favor field-level

outbreaks of center rot. To avoid increased temperatures, and promote air movement, growers have begun using different types of plastic mulch and cutting slits in the plastic mulch at bulbing (B. Gugino, pers. com.). In addition, silver colored plastic mulches may deter thrips. Western flower thrips (*Frankliniella occidentalis* Pergande) and onion thrips (*Thrips tabaci* Lindeman) have been identified as vectors of *P. ananatis* and *P. agglomerans* in Georgia (Dutta et al., 2012, 2014; Gitaitis et al., 2003; Wells et al., 2002), in addition to iris yellow spot virus (Gent et al., 2006), thus thrips management is important to reduce the potential spread of the bacterial pathogens.

As many of the pathogenic bacteria enter through wounds, it is important to minimize injury to maturing or harvested bulbs. At harvest, bulbs are dried as soon as possible. Crop rotation for two or more years along with the elimination of volunteer onions and weeds is recommended. *P. carotovorum* subsp. *carotovorum, P. ananatis*, *P. agglomerans,* and *P. marginalis* have been found as endophytes and epiphytes on many weed species occurring within and/or in close proximity to onion fields in PA (Pfeufer, 2014). Also, rep-PCR facilitated strain tracking of *P. ananatis* matched isolates from surface-disinfested weed tissue collected at mid-season with those of infected onion that had been stored for four months (Pfeufer, 2014). Although the pathogenicity of isolates collected from weeds have shown a high degree of variability ranging from pathogenic to non-pathogenic, weeds may play a role as a potential source of bacterial inoculum (Pfeufer, 2014).

Pfeufer (2014) isolated pathogenic *P. carotovorum* subsp. *carotovorum, P. agglomerans, P. ananatis*, and *P. marginalis* as epiphytes from the surface as well as endophytes from the inner tissues of transplants produced in Texas, Arizona, and locally in PA. The presence of bacterial pathogens on the surface of transplants and within surface-disinfested transplants may suggest that seeds may be a potential source of bacterial inoculum. In vegetable crops, many bacterial pathogens are known to be seedborne such as *Xanthomonas campestris* pv. *campestris* causing black rot of crucifers (Clayton, 1929; Cook et al., 1952; Monteith, 1921; Schaad et al., 1980), bacterial leaf spot of pepper (Ritchie, 2000), and bacterial canker of tomato (Burokiene et al., 2005;

Shaker, 2014; Strider, 1969). Although there is currently no conclusive evidence to support that the center rot pathogen is seed-borne in cv. Candy onion seed, Walcott et al. (2002) identified natural infestation of *P. ananatis* in onion cv. Sweet Vidalia seed. Therefore, growers should always purchase seed and transplants from reputable sources. Since previous data has shown that onion transplants can harbor bacterial pathogens, research is needed to identify transplant treatments that will reduce bacterial inoculum on transplants prior to planting in the field.

One of the major tools of integrated pest management is the selection of cultivars that are less susceptible to disease. To our knowledge, there are no known onion cultivars that are tolerant or resistant to bacterial diseases, including center rot. This may be especially challenging to identify in sweet onion cultivars because their high sugar content and low pungency may make them favorable hosts for bacterial pathogens. The high sugars provide a rich carbohydrate source and the low pungency compounds reduce what may be natural plant defenses. The risk of center rot can be reduced if the grower plants cultivars that mature earlier, reducing the time the onion is in the field and possibility of infection. The architecture of the onion varies with different cultivars, and may be important in identifying cultivars less susceptible to center rot. For instance, onions with a reduced neck diameter would help facilitate faster drying of the onion neck, possibly preventing *P. ananatis* and *P. agglomerans* from entering the bulb. The identification of less susceptible onion cultivars would provide growers with an additional tool to manage center rot.

Nutrients affect plant growth and yield, but may also have secondary effects on growth pattern, plant morphology, and anatomy or chemical composition, and may increase or decrease disease susceptibility. In general, optimal plant growth is obtained with a 'balanced' nutrient supply and is usually considered optimal for plant resistance to pathogens. Preliminary studies have found a negative relationship between soil NH_4 after transplanting and bacterial rot incidence at harvest (Pfeufer, 2014). Also, on-farm survey data indicated a strong negative relationship between leaf tissue nitrogen at mid-season and increased losses from bacterial rots at harvest (Pfeufer, 2014). More trials are needed

to confirm that this preliminary work finding a negative relationship between onion N and bacterial rot incidence at harvest is consistent. This relationship may exist because vigorously growing plants have a higher capacity to compensate for losses due to a bacterial infection (Marschner, 2012).

The goal of this thesis research is to develop targeted research-based management strategies that growers can implement as part of an IPM program to reduce losses due to bacterial rots of onion.

Specific objectives are to:

1. Evaluate the susceptibility of commercially available onion cultivars to center rot.
2. Evaluate the effect of the timing and rate of nitrogen application to reduce center rot incidence thus increasing marketable yield.
3. Evaluate the efficacy of pre-plant onion transplant treatments to manage *P. ananatis* and *P. agglomerans* colonizing the plant surface.

The results of this research will be disseminated to growers as a tool to reduce harvest and post-harvest losses. Ultimately, this research will increase the profitability of sweet onion production in PA.

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CHAPTER 2: IDENTIFYING ONION CULTIVARS WITH REDUCED SUSCEPTIBILITY TO CENTER ROT

Abstract

Onion growers in Pennsylvania (PA) are continually challenged by losses from center rot disease caused by the bacterial pathogens *Pantoea ananatis* and *P. agglomerans*. The objective of this research is to identify onion cultivars that are less susceptible to center rot than the commercial standard cv. Candy but still meet the criteria of the PA Simply Sweet Onion Marketing Program. Thirteen onion cultivars were evaluated between 2015 and 2016 for center rot susceptibility and select horticultural characteristics, including neck and bulb diameter. Cultivars were arranged in a randomized complete block design and plots were split by proximity to inoculated plants. Two onion leaves of select plants were inoculated with a mixture of *P. ananatis* and *P. agglomerans* isolates. At harvest, onions were graded by size, marketability and disease incidence. Sub-samples of asymptomatic onions were evaluated for soluble solids, pungency and post-harvest disease incidence. Center rot incidence at harvest was significant by cultivar in 2015 and 2016 at Rock Springs ($P=0.001$ for 2015 and $P=0.055$ for 2016), but not at Landisville ($P=0.621$). Center rot incidence post-harvest was significantly different by cultivar in both 2016 trials ($P\leq 0.001$ for Landisville and $P=0.047$ for Rock Springs) but not in 2015 ($P=0.241$). Early-, mid- and late-season neck diameter evaluations had a weak relationship with center rot incidence post-harvest. All cultivars met the minimum sugar criteria but pungency was not met in 2016 for the marketing program. Future on-farm trials will further investigate cultivars that show promise in sweet onion production in PA.

Introduction

Cultivar selection is one of the most important tools of any integrated pest management program. Cultivar selection is important for selecting desirable traits such as high yield, drought tolerance and/or disease resistance. Resistance is determined by the

ability of the host to limit penetration, development and/or reproduction of the pathogen. Tolerance is the ability of the host to maintain growth and exhibit little disease damage despite pathogen infection. Host resistance or tolerance to pathogens can be increased by changes in plant anatomy (e.g. higher lignification and/or silification) as well as physiological and biochemical changes that increase plant defense compounds and restricted nutrient transfer to the pathogen (Nürnberger et al., 2004; Walters and Bingham, 2007; Zeyen et al., 2002). Resistance can occur if the most susceptible stages of plant growth occur at a different time than the highest activity of the pathogen, known as 'escape from attack' or 'outgrowing' the pathogen (Huber, 1980). Species or strains of pathogens are always evolving, allowing them to evade or suppress plant defenses, making breeding for resistance or tolerance a challenge (Anderson et al., 2010). Although resistance and tolerance are genetically controlled, they are still influenced by environmental conditions (Marschner, 2012).

Bacterial bulb rots of onion and their causal pathogens include: sour skin, caused by *Burkholderia cepacia* Burkholder; slippery skin, caused by *Burkholderia gladioli* pv. *allicola* Burkholder; leaf streak, caused by *Pseudomonas viridiflava* Burkholder; leaf blight, caused by *Xanthomonas axonopodis* Kadota; soft rot, caused by *Pseudomonas marginalis* pv. *marginalis* Brown and *Pectobacterium carotovorum* subsp. *carotovorum* Jones; and center rot, caused by *Pantoea ananatis* Serrano and *Pantoea agglomerans* Beijerinck (Bull et al., 2010). Of these pathogens, *P. carotovorum* subsp. *carotovorum*, *P. agglomerans*, and *P. marginalis* pv. *marginalis*, are the most common bacterial pathogens in the PA onion cropping system (Pfeufer, 2014).

Center rot is often not identified in onion bulbs because the symptoms are subtle at the time of harvest and only consist of one or two soft, discolored rings in the neck when topped prior to lodging (bending or breaking the stalk) or drying down of the neck. This symptom can be difficult to observe because once the necks have lodged or dried in the field, symptoms are not apparent until the onion bulb is horizontally cut. Center rot pathogens are thought to initially enter the leaf through stomata or wounds and initially cause water-soaked, soft and bleached white lesions. Infected leaves can completely

collapse and as the pathogen spreads, multiple leaves may become symptomatic and/or wilt. It is thought that the bacteria move from the leaf to the bulb once the leaf is lodged (Carr et al., 2013). Due to the high center rot disease incidence in PA and challenge of rogueing infected onions at harvest, center rot is the focus of this research.

In PA, the majority of sweet onions are marketed through the PA Simply Sweet Onion Program, the state's only trademarked crop. Onions in the PA Simply Sweet Program are yellow, intermediate day, Spanish-type onions. They are summer-grown onions that are usually harvested in early to mid-July, totaling a three-month field season. They are characteristically sweet with at least 6% soluble solids (sugars). The PA Simply Sweet program requires low pungency, with concentrations between 1-4 µmol pyruvic acid/kg bulb tissue. Pungency can be categorized using the following scale: very mild sweet onion= 1-4 µmoles pyruvic acid/kg weight of bulbs; mild sweet onion= 5-7 µmoles; intermediate pungency= 8-10 µmoles; pungent= 11-15 µmoles; very pungent= > 15 µmoles (Orzolek, 2012). Onion bulbs in this program must meet a minimum size of 7.62 cm (3 in.) in diameter. PA Simply Sweet onions are marketed as fresh-slicing onions rather than dry, storage onions. The currently accepted cultivars in the PA Simply Sweet Onion Program are cvs. Candy, Expression and Enterprise, although Candy comprises the majority of the acreage.

Onion architecture can vary between different cultivars, and may be important in identifying cultivars less susceptible to center rot (Ćota et al., 2013). The risk of center rot could potentially be reduced if the grower plants cultivars that mature earlier, reducing the time the onion is in the field and thereby the possibility of infection. When bacterial disease is suspected, growers often harvest their bulbs early, sacrificing bulb size with the hope that once the onion neck is dried down, the bacterial pathogens can no longer move from the leaves into the bulb. However, there currently is no scientific evidence to support growers using this tactic to reduce losses. For instance, onions with a reduced neck diameter may be more challenging for bacteria to move through from leaf to bulb. Also, a reduced neck diameter would help facilitate faster drying of the onion neck and therefore movement of the bacteria from the neck into the bulb. In the end, the

identification of less susceptible onion cultivars would provide growers with an additional tool to manage center rot.

Objectives and Hypotheses

Onion growers in Pennsylvania (PA) are continually challenged by in-field and post-harvest yield losses due to several bacterial diseases. The identification of resistant or tolerant cultivars could help growers mitigate these losses. Cultivar Candy, the most common onion cultivar grown in the PA Simply Sweet Onion Marketing Program, is very susceptible to bacterial disease based on continued commercial losses (B. Gugino, pers. comm.). The goal of this research is to identify onion cultivars that are less susceptible to center rot than the standard cv. Candy and still meet the horticultural requirements of the PA Simply Sweet Onion Program. The identification of less susceptible cultivars would give growers an alternative option to cv. Candy. We hypothesized that the differences in onion neck diameter of different cultivars may play a role in the incidence of center rot disease in the bulbs at harvest and post-harvest.

Materials and Methods

Experimental Design

The onion cultivars evaluated included Lasso, Great Western, Aruba, Ovation, Dulce Reina, Spanish Medallion (Sakata Seed America, Morgan Hill, CA), Sedona, Crockett, BGS 280 F1, BGS 300 F1 Blush, Red Sky, Expression (Bejo Seeds, Inc., Oceano, CA) and Candy (Seedway, LLC, Hall, NY). The cultivars were selected with the guidance of Dr. Mike Orzolek, Emeritus Professor of Horticulture at Penn State. These cultivars were grown from seed in the greenhouse at the Plant Pathology Farm at the Russell E. Larson Agricultural Research Center (Rock Springs) in PA Furnace, PA. The seeds were sown into 200-cell flats containing Fafard® #2 soilless media and maintained in a greenhouse under ambient light. Plants were watered as needed and fertilized weekly with a soluble 25-8-18 fertilizer (Scotts Miracle-Grow, Marysville, OH). The insect-

parasitic nematode, *Steinernema feltiae* (Nemasys®, BASF Corporation, Research Triangle Park, NC) was applied bi-weekly to control thrips. The insecticides Malathion (3.9 mL/L, Loveland Products, Inc., Greeley, CO) and Conserve SC (0.6 ml/L, Dow AgroSciences, Indianapolis, IN) were also used to control thrips.

Twelve-week-old greenhouse-grown onion transplants were planted at Rock Springs on April 28, 2015 and April 19, 2016 and at the Penn State Southeast Agricultural Research and Extension Center in Manheim, PA (Landisville) on April 14, 2016. Onions were grown using standard black plastic with a double row of drip irrigation. Each treatment plot was 4 rows wide planted at 15 cm (6 in.) standard onion spacing. The 2015 plots were 6 m (20 ft) in length and 2016 plots were 4 m (13 ft) in length. Treatments were arranged in a randomized complete block design with four replications and split by inoculation proximity. A subset of plants in each plot was inoculated with a mix of *P. ananatis* and *P. agglomerans* isolates as the sub-plot (inoculation proximity). The inoculation proximity split-plot design was used to establish various levels of inoculum pressure. Inoculated, adjacent to inoculated, and uninoculated onions were rated separately for disease incidence and severity (Fig. 2.1).

Crop fertility, insects and weeds were managed using standard commercial practices. Weeds were managed using the pre-emergent herbicides Prefar 4-E (13.98 L/ha, Gowan, Yuma, AZ), GoalTender (0.584-1.167 L/ha, Dow AgroSciences, Indianapolis, IN) and Dual Magnum (1.167 L/ha, Syngenta Crop Protection, Greensboro, NC) between rows. A perennial rye cover crop was planted in drive rows, and hand weeding was performed within the bed. Insects were managed using Radiant SC (0.584-0.730 L/ha, Dow AgroSciences, Indianapolis, IN), Assail 30SG (0.42-0.56 kg/ha, United Phosphorus, Inc. King of Prussia, PA), Diazinon AG500 (0.02 mL/plant, Helena Chemical Company, Collierville, TN) and/or Warrior 1EC (0.219 L/ha, Syngenta Crop Protection, Greensboro, NC) and fungal diseases were managed using Fontelis (44.36 mL/305 m row through drip line and 1.168 L/ha, DuPont Crop Protection, Wilmington, DE), Endura (0.455 L/ha, BASF Corporation, Research Triangle Park, NC), Quadris Flowable (0.584- 0.876 L/ha, Syngenta Crop Protection, Greensboro, NC) and/or Bravo

Weather Stik (2.33 L/ha, Syngenta Crop Protection, Greensboro, NC) as necessary. Onions were fertilized with a 20-20-20 and urea ammonium nitrate (UAN) weekly with a total crop nitrogen supply of 179 kg N/ha (160 lb/A).

Inoculum preparation and inoculation procedure

Inoculum was prepared by streaking six isolates of *P. ananatis* (Pa1a, Pa1b, 2009-082) and *P. agglomerans* (2011-085, 2009-063, 2009-194) onto two large (15 x 100 mm) King's B (King et al., 1954) petri plates two days before preparing inoculum. These isolates were originally collected from symptomatic bulbs from PA between the years 2008 and 2011, and are part of the Gugino Lab bacterial isolate collection. These isolates have been shown to induce disease in pathogenicity tests (Pfeufer, 2014). Each plate was flooded with 4 mL of sterile MQ H_2O and scraped with a sterile plastic scraper to make a bacterial suspension. The suspension was pipetted into a sterile 50 mL tube, vortexed, and optical density (590 nm) was measured using a UV-Vis Spectrophotometer in 2015 (NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA) and a microplate reader in 2016 (EMax®, Molecular Devices, Sunnyvale, CA). Each isolate was adjusted to an optical density of 0.9 and 1 mL was removed from each of these isolates, combined into a sterile 50 mL tube, vortexed, and this mixture adjusted to an optical density of 0.047 to obtain an inoculum concentration of approximately 10^8 CFU/mL. One mL of the final inoculum was reserved to double check the viable cell concentration by dilution plating on KB media. The inoculum was aliquoted into sterile 1 mL Eppendorf tubes for field inoculations.

On June 24, 2015 onions were inoculated by puncturing one mature leaf approximately 5 cm from the leaf whorl with a sterile toothpick dipped in a 2.58 X 10^9 CFU/mL mixture of six *P. agglomerans* (Pa1a, Pa1b, 2009-082) and *P. ananatis* (2011-085, 2009-063, 2009-194) isolates. With this concentration, estimates suggest approximately 10 µL inoculum is delivered per puncture (Pfeufer, 2014). When symptoms did not appear within 1.5 weeks, a second leaf was inoculated with 1.92 X 10^{10} CFU/mL using the same parameters. In 2016, onions were inoculated following the same

procedure previously described, this time inoculating two leaves on June 15 with a 6.18 X 10^8 CFU/mL mixture of the same six isolates. Starting in mid-June through harvest, 10 plants per sub-plot were selected at random to evaluate for weekly disease severity post-inoculation. Disease severity was rated on a 0 to 7 visual disease scale [0 – no lesion, asymptomatic; 1 – local lesion, 2.5 cm x 2.5 cm or less; 2 – expanded lesion, but less than 1/4 of leaf; 3 – up to 1/2 of the inoculated leaf is chlorotic or bleached; 4 – more than 1⁄2 of the inoculated leaf is chlorotic or bleached, but uninoculated leaves do not show symptoms; 5 – entire inoculated leaf and a portion of an uninoculated leaf are symptomatic; 6 – multiple fully symptomatic leaves; 7 – ≥50% bleached and/or collapsed leaves]. Prior to harvest, plots at Landisville were rated a total of three times while those at Rock Springs were rated five and four times in 2015 and 2016, respectively.

Horticultural characteristic assessment

At three points during the season (May 25, June 17 and July 7 at Rock Springs in 2015; May 18, June 8 and June 30 at Landisville in 2016; May 24, June 17 and July 6 at Rock Springs in 2016 which correspond to early-, mid- and late-season) horticultural measurements were recorded for five representative onions from each sub-plot totaling 15 onion plants per replicate plot. These measurements included the bulb diameter (mm) at its widest point and neck diameter (mm) mid-way between the apical meristem and bulb.

Harvest evaluation

Rock Springs field trials were harvested on July 23, 2015 and July 19, 2016 and the Landisville field trial was harvested on July 14, 2016 totaling a three-month field season. Plots were harvested individually by sub-plot (inoculation proximity). The number and total weight (kg) of bulbs with center rot (onions with symptomatic neck scales) symptoms were recorded, and marketable and unmarketable bulbs were graded by size: small, < 6.4 cm in diameter; medium, 6.4 – 7.6 cm; jumbo, 7.6 – 10.2 cm; colossal, > 10.2 cm and weighed by size class. A subsample of 10 jumbo- and/or colossal-sized,

asymptomatic onions were analyzed for soluble solids (%) and pungency as determined by pyruvic acid content (Waters Agricultural Laboratories, Camilla GA). Another sub-sample of 20 asymptomatic jumbo- and/or colossal-sized onions from adjacent to inoculated and uninoculated sub-plots were comingled by replicate and cured under burlap (2015) or in a mesh bag (2016) in a greenhouse with forced air for two weeks. If 20 jumbo- or colossal-sized bulbs were not available, medium-sized bulbs were sampled. Cured onions were placed in 4°C storage and post-harvest center rot incidence (presence or absence of symptoms) was evaluated after three-months in storage by slicing each bulb in half longitudinally.

Data analysis

Data were analyzed using the one-way analysis of variance procedure in Minitab 17.2 (Minitab Inc., State College, PA). ANOVA with two or more factors were analyzed in SAS 9.4 (SAS Institute, Cary, NC). Center rot incidence was square root transformed in ANOVA to satisfy the assumption of normality. Post-hoc comparisons were completed using Fisher's LSD and Tukey's HSD, $\alpha = 0.05$. Using the General Linear Model procedure, cultivar, inoculation proximity and block (replicate) were input as class variables and block was labeled as random. Center rot incidence at harvest in 2015 did not have a normal distribution when analyzed as the split-plot design (n=120) despite transformation attempts so these data were compared between trials as the whole-plot (n=40), which was normally distributed when square-root transformed. Comparisons of severity distributions were conducted using the Kruskal-Wallis test in Minitab. Multiple linear regression was performed using the stepwise addition procedure with center rot incidence (%) at harvest and/or post-harvest analyzed as the dependent variable, which was the total split-plot incidence.

Results

Horticultural characteristics

Neck diameter significantly differed by cultivar early-season in the 2016 trials; mid-season at Landisville in 2016; and at the late-season evaluation date in all three trials (Table 2.1). Bulb diameter significantly differed by cultivar early-season in the 2016 trials, mid-season in all three trials, and late-season in all three trials (Table 2.2). The relationship between neck diameter as the predictor variable and bulb diameter as the dependent variable was explored for early-, mid-, and late-season data from all three trials. A positive, linear relationship existed between mid-season neck and bulb diameters ($P \leq 0.001$; $R^2 = 0.6448$) and this relationship was slightly improved when analyzed as a quadratic term (Fig. 2.2; $R^2 = 0.6492$). A similar relationship existed between early-season neck and bulb diameter ($P \leq 0.001$; $R^2 = 0.1923$), but not late-season neck and bulb diameter ($P = 0.2992$). Cultivars Spanish Medallion, Aruba, Blush, Red Sky, Lasso and Crockett had significantly smaller mid-season neck diameters compared to cv. Candy at Landisville. In both 2016 trials, cv. Crockett had the largest mean, late-season neck diameter and cvs. Candy, Spanish Medallion, Expression, Great Western and Red Sky had small late-season neck diameters that were not significantly different from each other. In 2015, late-season mean neck diameter was significantly larger for cvs. Aruba, Blush, Ovation, Sedona and Spanish Medallion compared to cv. Candy and cvs. Expression, Great Western, BGS 280 and Red Sky were comparable to cv. Candy (Table 2.1).

Marketable yield at harvest

In 2015 and 2016, marketable yield (≥ 7.62 cm bulb diameter) means ranged between 2.1 and 20.6 kg/ 10 m of harvested row (Table 2.3). Cultivar Expression had the highest marketable yield and percentage of bulbs that were jumbo or colossal in size (Table 2.4). Cultivar Candy had numerically fewer jumbo- and colossal-sized bulbs and lower total marketable yield compared to Expression in all three trials. Cultivars Ovation,

Great Western, Spanish Medallion and BGS 280 were not significantly different from Candy in terms of the percentage of jumbo and colossal-sized bulbs or marketable yield in all three trials. Cultivars Sedona, Blush, Dulce Reina, Crockett and Aruba had the lowest yields and percentage of jumbo- and colossal-sized bulbs in all three trials (Tables 2.3 and 2.4).

All cultivars evaluated met the minimum sugar criteria in all three trials but the pungency criteria for the PA Simply Sweet Onion Program were not met in the 2016 trials. The highest sugar values in all three trials were from the two red onion cvs. Blush and Red Sky. Compared to cv. Candy, cvs. Aruba, Sedona, Great Western and Crockett had higher sugar values in one or more trials. Pungency was lower in cvs. Blush, Great Western, Expression, Aruba, Spanish Medallion and Crockett compared to cv. Candy in one or more trials (Table 2.5).

Foliar disease assessment

One-week prior to harvest, foliar disease severity significantly differed between cultivars in the inoculated sub-plots in both Rock Springs trials ($P \leq 0.001$ in 2015; $P=0.002$ in 2016) but not Landisville ($P=0.203$). Compared to cv. Candy, median foliar center rot severity ratings of inoculated onions were lower for cvs. Lasso, Blush, Spanish Medallion and Expression at Rock Springs in 2016, Expression and Great Western at Rock Springs in 2015, and all cultivars except for Aruba at Landisville in 2016 within one-week prior to harvest (Fig. 2.3).

Center rot incidence

Center rot incidence at harvest was significantly different between cultivars in 2015 and 2016 at Rock Springs ($P=0.001$ for 2015 and $P=0.055$ for 2016), but not at Landisville ($P=0.621$) (Fig. 2.4). Landisville had significantly higher center rot incidence at harvest compared to Rock Springs in 2016 but 2015 Rock Springs was not significantly different from either trial ($P=0.0076$). There was a block effect at

Landisville where block 2 was significantly different from block 3 (P=0.0144). Center rot incidence at harvest was still not significant by cultivar at Landisville when these blocks where each removed separately and the data reanalyzed.

Center rot incidence post-harvest was significantly different by cultivar in both 2016 trials ($P \leq 0.001$ for Landisville and P=0.047 for Rock Springs) (Fig. 2.5). Onions stored from Landisville had significantly higher center rot incidence than both Rock Springs trials ($P \leq 0.001$). Cultivar Candy had the lowest center rot incidence post-harvest at Landisville, and cvs. Spanish Medallion, Great Western, Expression, Crockett and Blush were not significantly different than cv. Candy. Cultivar Lasso had the highest center rot incidence post-harvest at Landisville followed by cv. Aruba. Onion cultivars stored from Rock Springs in 2016 were not significantly different than cv. Candy and numerically, cv. Expression had the fewest symptomatic bulbs while cv. Lasso had the most. In 2015, center rot incidence was not significantly different between cultivars after three months in storage (P=0.241; data not shown).

There was a weak linear relationship between early-, mid-, and late-season neck diameters with post-harvest center rot disease incidence (P=0.0195, $P \leq 0.001$, P=0.0054 respectively; R^2=0.3993; Fig. 6). When bulb diameter was included in the analysis, mid-season neck diameter ($P \leq 0.001$) and late-season neck and bulb diameter measurements ($P \leq 0.001$ and P=0.0039, respectively) were significant, and the r-square value of this relationship slightly improved R^2=0.4139. When the same predictor variables were analyzed, this time with center rot incidence at harvest as the dependent variable, only early-season bulb diameter was a significant factor (P=0.0256) and the r-square value was extremely low (R^2=0.0415). When the dependent variable was changed to total center rot incidence as the sum of the percentages at harvest and post-harvest, the early-, mid- and late-season neck diameters (P=0.0043; $P \leq 0.001$; P=0.0024) were significant and the late-season bulb diameter was nearly significant (P=0.0876) (R^2=0.3819). Adding interaction terms to these models did not improve the relationship (data not shown). No multicollinearity was indicated among the neck and bulb diameter predictor variables (variance inflation factors [VIFs] < 1.5; data not shown).

Discussion

The positive relationship between mid-season neck diameter and mid-season bulb diameter suggests that the neck and bulb are enlarging at a similar rate. Also, there was not a relationship between late-season neck diameter and late-season bulb diameter, which may be due to rapid growth of the bulb at this stage when nutrients from the leaves are mobilized and translocated to the bulb, eventually leading to leaf senescence at bulb maturity (Sullivan et. al., 2011). Cultivars Expression, Great Western and Candy had consistently large early-, mid- and late-season bulb diameters. These results are similar to those at harvest where cv. Expression had the highest marketable yield and highest percentage of jumbo and colossal sized bulbs in all three trials and cvs. Great Western and Candy were not significantly different in marketable yield. Cultivar Expression also had lower median foliar severity compared to cv. Candy in all three trials (Fig. 2.3). Although cv. Blush had the overall lowest center rot incidence, it also had the lowest marketable yield, along with Sedona (Fig. 2.4; Table 2.3).

Cultivars Blush, Red Sky, Crockett, Sedona, Spanish Medallion, and BGS 280 had lower center rot incidence at harvest compared to cv. Candy in 2015 and 2016 trials at Rock Springs (Fig. 2.4). All cultivars evaluated at Landisville had lower center rot incidence at harvest compared to cv. Candy, although these differences were not significant (Fig. 2.3). In all three trials, the only cultivar to have lower center rot incidence than cv. Candy at harvest while still producing comparable marketable yields and percentage of jumbo and colossal-sized bulbs to cv. Candy was Spanish Medallion (Fig. 2.4; Table 2.3; Table 2.4).

Results for cv. Crockett at Landisville may have been skewed since a single row of the inoculated onions in block one were damaged by farm equipment, although an overall block effect was present for blocks two and three of this trial. Also, all cultivars were harvested on the same date, despite lodging. Onions bulbs rapidly develop in the last four weeks of the growing season, prior to full maturity, and once mature, onion foliage naturally lodges. Typically, growers will harvest the crop once 50% of the plants

have lodged in the field (J. Stoltzfus, pers. comm.). Observationally, some cultivars such as Candy, Expression and Red Sky had started to lodge prior to harvest while cvs. Crockett and Dulce Reina had not. In this study, we did not see a consistent difference in center rot incidence between cultivars that mature earlier such as Candy and Expression which take approximately 100 days to maturity and cultivars that take 120 days to maturity like Sedona and Dulce Reina. In future trials, it is important to use lodging as an indicator of bulb maturity for each cultivar as this could impact disease assessment. It is thought that *P. ananatis* moves from the leaf to the bulb once the leaf is lodged (Carr et al., 2013). Therefore, it may be that we are underestimating disease, particularly post-harvest disease incidence, in cultivars that did not lodge prior to harvest. Sacrificing bulb size by harvesting onions prior to lodging may be a tactic that growers can use to prevent the center rot pathogens from entering the bulb (Pfeufer, 2014).

Due to the variability in center rot disease incidence post-harvest, significant differences were not observed between cultivars in 2015. However in 2016, cvs. Aruba and Lasso consistently had the highest center rot incidence while Expression had the lowest, numerical incidence post-harvest at Rock Springs in 2016 (Fig. 2.4). Interestingly at Landisville, cv. Candy had the lowest center rot incidence post-harvest but had the numerically highest center rot incidence at harvest (Fig. 2.4; Fig. 2.5). Perhaps the center rot pathogens were present in the onion neck but not the bulb at the time of harvest. Therefore, curing of the onion bulbs dried the neck tissue, preventing bacterial movement into the bulb post-harvest. Consistently, cv. Spanish Medallion had low center rot incidence at harvest and post-harvest in all three trials (Fig. 2.4; Fig. 2.5).

In all three trials, all the cultivars evaluated met the sugar requirements of the PA Simply Sweet onion program. Variability in pungency values in this trial may be due to environmental conditions. Onion pungency is determined by genetics and environmental factors such as water supply, temperature, nitrogen and sulfur fertility (Randle, 1997). Although the onions evaluated in this trial were drip irrigated, overall environmental conditions were dry in both locations in 2016. In July, the average Pennsylvania

precipitation was 9.8 cm in 2016 and 11.0 cm in 2015, which may have contributed to the high pungency values (NRCC, 2016).

The significant, weak, linear relationship found between early-, mid- and late-season neck diameter measurements and center rot incidence post-harvest (Fig. 2.6) suggests that larger neck diameters may have higher center rot disease incidence post-harvest. This was observed in cultivars like Aruba that had consistently large neck diameters late-season along with high foliar disease severity and high center rot incidence at harvest and post-harvest (Table 2.1; Fig. 2.3; Fig. 2.4; Fig. 2.5). Therefore, it may be more important for PA onion growers to select cultivars that generally do not have larger neck diameters. Cultivars Aruba, Crockett and Dulce Reina had large neck diameters late-season in addition to high disease incidence and/or variable disease incidence at harvest and post-harvest. Center rot foliar severity ratings were also highest late-season around the time of harvest. Cultivars that have larger neck diameters at harvest may provide more favorable conditions for the movement of *P. agglomerans* and *P. ananatis* from the neck to the bulb, which is why higher center-rot disease incidence was observed in these onions post-harvest (Fig. 2.5). However, cv. Blush had consistently large neck diameters late-season and low disease incidence at harvest and post-harvest (Table 2.1; Fig. 2.4; Fig. 2.5), which explains why the relationship between onion neck diameter and center rot incidence post-harvest was weak.

It is likely that neck diameter is not the only factor contributing to center rot incidence at harvest and post-harvest. For example, Pfeufer (2014) found a linear, positive relationship between soil temperature and center rot incidence at harvest. In chapter three (Fig. 3.6; p. 63), we show a positive, quadratic relationship between total foliar nitrogen (%) and center rot incidence at harvest. The difference in the cuticle layer of each cultivar may also have a relationship with center rot disease incidence. The ketone hentriacontanone-16 is the main epicuticular wax of the cuticle layer on onion leaves (Damon et. al., 2014). Field studies conducted by Damon et. al. (2014) found that numbers of adult and larval thrips were significantly higher on waxy onions compared to glossy and semi-glossy types and there was significantly less ketone hentriacontanone-

16 on semi-glossy type onions compared to waxy ($P < 0.01$). The differences in ketone hentriacontanone- 16 and other epicuticular waxes may also play a role in *P. ananatis* and *P. agglomerans* infection and should be evaluated in future onion cultivar evaluations.

Conclusion

Center rot incidence at harvest significantly differed by cultivar and in all three trials, the only cultivar to have lower center rot incidence at harvest than cv. Candy while still producing comparable marketable yields and percentage of jumbo and colossal-sized bulbs was Spanish Medallion. Center rot incidence post-harvest was significantly different by cultivar in both 2016 trials and a weak, linear relationship was observed between neck diameter and center rot incidence post-harvest. Neck and bulb diameters were significantly different by cultivar and cv. Aruba had consistently large late-season neck diameters and high center rot incidence and foliar disease severity. Cultivars Spanish Medallion and Expression had comparable neck diameters to standard cv. Candy although they had lower center rot incidence post-harvest and/or lower median foliar disease severity and higher marketable yields and percentage of jumbo and colossal-sized bulbs compared to cv. Candy. The relationship between onion neck diameter and center rot will continue to be explored in future on-farm trials replicated throughout PA.

The identification of onion cultivars less susceptible to center rot than the standard cv. Candy will provide growers with another tool to manage bacterial disease. The use of less susceptible cultivars can also increase farm profitability. The increase in diversity of onion cultivars will complement our already-established diversified vegetable production system in PA. In the future, further cultivar evaluations should be conducted in on-farm trials in multiple locations and additional factors that may influence future breeding efforts, such as epicuticular waxes, can be compared between cultivars. These trials will build upon the current dataset under commercial production conditions with the overall goal of improving management recommendations for both PA Simply Sweet onion growers as well as those in similar onion production areas across the world.

Figures and Tables

Fig. 2.1. Inoculation diagram with locations of high (red, toothpick inoculated), medium (orange), and low (yellow) inoculum pressure sections of each plot.

Table 2.1. Mean neck diameter by cultivar. Five plants were measured per sub-plot. Data was analyzed separately by trials using a one-way ANOVA in Minitab and letters indicate statistically significant differences by Fisher's LSD (α = 0.05).

	Early-Season Neck Diameter (mm)		
Cultivar	Rock Springs, 2015	Rock Springs, 2016	Landisville, 2016
Candy	6.28 a	8.22 a	6.69 abc
Aruba	6.26 a	7.12 b	6.28 bc
Expression	6.25 a	7.46 b	6.81 ab
Great Western	6.25 a	7.53 ab	6.53 abc
Spanish Medallion	6.01 a	7.30 b	6.22 bcd
BGS 280	5.88 a	. .	. .
Red Sky	5.73 a	6.07 c	6.05 cd
Blush	5.65 a	6.86 b	5.59 d
Ovation	5.62 a	. .	. .
Sedona	5.32 a	. .	. .
Lasso	. .	7.46 b	6.27 bc
Crockett	. .	7.14 b	6.95 a
Dulce Reina	. .	6.96 b	6.36 abc
	P=0.530	P≤0.0001	P=0.008

Table 2.1. Continued.

Cultivar	Mid-Season Neck Diameter (mm)					
	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Expression	15.53	a	18.11	ab	14.06	a
Spanish Medallion	15.32	a	17.23	abc	12.01	de
Aruba	15.21	ab	16.83	bc	12.43	cde
Candy	14.72	abc	18.13	ab	13.62	ab
Great Western	14.77	abc	18.37	a	13.18	abc
BGS 280	14.18	abc	.	.	.	.
Ovation	14.30	abc	.	.	.	.
Blush	13.63	bc	17.63	ab	11.31	e
Red Sky	13.26	c	16.24	c	11.64	de
Sedona	13.49	c	.	.	.	.
Lasso	.	.	17.36	abc	12.28	cde
Crockett	.	.	17.84	ab	11.99	de
Dulce Reina	.	.	17.42	abc	12.57	bcd
	P=0.092		P=0.106		P=0.001	

Cultivar	Late-Season Neck Diameter (mm)					
	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Aruba	21.46	a	22.20	b	20.89	ab
Blush	20.86	a	22.70	b	20.82	ab
Ovation	20.72	a	.	.	.	.
Sedona	21.46	a	.	.	.	.
Spanish Medallion	20.55	ab	20.00	c	18.90	c
Candy	18.66	cd	19.82	c	18.64	c
Expression	18.75	cd	19.39	c	18.33	c
Great Western	17.46	d	19.22	c	18.39	c
BGS 280	19.04	bc	.	.	.	.
Red Sky	18.06	cd	19.52	c	18.59	c
Lasso	.	.	21.97	b	20.15	bc
Crockett	.	.	24.28	a	22.18	a
Dulce Reina	.	.	22.39	b	21.30	ab
	P≤0.0001		P≤0.0001		P≤0.0001	

Table 2.2. Mean bulb diameter by cultivar. Five plants were measured per sub-plot. Data was analyzed separately by trials using a one-way ANOVA in Minitab and letters indicate statistically significant differences by Fisher's LSD ($\alpha = 0.05$).

	Early-Season Bulb Diameter (mm)					
Cultivar	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Candy	9.29	a	10.50	a	12.21	abc
Great Western	8.87	a	9.69	ab	12.51	abc
BGS 280	8.35	ab	.	.	.	.
Expression	8.20	ab	9.42	abc	13.25	a
Spanish Medallion	8.13	ab	9.26	bc	12.59	ab
Red Sky	8.07	ab	7.07	e	10.80	cd
Aruba	7.62	ab	8.81	bcd	10.35	de
Blush	7.43	ab	7.92	de	9.02	e
Ovation	6.94	b	.	.	.	.
Sedona	6.50	b	.	.	.	.
Lasso	.	.	8.98	bcd	11.48	bcd
Crockett	.	.	9.04	bc	11.74	abcd
Dulce Reina	.	.	8.49	cd	11.45	bcd
	P=0.147		P≤0.0001		P=0.001	
	Mid-Season Bulb Diameter (mm)					
Cultivar	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Expression	22.87	a	31.71	a	19.79	a
Great Western	21.67	ab	30.77	a	16.95	bc
Candy	20.57	abc	30.07	a	17.73	b
Lasso	.	.	24.38	b	16.06	bc
BGS 280	19.77	abcde	.	.	.	.
Spanish Medallion	19.57	abcde	24.09	b	15.35	cd
Red Sky	16.57	de	20.45	b	13.91	d
Aruba	18.36	bcde	23.65	b	15.94	c
Blush	16.34	de	21.84	b	13.76	d
Ovation	18.04	cde	.	.	.	.
Sedona	15.99	e	.	.	.	.
Crockett	.	.	23.32	b	15.36	cd
Dulce Reina	.	.	23.97	b	15.86	c
	P=0.005		P≤0.0001		P≤0.0001	

Table 2.2. Continued.

	Late-Season Bulb Diameter (mm)					
Cultivar	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Expression	64.73	a	72.19	a	72.19	a
BGS 280	64.60	a	.	.	.	.
Candy	62.81	ab	72.68	a	72.68	a
Great Western	62.58	ab	71.22	a	71.22	a
Ovation	57.47	abc	.	.	.	.
Red Sky	53.31	abcd	58.81	cd	58.81	cd
Spanish Medallion	51.70	bcd	64.85	b	64.85	b
Aruba	47.41	cd	59.64	bcd	59.64	bcd
Blush	48.83	d	53.93	d	53.93	d
Sedona	41.24	d	.	.	.	.
Lasso	.	.	61.95	bc	61.95	bc
Crockett	.	.	54.51	d	54.51	d
Dulce Reina	.	.	54.09	d	54.09	d
	P=0.001		P≤0.0001		P≤0.0001	

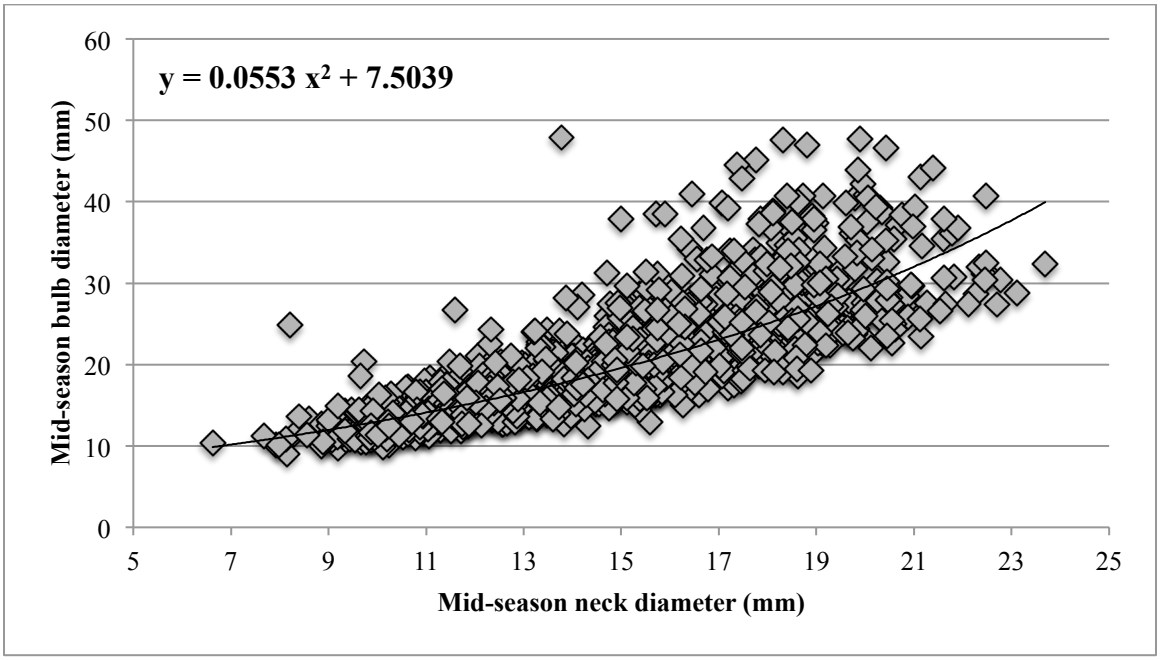

Fig. 2.2. Relationship between mid-season neck and bulb diameter combined data from 2015 and 2016 trials (n=1800). Five plants were measured per sub-plot and each point represents an individual plant ($P \leq 0.001$; R^2=0.6492).

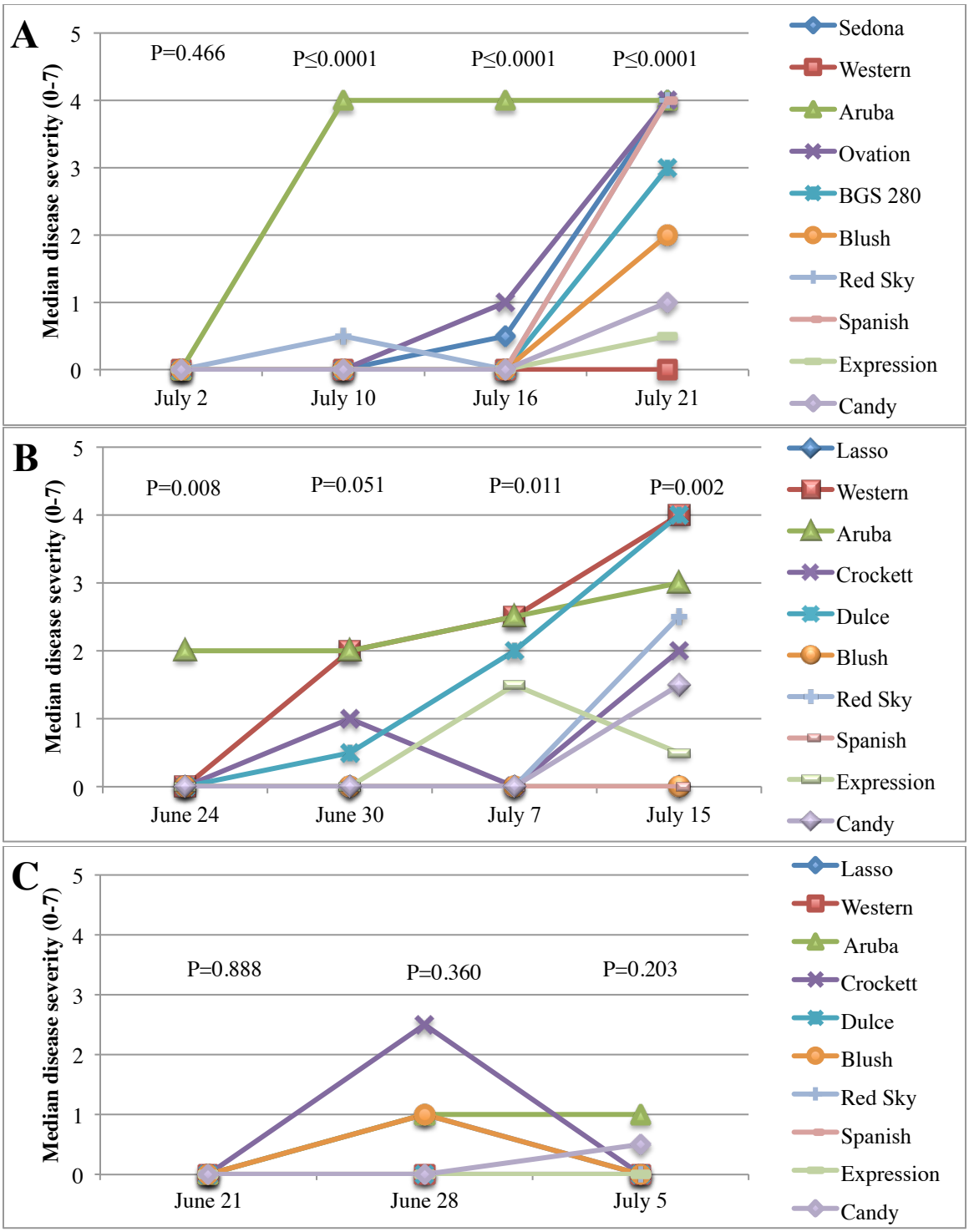

Fig. 2.3. Weekly foliar center rot severity ratings post-inoculation. A: Rock Springs, 2015; B: Rock Springs, 2016; and C: Landisville, 2016. For each treatment, 10 inoculated plants per plot were rated for disease severity using a 0-7 scale (p. 22). Data were analyzed separately for each week within a trial using Kruskal-Wallis in Minitab 17.3 and median values presented for each cultivar.

Table 2.3. Marketable yield of twelve cultivars compared to standard cv. Candy. The total marketable yield is based on mean asymptomatic bulb weight of jumbo and colossal sized-bulbs (≥ 7.6 cm diameter). Data were analyzed using ANOVA and when significant (α = 0.05), means were separated using Tukey's HSD (SAS 9.4).

Cultivar	**Total Marketable Yield (kg / 10 m row)**					
	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Expression	14.8	a	20.6	a	15.1	a
Candy	13.9	ab	18.5	a	10.9	b
Great Western	13.4	abc	20.4	a	12.9	ba
Ovation	12.3	abc	-	-	-	-
Spanish Medallion	11.6	abc	17.2	ab	9.2	bc
BGS 280	10.1	bcd	-	-	-	-
Red Sky	9.2	cde	12.0	dc	6.1	dce
Aruba	6.8	de	11.6	dc	6.5	dc
Blush	5.9	e	8.8	de	2.1	e
Sedona	5.9	e	-	-	-	-
Lasso	-	-	13.8	bc	6.4	dc
Dulce Reina	-	-	10.9	dce	4.7	de
Crockett	-	-	7.1	e	3.4	de
	P ≤ 0.001		P ≤ 0.001		P ≤ 0.001	

Table 2.4. Jumbo- and colossal-sized bulbs by cultivar compared to standard cv. Candy. The percentage of jumbo- and colossal-sized bulbs is out of the total asymptomatic bulbs harvested. Data were analyzed using ANOVA and when significant (α = 0.05), means were separated using Tukey's HSD (SAS 9.4).

Cultivar	**% Jumbo- and colossal- sized bulbs**					
	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
Expression	84.1	a	97.9	a	88.9	a
Candy	79.1	a	96.4	a	76.9	a
Ovation	74.4	ab	.	.	.	.
Great Western	73.4	ab	97.4	a	84.0	a
Spanish Medallion	65.3	abc	93.0	ab	62.1	ab
BGS 280	57.2	abcd	.	.	.	.
Red Sky	47.9	bcd	70.5	dc	46.0	bc
Aruba	42.9	cd	71.3	dc	49.2	bc
Sedona	32.5	d	.	.	.	.
Blush	30.4	d	54.3	fe	17.3	d
Lasso	.	.	80.8	bc	47.3	bc
Dulce Reina	.	.	65.7	de	35.0	dc
Crockett	.	.	43.6	f	23.6	dc
	P ≤ 0.001		P ≤ 0.001		P ≤ 0.001	

Table 2.5. At harvest sugar (%) and pungency (μmoles pyruvic acid/kg) by cultivar compared to the commercial standard cv. Candy. PA Simply Sweet Onion Program criteria are sugar ≥ 6.0% and pungency = 1-4 μmoles pyruvic acid/kg weight of bulbs.

Cultivar	**Sugar (%) and pungency (μmoles pyruvic acid/kg)**					
	Rock Springs, 2015		Rock Springs, 2016		Landisville, 2016	
	Sugar	Pungency	Sugar	Pungency	Sugar	Pungency
Blush	9.3	3.0	9.8	6.7	9.9	6.9
Red Sky	9.2	3.4	9.7	6.0	8.9	7.4
Aruba	8.1	3.7	8.5	6.4	8.3	6.5
Sedona	8.0	3.5	.	.	.	.
Candy	7.9	3.2	8.3	4.5	8.6	6.9
Expression	7.0	3.4	7.6	5.3	7.7	5.1
BGS 280	7.0	2.9	.	.	.	.
Ovation	6.7	3.5	.	.	.	.
Spanish Medallion	6.6	3.2	7.5	5.9	7.6	5.6
Great Western	6.2	3.1	9.3	7.0	7.3	6.2
Lasso	.	.	7.9	5.7	8.4	7.1
Dulce Reina	.	.	7.6	6.0	8.0	7.4
Crockett	.	.	9.9	5.3	9.3	5.8

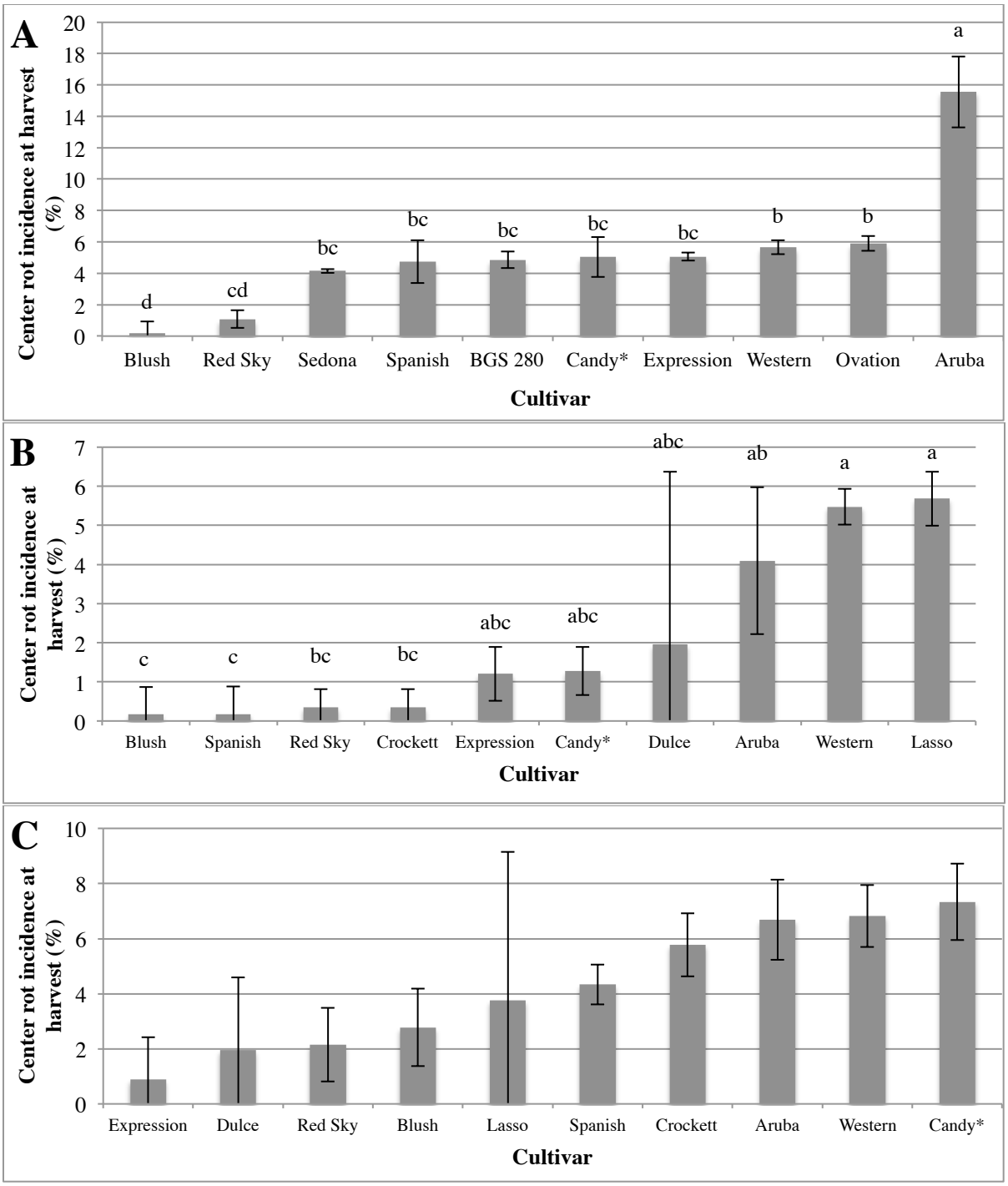

Fig. 2.4. Center rot incidence at harvest (%) by cultivar grown. A: Rock Springs, 2015 (P=0.001); B: Rock Springs, 2016 (P=0.055); C: Landisville, 2016 (P=0.621). Center rot incidence was the sum of center rot per replicate plot (n=40). * Identifies grower standard cv. Candy. Data plot data were analyzed using a one-way ANOVA in Minitab 17.3, error bars represent the standard deviation of the mean and letters above each bar indicate statistically significant differences by Fisher's LSD ($\alpha = 0.05$). Data were square-root transformed prior to analysis and figure contains de-transformed values.

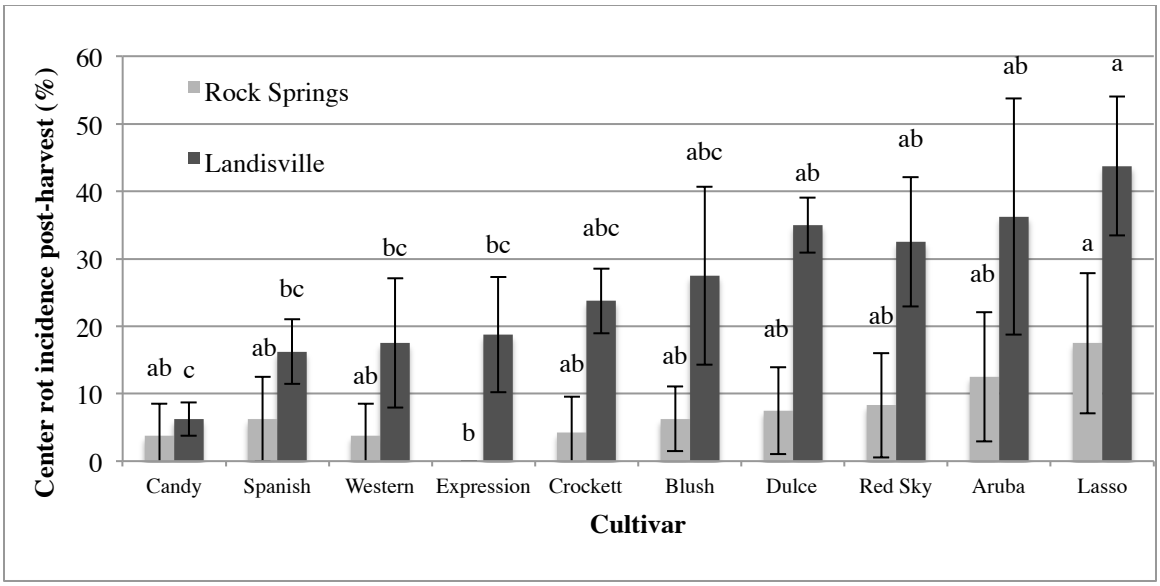

Fig. 2.5. Center rot incidence post-harvest by cultivar grown, from Rock Springs and Landisville, 2016 trials. Data were analyzed using a one-way ANOVA in Minitab 17.3, error bars represent the standard deviation of the mean and letters above each bar indicate statistically significant differences by Tukey's HSD ($\alpha = 0.05$). Analysis was performed separately for each trial ($P \leq 0.001$ for Landisville and P=0.047 for Rock Springs).

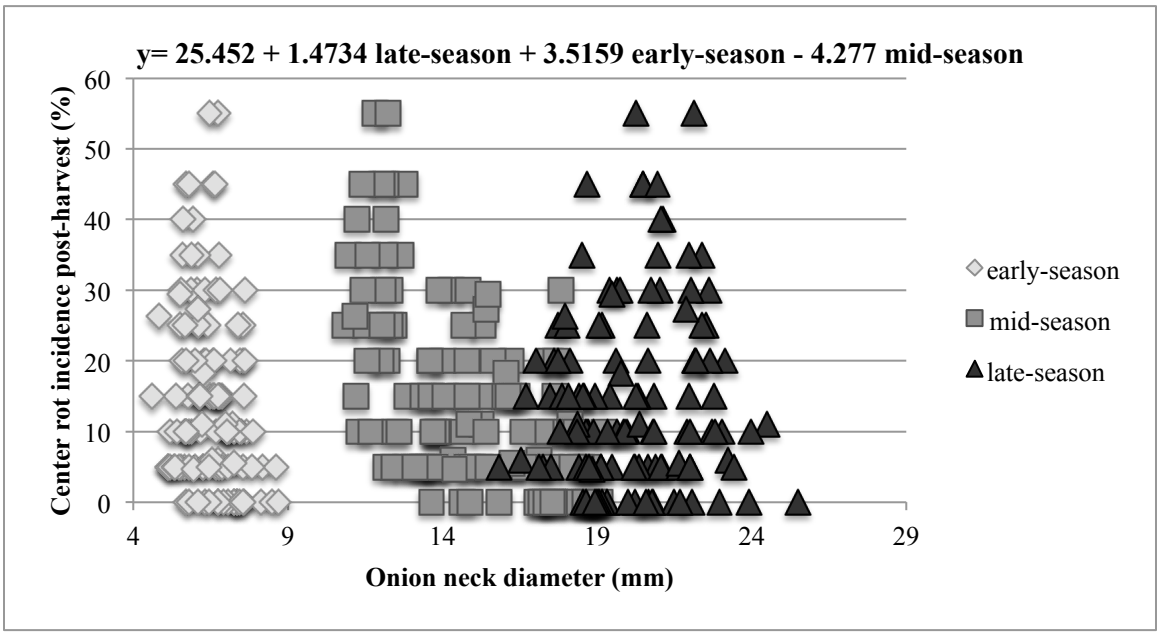

Fig. 2.6. Relationship between the predictor variables early-, mid- and late-season neck diameter (P=0.0195, $P \leq 0.001$, P=0.0054, respectively; R^2=0.3993) and the dependent variable center rot incidence post-harvest, combined data from 2015 and 2016 trials (n=120). Five plants were measured per sub-plot, averaged and analyzed as the mean value per whole plot. Center rot incidence is the percentage of total symptomatic bulbs post-harvest. Each point represents one treatment replicate per trial.

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CHAPTER 3: OPTIMIZING THE TIMING AND RATE OF NITROGEN APPLICATION TO REDUCE ONION CENTER ROT LOSSES IN PENNSYLVANIA

Abstract

Center rot disease of onion, caused by *Pantoea ananatis* and *P. agglomerans*, can cause losses up to 50% in Pennsylvania fields. Preliminary findings indicated that applying total crop nitrogen (N) prior to bulbing increased total yield and reduced bacterial disease incidence. The objective of this research was to evaluate the relationship between timing and rate of N application with disease incidence and marketable yield. In 2015 and 2016, three randomized complete block split-split plot field trials were conducted in Centre and Lancaster Co., to evaluate the weekly fertigation of urea ammonium nitrate at four rates (0, 56, 117 and 179 kg N/ha) with applications made either half-season before bulbing or full-season (whole plot). A subset of plants in each plot was inoculated with a mix of *P. ananatis* and *P. agglomerans* isolates as the sub-sub plot (inoculation proximity). Foliar N content, marketable yield, disease incidence and severity were evaluated. In 2016, inoculation proximity was highly significant in determining center rot incidence ($P \leq 0.0001$ for both trials) and there was a significant interaction between rate and timing of N application ($P=0.0417$; $P=0.0376$). In 2015, only N rate significantly affected disease incidence ($P \leq 0.0001$). A positive, quadratic relationship was found between foliar % N levels at bulbing and center rot incidence at harvest ($P \leq 0.0001$; $R^2=0.522$). In all three trials, marketable yield was not significantly different between the three N rates, excluding the control, and the recommended rate of 117 kg N/ha had the highest numerical marketable yield. The results of this research will be used to identify the optimum rate and timing of N application to reduce center rot losses and thus provide growers with higher precision disease management.

Introduction

Nitrogen (N) fertility is a major component of any crop fertility program and takes into account the appropriate amount of fertilizer to be applied based on plant requirements over time. The application of nutrients via synthetic fertilizers can be manipulated rather easily to mitigate biotic stresses, however, a well-defined or optimized N fertility program is an underutilized tool that can be used to manage disease (Huber and Wilhelm, 1988). The manipulation of fertilizer has a direct effect on plant growth and composition and can induce secondary and cascading effects on plant resistance and tolerance to pathogens. There are many ways in which plant nutrient supply or concentration can affect plant disease, including the alteration of plant growth and tissue composition, which can alter a host's pathogen susceptibility (Marschner, 2012). Depending on the pathogen and nutrient, adjusting nutrient supply may increase or decrease susceptibility to pathogen infection.

Nitrogen is the element needed in largest quantities by plants, second only to carbon. About 1 to 5% of total plant dry matter is made up of N (Marschner, 2012). Nitrogen is used by the plant to make proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. On earth, nitrogen is most abundant in the atmosphere as N_2, where it is unavailable for plants to uptake, with the exception of plants that are capable of forming symbiosis with N_2-fixing bacteria (Marschner, 2012). The forms of N most commonly taken up by plants are nitrate (NO_3^-) and ammonium (NH_4^+). Nitrate is usually present in higher concentrations (1-5 mM) and is more mobile in agricultural soil than ammonium (20-200uM) (Miller and Cramer, 2004; Owen and Jones, 2001). Generally, only 40-50% of the N fertilizer applied to crops is taken up by the crop, while the remaining N is lost to environmental factors or competition with biotic organisms (Sylvester-Bradley and Kindred, 2009).

Results may be inconsistent when evaluating N influence on disease development due to the supply of nutrients (e.g. low, optimal, excessive), form of N supplied (e.g. NO_3^- or NH_4^+, which are metabolized differently), or difference in pathogen nutrient

acquisition between obligate and facultative parasites (Hoffland et al., 2000; Kivilanan and Scheffer, 1958). For example, the susceptibility of tomato plants to powdery mildew caused by *Oidium lycopersicum*, an obligate parasite, increases as soil N application rates increase, whereas the susceptibility to Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopercisi*, a facultative parasite decreases with N supply (Hoffland et al., 2000). These differences are influenced by how the parasite obtains its nutrition. Obligate parasites assimilate nutrients supplied by living cells, whereas facultative parasites assimilate senescing tissue or release toxins that damage host cells before assimilation (Marschner, 2012).

Nitrogen fertility can play a role in bacterial diseases caused by facultative parasites, and can be divided into three types: leaf spot diseases, soft rots, and vascular diseases (Grossman, 1976). Bacterial leaf spot pathogens (e.g. *Xanthomonas oryzae*) usually enter the plant via the stomata and spread and multiply in intercellular spaces. The nutritional status of the host influences the spread and multiplication of bacteria and disease severity can increase with N deficiency (Huber and Thompson, 2007).

Pantoea ananatis Serrano and *Pantoea agglomerans* Beijerinck are facultative parasites and the causal agents of center rot. Due to their high incidence in Pennsylvania (PA) (Pfeufer, 2014), they are the focus of this research. Center rot is considered a devastating onion disease because the symptoms may not be readily visible at the time of harvest when it is easiest to cull symptomatic bulbs. Infected bulbs can be identified by a soft, discolored ring(s) in the neck. As the plants reach maturity, this symptom can be difficult to observe due to natural lodging and drying of the necks in the field prior to harvest. Center rot pathogens are thought to initially enter the leaf through stomata or wounds. As these lesions enlarge, the tissue often becomes bleached white in color. Infected leaves can become fully necrotic and collapse. As the pathogen spreads, multiple leaves may become necrotic and/or wilt. It is thought that once the infected leaf is lodged, the pathogen moves from the leaf to the bulb, where it can infect one or more scales (Carr et al., 2013).

Growers can contract with commercial fertilizer companies or consultants for fertigation programs, spending as much as $1600 per ha on crop fertility (J. Stoltzfus, pers. comm.; Pfeufer, 2014). However, data on the contribution of these fertility programs to increasing overall marketable yield is lacking. Gamiely et al. (1991) found that onions grown in a modified hydroponic system and supplied solely with NH_4 had reduced plant canopy, early onset of bulbing, reduced water usage, and low bulb weight. However, Pfeufer (2014) found a positive relationship between NH_4 in the soil and midseason foliar N of onion as well as the silt content of the soil in field grown onions. Although different N sources may play a role in critical N concentrations in the plant (Westerveld et al., 2003a; 2003b), no significant differences between N sources were found to influence overall yield (Pfeufer, 2014).

It was previously thought that a high N fertility program may increase bacterial disease in onion (Diaz- Perez et al., 2002; Mohan, 2008; Gitaitis et al., 2008; Hoepting et al., 2012; Pfeufer, 2014). Observations in small-plot, on-farm trials in PA found that onions growing in heavily manured soils at the bottom of a slope had up to 83% bacterial bulb decay at harvest (Hoepting, 2012). Also, bacterial bulb decay has been shown to be 1.5 times higher with high rates of N (100 kg/ha) compared to low rates (0 to 50 kg/ha) (Hoepting, 2012). However, preliminary studies in 54 onion fields in PA and New York (NY) suggested a strong negative relationship between foliar N at midseason and total incidence of onion bacterial rot at harvest and after storage (Pfeufer, 2014). Also, a weak negative relationship between soil NH_4 after transplanting and bacterial rot incidence at harvest has been observed in preliminary replicated field trials (Pfeufer, 2014). These findings suggest that the timing and rate of N application may play a role in bacterial disease development in onion.

In PA some growers fertilize (via drip irrigation) their onions weekly up until the summer solstice (bulb initiation), and then apply water without fertilizer from bulbing to harvest while others will chose to fertilize throughout the entire growing season. It is not yet known whether the timing or duration of fertilization (full-season or half-season) has an effect on the incidence of center rot of onion. Brewster and Butler (1989) found that

reduced yield and delayed bulb ripening only occurred when onions were under fertilized early in the season. Onions fertilized early in the season with N fertilizer, followed by an N reduction late in the season had no effect on marketable yields. Westerveld et al. (2003a) reported higher onion yields when evenly splitting N fertility concentration pre-plant and in-season. Wright (1993) found that onions fertilized late season had higher incidence of storage loss due to bacterial diseases.

Objectives and Hypotheses

The goal of this research is to identify the optimum timing and rate of N application to reduce potential center rot losses at harvest, and thus provide growers with higher precision disease management. Preliminary data suggested that applying total crop N prior to bulbing increased total yield and reduced bacterial disease incidence (Pfeufer, 2014). Also, low lying areas in heavily manured fields used for onion production have had up to 83% bacterial disease incidence in the bulbs at harvest (Hoepting et al., 2012). Based on this data, we hypothesize that N fertility affects center rot incidence and that a half-season reduced-rate N application will reduce center rot incidence and thus increase marketable yield. Our objective was to evaluate this relationship in more detail by evaluating the effect of four different rates of N applied either half-season (up until bulbing) or full season (until harvest), on center rot incidence at harvest.

Materials and Methods

Source of plant material

A total of three field trials were conducted to evaluate the effect of nitrogen application timing and rate on marketable yield and center rot incidence. Bare-root, onion transplants, cv. Candy (Dixondale Farms, Carrizo Springs, TX in 2015 and Sunbelt Transplant Inc., Buckeye, AZ in 2016), were planted at the Penn State Russell E. Larson Agricultural Research Center in PA Furnace, PA (Rock Springs) on May 4, 2015 and

April 19 (rep 1) and 20 (reps 2-4), 2016 and the Penn State Southeast Agricultural Research and Extension Center in Manheim, PA (Landisville) on April 13, 2016.

Field preparation, maintenance, and experimental design

Based on a soil test and following standard commercial production practices, soil nutrient levels other than N were adjusted according to commercial crop recommendations prior to planting based on a composite soil nutrient test (Penn State Agricultural Analytical Services Lab, University Park, PA). Phosphorus was applied at 56.05 kg P_2O_5/ha at Landisville, 249 kg P_2O_5/ha at Rock Springs in 2015 and 392.35 kg P_2O_5/ha at Rock Springs in 2016. Potassium was adjusted by adding 235.41 kg K_2O/ha at Rock Springs in 2016. Gypsum (21% calcium and 16% sulfur) was also added to the 2016 Rock Springs field at 896.8 kg/ha. Onions were grown using standard black plastic mulch with a double row of drip irrigation. Field trial plots were established on the native Hagerstown silt loam soil. The week of planting, composite soil samples consisting of approximately 15 cores were collected from each trial across the entire field using a soil probe 2.54 cm in diameter to a depth of 15.2 cm and homogenized by hand. Samples were stored at -20°C until analyzed by the Penn State Agricultural Analytical Services Lab for total % N combustion.

Liquid urea ammonium nitrate fertilizer (UAN, 30-0-0, sourced from Growmark, Pleasant Gap, PA) amended with Agrotain® Ultra 1.67 mL/L (1 qt /150 gal H_2O, Koch Agronomic Services, Wichita, KS), nitrogen stabilizer, was applied weekly through a fertigation system. The whole plot was the timing of N application either half-season (six week duration until bulbing) or full season (10 week duration until harvest). Within each whole plot, all N rate treatments (sub-plot) were arranged in a randomized complete block design and replicated four times. The N rate treatments consisted of the following: 1) high rate of 179 kg N/ha (160 lb/A), 2) recommended commercial production rate of 117 kg N/ha (105 lb/A), 3) reduced rate of 56 kg N/ha (50 lb/A), and 4) control (H_20). A subset of plants in each sub-plot was inoculated with a mix of *P. ananatis* and *P. agglomerans* isolates and included as the sub-sub plot (inoculation proximity). Each sub-

plot was 12.2 m (40 ft) long and approximately 1 m (3 ft) wide across the top of the raised bed. Onions were planted four rows wide at the standard 15.2 cm (6 in.) spacing within and across the rows. Two soil temperature sensors (HOBO Pendant Temperature Data Logger; Onset, Pocasset, MA) were placed in two of the four reps at a 15 cm soil depth beneath the plastic mulch. In 2015, a Dosatron® (Clearwater, FL) set to 64:1 was used to apply N treatments diluted in 3.8 L H_2O into the drip irrigation system. In 2016, a modified fertigation system was used to apply the N treatments diluted in 1.9 L H_2O starting the second week of fertilization (Fig. 3.1). Pressure was maintained between 12-15 psi while applying treatments. Orifices were plumbed into the system after week three to reduce the flow rate of the fertilizer and ensure even distribution of fertilizer. Each treatment plot could be individually controlled through the use of multiple rows of head tape and shut-off valves.

Weeds were managed using the pre-emergent herbicides Prefar 4-E (13.98 L/ha, Gowan, Yuma, AZ), GoalTender (0.584-1.167 L/ha, Dow AgroSciences, Indianapolis, IN) and Dual Magnum (1.167 L/ha, Syngenta Crop Protection, Greensboro, NC) between rows. A rye cover crop was planted in drive rows, and hand weeding was performed within the bed. Insects were managed using Radiant SC (0.584-0.730 L/ha, Dow AgroSciences, Indianapolis, IN), Assail 30SG (0.42-0.56 kg/ha, United Phosphorus, Inc. King of Prussia, PA), Diazinon AG500 (0.02 mL/plant, Helena Chemical Company, Collierville, TN) and/or Warrior 1EC (0.219 L/ha, Syngenta Crop Protection, Greensboro, NC) and fungal diseases were managed using Fontelis (44.36 mL/305 m row through drip line and 1.168 L/ha, DuPont Crop Protection, Wilmington, DE), Endura (0.455 L/ha, BASF Corporation, Research Triangle Park, NC), Quadris F (0.584- 0.876 L/ha, Syngenta Crop Protection, Greensboro, NC) and/or Bravo Weather Stik (2.33 L/ha, Syngenta Crop Protection, Greensboro, NC) as necessary.

Horticultural characteristic assessment

Representative onion plants from each treatment plot were destructively harvested, comingled and sent to the Penn State Agricultural Analytics Lab for total % N

combustion analysis of leaf tissue in the three trials as follows. In 2015, three representative onion plants were sampled separately from each sub-sub plot totaling 15 onion plants per sub-plot and in 2016, three representative onion plants were sampled from the uninoculated sub-sub plot sections only totaling three onion plants per sub-plot. These samples were collected at five time points in 2015 (June 3, June 16, July 1, July 15 and July 27) and four time points in 2016 (May 11, June 2, June 21 and July 11 at Landisville; May 16, June 7, June 29 and July 19 at Rock Springs) to represent in early-, mid-season, bulb initiation and late-season foliar N content. Samples collected in 2016 at the final time point from rep 3 (16 samples total for each trial) had an additional acid digestion analyses performed for P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, Na, and S nutrients. Dry weights were recorded for all foliar samples and fresh weights were recorded for the 2015 samples only. At three points in the season (June 16, July 1 and July 15 at Rock Springs in 2015; May 18, June 2 and June 21 at Landisville in 2016; May 25, June 7 and June 29 at Rock Springs in 2016) horticultural measurements including bulb diameter (mm) at its widest point (2015 and 2016) and neck diameter (mm) mid-way between the apical meristem and bulb (2015 and 2016) were recorded for five representative onions from each sub-sub plot totaling 15 onion plants per replicate plot.

Inoculation

Onions were inoculated using the same parameters described in chapter two (p. 21). The final inoculum concentration was 2.58 X 10^9 CFU/mL in 2015. On June 24, 2015 onions were inoculated by puncturing one mature leaf approximately 5 cm from the leaf whorl with a sterile toothpick dipped into inoculum. When symptoms did not appear within 1.5 weeks, a second leaf was inoculated with 1.92 X 10^{10} CFU/mL using the same parameters. In 2016, onions were inoculated following the same procedure previously described, this time inoculating two leaves on June 14 at Rock Springs and June 15 at Landisville with a 6.18 X 10^8 CFU/mL mixture of the same six isolates.

Foliar disease assessment

Beginning in early to mid-June, 10 onion plants per sub-sub plot were scouted weekly for disease severity, which was rated on a 0 to 7 scale (Chapter 2, p. 22). Prior to harvest, plots at Landisville were rated a total of three times while those at Rock Springs were rated five and four times in 2015 and 2016, respectively.

Harvest evaluation

Rock Springs field trials were harvested on July 28, 2015 and July 20, 2016 and the Landisville field trial was harvested on July 12, 2016 totaling a three-month field season. At harvest, plots were harvested individually and separated by the split-split plot design. The number and total weight of bulbs with center rot (onions with symptomatic neck scales) and surface rot (symptomatic onion bulbs with soft scales on the outside of the bulb) symptoms were recorded, and marketable and unmarketable bulbs were graded by size: small, < 6.4 cm in diameter; medium, 6.4 – 7.6 cm; jumbo, 7.6 – 10.2 cm; colossal, > 10.2 cm.

Data analysis

Data were analyzed using the one-way analysis of variance procedure in Minitab 17.2 (Minitab Inc., State College, PA). ANOVA with two or more factors were analyzed in SAS 9.4 (SAS Institute, Cary, NC). Center rot incidence was square root transformed in ANOVA to satisfy the assumption of normality. Post-hoc comparisons were completed using Tukey's HSD, $\alpha = 0.05$. Using the General Linear Model procedure, fertilizer timing, fertilizer rate, inoculation proximity and block (replicate) were input as class variables and block was labeled as random. Comparisons of severity distributions were conducted using the Kruskal-Wallis test in Minitab. Multiple linear regression was performed using the stepwise addition procedure with center rot incidence at harvest (%) analyzed as the dependent variable, which was the total split-plot incidence.

Results

Horticultural characteristic assessment

Total soil % N was 0.10 at Rock Springs in 2015, 0.12 at Rock Springs in 2016 and 0.11 at Landisville in 2016. When comparing destructively harvested leaf dry weights of uninoculated onions at the time of harvest (last sampling), Landisville had significantly higher dry weights, subsequently followed by 2016 Rock Springs and 2015 Rock Springs ($P \leq 0.0001$). Foliar dry weight was significantly different by N rate ($P \leq 0.0001$ for 2015 and 2016 Rock Springs; and $P=0.0228$ for Landisville), full season treatments were significantly higher than half season in 2015 ($P \leq 0.0001$), and a significant interaction existed between rate and time in 2015 ($P=0.0061$). In this analysis, the 117 kg N/ha rate had significantly higher dry weights than all other rates at Landisville in 2016 and although not significant, this N rate had the highest numerical dry weight and fresh weight at Rock Springs in 2015. Also, inoculation proximity was not a significant factor determining dry weight ($P=0.6435$) or fresh weight ($P=0.5926$) in 2015. Fresh weight data from 2015 revealed similar findings in which N rate was significant ($P \leq 0.0001$), full season treatments were significantly higher than half season treatments ($P \leq 0.0001$) and a significant interaction existed between rate and time ($P=0.0026$).

Neck and bulb diameters were consistently influenced by N rate at all evaluation dates ($P \leq 0.05$) in all three trials, except for at the first evaluation date in Landisville and in 2016 Rock Springs early-season neck diameter only. A significant interaction ($P \leq 0.05$) between rate and time occurred for the mid- and late season neck and bulb diameters in the 2015 Rock Springs trial as well as in 2016 Landisville trial except for the late season neck diameter. Also the 56, 117 and 179 kg N/ha rates did not significantly affect late-season neck or bulb diameters.

The complete nutrient analysis of 2016 leaf samples taken at harvest was similar for both Landisville and Rock Springs samples and nutrient values across treatments were

similar. Comparisons of nutrient analysis results with the reference sufficiency ranges (Penn State Agricultural Analytical Services Lab, University Park, PA) for onion tissue analysis are presented in Table 3.5. One noteworthy difference between trials was the high level of sodium in the onion foliar tissues at Landisville (144-418 mg/kg) compared to Rock Springs (31-48 mg/kg).

Foliar disease assessment

Median foliar disease assessment ratings ranged between 0 and 4 depending on the N rate in Landisville and the N application rate was significant on all disease assessment dates ($P=0.004$; $P=0.009$; $P\leq0.0001$). Foliar disease assessment at harvest was significant by inoculation proximity (sub-sub plot) in all three trials ($P\leq0.0001$ for Landisville and 2016 Rock Springs; $P=0.042$ for 2015 Rock Springs). Within each N application rate at Landisville, median disease severity ratings decreased as distance from the inoculated sub-sub plots increased. All rates had median ratings of 5 and 6 for inoculated sub-sub plots, all rates except for the control had median ratings of 4 for adjacent sub-sub plots and the 117 and 179 kg N/ha had median ratings of 4 for the uninoculated sub-sub plots (Fig. 3.2). A similar rate affect was present in the inoculated sub-sub plots of the 2016 Rock Springs trial where the 117 kg N/ha had a median severity of 3 and rate 179 kg N/ha had a median severity of 4 by the final rating assessment date. Within nitrogen rate, inoculation proximity did not affect the median foliar disease severity rating in the 2015 Rock Springs trial.

Center rot incidence at harvest

Center rot incidence in sub-sub plots (inoculation proximity) at harvest ranged from 0-11.1% in 2016 Rock Springs, 0-22.8% in 2015 Rock Springs and 4.8-72.6% in Landisville trials. Surface rot incidence in sub-sub plots at harvest ranged from 0-5.3% in 2016 Rock Springs, 0-22.1% in 2016 Landisville and 0-4.7% in 2015 Rock Springs trials. Center rot incidence at harvest was significantly different by field trial location ($P\leq0.0001$) with Landisville having significantly higher disease incidence than both Rock

Springs trials. In 2016, inoculation proximity was highly significant in determining center rot incidence and there was a significant interaction between N rate and timing of N application (Fig. 3.4; Table 3.2; Fig. 3.5; Table 3.3). It is important to note that Landisville data were subject to a blocking effect such that the area of the field with greater weed pressure (block 4) was significantly different from blocks 1, 2 and 3. However, whenever block 4 was removed from the data analysis, the significant interaction between N rate and application timing still existed (P=0.0228). In 2015, only N rate significantly affected center rot incidence (Fig. 3.3; Table 3.4). The 2015 Rock Springs data was subject to a blocking effect in which block 2 was significantly different from blocks 1, 3 and 4. When block 2 was removed and the data re-analysized, rate was still significant (P≤0.0001). When sub-plots were split by inoculation proximity, the significant interaction between rate and time persisted when adjacent and uninoculated sub-sub plots were pooled (P=0.0004 for 2016 Rock Springs and P=0.0019 for Landisville). However, this interaction did not exist when evaluating the inoculated sub-sub plots in either trial and only rate was significant in the Rock Springs 2016 trial (P=0.046).

Foliar % N significantly differed by N rate and field trial location; with the highest N rate (179 kg N/ha) and the Landisville trial location having the highest mean foliar % N values (P≤0.0001 for both). When evaluating foliar % N data separately for each trial (n=32), both Rock Springs trials had significant, positive relationships with % center rot at harvest, while no significant relationship was observed at Landisville. The foliar % N sampling taken at bulb initiation was the only significant factor predicting center rot at harvest in the 2016 Rock Springs trial (P=0.0007; R^2= 0.3224) while the early-season foliar % N sampling was the only significant factor predicting center rot at harvest in the 2015 Rock Springs trial (P=0.0002; R^2= 0.3681).

Foliar % N data collected from 2015 Rock Springs at sampling two (week 6) and three (week 8) were averaged and included in the analysis as an approximation for the mid-season sampling date collected at week 7 in the 2016 trials. When foliar % N sampling data from all three trials were collectively analyzed (n=96), only bulb initiation

foliar % N sampling (week ten) was a significant factor predicting center rot at harvest ($P \leq 0.0001$; $R^2 = 0.5046$). This relationship slightly improved when analyzed as a quadratic term ($P \leq 0.0001$; $R^2 = 0.5225$; Fig. 3.6). When dry weight data was included in this analysis, the relationship slightly improved and mid-season foliar % N ($P \leq 0.0001$), bulb initiation foliar % N ($P = 0.0078$) and bulb initiation dry weights ($P = 0.002$) were significant factors predicting center rot at harvest (Model $P \leq 0.0001$; $R^2 = 0.6114$). When neck diameter and bulb diameter measurements from uninoculated sub-sub plots were added to the analysis, the mid-season and bulb initiation foliar % N samplings remained in the model but the bulb initiation dry weight variable was removed and the late-season neck and bulb diameter ($P = 0.0019$ and $P = 0.0077$ respectively) and mid-season bulb diameter ($P = 0.0331$) variables were added (Model $P \leq 0.0001$; $R^2 = 0.6885$). Adding interaction terms to these models did not improve the relationship (data not shown). No multicollinearity was indicated among sampling mid-season foliar % N, bulb initiation foliar % N, bulb initiation dry weight, late-season neck diameter and mid- and late-season bulb diameter variables (variance inflation factors [VIFs] < 1.5; data not shown).

Marketable yield

When marketable yield data was pooled across inoculation proximity (sub-sub plots), marketable weight of jumbo and colossal sized bulbs ranged from 21.8 to 73.7 kg for 2016 Rock Springs, 24.8 to 40.5 kg for 2016 Landisville and 0.2 to 39.8 kg/18 m row length harvested at 2015 Rock Springs trials (Table 3.1). In all three trials, total marketable yield was not significantly different between the three N rates, excluding the control, and the reduced rate of 117 kg N/ha had the highest numerical marketable yield. When marketable yield data was split by inoculation proximity, N application timing was significant in the 2015 Rock Springs trial ($P = 0.0166$), nearly significant at Landisville ($P = 0.0958$), and a significant interaction occurred between rate and time ($P = 0.0112$ for Landisville and $P = 0.0281$ for 2015 Rock Springs). Marketable yield was significantly lower in the inoculated sub-sub plots than adjacent and uninoculated sub-sub plots at Landisville ($P \leq 0.0001$) but not Rock Springs ($P = 0.3765$ for 2016 and $P = 0.2030$ for 2015).

Discussion

Two separate inoculations were required in 2015 to establish center rot disease. When 2015 onions were first inoculated, the following week's nighttime temperatures ranged between 16 and 21 °C. When re-inoculated on July 7, nighttime temperatures ranged between 22 and 24 °C for the week following. This increase in temperature was enough to establish disease in 2015. Unfortunately, this was only three weeks prior to harvest, which did not provide very much time for disease spread, and most likely contributed to low levels of disease pressure.

Overall, the mean foliar % N decreased in all trials over time. The reference values (recommended value) for complete nutrient content in onions are benchmarked for whole tops/shoots (not the whole plant) when onions are 1/3 to ½ mature. Since the complete nutrient analysis was performed at harvest, nutrient levels that are below the reference value may not be considered deficient since the plants have already been reallocating resources (Table 3.5). Nutrient concentrations in the leaf tissue decline as the onion reaches maturity and nutrients are being remobilized and translocated to the bulb (Sullivan et. al., 2011).

In all three trials by the end of the season, neck and bulb diameters did not differ between the treatments receiving nitrogen (control excluded). Furthermore, the plants grown under the Penn State recommended rate of 117 kg N/ha had higher dry weight and marketable yields at harvest compared to the other N rates, including even the high rate of 179 kg N/ha. Also, marketable yields were not significantly different between the three N rates in all three trials. Therefore, growers may be able to apply N rates as low as 56 kg N/ha and still achieve comparable yields to rates as high as 179 kg N/ha. Similarly, Hoepting and Beer (2012) found that reduced rates of 50 kg N/ha (45 lb N/A) produced comparable marketable yields to the higher 101 kg N/ha (90 lb N/A) N rate in New York onion fields.

Median center rot disease severity ratings were higher in the plots receiving higher rates of N (Fig. 3.2). Compared to the inoculated sub-sub plots, adjacent and uninoculated plots had lower median foliar disease severities at the lower nitrogen application rate in Landisville (Fig. 3.2) and 2016 Rock Springs trials. This may mean that under high disease pressure, the rate of nitrogen did not have as much of an impact on median foliar disease severity compared when disease pressure was lower as observed in the adjacent and uninoculated sub-sub plots. Based on these findings, manipulating N rate may only be beneficial to growers in low or medium disease pressure situations and these factors may not be as influential under high disease pressure. Therefore, further studies are needed to evaluate the total amount of N and timing of application under high disease pressure to validate these findings.

Surface bulb rot incidence was extremely low in both Rock Springs trials compared to Landisville. The bacterial pathogens causing surface rot have yet to be classified but it is suspected that *Pseudomonas marginalis* pv. *marginalis* Brown and *Pectobacterium carotovorum* subsp. *carotovorum* Jone which cause a soft rot in onion may be associated with outer scale bulb rots based on symptomatic bulb isolations (Mansfield, M., pers. comm). It was observed that the majority of the onion bulbs remained secured under the plastic at harvest in Landisville. Typically as the onion bulbs enlarge they will break through the plastic so that the majority of the bulb is above the plastic and subject to more air circulation. During onion bulb maturation, soil temperatures can be quite high under black plastic and Pfeufer (2014) found a positive relationship between soil temperatures under the plastic mulch within three weeks of harvest and bacterial disease incidence at harvest. When comparing the Landisville soil temperatures during harvest to Rock Springs, Landisville daytime high soil temperatures ranged between 24-33°C, 2015 Rock Springs between 23-29°C, and 2016 Rock Springs between 22-27°C. Perhaps the higher soil temperatures at Landisville may have contributed to the higher surface rot incidence at harvest. This could have also contributed to increased center rot disease incidence at Landisville as well.

An inconsistent interaction between the rate and timing of N application was observed in 2016 between the two site locations. These differences may be attributed to the high disease pressure at Landisville. Although there was no interaction between rate and time in the 2015 Rock Springs trial, rate alone was a significant factor (Fig. 3.3). The N content in the soil was similar for the three locations at the time of planting although the foliar % N levels were highest at Landisville. The high foliar % N levels may also have been contributing to higher center rot incidence observed at Landisville. Bulb initiation foliar % N was the most significant factor predicting center rot at harvest (Fig. 3.6) compared to the other sampling times. This sampling occurred approximately three weeks before harvest during the 7 to 9 leaf stage. This is also the start of bulb initiation when nutrients are being reallocated from the leaves to the bulb and may indicate that bulb initiation is the most critical time point in plant development to make sure N tissue levels are sufficient. This coincides with in-season N fertilizer recommendations which state that onions take up N most efficiently during bulb initiation (Sullivan et. al., 2011).

All significant multiple regression models with center rot incidence as the dependent variable included the bulb initiation (week 10; 7-9 leaf stage) foliar % N as a predictor variable. Since this variable alone explains 52% of the change in center rot at harvest, it was chosen as the best model for this dataset (Fig. 3.6). Although these analyses evaluated center rot incidence at harvest as the dependent variable, the foliar % N data may have a stronger relationship with post-harvest center rot incidence. Perhaps N rate and time would have a delayed affect in storage just as Wright (1993) found that onions fertilized late season had higher incidence of storage loss due to bacterial diseases. Therefore, future studies evaluating the relationship between N fertility and center rot disease should include post-harvest evaluations.

Conclusion

Consistently across all three trials, the marketable yields obtained applying 56 kg N/ha were not significantly different from those when 117 or 179 kg N/ha was applied. This may mean that growers can apply lower rates of N on their onions and still obtain

similar marketable yields. Under lower disease pressure, lower nitrogen application rates led to reduced disease incidence at harvest. However a significant interaction with application timing complicates interpretation of the results. Across multiple datasets, foliar % N, foliar dry weight, neck diameter, and bulb diameter at bulb initiation (7 to 9 leaf stage) had a positive relationship with center rot incidence at harvest. As this is a time of rapid growth for the onion, a balanced N fertility program at this time is an important part of any integrated pest management program.

In this chapter, we report findings that vary by location and warrant further study. With this said, it is important to further investigate the relationship between N rate and application timing on center rot disease incidence at more locations before augmenting current management recommendations for PA onion growers. The current, Penn State recommended rate of 117 kg N/ha had lower center rot foliar disease severity ratings and disease incidence at harvest compared to the highest rate in both Rock Springs trials. Under high disease pressure, center rot severity was not significantly different between N rates but as inoculum pressure decreased in adjacent and uninoculated sub-sub plots of this trial so did center rot disease incidence. Since the findings were variable in terms of location, it is important that growers experiment with N rate and application timing on their own farms. Under medium or low disease pressure situations, reduced, half season rates of N may result in low center rot incidence and thus, increased marketable yields. In the meantime, further experimentation with N rate and time under high disease pressure is warranted and these studies will lead to specific recommendations.

The manipulation of N rate and time has the potential to manage center rot disease, and potentially increase farm profitability. Although beyond the 2-year duration of this project, the relationships between N fertility and center rot incidence identified in this study can be further evaluated to determine why this relationship occurs. This study has been designed for PA onion growers but could be applicable to other onion production systems throughout the world. Through the study of N application, we can provide onion growers with higher precision disease management.

Tables and Figures

Fig. 3.1. Modified fertigation system used in 2016 field trials.

Table 3.1. Marketable yield by rate compared to recommended rate of 117 kg N/ha. Total marketable yield is mean, asymptomatic bulb weight of onions ≥ 7.6 cm diameter as the sum of a split-plot. Data were analyzed using ANOVA in SAS 9.4 and letters indicate statistically significant differences by Tukey's HSD (α = 0.05).

N rate	Total Marketable Yield (kg/18 m row length harvested)		
	Rock Springs, 2015	Rock Springs, 2016	Landisville, 2016
117 kg N/ha	41.6 a	63.5 a	35.1 a
179 kg N/ha	40.9 a	63.5 a	34.1 ab
56 kg N/ha	35.0 ab	56.3 a	32.8 ab
0 kg N/ha	22.6 b	35.2 b	29.9 b
	P = 0.0012	P ≤ 0.0001	P = 0.0503

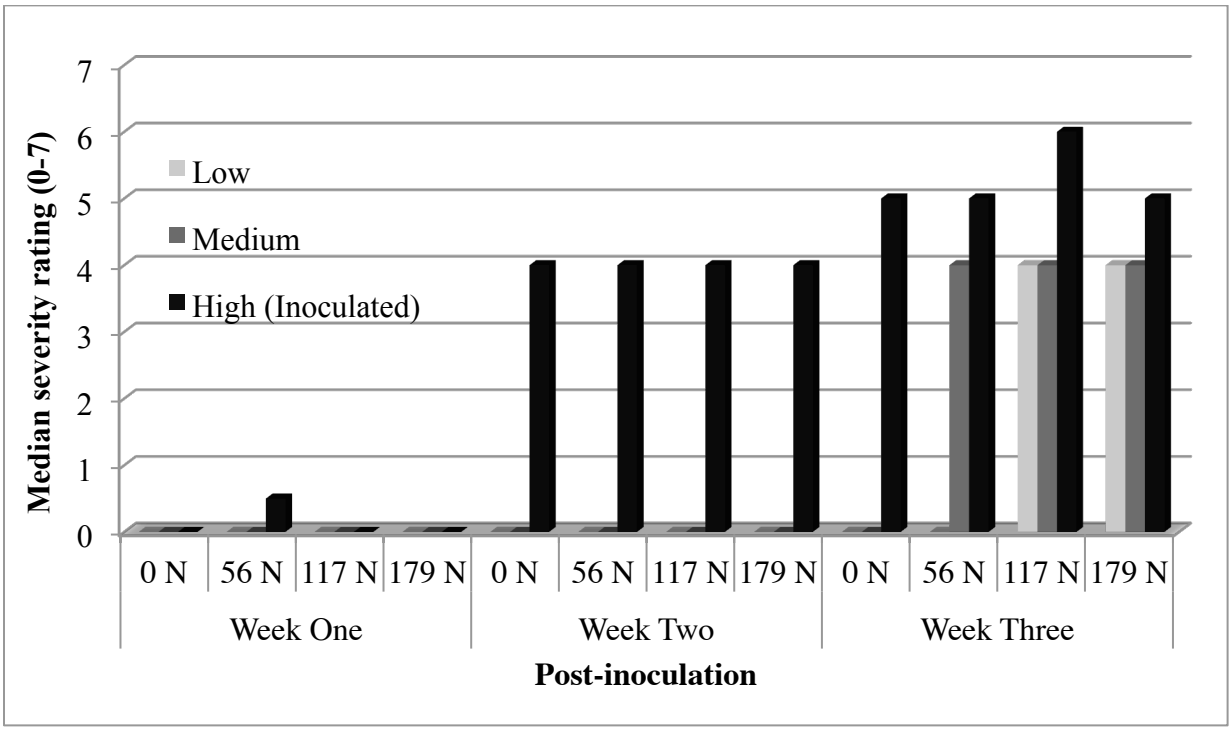

Fig. 3.2. Weekly foliar center rot severity ratings post-inoculation, from Landisville, 2016. For each treatment, 15 inoculated plants per plot were rated for disease severity using a 0-7 scale (Chapter 2, p. 22). Data were analyzed using Kruskal-Wallis in Minitab 17.3 and median values presented for each cultivar. Severity was significantly different ($\alpha = 0.05$) by N rate on week three for high ($P \leq 0.0001$), medium ($P=0.047$) and low ($P=0.004$) disease pressure sub-sub plots, and for high disease pressure sub-sub plots on week two ($P=0.008$) and week one ($P=0.015$).

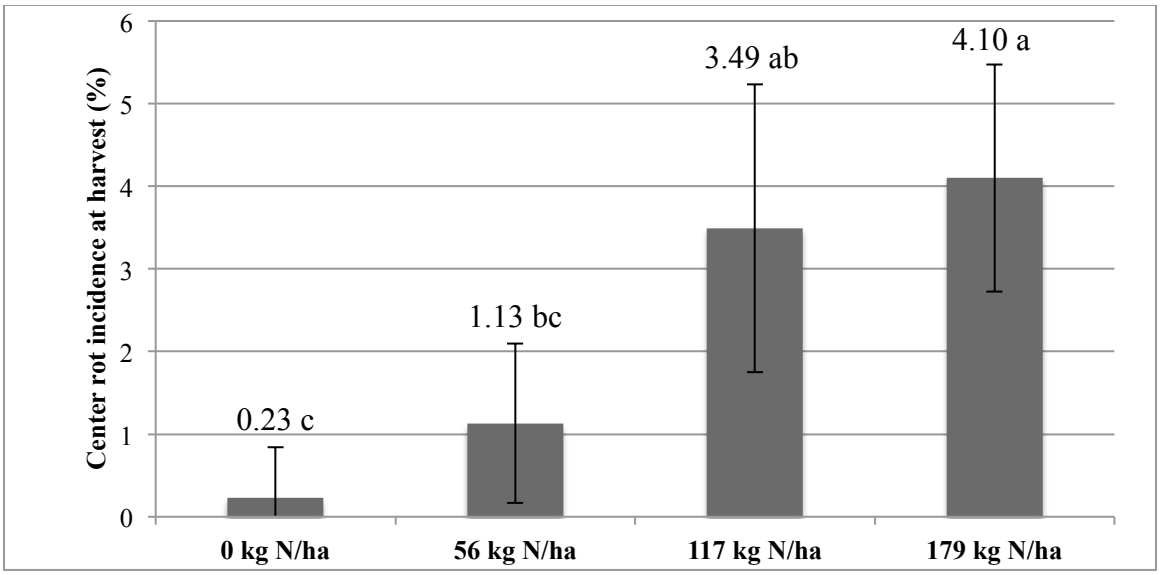

Fig. 3.3. Center rot incidence (%) at harvest by N rate, from Rock Springs, 2015. Data were analyzed using a one-way ANOVA in Minitab 17.3, error bars represent the standard deviation of the mean, and letters above each bar indicate statistically significant differences by Tukey's HSD ($\alpha = 0.05$). Values were square root transformed prior to analysis and figure contains de-transformed values ($P \leq 0.0001$).

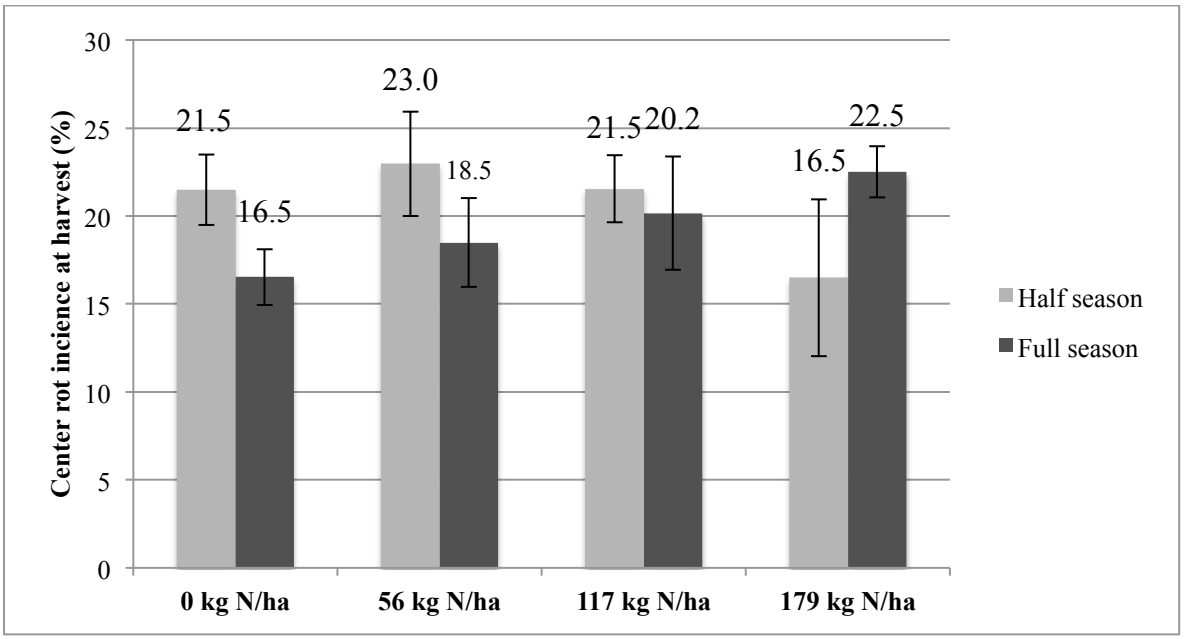

Fig. 3.4. Center rot incidence (%) at harvest by N rate and time, from Landisville, 2016. Data were analyzed using proc glm in SAS 9.4, error bars represent the standard deviation of the mean. Values were square root transformed prior to analysis and figure contains de-transformed values ($P = 0.0417$).

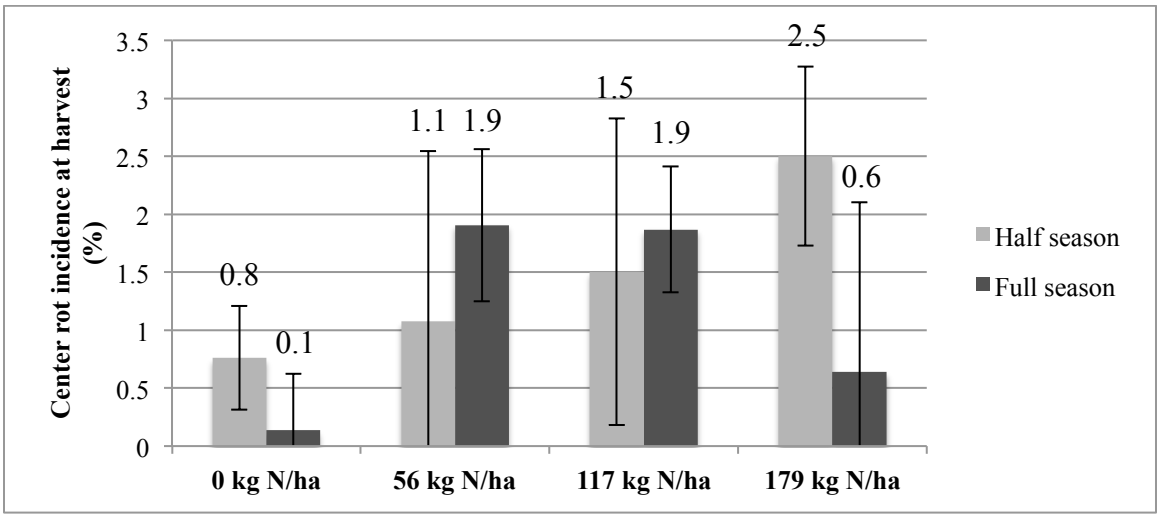

Fig. 3.5. Center rot incidence (%) at harvest by N rate and time, from Rock Springs, 2016. Data were analyzed using proc glm in SAS 9.4, error bars represent the standard deviation of the mean. Values were square root transformed prior to analysis and figure contains de-transformed values (P=0.0376).

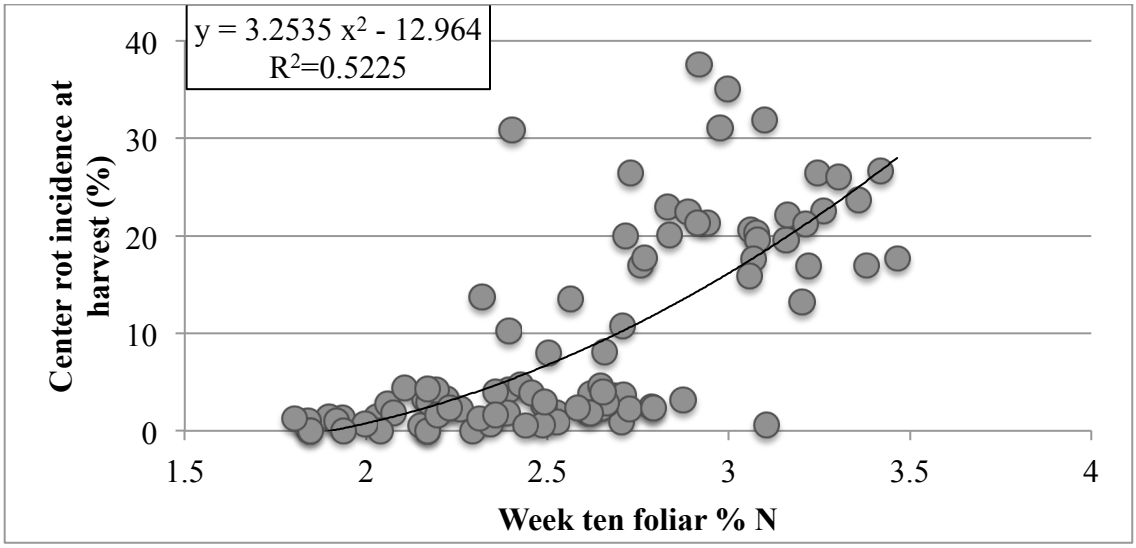

Fig. 3.6. Relationship between week ten (bulb initiation) foliar % N and center rot incidence at harvest, combined data from 2015 and 2016 trials. Three plants were destructively harvested per sub-plot, comingled and analyzed for total N via dry combustion. Center rot incidence is the sum symptomatic bulbs in a split-plot as a percentage of total bulbs harvested per plot. Each point represents one split-plot. (P≤0.0001).

Table 3.2. Center rot incidence at Landisville by factor (n=96). Data was analyzed by sub-sub plot. Values were square root transformed prior to analysis. Using the General Linear Model procedure, fertilizer timing, fertilizer rate, inoculation proximity and block (replicate) were input as class variables and block was labeled as random.

Factor	df	F value	P-value
rate	3	0.36	0.7813
time	1	0.55	0.4597
inoculation proximity	2	107.98	≤0.0001
replicate	3	8.15	0.0001
rate*time	3	2.89	0.0417
rate*inoculation proximity	6	0.77	0.5942
time*inoculation proximity	2	0.90	0.4113
rate*time*inoculation proximity	6	1.21	0.3096

Table 3.3. Center rot incidence at Rock Springs in 2016 by factor (n=96). Data was analyzed by sub-sub plot. Values were square root transformed prior to analysis. Using the General Linear Model procedure, fertilizer timing, fertilizer rate, inoculation proximity and block (replicate) were input as class variables and block was labeled as random.

Factor	df	F value	P-value
rate	3	4.04	0.0105
time	1	1.69	0.1973
inoculation proximity	2	25.42	≤0.0001
replicate	3	0.57	0.6349
rate*time	3	2.97	0.0376
rate*inoculation proximity	6	0.79	0.5815
time*inoculation proximity	2	0.02	0.9826
rate*time*inoculation proximity	6	2.09	0.0652

Table 3.4. Center rot incidence at Rock Springs in 2015 by factor (n=96). Data was analyzed by sub-sub plot. Values were square root transformed prior to analysis. Using the General Linear Model procedure, fertilizer timing, fertilizer rate, inoculation proximity and block (replicate) were input as class variables and block was labeled as random.

Factor	df	F value	P-value
rate	3	15.47	≤0.0001
time	1	2.07	0.1543
inoculation proximity	2	1.04	0.3587
replicate	3	15.54	≤0.0001
rate*time	3	0.73	0.5402
rate*inoculation proximity	6	1.26	0.2868
time*inoculation proximity	2	1.32	0.2728
rate*time*inoculation proximity	6	0.17	0.9838

Table 3.5. Comparisons of nutrient analysis results with the reference sufficiency ranges. Sufficiency ranges are based on recommendations from the Penn State Agricultural Analytical Services Lab, University Park, PA. Three plants were destructively harvested per sub-plot and comingled. Samples from rep 3 in 2016 were subject to acid digestion analyses for P, K, Ca, Mg, Mn, Fe, Cu, B, Al, Zn, Na, and S nutrients.

	Reference sufficiency ranges			2016 field trial results	
	low	normal	high	Landisville	Rock Springs
P (%)	0.25	0.35	0.51	0.17-0.25	0.16-0.60
K (%)	3.50	4.00	5.51	2.17-2.99	1.98-3.09
Ca (%)	0.80	1.00	2.01	1.39-2.06	1.91-2.91
Mg (%)	0.22	0.25	0.41	0.28-0.37	0.27-0.35
S (%)	0.30	0.50	1.01	0.20-0.37	0.35-0.65
Mn (mg/kg)	30	50	251	93.0-136.0	86.0-151.0
Fe (mg/kg)	50	60	301	56.0-115.0	73.0-204.0
Cu (mg/kg)	8	15	36	4.0-6.0	3.0-5.0
B (mg/kg)	18	22	61	15.0-23.0	16.0-22.0
Al (mg/kg)	.	.	.	26.0-78.0	39.0-124.0
Zn (mg/kg)	20	25	101	10.0-15.0	9.0-12.0
Na (mg/kg)	.	.	.	144.0-418.0	31.0-48.0

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CHAPTER 4: EVALUATING TRANSPLANT TREATMENTS IN MANAGING CENTER ROT PATHOGENS COLONIZING THE ONION LEAF SURFACE

Abstract

Onion losses at harvest due to bacterial diseases have been as high as 50% in recent years in Pennsylvania. Pathogenic *Pantoea ananatis* and *P. agglomerans*, the onion center rot pathogens, have been isolated from onion transplants. The goal of this research is to identify potential pre-plant transplant treatments that could potentially be used to reduce *P. ananatis* and *P. agglomerans* populations on the surface of onion transplants and thus provide growers with another management tool. Culture-based *in-vitro* and transplant assays were conducted to evaluate the efficacy of the commercially available products OxiDate (hydrogen dioxide), OxiPhos (hydrogen peroxide with mono- and di-potassium salts of phosphorus acid), FireWall (streptomycin sulfate), MasterCop (copper sulfate pentahydrate) and Actinovate (*Streptomyces lydicus*). *In-vitro* assays evaluated optical density and transplant assays evaluated CFUs/mL using LB and KB medias, respectively. OxiDate, OxiPhos, Firewall and MasterCop all had significantly lower optical densities and CFUs/mL compared to the positive control. Optical density was not significantly different than the positive control when Actinovate was filter sterilized, and both filter-sterilized and non-filter-sterilized Actinovate had significantly higher optical density and CFUs/mL values compared to the negative control. These culture-based assays may have been limiting for the evaluation of Actinovate and further analysis including field evaluations are needed to evaluate product efficacy.

Introduction

Pennsylvania (PA) onion growers are continually challenged by bacterial diseases, despite ongoing management efforts. Bacterial rots of onion bulbs include: sour skin, caused by *Burkholderia cepacia* Burkholder; slippery skin, caused by *Burkholderia gladioli* pv. *allicola* Burkholder; leaf streak, caused by *Pseudomonas viridiflava* Burkholder; leaf blight, caused by *Xanthomonas axonopodis* Kadota; soft rot, caused by

Pseudomonas marginalis pv. *marginalis* Brown and *Pectobacterium carotovorum* subsp. *carotovorum* Jones; and center rot, caused by *Pantoea ananatis* Serrano and *Pantoea agglomerans* Beijerinck (Bull et al., 2010). In addition, symptomatic bulbs are often infected by more than one of these pathogens. Of these pathogens, *P. carotovorum* subsp. *carotovorum*, *P. agglomerans*, and *P. marginalis* pv. *marginalis*, are the most common onion pathogens in PA. Since *P. ananatis* and *P. agglomerans* incidence has recently increased in PA, they can be considered emerging pathogens of onion (Anderson et al., 2004). Center rot is also of particular concern because it often goes unidentified at harvest due to its discrete symptoms. These symptoms include a soft, discolored ring in the neck of the onion, which is often masked by plants that have lodged or the necks have been dried in the field prior to harvest.

Many of these bacterial pathogens, including those that cause center rot, enter through the leaf stomata or wounds. As leaf lesions enlarge, the tissue can become water-soaked, soft and bleached white. Infected leaves collapse alongside the onion neck. Severe symptoms may include complete wilting and bleaching of all leaves. Carr et al. (2013) described transmission of *P. ananatis* from the leaves to the bulb as occurring when the infected leaf lodges. Center rot bulb symptoms typically include one or a few discolored scales with macerated tissue apparent when the bulb is cut in half. Infected bulbs are usually odorless unless another pathogen is also infecting the bulb.

Extensive survey research done on 28 farms in 2011 and 26 farms in 2012 by Pfeufer (2014) found that there might be multiple sources of inoculum contributing to bacterial disease incidence in PA onion fields. These sources of inoculum include onion transplants and weeds. For example, *P. carotovorum subsp. carotovorum, P. ananatis*, *P. agglomerans, and P. marginalis* have been found to be present in many weed species occurring on and in close proximity to onion fields in PA (Pfeufer, 2014). Also, rep-PCR facilitated strain tracking of *P. ananatis* matched isolates from surface sterilized weed tissue collected at mid-season with those of infected onion that had been stored for four months (Pfeufer, 2014). It is important to note that the pathogenicity tests of these

isolates, especially from weeds and soil, have shown a high degree of variability, and range across a continuum of nonpathogenic to pathogenic (Pfeufer, 2014).

Pfeufer (2014) isolated pathogenic *P. carotovorum* subsp. *carotovorum, P. agglomerans, P. ananatis*, and *P. marginalis* as epiphytes from the surface as well as endophytes from the inner tissues of transplants produced in Texas, Arizona, and locally in PA. These results imply that seed may play a role as the source of bacterial inoculum, as shown by Walcott et al. (2002) with cv. Sweet Vidalia. What can be concluded from this data is that transplants from both out of state and in-state can potentially harbor bacterial pathogens of onion. PA onion transplants are typically sourced from transplant producers in Arizona or Texas, or from local transplant growers who start their own seeds in December through January. Transplants are usually 10 to12 weeks old when planted into PA fields in late March through April. Field-grown transplants shipped from out of state arrive bundled, topped, and bare root and are often intermingled with soil in the box or crate. Wounding during harvest and shipping could make for ideal sites for bacteria to enter plant tissue.

To our knowledge, there are no known bactericides or broad-spectrum chemical treatments labeled as pre-plant transplant dips/drenches for the management of bacterial pathogens on onion. Eliminating the bacteria prior to planting through use of a transplant treatment could reduce a potential source of inoculum. Growers primarily manage bacterial disease by using copper mixed with ethylene-bis-dithiocarbamate fungicides such as mancozeb. However, these products have been reported to have low efficacy and copper-tolerant strains of *P. ananatis* have been identified in Georgia onion fields (Nischwitz et al., 2007; Pfeufer, 2014). In addition, these products are used as an in-season management tool rather than a preventative transplant dip. Thus the need exists to identify products that could disinfest or reduce inoculum on the transplant leaf surface prior to planting.

The following treatments may prove effective at reducing bacterial populations on leaf surface of onion transplants. Hydrogen dioxide (OxiDate® 2.0, BioSafe Systems,

Glastonbury, CT) and hydrogen peroxide with mono- and di-potassium salts of phosphorus acid (OxiPhos®, BioSafe Systems, Hartford, CT) act as broad spectrum bactericides/fungicides labeled for onion for the management of *Botrytis*, downy mildew, powdery mildew, and some efficacy data is available for management of *Xanthomonas* leaf blight. In addition, the product label lists two concentrations for product use on onion, 'preventative,' and 'curative,' both on a 5 to 7 day spray interval; curative is simply the maximum concentration, while preventative is a reduced concentration. Many PA growers already use OxiDate and OxiPhos products in their onion fields, although sufficient data has not been generated in terms of their efficacy. Pfeufer (2014) showed that hydrogen dioxide was as effective as the grower standard copper-mancozeb treatments in managing center rot of onion under low inoculum pressure. Hydrogen peroxide and dioxide are general disinfectants. They kill microorganisms they come in contact with by stealing electrons through oxidation. In this process, the cell membrane is oxidized, disrupted and the cell breaks and dies (Caldwell et al., 2013). Hydrogen peroxide is reactive and short- lived and no residual activity has been reported in the environment (HERA, 2005).

Copper sulfate pentahydrate (MasterCop®, ADAMA, Raleigh, NC) is a fungicide/bactericide, which disrupts bacterial protein function. Copper ions are toxic to microorganisms and are most effective when absorbed by germinating spores, and often require reapplication to prevent disease establishment. When they are absorbed into the fungus or bacterium, the copper ions bind to various protein groups (e.g., imidazoles, phosphates, sulfhydryls, hydroxyls) and denature the cellular protein (Caldwell et al., 2013). Although copper is the PA grower standard, research has not been conducted to determine whether copper is an effective pre-plant dip for managing epiphytic *P. agglomerans* and *P. ananatis*. MasterCop is labeled for onion and includes control recommendations for bacterial blight.

Streptomycin sulfate (FireWall™ 17 WP, AgroSource, Mountainside, NJ) can be used as a treatment to soak ornamental cuttings prior to planting. Vegetable crops currently on the FireWall label include celery, pepper, potato and tomato. Since onion is

not included on this label, label applications for soaking seed pieces of potato to control soft rot blackleg (*Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*) may be applicable to onion. Streptomycin sulfate can inhibit the growth of bacteria by blocking protein synthesis through binding to the small 16S rRNA of the 30S subunit of the bacterial ribosome (McManus and Stockwell, 2001). There is a precedent set for use of antibiotics such as Agri-Mycin® 17 (Nufarm Agricultural Products, Alsip, IL), also streptomycin sulfate, during transplant production of celery, tomatoes and pepper for the management of bacterial diseases. However, there are implications related to the extensive use of antibiotics like the development of resistant strains that should be considered when recommending antibiotics for use in agriculture (Gullberg et al., 2011).

Streptomyces lydicus (Actinovate® AG, Novozymes BioAg, Brookfield, WI) is a biological fungicide used to suppress and control bacterial pathogens under the same properties as streptomycin-based products. This product is labeled to control pathogens such as *Erwinia* and *Xanthomonas perforans* in addition to various fungi and oomycete pathogens. Actinovate® AG is labeled for onion and includes pre-planting recommendations for application on bulbs that could be applicable to onion sets. *Streptomyces lydicus* is a ubiquitous, naturally occurring bacterium that is commonly found in soil. The isolate WYEC 108 has been commercialized and is available as a soluble powder that contains *S. lydicus* spores and proprietary inert ingredients. *S. lydicus*'s mode-of-action is to colonize the plant surfaces and outcompete pathogens for physical space and nutrients exuded by the plant. Additional modes-of-action may include parasitism of fungal plant pathogens and the production of antibiotics, antifungal compounds, and enzymes that digest the cell walls of fungi (Caldwell et al., 2013).

Objectives and Hypotheses

The goal of this study is to identify potential pre-plant transplant treatments that can reduce populations of the center rot pathogens (*P. ananatis* and *P. agglomerans*) on onion transplants and thus provide growers with another management tool. We evaluated the hypothesis that exposure to the bactericide would lead to reduced epiphytic

populations of *P. ananatis* and *P. agglomerans.* Our milestones for this study included an *in-vitro* plate assay for product screening and a transplant assay for screening products to manage epiphytic populations of *P. ananatis* and *P. agglomerans* on onion transplants.

Materials and Methods

In-vitro plate assay product screening

The bacterial isolates *P. agglomerans* (09-194, 09-63, 11-85, 12-14, 15-78) and *P. ananatis* (Pa1a, Pa1b, 09-82) were used in this study. These isolates were originally isolated from symptomatic bulbs originating from PA production fields or directly from onion transplants prior to field planting between the years 2008 and 2015, and are part of the Gugino Lab bacterial isolate collection. These isolates have been shown to induce disease in pathogenicity tests (Pfeufer, 2014). The isolates were grown for 48 h at 25°C, flooded with 4 mL of sterile MQ H_2O, and scraped with a sterile plastic scraper. This suspension was decanted into a sterile 15 mL tube for each isolate. Each concentrated bacterial stock was adjusted to an optical density (OD) of 0.047 at a 590nm absorbance for a 5.7 x 10^8 CFU/mL solution of inoculum using a microplate reader (EMax®, Molecular Devices, California, USA). One mL of each bacterial stock was transferred into sterile centrifuge tubes containing 9 mL sterile MQ H_2O until an approximate inoculum concentration of 10^4 CFU/mL was reached for each isolate. One mL of each isolates' final inoculum was reserved to double check the viable cell concentration by dilution plating on King's B (KB) media (King et al., 1954). Preliminary assays evaluated the use of 10^2 to 10^8 CFU/mL for this assay and identified 10^4 CFU/mL to have the uniform optical density values across different isolates and replicates. Final inoculum ranged between 1.1 X 10^3 and 4.4 X 10^5 CFU/mL in this study.

Each product was incorporated into autoclaved, Luria-Bertani broth media (LB; Difco Luria-Bertani broth, Miller, Becton, Dickinson and Co., Sparks, MD, USA). This was done by making a stock solution (SS) of 1 g or 10 mL product in 100 mL of MQ H_2O. Flasks containing 250 mL of LB media were amended with four different

concentrations of product by aliquoting the SS into each flask of LB media using a 10 mL syringe to result in the desired final concentrations (100%, 75%, 50%, 25% of the label rate). The label rate for Actinovate AG (*Streptomyces lydicus*) was 0.90 g/L H_2O (12 oz/100 gal), the recommended rate for onion foliar spray in field. For Firewall (streptomycin sulfate) it was 0.60 g/L H_2O (4 oz/50 gal), the recommended rate for soaking potato seed to control soft rot blackleg (*Erwinia carotovora* subsp. *atroseptica* and *Erwinia carotovora* subsp. *carotovora*). For MasterCop (copper sulfate pentahydrate) the label rate was 1.77 L/ha (1.5 pt/A or 20 gal H_2O), the recommended field rate for onion and bacterial blight. The label rate for OxiDate (Hydrogen dioxide) was 10.01 mL/L H_2O (128 fl. oz/100 gal), the curative field rate for onion diseases. For OxiPhos (Mono-and di-potassium salts of phosphorus acid in combination with hydrogen peroxide) the label rate was 12mL/L H_2O (5 qt/100 gal), the field rate for onion bacterial soft rot and bacterial leaf blight. Products that went into solution were filter-sterilized using a 0.2 µm. Additional concentrations (15%, 10%, and 5% of the label rate) were evaluated for the products OxiDate, OxiPhos, and Firewall. One 500 mL flask of LB media was prepared for the positive and negative control and was not amended with product. A minimum of two assays were performed per product.

Products were evaluated in a 96-deep well, autoclavable plate with autoclavable, sealing mats (VWR®, Radnor, PA, USA). Two milliliters of amended or non-amended LB media was dispensed into its respective wells (Fig. 4.1). For plate inoculation, 10 µL of 10^4 CFU/mL of each isolate was transferred into its corresponding well using a multichannel pipette. For each plate, columns 1-9 were inoculated, leaving columns 10-12 uninoculated (10 µL of sterile MQ water) for the negative control. Each row was inoculated with one bacterial isolate. A total of three replications were present for each treatment. After all wells had been inoculated, plates were placed in an orbital shaker at 150 rpm for 1 min to mix treatments prior to taking the time 0 hour reading. After time 0 hour samples were collected, mat lids were replaced and plates were maintained at 30°C, in an orbital shaker at 150 rpm. OD reading samples were prepared using a multichannel pipette to transfer 200 µL of solution from each well to the same corresponding well of a 96-well ELISA plate (Falcon®, Corning Incorporated Tewksbury, MA, USA) capable of

holding 250 µL of liquid. OD readings were taken at 0, 12, 24, 36, and 48 h intervals using a microplate reader. Material was discarded after each reading.

Culturing from the biological product Actinovate

LB flasks amended with Actinovate AG were placed in the incubator/shaker at 30°C and at 150 rpm. Once turbid growth was observed, 10 µL of the solution was transferred to KB media. All bacterial isolates had white, creamy phenotypic characteristics on KB media. Representative colonies were subjected to direct-colony PCR. The PCR template was a sterile pipette-tip touched, single bacterial colony. Three representative colonies of these unknown bacterial isolates were amplified for 16S rDNA sequencing, using the primer sequences 530F and 1492R (Borneman et al., 1996). PCR reactions were cleaned using ExoSAP-it (USB®, Cleveland, OH, USA), then 5 µL of reaction mixed with 1 µL of each primer was submitted to the Penn State Nucleic Acid Core Facility for sequencing. Sequence data was edited, then NCBI BLAST was used to identify the bacterial isolates to genus.

Transplant assay

This assay was designed as a randomized complete block design with four replications. Cultivar Candy onion transplants for these assays were sourced from an out-of-state transplant producer (Sunbelt Transplant Inc., Buckeye, AZ). The transplant treatments were as follows: 1) negative control (70% EtOH surface sterilized); 2) positive control (70% EtOH surface sterilized + inoculated); 3) Oxidate; 4) OxiPhos; 5) Firewall; 6) Actinovate; 7) MasterCop. All plants assigned to a bactericide treatment were treated as the positive control treatment until day 2 when the bactericide was applied. All bactericide products were applied at the 100% label rate used in the *in-vitro* plate assay experiment.

On day 0, all plants were treated with 70% EtOH using an agitator sprayer in a biofume hood. Treatments were applied to one side of the onion transplant until run-off,

allowed to air dry, and then plants were flipped over to apply the same treatment on the opposite side of the plant to run-off. Plants assigned to the positive control and bactericide product treatments were also inoculated with a mixture of *P. ananatis* (09-082) and *P. agglomerans* (09-063) isolates using the same agitator sprayer method until run-off. Inoculum was prepared by streaking isolates onto two large (15 x 100 mm) KB plates two days before preparing inoculum. Each plate was flooded with 4 mL of sterile MQ H_2O and scraped with a sterile plastic scraper to make a bacterial suspension. The suspension was pipetted into a sterile 50 mL tube, vortexed, and optical density (590 nm) was measured using a microplate reader. Each isolate was adjusted to an optical density of 0.9 and one mL was removed from each of these isolates, combined into a sterile 50 mL tube, vortexed, and this mixture adjusted to an optical density of 0.047 to obtain an inoculum concentration of approximately 10^8 CFU/mL. One mL of the final inoculum was reserved to double check the viable cell concentration by dilution plating on KB media. The final inoculum concentration ranged between 3.10 X 10^8 and 6.2 X 10^8 CFUs/mL.

Once plants were completely dry on day 0, they were placed into sterile glass culture tubes containing 4 mL sterile MQ H_2O in groups of three. The blocks (racks) of culture tubes were maintained on a lab bench at room temperature (21°C) under ambient light. On day 2 of the experiment, plants assigned to a bactericide product treatment were treated. Each product was pre-mixed in 250 mL sterile MQ H_2O to obtain the previously calculated 100% label rate. Each bundle of 3 plants were removed from their culture tube using sterile forceps, inverted and dipped into their respective bactericide treatment for 5 sec. The bundle was immediately returned to its original culture tube and the process repeated for each treatment. Positive and negative control treatments were not treated on day 2.

Each day of the experiment (day 0-7), one group of 3 plants per treatment had their roots abscised with surface sterilized scissors approximately 2.5-5.0 cm above the point where the roots met the bulb. On days 0 and 1, only the positive and negative control treatments were destructively sampled. The leaves were placed in a sterile 50 mL

centrifuge tube containing 25 mL sterile MQ H_2O and placed in an orbital shaker at 150 rpm and 30°C for 1 h. Serial dilutions were performed with the onion suspension in a flat-bottom serial dilution plate; one series per replicate. A total of 10 µL of each solution was placed on KB media and grown at room temperature 21°C. Colonies were counted approximately 36-48 h after plating.

Data analysis

For the *in-vitro* plate assay, changes in optical density over time were evaluated using proc mixed repeated measures SAS 9.4 (SAS Institute, Cary, NC). Optical density did not vary by isolate or experiment so data were pooled and the mean value was used for analysis, except for the Actinovate treatment data. The autoregressive covariance structure ar (1) was used for this analysis since it had the lowest Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC) values compared to other covariance structures. Transplant CFU/mL data were analyzed using proc mixed in SAS 9.4 and a one-way ANOVA in Minitab 17.3 (Minitab, State College, PA, USA), in addition to post-hoc mean comparisons using Tukey's HSD ($\alpha = 0.05$).

Results

***In-vitro* plate assay**

OxiDate, OxiPhos FireWall and MasterCop treated media had significantly lower optical density for all product concentrations over time compared to the positive control (Fig. 4.2). All bactericide treatments and negative control had a significantly lower optical density than the positive control at $\alpha = 0.05$, except for Actinovate filter sterilized at the 25% and 50% concentrations (P=0.4595; P=0.3311; Fig. 4.2F). The optical density of OxiDate at the 5% concentration was significantly higher than the 100% and 75% label rate over time (P=0.0303; P=0.0322, respectively) but no significant differences were present between the other OxiDate concentrations and negative control (P>0.05; Fig. 4.2A). The optical density of OxiPhos at the 5% label rate was significantly higher

than the 100% and 75% label rate (P=0.0361; P=0.0464, respectively) and the 50% and 100% label rates were significantly lower than the negative control over time (P=0.0189; P=0.0459, respectively; Fig. 4.2B). The only concentrations of FireWall that were not significantly different by optical density over time were 100% with 75%, 15%, and 5%, 75% with 5% and 15%, 50% with 25% and 10%, 15% with 5%, and 10% with 25% and the negative control (P>0.05; Fig. 4.2C). The only concentrations of MasterCop that were not significantly different by optical density over time were 100% and 25% (P=0.3895; Fig. 4.2D).

When each time point was analyzed separately, all concentrations of OxiDate and OxiPhos were significantly different from the positive control but not the negative control by 24 h (Table 4.1). By 48 h, FireWall at the lowest concentration (5%) had a significantly higher optical density compared to the 50 to 100% FireWall concentrations and negative control and concentrations 10 to 25% were not significantly different than the 5% (Table 4.1). A similar trend was seen with low concentrations of MasterCop. The MasterCop product is dark in color and as product concentration decreased, so did the optical density value (Table 4.1). However, by time point 24 h, the lowest concentration of MasterCop (25%) had significantly higher optical density compared to the higher product concentrations (Table 4.2).

Optical densities from Actinovate treated media were highly variable and optical densities from non-filter sterilized Actinovate significantly differed by trial (P≤0.0001; Fig. 4.2E). Optical densities within this treatment were significantly higher when the Actinovate was filter sterilized (P=0.020; Table 1). Filter-sterilized Actinovate at the 25% and 50% levels were not significantly different from the positive control over time (Fig. 4.2F) but non-filter sterilized Actinovate was significantly different from the positive control over time (Table 4.1).

Culturing from the biological product Actinovate

Destructive samples plated from the non-filtered Actinovate amended media resulted in two organisms including *Pantoea agglomerans* (identified based on morphological characterization) and an unknown white pigmented colony with undulated margins and umbonate elevation (Fig. 4.4). Direct colony PCR and sequence analysis of five individual colonies suggested that the unknown white pigmented colonies were *Bacillus* sp. with 99% sequence identity and 100% query coverage for a number of accessions in GenBank including KU877666, KX839268, FJ608704 and CP017747.

Transplant assay

Epiphytic CFUs/mL were significantly different by product treatment on day 2, 3, 6 and 7 and nearly significant on day 5 (Table 2). FireWall, MasterCop, OxiPhos and OxiDate were not significantly different from one another at most time points (Table 2). FireWall had consistently low CFUs/mL compared to all other treatments, even on the final sampling day 7 (Table 4.2). On day 3, FireWall and the negative control treated plants had significantly lower CFUs/mL compared to the positive control (P=0.0055; P=0.0097) and FireWall was also significantly lower than OxiDate and OxiPhos (P=0.0222; P=0.0341; Fig 4.3). Actinovate had numerically higher CFUs/mL than the positive control on days 3, 4 and 7 and was significantly higher than the other bactericides on day 2 (Table 4.2). On day 5, all bactericide treated plants and the negative control had significantly lower CFUs/mL compared to the positive control ($P \leq 0.05$; Fig. 4.3).

Discussion

OxiDate, OxiPhos, Firewall and MasterCop all had significantly lower optical densities and CFUs/mL compared to the positive control and show promise as transplant treatments for reducing epiphytic pathogen populations thus reducing a potential inoculum source and contributing to the management of onion center rot (Fig. 4.2; Table

4.1; Table 4.2; Fig. 4.3). OxiDate and Actinovate AG are Organic Materials Review Institute (OMRI) approved products and can be used in certified organic production. Some copper products are OMRI approved, although the MasterCop product used in this study is not. Therefore some of the options explored here may be potential center rot management strategies for organic growers. Of the products screened in this study, FireWall had the lowest CFU/mL at all time points and a consistently low optical density. Although some of the products like OxiPhos and OxiDate performed well at concentrations as low at 5% of the label rate, FireWall did not (Table 4.2). MasterCop at its lowest concentration of 25% also had high optical density values just under the positive control, indicating that *P. agglomerans* and *P. ananatis* growth was not inhibited at this concentration. Perhaps at low concentrations of FireWall and MasterCop, there is not enough active ingredient for the product to effectively reduce populations of *P. agglomerans* and *P. ananatis.* The selection for antibiotic resistance has been shown to occur at low concentrations (Gullberg et al., 2011). Also, copper-tolerant strains of *P. ananatis* have been identified and perhaps further isolate screening in this assay would reveal similar findings (Nischwitz et al., 2007; Pfeufer, 2014). The lower concentrations that were effective at maintaining a low optical density in the *in-vitro* assays would need to be tested on onion transplants for efficacy as lower concentrations of product may have insufficient coverage of active ingredient on the plant surface. Each product was applied for a duration of 5 sec per plant, but future analysis could explore longer durations and their efficacy.

On day four of the transplant assay, the positive control was not significantly different from the bactericide treated plants (Fig. 4.3; Table 4.2). However, CFUs/mL for the positive control were higher on day 5. Perhaps the low CFUs/mL values for the positive control was a result of depleted resources on the onion transplant surface at this time. A similar trend has been reported with *Pseudomonas syringae* cells on bean leaf surfaces (Montier and Lindow, 2003). The increase in CFUs/mL for this treatment may have been the re-colonization of the leaf surface by secondary bacteria in the environment, which would not have been distinguished in this assay. Future screening of

products on onion transplants should consider this possibility and designed to distinguish the inoculated *Pantoea* from other bacteria.

Actinovate *in-vitro* assays were conducted with one-year old and newer product that was less than one-year old. The product label indicates that after one-year, product efficiency may severely decrease. This may explain why optical density was significantly higher in old, non-filter sterilized Actinovate compared to new, non-filter sterilized Actinovate. Perhaps the old product was not as effective at reducing bacterial growth compared to the new product. The filter sterilized Actinovate was not significantly different between experiments using old and new product. The vegetative hyphae of *Streptomyces* range in size from 0.5 to 2.0 micrometer (Chater, 1984). Therefore, it is likely that using a 0.2 μm filter sterilization filtered out the *S. lydicus* from the product solution making the product ineffective, and the growth measured via optical density in this study was from *P. agglomerans* and *P. ananatis*.

The comparison of sequences from isolates cultured from the Actinovate product to known *Bacillus* groups suggested these isolates belong to the *Bacillus cereus* and *Bacillus subtilis* groups. Further sequence analysis needs to be conducted in order to identify the species and/or strains of *Bacillus* that were isolated from Actinovate. The active ingredient (*S. lydicus*) of Actinovate was not able to be isolated and sub-cultured in LB and KB medias. To further characterize the Actinovate product, additional medias like *Streptomyces* selective agar (Hayakawa and Nonomura, 1987) should be used. *Bacillus* sp. is not listed as an active or inert ingredient on the Actinovate label and to our knowledge, there have been no previous reports on *Bacillus* isolation from Actinovate. Perhaps the manufacturing of the biological product also selects for *Bacillus* sp. Another explanation could be a contamination error in our study. The *in-vitro* and transplant assays performed in this study were not designed appropriately for a culturable, biological bactericide. Although we were not able to culture *S. lydicus*, the growth of *Bacillus* disrupted our quantification of *P. agglomerans* and *P. ananatis*. Therefore, additional assays that distinguish *P. agglomerans* and *P. ananatis* from other bacteria such as green fluorescent protein (GFP) transformation, rep-PCR facilitated strain

tracking, quantitative-PCR or *P. agglomerans* and *P. ananatis* selective media would need to be used for a more accurate quantification of population. Although a semi-selective *Pantoea ananatis* media exists (Goszczynska et al., 2006), common onion epiphytes such as *Pseudomonas marginalis* can grow on this media (Mansfield, pers. comm.).

Conclusion

OxiDate, OxiPhos, Firewall and MasterCop all had significantly lower optical densities and CFUs/mL compared to the positive control and should be included in future analysis of transplant treatments for managing onion center rot. It is likely that the 100% label rate application of these products may prove most effective in a field evaluation as this concentration proved effective in our *in-vitro* and transplant assays. The culture-based assays conducted in this study were not appropriate for the quantification of *P. agglomerans* and *P. ananatis* in the presence of the biological product Actinovate. *Bacillus* spp. was consistently isolated from Actinovate, although *S. lydicus* is the active ingredient of the product. Further characterization of the *Bacillus* spp. and the Actinovate product needs to be conducted before further evaluating its efficacy on onion transplants.

The work presented here is preliminary and the bactericides that performed well in these assays will later be evaluated in field assays to further evaluate their efficacy at reducing epiphytic populations of bacterial pathogens on onion transplants and their potential to reduce bacterial disease incidence at harvest. Products can be evaluated on both out-of-state and locally produced transplants. Transplants can be inoculated, treated with product and transplanted into the field. By using rep-PCR facilitated strain tracking, intensive samplings of the onions can track the isolates used in inoculation and document survival after product application.

Many of the treatments evaluated in this study are already labeled for use in onion production, therefore minimal adjustments would need to be made to the product label to encompass a pre-plant application. The results of this research are widely applicable to

other onion production systems beyond PA. Identifying pre-plant onion transplant treatments that may be able to manage bacterial pathogen populations colonizing the plant surface can provide growers with another tool to incorporate into an integrated program for the successful management of bacterial disease of onion. Successful treatments can aid in reducing one of the most important sources of inoculum early in the season.

Figures and Tables

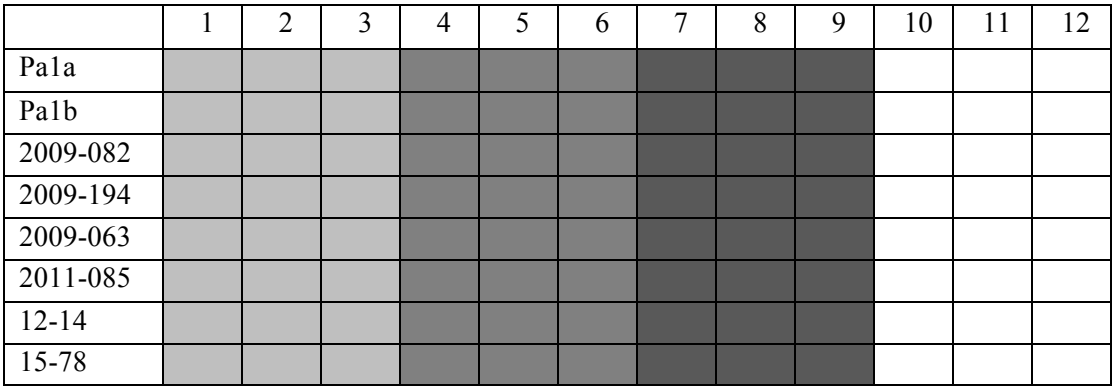

Fig. 4.1. Diagram of *in-vitro* plate-assay design. Well columns 1, 2, 3, 10, 11 and 12 contained 0% product. Well columns 4, 5 and 6 contained 5%, 10%, 25% or 75% product. Well columns 7, 8 and 9 contained 15%, 25%, 50% or 100% product. Columns 1-9 were inoculated, leaving columns 10-12 uninoculated (10 µL of sterile MQ water) for the negative control. Each row was inoculated with one bacterial isolate identified in the outer left column.

Table 4.1. Means comparison of optical density from product amended media. Each data point represents a mean of two or more experiments, eight bacterial isolates and three replicates (n=135 for OxiDate, OxiPhos and FireWall; n=90 for MasterCop and Actinovate). Data was analyzed separately by each time point and product treatment using a one-way ANOVA in Minitab and letters indicate statistically significant differences by Tukey's HSD ($\alpha = 0.05$).

Treatment	Time (h)				
	0	**12**	**24**	**36**	**48**
negative control	0.053 a	0.052 b	0.053 b	0.054 b	0.055 b
100% OxiDate	0.043 c	0.041 d	0.042 b	0.042 b	0.041 b
75% OxiDate	0.044 c	0.041 d	0.041 b	0.042 b	0.042 b
50% OxiDate	0.043 c	0.042 d	0.043 b	0.044 b	0.042 b
25% OxiDate	0.048 b	0.046 c	0.046 b	0.046 b	0.045 b
15% OxiDate	0.051 ab	0.048 c	0.048 b	0.049 b	0.047 b
10% OxiDate	0.054 a	0.053 ab	0.053 b	0.050 b	0.049 b
5% OxiDate	0.053 a	0.053 ab	0.054 b	0.054 b	0.052 b
positive control	0.053 a	0.055 a	0.336 a	0.564 a	0.685 a
	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001
negative control	0.052 a	0.054 b	0.055 b	0.053 b	0.052 b
100% OxiPhos	0.047 e	0.043 f	0.040 b	0.041 b	0.042 b
75% OxiPhos	0.043 e	0.043 f	0.041 b	0.041 b	0.043 b
50% OxiPhos	0.044 de	0.044 ef	0.045 b	0.043 b	0.042 b
25% OxiPhos	0.046 cd	0.047 de	0.049 b	0.046 b	0.045 b
15% OxiPhos	0.049 bc	0.050 cd	0.050 b	0.046 b	0.048 b
10% OxiPhos	0.051 ab	0.052 bc	0.050 b	0.052 b	0.049 b
5% OxiPhos	0.051 ab	0.053 bc	0.052 b	0.052 b	0.052 b
positive control	0.054 a	0.057 a	0.338 a	0.563 a	0.745 a
	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001
negative control	0.052 f	0.052 d	0.054 c	0.053 d	0.056 c
100% FireWall	0.073 ab	0.068 a	0.074 bc	0.102 b	0.059 c
75% FireWall	0.077 a	0.067 ab	0.069 bc	0.101 b	0.058 c
50% FireWall	0.064 cd	0.065 ab	0.064 c	0.066 bcd	0.055 c
25% FireWall	0.061 d	0.062 ab	0.072 bc	0.064 cd	0.061 bc
15% FireWall	0.068 bc	0.067 ab	0.106 b	0.071 bcd	0.068 bc
10% FireWall	0.058 de	0.061 bc	0.059 c	0.064 bcd	0.060 bc
5% FireWall	0.059 d	0.060 bc	0.062 c	0.095 bc	0.118 b
positive control	0.053 ef	0.056 c	0.345 a	0.538 a	0.668 a
	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001

Table 4.1. Continued

Treatment	Time (h)				
	0	**12**	**24**	**36**	**48**
negative control	0.057 e	0.057 d	0.058 e	0.058 f	0.058 e
100% MasterCop	0.314 a	0.375 a	0.205 c	0.347 c	0.210 d
75% MasterCop	0.243 b	0.226 b	0.189 c	0.248 d	0.180 d
50% MasterCop	0.175 cd	0.198 b	0.137 d	0.189 e	0.265 c
25% MasterCop	0.127 d	0.140 c	0.263 b	0.470 b	0.512 b
positive control	0.056 e	0.062 d	0.384 a	0.583 a	0.714 a
	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001
negative control	0.054 a	0.052 c	0.052 b	0.052 c	0.057 c
100% Actinovate non-filtered	0.055 a	0.071 ab	0.220 a	0.297 b	0.338 b
75% Actinovate non-filtered	0.057 a	0.073 a	0.215 a	0.285 b	0.369 b
50% Actinovate non-filtered	0.054 a	0.067 ab	0.218 a	0.282 b	0.342 b
25% Actinovate non-filtered	0.054 a	0.065 b	0.207 a	0.249 b	0.308 b
positive control	0.057 a	0.052 c	0.201 a	0.402 a	0.564 a
	P=0.352	P≤0.0001	P≤0.0001	P≤0.0001	P≤0.0001
negative control	0.052 a	0.057 b	0.055 c	0.057 b	0.054 c
100% Actinovate filtered	0.051 a	0.063 a	0.257 ab	0.484 a	0.309 b
75% Actinovate filtered	0.052 a	0.057 ab	0.233 b	0.440 a	0.257 b
50% Actinovate filtered	0.052 a	0.058 ab	0.254 ab	0.504 a	0.599 a
25% Actinovate filtered	0.052 a	0.059 ab	0.278 a	0.479 a	0.560 a
positive control	0.052 a	0.057 ab	0.244 ab	0.454 a	0.583 a
	P=0.910	P=0.059	P≤0.0001	P≤0.0001	P≤0.0001

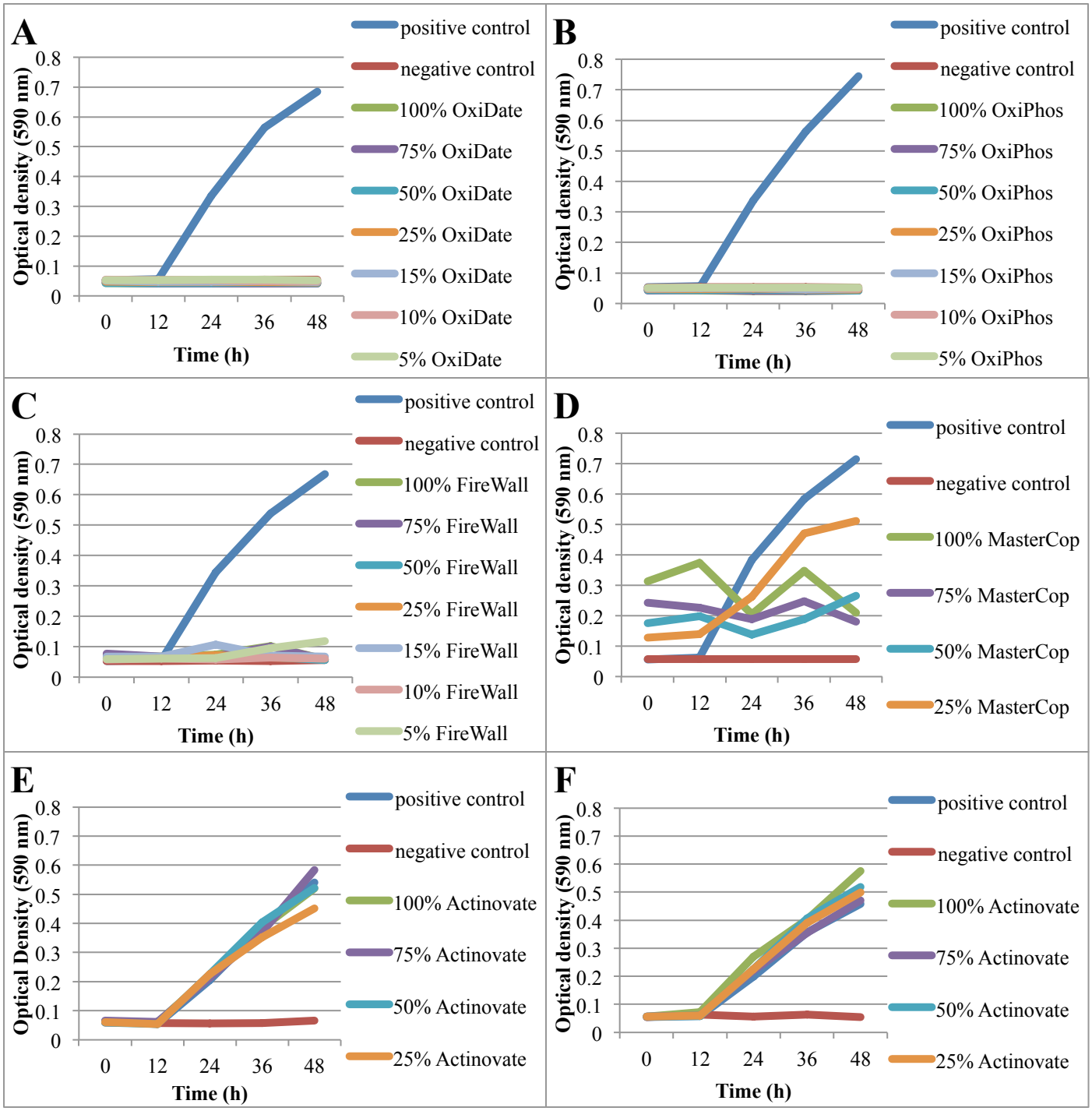

Figure 4.2. *In-vitro* assays comparing optical density of bactericide treated media over time. A: OxiDate (n=135); B: OxiPhos (n=135); C: FireWall (n=135); D: MasterCop (n=90); E: Actinovate non-filter sterilized (n=90); F: Actinovate filter strerilized (n=90). Data was pooled by isolate and replicate. Analysis is based on repeated measures analysis using proc mixed and means separation using Tukey's HSD (SAS 9.4).

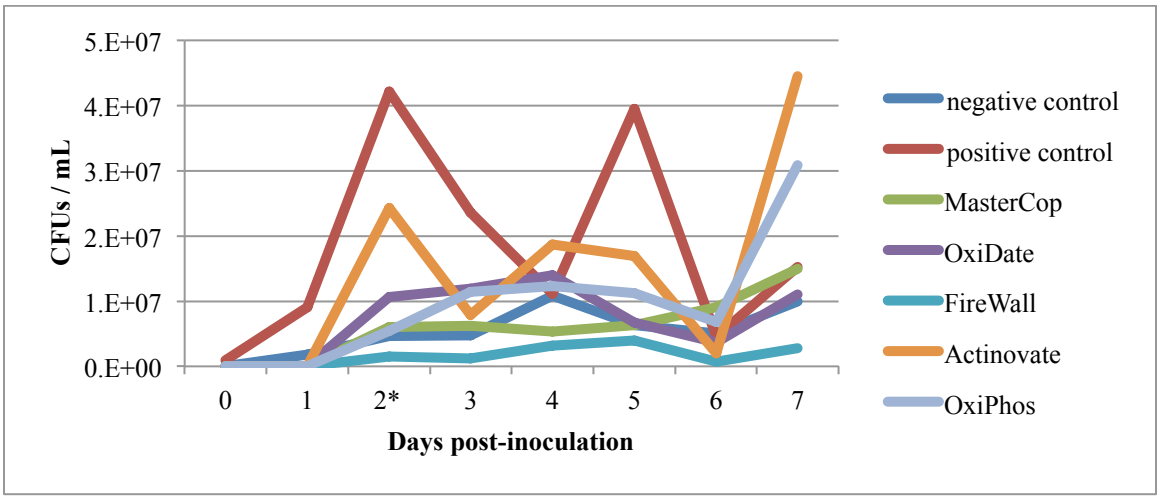

Fig. 4.3. Transplant assay comparing mean epiphytic CFUs/mL over time from product treated plants. Three plants per treatment replicate were destructively harvested and epiphytic wash was plated for CFU/mL assessment. Each data point represents a mean of two experiments and four replicates (n=8). Plants were inoculated on day 0 and products were applied on day 2*. The data presented for days 0 and 1 is from positive and negative controls only. Product treated plants were not destructively harvested until day 2. Data was analyzed using proc mixed and Tukey's HSD (SAS 9.4).

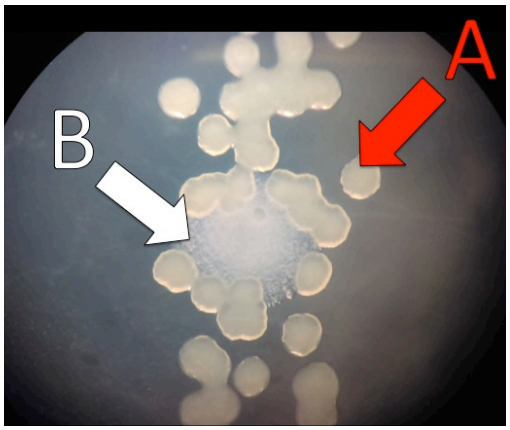

Fig. 4.4. Comparison of *P. ananatis* and *Bacillus* sp. colonies. A: *P. ananatis*; B: *Bacillus* spp. Destructive samples were taken from *in-vitro* plate assay wells containing 100% non-filter sterilized Actinovate and *P. ananatis* isolate Pa1a. Each 50 µL sample was serial diluted and 10 µL of each dilution was plated onto KB media. The dilution presented here is 10^{-6}.

Table 4.2. Means comparison of epiphytic CFUs/mL from product treated transplants. Three plants per treatment replicate were destructively harvested and epiphytic wash was plated for CFU/mL assessment. Each data point represents a mean of two experiments and four replicates (n=8). Data was analyzed by a one-way ANOVA in Minitab and letters indicate statistically significant differences by Tukey's HSD ($\alpha = 0.05$).

Treatment	Day					
	2	3	4	5	6	7
FireWall	1.5E+06 b	1.3E+06 b	3.2E+06	4.0E+06	7.5E+05 b	2.7E+06 b
neg. control	4.7E+06 b	4.7E+06 b	1.1E+07	6.3E+06	5.1E+06 ab	9.9E+06 ab
OxiPhos	5.5E+06 b	1.1E+07 ab	1.2E+07	1.1E+07	6.8E+06 ab	3.1E+07 ab
MasterCop	6.0E+06 b	6.2E+06 ab	5.3E+06	6.3E+06	9.1E+06 a	1.5E+07 ab
OxiDate	1.1E+07 b	1.2E+07 ab	1.4E+07	6.7E+06	3.7E+06 ab	1.1E+07 ab
Actinovate	2.4E+07 a	7.9E+06 ab	1.9E+07	1.7E+07	2.0E+06 ab	4.5E+07 a
pos. control	4.2E+07 a	2.4E+07 a	1.1E+07	3.9E+07	4.6E+06 ab	1.5E+07 ab
	P≤0.0001	P=0.017	P=0.347	P=0.069	P=0.028	P=0.028

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CHAPTER 5: DEVELOPING EDUCATIONAL MATERIALS TO INCORPORATE INTO TRAINING FOR WESTERN HONDURANS WORKING TOWARDS WOMEN EMPOWERMENT THROUGH HORTICULTURE

Abstract

Women's participation in horticultural production in the Western highlands of Honduras is continually challenged by multiple factors that limit participation. One of these factors is disease driven yield loss. Initial research conducted through the Women in Agriculture Network (WAGn): Honduras project has identified obstacles that limit the participation of women, in an effort to develop technologies and practices that increase household income and nutrition. These technologies will be promoted in Farmer Field Schools conducted at the Horticulture Innovation Lab Regional Center at Zamorano University. In order to enhance these programs, educational materials need to be developed for the Farmer Field School leaders. In this project, we designed a horticultural production guide to aid professionals and growers in low-input horticultural practices and pest management options. We traveled to Honduras to connect with Extension professionals and growers and identified culturally appropriate production recommendations. This horticultural production guide will provide a linkage between current research and extension information, while in the process reducing yield loss and enhancing household nutrition. The text of this document was terse, as recommended, so that growers with minimal education could easily understand the content. In combination with the Farmer Field Schools, these materials have the ability to increase the productive capacity of growers through a multi-dimensional, interdisciplinary, and holistic approach to managing diseases.

Introduction

In the Central American country of Honduras, the median per capita daily expenditure is $1.30 (IFPRI, 2013), 67% of the country is living in poverty (J. Lansdale, pers. comm.) and there is limited access to inputs, credit, markets, and technical

assistance for women, resulting in low agricultural productivity. The Western highlands of Honduras have long been characterized by subsistence farming, poor diets and poverty (J. Lansdale, pers. comm.). Women must move to higher value agricultural production to increase income and improve nutrition in their household. There are limited economies of scale in horticultural production, allowing smallholder farmers to effectively compete in the market (Chalmers et al., 2012). Horticulture provides greater demand for labor and opportunities for value-addition, as well as potential improvements in nutrition and dietary diversification. However, horticulture can be difficult for women and other resource poor farmers in Western Honduras to participate in due to necessary investments in technologies such as drip irrigation, solar dryers, on-farm storage and greenhouses, as well as access to credit or other financing options.

Research conducted through the Women in Agriculture Network (WAGn): Honduras project (P.I. Dr. Janelle Larson), a collaboration between the Penn State College of Agricultural Sciences and the Horticulture Innovation Lab Regional Center at Zamorano (funded by USAID/Horticulture Innovation Lab Initiative), seeks to empower women in agriculture and other marginalized populations through the horticultural value chain. WAGn Honduras will identify barriers to participation of women in the horticultural value chain, and the returns to that participation. In collaboration with the Regional Centre at Zamorano, WAGn will design and implement research activities in the form of Farmer Field Schools. Famer Field Schools provide field-based, season-long training delivered by an extension specialist and have proven to be a successful, cost-effective method of integrating small farmers and women in learning activities (Davis et al., 2012; Collinson et al., 2013). Zamorano has provided thousands of Farmer Field Schools to Central American growers in the past 15 years. These Farmer Field Schools will serve as a technology transfer system using experiential, educational methods, enhanced by educational materials. The Farmer Field Schools will have a field-based, season-long training program with regular meetings nearby the participating farmers. In this research, we developed educational materials to use in these Farmer Field Schools.

Approach

We proposed to develop educational materials that could be used to train Farmer Field School participants associated with the WAGn Honduras project. It was important to travel to meet the Extension professionals and growers of the Western highlands of Honduras and partner with them to develop appropriate materials for the Farmer Field Schools that are tailored to regional needs. We travelled to Zamorano to learn about the current research and outreach being conducted through the USAID/UC Davis Horticulture Innovation Lab Regional Center at Zamorano, as well as the pest management challenges faced by growers in Honduras specifically those due to insect pests and diseases. Our hosts were the Horticulture Innovation Lab staff members Patricia Azucena Arce Valladares, Gabriela Suyapa Hernandez Casco and student translator Emmanuel Villeda Rivera (Fig. 5.1).

Using the knowledge and experiences gained from this trip, my overall goal was to develop a detailed outline with content focusing on low-input production and pest management that can serve as the foundation of a horticulture production guide used in Farmer Field Schools. This was accomplished through the following proposed objectives: 1. Identify current materials and teaching aids being used in horticultural production, and the effectiveness of these materials; 2. Define appropriateness of material content given technical feasibility, economic viability, socio-cultural acceptability, and environmental sustainability; and 3. Develop curricula in the form of a guide to aid professionals and growers in low-input horticultural production.

Summary of trip (Objectives 1 and 2)

In order to gain first-hand knowledge of the horticultural production in Honduras, I travelled to Zamorano University, a Panamerican Agricultural School, located in the valley of the Yeguare River, Honduras, March 20-23, 2016 with my advisor, Dr. Beth Gugino, Associate Professor of Vegetable Pathology. We visited the Horticulture Innovation Lab where courses, workshops and conferences are held for growers, NGOs,

government representatives, university and horticultural professionals. This area serves as a living lab for teaching new technologies and post-harvest practices developed by senior students from Zamorano and adapted for small-scale growers. Some of the specific technologies currently being demonstrated at the Innovation Lab included mesh houses for insect pest exclusion during crop production, zero energy cool chambers for post-harvest storage of fresh produce, vertical hydroponic production systems for urban agricultural production of crops such as lettuce and traditional dryers for food preservation. We also visited the organic and horticulture production areas at Zamorano, which are maintained by the undergraduate students as part of their curriculum (Fig. 5.2). In addition, Zamorano also has a student-run post-harvest facility to evaluate post-harvest technologies. Which further demonstrates the "learning by doing" modality of Zamorano. Each year, the Horticulture Innovation Lab hosts a post-harvest short course for the region's producers.

Through meetings with the Extension professionals and growers of Western Honduras, I observed first-hand the needs and challenges growers face in horticultural crop production. Our meeting with Ing. Ivanna Vejarano, Instructor of Integrated Crop Management and Climate Change, was most beneficial in providing direction for the creation of the horticulture guide used in the WAGn Honduras Farmer Field Schools. Vejarano has led similar Farmer Field Schools for women in Guatemala where women expanded their home gardens from approximately 10 m^2 to 200 m^2 in size. Through this expansion, women were able to retain 30% of their produce for household consumption and sell the remaining 70% at market (Vejarano, pers. comm.). These gardens were designed with a diversity of common garden crops such as tomato, onion, pepper, lettuce and carrot with only one or two of these crops being expanded in quantity to sell to market. Based on Vejarano's experience, multiple Farmer Field School sessions should be conducted with the participants to ensure information adoption. With one year of Farmer Field School participation, it is expected that 60% of the knowledge will be retained, two years will have 80% retention and three years will have 100% retention (Vejarano, pers. comm.). The education level of the women participating in Farmer Field Schools varies; many have received minimal formal education. For this reason, it is

important that our horticulture guide included minimal text with a greater focus on pictures and diagrams (Vejarano, pers. comm.). For example, bokashi composting is a very common composting technique used in this region and instead of describing it in text; a diagram should be used to illustrate how to prepare a bokashi (Luiz et al., 2013).

Through meetings with growers, we were able to observe the impact that collaboration with Zamorano has provided them. We visited two small producers, located in Galeras and Teupasenti. The growers had similar production practices. Both used a lagoon to store irrigation water, which was pumped either directly from the river or a canal. Within the lagoon, both producers farmed red tilapia, a preferred breed for consumption in Honduras. Both growers used mesh houses for insect exclusion during crop production. This barrier is particularly important for protecting crops from insects like the whitefly (*Aleyrodidae* spp.), which can vector viruses. Drip irrigation was used on both farms as it provides the most efficient use of water and best environmental conditions for minimizing disease incidence. The farms varied in the crops produced. The grower in Galeras had a more diversified vegetable farm growing lettuce, bean, onion, peanut, cassava and flor de Jamaica whereas the growers in Teupasenti focused on few crops such as onion, corn and sweet potato. Both growers had one or more trees for fruit production for example mango, banana, plantain, tamarind and coconut. Both farms were predominantly male run. The Galeras grower was widowed so we were unclear of what role his former wife had in production. The woman of the Teupasenti farm participates in production but her main focus was running her soap production business and selling the soap at local markets and stores.

Based on our experiences with growers and meetings with professionals at Zamorano, we decided the crops of focus for the horticulture production guide should be onion, tomato, pepper, carrot and lettuce since these crops have the ability to increase income and improve household nutrition (Vejarano, pers. comm.). It is anticipated that this guide will be used in the WAGn Honduras project to empower women in agriculture and other marginalized populations in Western Honduras through the horticultural value chain. This horticultural production guide emphasizes low-cost and low-risk technologies

that reduce yield losses due to diseases. By encouraging these technologies, we expect to see an increase in crop yields and thus income, which can lead to nutritional enhancements in the diets of low-income households. These materials were designed based upon our understanding of the region and the list of pests was determined in collaboration with Zamorano staff. The goal of this guide is to provide straightforward, simplistic information for Western Honduran women with minimal education. The content of this guide is presented below and the finished product will be supplemented with images of local Honduran plants and diagrams from Zamorano University. Although this guide was developed in English, it will be translated to Spanish with the help of a translator. This material may also be appropriate to reach Spanish-speaking smallholder growers in other regions as well, including Guatemala and El Salvador. These materials are widely adaptable and may even be used on larger scale production systems in other regions such as Pennsylvania.

Anticipated impact of research

The development and dissemination of these educational materials will have an immediate and direct impact on all participants in the Farmer Field Schools by increasing the number of resources available to incorporate into an integrated program for the successful management of disease. The creation of a horticultural production guide used in the Farmer Field Schools is synchronous with the already-established objectives of the WAGn Honduras project. As we succeed in managing diseases of horticultural crops in Western Honduras, growers will be able to sustain and/or increase their yield per hectare, thus building the fresh market industry in Honduras, and increasing household nutrition. Practices adopted as a result of the participation in the Farmer Field Schools and use of the curricula will be evaluated by the WAGn Honduras project. The materials designed in this project may also be applicable for other similar production systems, like those located in Guatemala and El Salvador. These materials are widely adaptable and may even be used on larger scale production systems in other regions of the world. The general public of Honduras will also benefit from having access to an increased supply of affordable, locally-grown horticultural produce. In combination with the Farmer Field

Schools, the materials designed in this research have the ability to increase the productive capacity of growers through the sustainable management of pests.

A horticultural production guide for Western Hondurans (Objective 3)

Selecting a Garden Site

The best garden sites have good exposure to sunlight. Vegetables need a minimum of 6 hours of full sun each day although 8-10 hours are preferred to produce large yields. Soil type is also important to consider for drainage and fertility. Heavy clay soils have poor drainage, which can favor disease, and heavy sand soils have excessive drainage, which may lead to plant drought stress. Soil can be amended with organic matter to retain moisture and create fertile, healthy soil. Soil tests can be taken to determine the fertility status of the soil (see Zamorano for soil test instructions). Soil testing can provide information about the soil pH, nutrient levels, organic matter content and soluble salt levels and recommendations for the site based on specific crop requirements.

Preparing the site

Healthy soil is the foundation of growing plants. Healthy soil can be built by adding organic matter such as manure, cover crop residues and various types of compost including bokashi compost and vermicompost. For green materials, it is best to incorporate them into the soil with enough time to decompose before planting crops. Organic mulch and cover crop residues can be spread on top of soil to retain soil moisture, manage weeds, prevent erosion, moderate soil temperature and more.

Irrigating

Drip irrigation will provide the most efficient use of water. It places water in the rooting zone without wetting the foliage, which can reduce disease. Water-soluble

fertilizers can be applied through drip irrigation using fertigation systems. Nutrient loss (leaching) is reduced when using drip irrigation compared to other methods. If using overhead irrigation sprinklers or a hose, the best time to irrigate is the morning and if possible apply the water at the base of the plants to reduce leaf wetness, which can favor disease. The leaves can dry before the sun sets and dew forms.

5 Tips for Conserving Water

1. Use a rain gauge. Generally, vegetables need 2.5 cm of water a week. Only use supplemental irrigation if rainwater is low.
2. Add organic matter and use mulches.
3. Use drip irrigation.
4. Plant drought-resistant cultivars.
5. Manage weeds since they compete with crops for water.

Selecting plant material and cultivars

Cultivar selection is important for selecting desirable traits such as high yield. It can be helpful to ask neighbors and friends about the cultivars they grow that perform well in the area. Cultivar traits can include but are not limited to disease resistance, drought tolerance, high yield and improved nutrition. In general, brighter colored fruits and vegetables tend to have higher phytonutrient content. For example, brightly colored tomatoes and peppers have higher lycopene, an antioxidant linked to health benefits. When selecting transplants, pick healthy, vigorous plants. Avoid selecting plants that are flowering or are pot-bound. The ideal age of transplants for tomatoes are 6–8 weeks, peppers 8–10 weeks and onion 9–12 weeks.

Managing pests

Just like people, plants can get sick too. Sick plants do not grow well or produce good quality food. Plant diseases can be caused by viruses, bacteria, fungi, protozoa and nematodes. Plant pathogenic microorganisms often favor plants that are stressed. Stresses

for plants can include too many or too few nutrients, moisture, and light. Plants also experience stress from toxic chemicals, air pollution, and competition with other plants (usually weeds). Insects like pollinators can be beneficial to crops (pollination) while others are pests. Damage to plants via insects, animals, or harsh environmental conditions can leave open wounds for pathogens to enter the plant and cause disease as well. Weeds can also be pests since they compete with crops for moisture, light and nutrients. Weeds should be removed when they are young and never allowed to set seed. Mulches can help prevent weed seeds from germinating and conserve moisture.

There are many common practices that you can do to reduce pests, including:

- Purchasing pathogen-free seed
- Only plant healthy looking transplants
- Selecting cultivars resistant to common pests
- Applying proper amounts of fertilizer and irrigation water
- Adding organic matter to increase beneficial microorganisms in the soil
- Removing and destroying weeds
- Pruning and removing diseased plant material
- Rotating between different plant species and promote diversity within plantings with multiple crops like onions, lettuce and carrot
- Promoting good air circulation to promote drying
- Creating habitat for beneficial insects
- Identifying the pest early before it causes damage

A successful pest management program is dependent upon regular scouting for early detection of pests and the correct identification of the pests. The selection of the appropriate pesticide and its rate, time of application and weather conditions for application contribute to its success or failure. Always follow the directions on the container or package when mixing and applying pesticides. Home remedies like soaps, detergents and compost teas can serve as alternatives to pesticide use.

Onions

Onions form bulbs in response to day length and can be classified as short-day, intermediate, long-day, or day-neutral. Any of these types of onions can have skin or flesh that is yellow, white, or red in color. Onions can be planted in the field as seed, sets or transplants. Bulb onions should be planted at 15 cm spacing. Optimum growth for onions requires at least 4 cm of water per week either through irrigation or rainfall. Inadequate watering will reduce bulb size and increase pungency. Medium-sized onion sets, 1 to 2 cm in diameter, are best for producing mature onions. Pinch off seed stalks as soon as they develop, or else thick, double-neck onions will likely be produced and are unmarketable.

When about half or more of the onion tops have fallen and started to turn brown and dry down, they are mature and ready to be harvested. Onion leaves should be removed and at least 3 to 5 cm of neck tissue should remain to avoid bulb rot during storage. Onion bulbs need to be dried (cured) before storage, which can be done by spreading them out in a well-ventilated area protected from direct sunlight and with good air movement. Onions can be stored in a mesh bag or slatted container with good air circulation.

Diagnosing onion pests

Thrips (*Thrips tabaci*)- Thrips are small, yellow to dark brown insects that are about 1.3 mm in size. Their feeding damage looks like silvering and flecking on leaves.

Alternaria (*Alternaria porri*)- Alternaria is a foliar disease with small, water-soaked spots that turn brown. As the lesions enlarge they turn purple and a target spot pattern often forms surrounded by a yellow-red zone. During moist weather, brown to black masses of fungal spores form on the lesions.

Downy mildew (*Peronospora destructor*)- Downy mildew is a foliar disease with pale-green, yellowish to brownish, oval lesions on leaves or seed stalks of onion. Fuzzy grey-purple spore masses form in lesions.

Botrytis (*Botrytis* sp.)- Botrytis is a fungal disease that causes small, yellow to white, oval, sunken spots or flecks on leaves and/or areas as low as the soil-line.

Pink root (*Phoma terrestris*)- Pink root is a root rot disease. Diseased roots will be pink in color. Above ground symptoms include small, stunted plants with dieback.

Fusarium basal rot (*Fusarium oxysporum* f.sp. *cepae*)- Fusarium basal rot is a root and basal plate rot disease. Diseased bulbs will often form pink mold in storage. Above-ground symptoms include yellowing and dieback of leaf tips.

Bacterial disease- There are many different types of bacterial diseases in onion. Some bacterial pathogens enter the plant leaves and cause yellowish-greyish discolored, water-soaked lesions. Eventually, infected leaves wilt and some bacteria will spread into the center of the onion bulb while leaving the outer bulb scales firm. Internal bulb decay can be detected at harvest by looking for a brown discoloration of scales in the onion neck. Diseased bulbs will rot in storage and often have a foul-smell.

Leafy vegetables

Leafy vegetables, particularly lettuce and spinach perform best in cool weather (7–18°C is ideal). Spinach, head lettuce and most leaf lettuce cultivars may set seed during the long, warm days. Some cultivars are tolerant of warmer temperatures. Lettuce is divided into two types, head and leaf. Harvest head lettuce when the heads are firm but not over mature. Unlike leaf lettuce, a new head will not grow once head lettuce is harvested. Spinach and leaf lettuce can be continually harvested any time after the outer leaves are 10 to 15 cm long. Leafy vegetable seed can be sown directly into the soil.

Certain cultivars require light for germination. Sandy, loam soils that are loose and fertile are ideal for growing leafy vegetables.

Diagnosing lettuce pests

Leafminers (*Liriomyza* sp.)- Leafminers are small, gray-black flies with yellow markings. Damage is caused by larval feeding causing winding, whitish tunnels or mines within the leaf tissue.

Aphids (Aphididae Family)- Aphids are small, soft bodied insects with piercing, sucking mouthparts. They have pear or oval shaped bodies with long legs and antennae. They have a pair of tube structures projects from their rear. Aphids are found in dense groups, and can be distinguished because they do not fly away when disturbed. Damage is caused inside the leaves or at the heart of a leaf lettuce.

Bean slug (*Sarasinula plebeia*)- Bean slugs are soft bodied, elongate plant pest that leave a film behind as they move. They have tube-like protrusions on the head. Plant damage is ragged holes in new shoots and leaves, or new growth completely eaten.

Xanthomonas bacteria (*Xanthomonas* sp.)- Xanthomonas bacteria cause a foliar disease with water-soaked spots that are typically angular in shape. Lesions become black and eventually leaves will collapse and become papery.

Erwinia wilt (*Erwinia* sp.)- Erwinia wilt is a foliar disease that causes leaf wilting. Light brown to red lesions in the vascular tissue can be viewed from the cut stem end. As the disease advances, the middle of the stem becomes water-soaked, macerated, and greenish. Wilting of the mature head is associated with extensive rotting of the stem.

Alternaria leaf spot (*Alternaria* sp.)- Alternaria leaf spot is a foliar disease with brown-black, small spots on leaf blades Leaf-spotting on margins of leaf blades can be

confused with black rot symptoms. Old leaf spots become papery in texture and may tear and display a shot-hole effect.

Downy mildew (*Bremia lactucae*)- Downy mildew is a foliar disease that causes light green to yellow angular spots on the upper surfaces of leaves. White fluffy growth often develops on the lower sides of these spots. Older leaves are attacked first.

White mold (*Sclerotinia* sp.)- White mold is a fungal disease that affects stems and foliage at the base of cole crops and lettuce plants. Diseased tissue is soft, and watery and white, cottony mold often forms on the plant surface. Plants eventually wilt and hard black structures called sclerotia may form on dead stems.

Root rot (*Rhizoctonia solani*)- Root rot disease or damping-off of lettuce can wilt and even kill plants before or just after emergence. Diseased seedlings have rotted roots and stem lesions.

Carrots

Carrots require loose soils to develop good quality, sizable roots. Well-tilled, loose soil is important for forming carrots. Soil can be amended with compost, organic matter or vermiculite to make it lighter. Carrots will often be misshapen and have poor-quality roots if transplanted so direct sowing of the seed is recommended. Seeds should be planted 6 to 12 mm deep and 2 to 7 cm apart. Germination can be slow. To minimize soil crusting, place a thin band of vermiculite, sand, or perlite over the seed row. Do not overwork heavy garden soils, especially when wet. To prevent carrot tops from greening, make sure that the carrot roots are covered with soil at all times. Carrots should be stored at temperatures near 0°C with a relative humidity of 95 percent.

Diagnosing carrot pests

Pythium dieback (*Pythium* sp.)- Pythium dieback is a root rot disease, which causes rusty-brown lesions, lateral root formation, forking and stunting on carrots.

Nematodes (*Meloidogyne* sp.)- Nematodes are microscopic worms that feed on carrot roots. Their feeding causes extreme forking, stubbing and bunching of the carrot root and foliage will often wilt. Some nematodes create galls on feeder roots.

June Beetles (*Phyllophaga* sp.)- June beetles are insects that vary in size, color and identification, however most are indiscriminate feeders and will eat foliage, flowers and fruit of lettuce. Damage can be skeletonized leaves.

Peppers

Pepper seeds germinate best in soil temperatures between 26 to 29°C. Sow seed 6 to 12 mm deep in peat pellets or other growing media about 7–8 weeks before planting in the garden. Transplants should be healthy looking, approximately 15 to 23 cm tall, with a sturdy stem. The best conditions for transplanting are cool, cloudy days. In sunny weather, it is best to wait until late afternoon or evening to transplant. Plants should be placed 30 to 45 cm apart. Bell (sweet) peppers can be harvested once they reach full size or after they turn red (yellow, orange, white, or purple in some cases). Hot peppers vary in size and shape based on cultivar. Both sweet and hot pepper fruit are edible in all growth stages.

Diagnosing pepper pests

Pepper weevil (*Anthonomus eugenii*)- Weevils are black and oval shaped insects, with a snout used for feeding. They damage peppers by feeding on blossoms and immature fruit, inhibiting bud development.

Whitefly (Aleyrodidae Family)- Whiteflies are insects that feed on plant sap. They can be identified on pepper by looking on undersides of leaves for small white-yellow flies. The adults deposit sticky excrement, which will eventually be covered by a black mold called sooty mold.

Leaf spot (*Xanthemonas campestres*) - Bacterial leaf spot is a foliar disease that causes many small, dark spots to appear and the spots can be surrounded by a yellow, water-soaked halo. This disease also occurs on tomato.

Powdery mildew (*Leveillula taurica*)- Powdery mildew is a foliar disease. Infected plants will have patchy, white, powdery growth on the leaf surface. Yellowish or brownish discoloration may form on the upper leaf surface.

Erwinia wilt (*Erwinia carotovora* pv. *carotovora*)- Erwinia wilt is a bacterial disease that causes wilting in pepper. It begins as dark veinal leaf tissue followed by leaf yellowing and browning. Eventually, the stems may show internal dark brown discoloration and sunken stem lesions called cankers develop.

Root rots (*Fusarium* sp., *Phytopthora capsici*, *Rhizoctonia solani*, etc.)- Fungal root rot diseases cause wilting and death of pepper plants. Roots will turn brown and mushy and die.

Leaf spot (*Cercospora* sp.)- Cercospora leaf spot is a foliar disease that causes spots with grey centers and reddish-brown margins. Spots become tan with a dark ring and yellow halo around the ring.

Cucumber mosaic virus (CMV)- CMV is a virus, which causes molting, yellowing and/or ring-spot patterns on leaves. Overall, infected plants will become stunted. Brown lesions may also form on foliage or fruit.

Tomatoes

If producing your own transplants, start seeds 6-8 weeks before planting in the garden. Transplants should be healthy looking, approximately 15 to 23 cm tall, with a sturdy stem. The best conditions for transplanting are cool, cloudy days. In sunny weather, it is best to wait until late afternoon or evening to transplant. Plants should be placed 38 to 61 cm apart if staking or trellising tomatoes and 1 to 1.5 m apart if not staking. Tomato side shoots (suckers) can be pruned, removed and discarded. Blossom end rot, a calcium deficiency in fruit, can be common on some tomato cultivars and during drought. To avoid this, ensure that the plant is receiving proper irrigation.

Diagnosing tomato pests

Aphids (Aphididae family)- Aphids are small, soft bodied insects with piercing, sucking mouthparts. They have pear or oval shaped bodies with long legs and antennae. They have a pair of tube structures projects from their rear. Aphids are found in dense groups, and can be distinguished from other insects because they do not fly away when disturbed. Damaged plants appear stunted and yellow.

Whitefly (Aleyrodidae Family)- Whiteflies are small flies that live on the underside of leaves and will fly away if disturbed. Damage is caused by adults feeding on plant sap, causing yellow leaves and curling. After feeding, there is often a sooty, black fuzzy growth called honeydew.

Yellow leaf curl virus (Geminiviridae)- Yellow leaf curl is a plant virus. Infected tomato plants are stunted and have a bushy appearance. Leaves of infected plants are small and curl upward. Leaves often have yellowing between leaf veins.

Bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*)- Bacterial leaf spot is a foliar disease that causes many small, dark spots to appear and the spots can be

surrounded by a yellow, water-soaked halo. Dead leaves usually stay hanging on tomato plants.

Powdery mildew (*Erysiphe* sp., *Leveillula taurica*)- Powdery mildew is a foliar disease that first appears as white, powdery spots that may form on both surfaces of leaves, on shoots, and sometimes on flowers and fruit. These spots gradually spread over a large area of the leaves and stems.

Early blight (*Alternaria solani*)- Early blight is a foliar disease that causes lesions, which begin as brown circles with yellow halos. As they expand, they may form concentric rings in a target spot pattern. Early blight may spread to unripe fruit forming dark, sunken lesions.

Septoria leaf spot (*Septoria lycopersici*)- Septoria leaf spot is a very common disease of tomato often confused with early and late blight. On tomato plants, Septoria lessions tend to develop on the bottom leaves first. Lesions are tan with small brown/black dots inside.

Late blight (*Phytophthora infestans*)- Late blight is a very well known disease due to its ability to cause complete destruction of potato and tomato plants if left unmanaged. The late blight pathogen requires living tissue to survive. Lesions are brown surrounded by a pale-green halo. Fuzzy white signs of the pathogen can often be found on the underside of an infected leaf.

Adapted and revised by Jennie D. Mazzone, master's student of plant pathology, Penn State. Original article, Penn State Vegetable Gardening: Recommendations for Home Gardeners (2010), Prepared by E. S. Sánchez, associate professor of horticultural systems management; P. A. Ferretti, professor emeritus of vegetable crops; T. E. Elkner, senior extension educator in Lancaster County; S. M. Bogash, VP Marketing and New Product Development at ISP Technologies; S. J. Fleischer, professor of entomology; B. K. Gugino, associate professor of plant pathology; W. J. Lamont Jr., professor of vegetable crops; M. D. Orzolek, professor emeritus of vegetable crops; and G. Pryor, master gardener coordinator.

Figures

Fig. 5.1. Gugino and Mazzone (center) with Horticulture Innovation Lab workers Patricia Azucena Arce Valladares and Gabriela Suyapa Hernandez Casco (left) and student translator Emmanuel Villeda Rivera (right).

Fig. 5.2. Mazzone and Valladares identify bacterial disease on onions in horticulture production area.

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APPENDIX: FIRST REPORT OF TOMATO FOLIAR BLIGHT CAUSED BY *RHIZOCTONIA SOLANI* IN PENNSYLVANIA

In July 2014, a foliar blight on tomato was observed in a 4A commercial production field in Union County, Pennsylvania on cvs. Mountain Fresh Plus, Mariana and Biltmore. Symptoms of the foliar blight were primarily located in the mid- to upper plant canopy and included brown, necrotic lesions and in severe cases, blighting of entire leaves. Signs of white mycelial growth were observed on the abaxial surface of the leaf. Incidence of the foliar blight in the field was estimated at 25% and disease severity per plant ranged from 5 to 30%. Microscopic observation of the leaf lesions identified basidiospores and mycelium with 90° branching angles characteristic of *Rhizoctonia* spp. (Butler and Bracker, 1970). Isolations were made from the margin of leaf lesions onto potato dextrose agar (PDA). Mycelium was also lyophilized, and DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD). PCR amplification was performed with ITS4 and ITS5 primers and sequence analysis of the 667 bp (GenBank accession KT758847) internal transcribed spacer region (ITS) indicated 99% identity with 100% of the query coverage to accessions of *Rhizoctonia solani* Kühn (teleomorph = *Thanatephorus cucumeris* (A.B. Frank) Donk) (eg. GQ885147). Tomato foliar blight caused by *R. solani* anastomostis group AG3 has been reported in Japan and North Carolina (Date et al., 1984; Ivors et al., 2009). In order to confirm anastomosis identity, macroscopic and microscopic somatic hyphal interactions were conducted according to Bartz et al. (2010). All pairings of the Pennsylvania isolate with tester isolates RHS1AP (AG3) and Tom7b (AG3) anastomosed, suggesting that our isolate belongs in the *R. solani* AG3 group. Pathogenicity tests on the tomato cv. Mountain Fresh Plus were conducted according to the protocol described by Bartz et al. (2010). Although the pathogenicity test was conducted three times, no basidiospores were observed, and only one out of twelve plants developed symptoms similar to the tomato foliar blight originally observed in the field. However, we were able to fulfill Koch's postulates using an alternate inoculation method by placing a 5mm diameter PDA plug onto the third or fourth fully expanded leaflet of five, six-week-old tomato plants. A sterile agar plug was used as a control for five additional plants. Plastic bags were placed over each tomato

plant and placed in a growth chamber maintained at 25°C and illuminated by 40-W cool, white fluorescent bulbs with a 12h photoperiod. Plants were watered with deionized water as necessary. Within one to five days, mycelium had grown from the agar plug onto the tomato leaflet, and within six days after inoculation, leaflets surrounding the inoculated leaf were blighted with signs of white mycelial growth, similar to those originally observed in the field. All five plants inoculated with *R. solani* showed blighting, and the pathogen was reisolated from symptomatic tissue and confirmed to be *Rhizoctonia solani* using the molecular techniques as previously described. When this experiment was repeated, four of the five inoculated plants showed blighting. No blighting was observed on control plants. This first report of *Rhizoctonia solani* AG3 causing foliar blight on tomato in Pennsylvania has increased the collective knowledge on foliar diseases of tomato and can be used in identification for more accurate diagnostics.

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