Factors Affecting Bulb Decay in Storage, and a DNA Macroarray Technique as a Tool for Early Pathogen Detection

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Plant Science

in the

College of Graduate Studies

University of Idaho

by

Guillermo Pugliese

Major Professor: Michael K. Thornton, Ph.D.

Committee Members: Brenda K. Schroeder, Ph.D.; John K. Fellman, Ph.D.

Department Administrator: Paul McDaniel, Ph.D.

May 2016

Authorization to Submit Thesis

This thesis of Guillermo Pugliese, submitted for the degree of Master of Science with a Major in Plant Science and titled "Factors Affecting Bulb Decay in Storage, and a DNA Macroarray Technique as a Tool for Early Pathogen Detection", has been reviewed in final form. Permission, as indicate by signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____

Michael K. Thornton, Ph.D.

Committee Members: _____ Date: _____

Brenda K. Schroeder, Ph.D.

_____ Date: _____

John K. Fellman, Ph.D.

Department

Administrator: _____ Date: _____

Paul McDaniel, Ph.D.

Abstract

Onion is an ancient crop with around 5000 years of human cultivation. Nowadays, onions are cultivated worldwide with approximately 84.7 million metric tons of production in over 170 countries. The USA is the third largest producer of onions, behind China and India. The Pacific Northwest (PNW) is the most important production area within the USA in terms of dry onion production for storage. In this area, onions are grown for storage for 1 to 6 months, during the winter when onions are not readily available. In most years, losses due to decay in storage are not important, however in some cases pathogens can cause decay in storage and cause significant losses. In the past few years, the PNW onion industry has been concerned about the level of bacterial rot in storage, and trying to understand the field conditions that could increase losses due to decay in storage. Therefore, field trials were conducted to modify field conditions in order to determine which increase the incidence of storage decay and evaluate a DNA macroarray technique as a predictive tool for the development of onion bulb rot during long term storage.

Acknowledgments

My utmost gratitude goes to my advisors Dr. Michael Thornton and Dr. Brenda Schroeder who trusted in me, and they always supported me in all the aspects.

My thanks and appreciation also goes to my other thesis committee member Dr. John Fellman for his precise advice, and for his contribution in this research.

I would like to thank Oksana Adams and Ransey Portenier who did a great job in conducting the field and storage procedures for this research.

I would like to express my gratitude to Robert Simerly and McCain Foods who supported this research, and also Robert was always predisposed to collaborate.

Finally, I want to the thanks to Fulbright and the San Juan government which supported my scholarship expenses.

Dedication

This thesis is dedicated to my wife Coty who supported me in this experience of studying abroad.

Also, I would like to dedicate this thesis to my parents that have been always supporting me in everything. And finally, I could not exclude my brothers and friends who have been always with me.

Table of Contents

List of Tables

List of Figures

CHAPTER 1: INTRODUCTION

1.1. Worldwide importance of the crop

Onions (*Allium cepa*) are native to the mountains of Turkmenia, Uzbekistan, Tajikistan and northern Iran, Afghanistan and Pakistan; and have been cultivated for over 5000 years. In fact, onion has been domesticated for such a long time that it is not present as a wild species (Brewster 2006). There are many similar ancient species such as *A. oschaninii* or *A. vavilovii*, but they have small bulbs or a long juvenile growth period (Brewster 2006). Examples of the historical importance of onion as a food crop include the paintings inside Egyptian pyramids and other tombs of the New and Old Kingdoms dating back to 3500 B.C., evidence of cultivation in China 5000 years ago, and onions are also mentioned in Vedic (Hinduism) and Sumerians (2500 B.C.) writing [National Onion Association (NOA) 2011]. Nowadays, onions are cultivated throughout the world, with approximately 170 countries where onions are produced (NOA 2011). According to FAO's statistics 84.7 million metric tons of dry bulb onions were harvested in 2013 (FAOSTAT 2015). Asia has long been the region with highest production due to the popularity of this crop in China and India. For example, in 2013 China produced 22.3 million metric tons and India produced 19.3 million metric tons, being number 1 and 2 in worldwide production, respectively. In third place in 2013, with a production of 3.2 million metric tons, was the USA (FAOSTAT 2015). In terms of yield, the USA ranked fourth with 54 metric tons/ha, whereas Ireland ranked first with 68.7 metric tons/ha (FAOSTAT 2015). In terms of consumption, onions are the third most popular fresh vegetable in the USA, with an average of 10 kilograms per capita (NOA 2011).

In summary, onions, which were initially cultivated in the mountains of Asia over 5000 years ago, have become a significant crop around the word, including in the USA, where production and consumption are important.

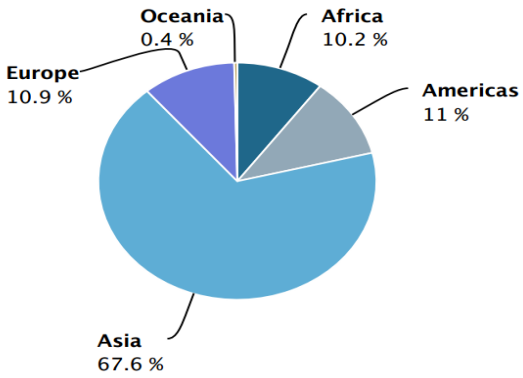


Figure 1: Onion production distribution in 5 continents. Extracted from FAOSTAT.

1.2. USA production

Within the USA, the dry bulb onion business produces 1 billion (USD) at the farmgate, and 5 to 7 billion at the consumer level. The USA onion production represents 4% of the word supply (NOA 2011), and this vegetable is produced on around 53,800 hectares (NASS 2016). The Pacific Northwest (PNW) is the principal producer of dry bulb onions for storage, with around the 50% of the total USA production and 63% of the production of dry summer onions for storage [National Agriculture Statistic Service (NASS) 2016]. According to USDA statistics (NASS 2016) for 2015, Washington grew close to 700,000 metric tons and was the most productive state, followed closely by Oregon with 662,000 metric tons, California with 655,350 metric tons, and Idaho with 292,621 metric tons. Summer onion production for storage in these four states in the year 2015 represented approximately 67% of the total dry onions harvested in the USA (NASS 2016).

Onion production in the USA is also distributed by date of planting, where there are both fall and spring planted production regions. Short-day length and intermediate-day length varieties are fall planted in southern California, Georgia, Texas, Arizona, New Mexico, New York, Colorado, Washington; with limited production Florida, South-North Carolina and Hawaii (NOA 2011), [Idaho Easter Oregon Committee (IEOOC) 1999]. These onions are generally not stored for more than a few weeks, and are marketed from April to August. On the other hand, mostly long-day length varieties are planted in the spring and marketed from August to April, often following extended periods of storage. The principal states for production of spring planted onions are the PNW (Idaho, Oregon, and Washington), Colorado, New York,

Michigan, Minnesota, Wisconsin, central California, Utah, Nevada, North Dakota, Illinois, Indiana, Iowa, Ohio, and limited production in Pennsylvania and Massachusetts. (IEOOC 1999, NOA 2011).

1.3. Classification of onion varieties

As mentioned above, dates of planting and area of production are associated with onion varieties where the day length is one of the most important features that determines adaptability (Brewster 2006). Thus, onions respond to the length of the day which stimulates bulb development of the onion plants. In this way, short-day types begin to develop bulbs when the day length surpasses 11-12 hours, intermediate-day types need day length of about 13-14 hours, and long-day types need more than 16 hours (Brewster 2006). Although, the length of the day determines the initiation of the bulb, temperature also plays an important role in the beginning of the bulb development (Brewster 2006).

Other features that differentiate onion varieties are shape and skin color (Brewster 2006). In the USA, yellow onions are the most widely produced type, with 87% of the total production, about 8% red onions, and 5% white onions (NOA, 2011). In the PNW, most of the onion production is based on long-day yellow varieties that are spring planted (IEOOC 1999). The onions from this region are marketed fresh from the field (without storage) during August to October; and then onions from storage are sold from November through April (USDA 1997).

1.4. PNW production practices

In the spring (March to April) onions are directly sowed with precision planters. A few growers in this region will initiate onion production by planting transplants, mainly to speed crop production and allow early harvest (Schwartz et al. 2012). Most onion seed used in this region is coated to improve the precision of the planting operation, and the seed coating contains pesticides (fungicides for damping-off and insecticide for maggots) and fertilizer (Schwartz et al. 2012) along with an adhesive or binder that give a spherical shape to the seed (Taylor et al. 2000). For germination, the seed needs a temperature greater than 1-3ºC with an optimum above 11ºC (Sullivan et al. 2001). After emergence, the onion plants grow very slowly until the third leaf stage, when new and larger leaves increase the growth rate. Growth and development is closely related to temperature and is stimulated by temperatures above

4.5ºC and below 27ºC (Sullivan et al. 2001). At the same time as leaf development, new roots are produced from the basal plate, generating a shallow, sparsely branched root system (Sullivan et al. 2001). New leaves are produced until the length of day stimulates growth of the bulb. In general, the onion plants have six to eight leaves when bulbing starts (Schwartz et al. 2012, Sullivan et al. 2001). Temperature and light quality regulate bulb growth, however they are not as important as day length (Brewster 2006, Sullivan et al. 2001). This short description of onion crop development doesn't address many procedures such as pest control, fertilization, irrigation and others where each action has its own criteria.

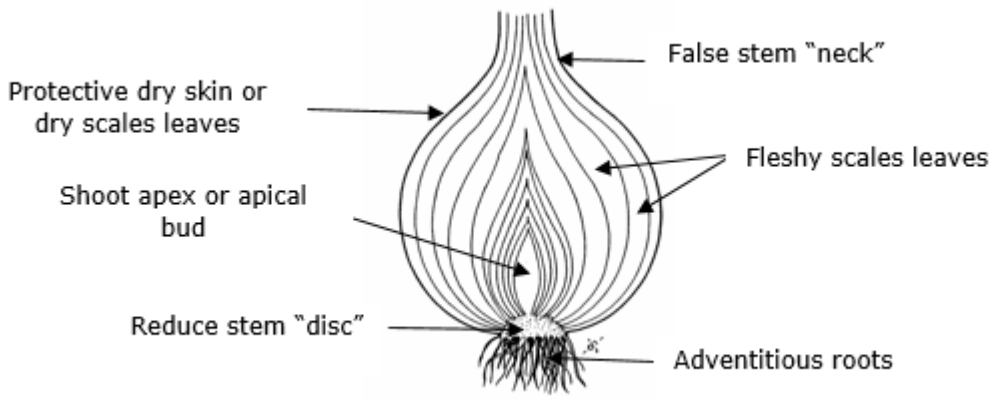


Figure 2: Diagram of the onion bulb parts. Shown are the disc which is a modified stem, the fleshy scale and the dry scale which are modified leaves, the adventitious roots, the false stem or neck and the apical shoot.

1.5. Curing practices

With time, depending on weather, marketability or storage schedule, onions are ready to harvest (Sullivan et al. 2001). In general, lifting (undercutting) is done after 50-75% of the plants have fallen over (Schwartz et al. 2012). After lifting and before the harvest, the onions remain in the field for 10 to 20 days for the purpose of "field curing". This process dries and closes the "neck" of the onion, and at least one or two outer scales dry down which is important for protection and to avoid dehydration during storage. Drying the neck prevents the growth of some pathogens (Schwartz et al. 2012), and the movement of bacteria and fungi through the neck into the bulb (Brewster 2006). After field curing, the onions are typical mechanically harvested and placed in storage facilities where postharvest curing can be done.

In general, the semiarid weather conditions of the production areas of the western USA allow good field curing (Schroeder et al. 2012). Nevertheless, in some seasons late rainfall or wet conditions could occur at the end of crop development and/or during field curing. This condition could lead to more bacterial rot in storage (Schwartz and Mohan 2008, Wright et al. 1993), and an increase in *Botrytis* infections can be seen when temperatures are between 10ºC and 25ºC with the wet conditions during the end of the season (Schwartz and Bartolo 2011).

Once in storage, the purpose of the postharvest curing is to dry the wounds produced during the harvest, and for the final drying of the onion neck (Schwartz et al. 2012). During this step some storage managers, depending on the year, use supplemental heating to raise bulb temperatures to about 35ºC in an effort to reduce storage rot (Schwartz et al. 2012). Other managers only use ambient air for curing (Vaughan et al. 1964). After curing, the storage temperature is ramped down to the holding temperature of 5ºC with 70% relative humidity (Schwartz et al. 2012).

1.6. Disease losses in storage

During storage there can be significant losses due to decay by pathogenic organisms. Some empirical data report losses of 10 to 40% in storage by onion bulb rot pathogens (Schwartz et al. 2012). A study of onion shipments to the New York market (Ceponis et al. 1986) found that about 16% of the inspected shipments during a 12-year period had some level of storage disease or disorder.

There are many factors that can increase susceptibility to pathogen infection and increase the incidence of decay in storage. Some of these factors can occur during the production season, such as wounds from nematode or insect feeding, hail damage to the leaves or bulbs, or weather conditions that favor pathogen infection. Other factors occur during storage, such as poor conditions for curing and holding which can significantly impact the incidence of storage rot.

According to the Compendium of Onion and Garlic Disease and Pests (Schwartz and Mohan 2008) there are eleven fungal diseases that can affect onion bulbs in storage. These diseases include: neck rot (*Botrytis allii and B. byssoidea*), brown stain (*Botrytis cinerea*), other Botrytis rots, black mold (*Aspergillus niger*), blue mold (*Penicillium sp*.), Diplodia stain

(*Diplodia natalensis*), mushy rot (*Rhizopus microsporues* and *R. stolonifer*), smudge (*Colletotrichum circinans*), Fusarium basal rot (*Fusarium oxysporum f sp. cepae*), Fusarium bulb rot (*Fusarium proliferatum*), and white rot (*Sclerotium cepivorum*). The latter three diseases are primarily important in the field, but also can affect onion bulbs in storage. Finally, there is one yeast, *Kluyveromyces marxianus var. marxianus* (Schwartz and Mohan 2008), that can cause onion decay in storage. According to Schwartz and Mohan (2008) there are twelve bacteria that are documented to cause onion bulb decay. These include *Burkholderia cepacia, B. gladioli pv. alliicola, Dickeya chrysanthemi, Enterobacter cloacae, Erwinia rhapontici, Pantoea agglomerans, P. allii, P. ananatis, Pectobacterium carotovorum subsp carotovorum, Pseudomonas aeruginosa, P. marginalis pv. marginalis,* and *P. viridflava*.

The causal agents of the symptoms that are produced by these various pathogens are often difficult to determine by visual inspection. Fungal rots can be somewhat easier to identify when the signs (sclerotia, hyphae, etc.) are present. However, bacterial rots are particularly difficult to identify by visual symptoms. Therefore, a tool that can provide an accurate identification would be very useful.

As a summary, the following figure shows a speculative disease triangle for onion bulb decay in storage where, beginning with the host, there is limited resistance to bulb rot pathogens (Schwartz and Mohan 2008). However some differences among onion varieties in decay incidence due to neck rot have been observed (Shock et al. 2014). As for the conducive environment, the moisture in the field at the end of the season (Schroeder et al. 2012, Schwartz and Bartolo 2011), prolonged heat curing in storage (Schroeder and du Toit 2010), and moisture in storage (Matson et al. 1985), can separately or in combination increase onion bulb decay in storage. Of course, only virulent pathogens produce onion decay in storage, including both fungi and bacteria. These pathogens could be seed borne (Maude and Presley 1976, Walcott et al.2002) or could produce infection from soil and air borne propagules (du Toit et al. 2004). The pathogens could be found in plant debris or soil or epiphytic on weeds (Gitatis et al. 2002a). Finally, some bulb rot pathogens could be vectored by thrips (Gitatis et al. 2002b). In short, seed soil and air borne pathogens could produce onion decay in storage.

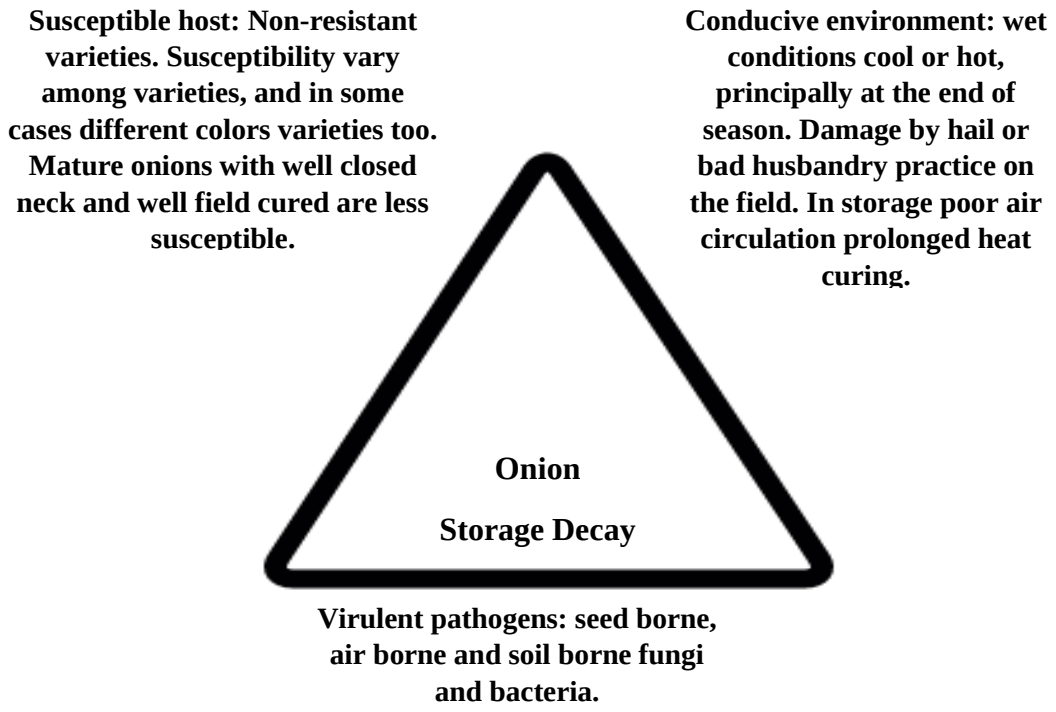


Figure 3: Onion storage disease triangle with a short summary of the three components susceptible host, conducive environment and virulent pathogens.

1.7. Incidence of major storage diseases

Obviously, some pathogens produce the most important losses. The most important losses by postharvest disease in onion in temperate zone regions are neck rot caused by *Botrytis allii* and *B. byssoidea*, (Schwartz and Mohan 2008). This disease can often cause 30% or more of losses in production regions as diverse as Australia, Canada, Europe, Israel, Japan, New Zealand and the USA (Schwartz and Mohan 2008). Ceponis et al. (1986) noted that depending on the onion variety shipped, incidence of gray mold (*Botrytis sp.*) ranged from 20 to 35%.

The PNW is one of the production regions where neck rot is an important postharvest disease. Due to the prevalence of this disease, early research efforts were conducted to find management practices that could reduce losses. Thus, it was reported that curing with forced air at 30 to 46ºC during early storage reduced the incidence of neck rot (Vaughan et al. 1964). This heat treatment is effective for *Botrytis* (Stanger et al. 1987), but at the same time could predispose the bulbs to other important rots, such as bacterial rot (Schroeder and du Toit 2010, Schroeder et al. 2012) or black mold (*Aspergillis niger*) (Crowe 2000). These diseases were

reported by Ceponis et al. (1986) to affect onion shipments to New York, with an incidence of bacterial decay averaging 25% (in some cases up to 50%), and incidence of black mold ranging from 3 to 12%.

Also there are some diseases, such as Iris Yellow Spot caused by *Iris Yellow Spot Virus* (IYSV), which growers have suggested predispose onion bulbs to more decay in storage. Likewise, weather conditions during the field curing phase, such as rainfall before the harvest (Schwartz and Mohan 2008), may increase the probability of rot in storage. All these topics will be addressed in the following chapter.

1.8. Identifying organisms responsible for decay

A major challenge for onion storage managers is that onion bulbs can be latently infected at harvest (Kritzman et al. 1981, Schwatz and Mohan 2008). That is the onion bulbs could look healthy at harvest, but with a pathogen already present in the bulb tissue, once in storage the pathogen could grow throughout the bulb causing storage rot. Knowledge of which pathogens are present in onion bulbs at harvest and their incidence could help to predict which crops are more at risk for developing rot in storage. Typically, identification of pathogens has been done by culture-based techniques which require time to isolate and grow the organism; as well as knowledge of fungal and bacterial taxonomy. Some new techniques have been developed that use polymerase chain reaction (PCR) amplification with a DNA oligonucleotide array. These techniques could result in a very sensitive, specific tool for pathogen detection and identification (Lievens and Thomma 2005). Thus, a technique called a DNA macroarray seems to be a suitable technique for pathogen identification (Lievens et al. 2012) that could be used after the onions harvest, achieving early detection and identification of bulb rot pathogens. This topic will be addressed in the third chapter.

The first objective of this research project was to analyze field and curing conditions that affect the storability of onions, with the principal targets being the rate of IYSV infection, rainfall before harvest, and curing temperatures. The second objective was to evaluate the use of a DNA macroarray technique as a method to accurately identify pathogens present in onion bulb tissue sampled at harvest or post-curing prior to storage that may lead to the development of decay in storage. If this latter approach is successful, it is possible that postharvest curing practices could be adjusted to improve onion storability.

CHAPTER 2: FIELD CONDITIONS AND POST-HARVEST CURING PRACTICES AFFECTING ONION STORABILITY

2.1 Abstract

Onion (*Allium cepa*) bulb storage is possible in the Pacific Northwest because of good weather conditions at the end of the onion crop season. However, in some years, the development of onion bulb decay in storage is extensive causing big losses to stakeholder. Numerous factors can influence the development of onion bulb decay in storage, including thrips and virus infection on the field, moisture or rain events during or at the end of the season, no pesticide application, and misled field and/or storage curing parameters. In general, these factors increase the incidence of onion bulb decay in storage. Therefore, these factors could impact the crop prior to storage, or once the onion bulbs are in storage. For example, it is known that increased moisture before harvest could increase decay in stored onion bulbs. In addition, there are reports that *Iris Yellow Spot Virus* (IYSV) infection in the field increases the incidence of decay in storage, but this has not been demonstrated. Finally, once onions are stored, the curing parameters can significantly impact the development of onion bulb decay in the storage facilities. The challenge is to manage each of these risks to attain the desired outcome of long term onion bulb storage.

2.2. Introduction

Generally, in the PNW, the weather is dry and warm at onion harvest time, which are conditions that favor onion bulb storability and losses range between 2 to 10% (Vaughan et al. 1964). However, occasionally losses can range between 20 to 50% (Vaughan et al. 1964), and in some cases losses can be up to 100% in individual facilities (Pelter and Sorensen 2004). Therefore, it is likely that field conditions during onion crop production can increase the susceptibility of onion bulbs to decay in storage.

One of these field conditions could be infection of the onion plants with IYSV. There are currently no reports that directly tie IYSV infection to incidence of decay in storage. However, a relationship between virus infection and decay can be hypothesized based on the fact that IYSV causes necrotic lesions to form on onion leaves (Waters 2014) and it is known that pathogens such as *Botrytis allii* can colonize dead or senescent tissue (Jones and Mann 1963).

Bacteria that cause storage decay have also been reported to infect wounded tissue (Schroeder and du Toit 2010). Likewise onion thrips (*Thrips tabaci*), which vector IYSV could also vector bacteria that cause storage decay (Dutta et al. 2014). IYSV is associated with the relatively new problem called internal dry scale where part of one of the fleshy scales becomes dry and papery inside the bulb close to the onion neck, and this scale could be infected by fungi or bacteria resulting in onion bulb decay (Reitz 2014b). Lastly, it has been observed that IYSV infected plants do not mature in the same way as non-infected ones, remaining upright instead of falling over at the end of the season (M. Thornton, *personal communication*). This change in maturity could increase the plant's exposure to pathogens such as *Botrytis* (Vaughan et al. 1964).

Another field condition that could affect the incidence of decay in storage is moisture at the end of the season. Bacterial bulb decay in storage has been reported to increase with rain fall and/or hot temperatures prior harvest (Schwartz and Mohan 2008, Bishop 1990). Likewise, excess overhead irrigation at the end of the season was reported to increase bacterial decay in storage (Teviotdale et al. 1990, Wright et al. 1993). Decay due to fungal pathogens such as *Botrytis sp.* can be increased by exposure of onion bulbs to moisture at the end of the season (Schwartz and Bartolo 2011, Kritzman et al. 1981, Schwartz et al. 2013, Vaughan et al. 1964).

The final field condition that will be addressed is the application of fungicides and bactericides during the growing season to prevent storage decay. In general, it is recommended to apply products containing copper and/or EDBC fungicides as a preventive measure to reduce bacterial diseases in the field and storage (Schwartz 2013, Schwartz and Gent 2007). Acibenzolar-S-methyl has shown promise for controlling bacterial diseases (Gent and Schwartz 2005, Beer et al. 2014). Another indirect control is the suppression of thrips, which can vector bacteria and introduce them into plants during feeding (Dutta et al. 2014). For fungal diseases, the most significant control is application of a seed treatment fungicide which decreases the seed borne *Botrytis* sp. infection that is related to the incidence of neck rot in storage (Maude and Presly 1976, Schwartz 2013, Beaver and Devoy 1988). Foliar fungicide applications are recommended for preventing neck rot when climatic condition are favorable for disease development (Schwartz 2013, Schwartz and Bartolo 2011). Nevertheless, pesticide applications at the end of the season have not been shown to be consistently effective against

bacterial and fungal decay in storage (Beaver and Devoy 1986, Beaver and Devoy 1988, Thornton 2012, Schroeder et al. 2012, Crowe 2000).

Once harvested, onion bulbs generally are cured in storage in the PNW. This curing is done to remove excess moisture, heal wounds, and/or suppress rot in storage (Schwarz et al. 2012). It is well know that heat curing can reduce neck rot incidence (Vaughan et al. 1964, Harros and Harris 1969, Stanger and Ishida 1987-1988, Maw et al. 2004). However, heat curing can increase bacterial rot (Schroeder et al. 2012, Schroeder and du Toit 2010), and black mold (Crowe 2000, Thornton 2012). The reports of decay being increased by heat curing could be a problem of curing times, as there is evidence that long exposure to high temperature could increase bacterial rot (Vahling-Armstrong et al. 2015, Schroeder et al. 2012, Schroeder and du Toit 2010).

2.3. Thrips and IYSV

Onion producers have observed a correlation between a high incidence of IYSV infection and increased losses due to decay pathogens in storage (M. Thornton, *personal communication*). IYSV is a member of the *Tospovirus* genus, a group of viruses that is known to be vectored by insects, primarily thrips (Crowe and Pappu 2005). Although onion thrips (*Thrips tabaci*) is the most common species found infesting onion crops throughout the world, there are several other species that are found feeding on onion leaves (*Franklinella occidentalis, F. schultzei, F. tenuicornis, F. fusca*) (Schwartz and Mohan 2008).

In general, thrips feed by puncturing the leaf surface with their rasping mouth parts, and sucking out the cell contents (Waters 2014, Reitz 2014a). They have the behavior of feeding near the base or “neck” of the onion plants where new leaves are emerging. For that reason, thrips damage is observed when leaves expand, and this damage takes on a “silver” appearance (Reitz 2014a). Thrips feeding reduces the photosynthesis capacity of the leaves, and when the populations are large, the result is smaller onion bulbs and lower yield (Waters 2014, Reitz 2014a).

All thrips cause the same type of feeding damage, however, IYSV is only vectored by onion thrips. *Franklinella occidentalis* and *F. schultzei* do not vector IYSV in onions, but they are broad and efficient vectors of other viruses of the same genus (Crowe and Pappu 2005). IYSV

produces chlorotic, straw-colored, diamond shape lesions on the leaves and/or flower stalks (Schwartz and Mohan 2008). In some lesions, a green spot is apparent in the center of the infected patch (Schwartz and Mohan 2008, Gent et al. 2006). Finally, severe thrips infestation and the resulting IYSV infection could cause the onion plants to dry up and die (Waters 2014, Reitz 2014a).

IYSV was first reported in the south of Brazil in 1981 where it was associated with the Tomato Spotted Wilt Virus (TSWV), which causes symptoms similar to those described above on inflorescence stalks which could lodge producing big losses in onion seed production (Gent et al. 2006). In 1989, similar symptoms were observed in the Treasure Valley of western Idaho and eastern Oregon (Gent et al. 2006, Hall et al. 1993), and then the presence of a Tospovirus was observed in an electronic micrograph of affected tissue (Hall et al. 1993). By 1992-93, the virus had been observed in Arizona, California, Idaho, and Oregon (Gent et al. 2006). Finally, IYSV was identified and described in the Netherlands in 1998 affecting *Iris hollandica* leaves (Cortes et al. 1998). In the following years, the symptoms observed in 1989 in the USA were demonstrated to be IYSV (Gent et al. 2006).

Figure 4: Picture of IYSV infection. On the right: (A) Adult onion thrips (*Thrips tabaci* Lindeman). (B) Electron micrograph of *Iris yellow spot virus* (IYSV) from a thin section of infected *Nicotiana benthamiana* (photograph credit: Dr Abed Gera, Volcani Center, Bet Dagan, Israel). (C) Symptoms of IYSV in onion. (D) Reproduction of symptoms characteristic of IYSV infection in mechanically inoculated onion plants under controlled conditions (Bag et al. 2015). On the left: Severe outbreak of iris yellow spot in an onion bulb crop in Colorado (Gent et al 2006)

The relationship between IYSV infection and decay in storage can be explained by evaluating some of the characteristics of onion pathogens and the plant symptoms associated with virus infection. First, one of the symptoms of IYSV is necrotic leaf lesions. *Botrytis allii*, as representative of neck rot decay, can directly infect green tissue via stomata when temperature and humidity conditions are appropriate (Kritzman et al. 1981), however this pathogen also infects onion through dead or senescent tissue in the neck (Jones and Mann 1963). Therefore, premature die back of the onion leaves could increase the potential window of infection by *Botrytis* when climatic conditions are not conducive. Alternatively, IYSV infected plants have been reported to have delayed or altered maturity, as evidenced by the fact that the necks do not soften and fall over in the same time frame as in non-infected crops (M. Thornton, *personal communication*). *Botrytis* could infect the plant through the upright neck tissue, especially if this is associated with a delay in drying of the necks.

Another fungus that could be associated with IYSV infection is *Aspergillus niger,* which apparently does not grow in green tissue, but it grows well in dead and senescent tissue when temperatures are higher than 25ºC with appropriate levels of humidity (Hayden and Maude 1992, Nagerabi and Ahmed 2003). Again, IYSV lesions and premature die back could provide a window for *Aspergillus niger* to infect the dying tissue. Also, if the canopy condition of the crop is without green tissue that can transpire to evaporate water and decrease localized temperature, this condition could increase of the relative temperature and favor the disease.

Lastly, *Fusarium proliferatum* onion decay in storage has recently been associated with internal dry scale (Reitz 2014b) and IYSV infection (du Toit 2014). *F. proliferatum*, which has been detected in other countries, is known as a very good saprophytic fungus (Carrieri et al. 2013, Stankovic et al. 2007) that can grow in maize at temperatures between 25ºC and 30ºC when kernels have between 20 to 25% water content (Marin et al. 1995). Therefore, conditions produced by IYSV or dry scale might provide a suitable environment in the onion neck for this fungus to grow.

A possible relationship between IYSV infections and bacterial decay could be associated with the lesions produced by the virus and thrips feeding, which act as wounds which are an important pathway for bacterial infection (Schwartz and Gent 2007, Schroeder et al. 2012, Schwartz 2013). Also, IYSV lesions could be infected by bacteria such as *Pantoea*

agglomerans and *P. ananatis,* which have been demonstrated to be vectored by onion thrips (Dutta et al. 2014). Lastly, in the last few seasons, internal dry scale has been associated with increased incidence of bacterial decay (Reitz 2014b). In this way, premature death of severely IYSV infected plants could increase the occurrence of dry scale in storage and the subsequent bacterial rots (Waters 2014)

2.4. Moisture conditions during production and field curing

Another field condition that could affect onion storability is rain fall events at harvest. It is well known that onions produced under sprinkler irrigation systems can experience storability issues, so rain events at harvest could potentially cause the same problems. Research conducted in California (Teviotdale et al. 1990) evaluated four different irrigation regimens (furrow, sprinkler until bulbing and furrow for the remainder, sprinkler until 30 days past bulbing and furrow for the remainder, and sprinkler all season) in combination with spray inoculation of the sour skin pathogen, *Pseudomonas cepacia* (*Burkholderia cepacia*). Their results showed that the incidence of sour skin was significantly less in the two regimens with a reduced amount of sprinkler irrigation, regardless of bacterial inoculation. Likewise, research from New Zealand (Wright et al. 1993) evaluated the effect of lifting dates (50-70% of plants top-down, and 90% plants top-down), period of curing (15 and 28 days), and sprinkler irrigation on the incidence of decay. Bulbs from the sprinkler irrigation treatment had a significantly higher soft rot incidence than the other treatments. These studies show that sprinkler irrigation (and possibly rainfall) during the latter part of the crop season can increase the incidence of storage rots, principally bacterial decay.

Many other research reports indicate that rain or sprinkler irrigation at the end of the season is conducive for bacterial decay (Schwartz and Mohan 2008). Also, rain and moisture at the end of the season have been reported to increase neck rot in storage (Schwartz and Bartolo 2011, Crowe 2000). It is possible that pathogens such as *Botrytis* take advantage of rain or irrigation by penetrating the green leaf tissue without showing symptoms, and present in the bulb as a latent infection (Kritzman et al. 1981). Something similar could happen with bacteria, for example *Enterobacter cloacae* seems to be able to colonize the onion plant during the season and then remain latent before causing decay in storage (Schwartz and Mohan 2008). Lastly, rainfall and sprinkler irrigation could splash disperse bacterial propagules as bacteria generally

need the water source for dispersal and infection (Teviotdale et al. 1989). However, in some cases the role of water dispersal is not very clear (Gitatis et al. 2004)

2.5. Fungicide and bactericide application

In general, application of fungicide and bactericide products are recommended at the end of the growing season principally when rainfall occurs (Schwartz and Bartolo 2011, Schwartz 2013). The products recommended for control of fungal onion pathogens are broad spectrum fungicides such as chlorothalonil, mancozeb, metalaxyl, dimethomorph, ciprodimil + fludioxonil, boscalid, azoxystrobin, pyraclostrobin + boscalid (Schwartz and Bartolo 2011). These fungicides target in general *Botrytis*, downy mildew (*Peronospora destructor*) and purple blotch (*Alternaria porri*). In the case of bacterial pathogens the most recommended products are copper and EBDC containing fungicides (Schwartz and Gent 2007). Other products recommended for bacteria which promote the plants defense include as Acibenzolar-S-methyl (Actigard®) (Beer et al. 2013). Also, a new product based on a combination of hydrogen dioxide, peroxyacetic acid and acetic acid has been recommended for bacterial leaf blight and *Botrytis* (http://www.biosafesystems.com/product-ag-oxidate.html).

Even though all these products are recommended the results obtained are often inconsistent. Therefore, fungicide and bactericide applications do not ensure a reduction in storage decay (Crowe 2000, Beaver and Devoy 1986, Beaver and Devoy 1988). Other research indicates that consistency of copper applications against bacteria can be improved when the product is applied at the correct timing (Schwartz et al. 2002).

2.6. Post-harvest curing

Immediately after harvest, onion bulbs that are not immediately marketed are placed into storage and a postharvest curing is typically done in storage (Schwartz et al. 2012). As mentioned above, this storage curing helps to dry the neck and cure wounds, and a heat treatment can reduce the incidence of *Botrytis* sp. (Vaughan et al. 1964, Harrow and Harris 1969). In this way, the work of Vaughan et al. (1964) in Oregon showed a decrease in the incidence of neck rot when heat (35ºC to 45ºC for 24 to 48 hours) was applied. In addition, it was demonstrated that the use of heat curing decreased the presence of neck rot and increased the color of the dry scales (Stanger et al. 1987).

The control of *Botrytis* obtained by heat curing can be explained by a few factors that affect the fungi. First, taking *Botrytis allii* as the representative fungus in neck rot, the growth rate of this pathogen is decreased drastically by temperatures higher than 25ºC, and the fungus almost stops growing at 30ºC (Ramsey and Lorbeer 1985) (Figure 5). Therefore the reduction in fungal growth rate, plus the desiccation that heat curing produces in the neck reduces fungal colonization (Schwartz et al 2012). Another consideration is that *Botrytis allii* mycelium is killed when exposed to temperatures above 36ºC for 7 days, however, the spores remain alive (Harrow and Harris 1969). It is important to mention here that the other temperature extreme where the fungus stops growing is below 5ºC. Onions are commonly stored at temperatures below this point after heat curing.

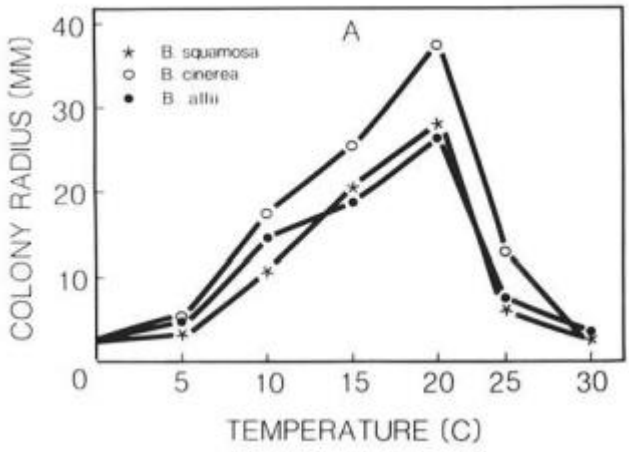


Figure 5: Growth rate of *Botrytis squamosa, B. cinerea* and *B. allii*, at different temperatures. Extracted from Ramsey and Lorbeer 1985.

Although these heat curing practices can reduce the incidence of some diseases, specifically neck rot, they also provide conditions favorable to other decay pathogens. In this way, slippery skin (*Burkholderia gladioli pv. alliicola*) and sour skin (*B. cepacia*) could affect the onions after heat curing (Schroeder et al. 2012). Similarly, it was demonstrated that the impact of *E. cloacae* bulb decay in onion could be increased by high temperature (more than 35ºC) and long periods of curing (Schroeder and du Toit 2010). Also, some fungi, such as *A. niger*, which causes black mold, grow better at 28-34ºC (Schwartz and Mohan 2008). Given that these temperatures are similar to the heat curing temperatures, black mold incidence might increase following heat curing (Crowe 2000, Anonymous 2012). On the other hand, at

temperatures of 20-25ºC and relative humidity of 70-80% (similar conditions of ambient curing) black mold contamination is significantly reduced (Nagerabi and Ahmed 2003). Thus, curing against neck rot with heat treatment could increase the incidence of other bacterial and fungal decays. In contrast, curing at ambient conditions may increase the incidence of neck rot. Thus, storage managers have to make decisions at harvest about curing conditions, based on their best guess as to which pathogens pose the most risk.

Even though the association of heat curing with some storage decay pathogen seems to be related to temperature, some research implies that exposure time could also be an issue. Postharvest curing at high temperatures could take between 2 to 3 weeks to ramp the temperature up to the desired level, and then cool the onions back to ambient conditions (Schroeder et al. 2012). These periods of time differ from the research reports on heat curing where heat was applied for 1 days to 10 days (Vaughan et al. 1964, Harrow and Harris 1969). Others have reported increased decay incidence when high temperatures (41ºC to 46ºC) were applied (Vaughan et al. 1964, Harrow and Harris 1969). Those high temperatures produced damage in the neck tissue, and after some time this affected area was infected by bacteria (Harrow and Harris 1969).

Another important factor that was observed in previous research, was the control of relative humidity during heat curing where the onions drying could cause weight loss and/or water condensation (Vaughan et al. 1964, Matson et al. 1985). The control of relative humidity during heat curing is not always an easy task as the bulbs release considerable quantities of moisture during curing (Anonymous 2012). In this way, the high humidity and increased temperatures associated with heat curing are conducive to infection by some pathogens, as noted previously.

2.7. Research approach

Consequently, the objectives of the research were first to determine if high levels of thrips feeding injury and resulting IYSV infection increase the incidence of decay in storage; second to determine if a simulated rain event before harvest increases the incidence of decay in storage; and third evaluate heat curing directly following onion harvest as a management practice to reduce decay. An additional approach was to evaluate if fungicide and bactericide applications could decrease storage decay.

It is hypothesized that higher thrips populations and IYSV infection lead to more onion decay in storage. It is also hypothesized that rain events before harvest would increase onion decay in storage (principally bacterial rot). Third, it is hypothesized that heat curing would increase bacterial decay and black mold, while decreasing the incidence of neck rot. Lastly, it is hypothesized that pesticide application would not decrease storage decay.

2.8. Materials and methods

2.8.1. Trial 2014

Field Trial: Field plots were established at the University of Idaho Parma Research and Extension Center on 15 March, 2014. The soil at the Parma station is a mix between the Greenleaf series (65%) and the Owyhee series (35%) characterized by a silt-loam texture in the top 20 centimeters, a silt-clay-loam to silt-loam in the following 25 centimeters, and silt to silt-loam 1.5 meters depth. The soil is well drained and deep, and is characterized by a slope of 0 to 1 %, coming from terrace land form where alluvium, lacustrine and loess deposit are the parent materials (consulted from NRCS USDA http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx). The onion variety planted was Vaquero which is a yellow medium storage, long day, globe variety, and its production cycle is around the 120 days (http://www.nunhems.com/www/NunhemsInternet.nsf/id/US_EN_Onion). The variety selection was based on the long period that this variety has been grown in the area (20 years) and for its importance in the fresh and processing markets (consulted from SeedQuest® http://www.seedquest.com/news.php?type=news&id_article=29279). The plots were direct sowed (coated seed) with a Mel Beck 6-row planter. Each plot was 15.24 meters long by 3 beds (3.35 meters wide). The field was irrigated with a drip irrigation system scheduled to maintain available soil water content above 65-70%. All herbicides and fertilizers were applied according to University of Idaho guidelines (Appendix).

The experimental design was a Completely Randomized Factorial Design with four replications. The field treatments are outlined in Table 1.

Table 1: Field treatments applied to Vaquero onions grown at Parma, ID during 2014.

Treatment	Thrips control	Simulated rain	Fungicide
1	**No**	**Yes**	**No**
2	**No**	**No**	**No**
3	**Yes**	**Yes**	**No**
4	**Yes**	**No**	**No**
5	**Yes**	**Yes**	**Yes**

These treatment combinations (as outlined in Table 1) were designed to provide onion bulbs with a wide range of decay potential.

Thrips control: Thrips management programs were selected to either promote or minimize insect feeding damage and IYSV infection. Treatments 1 and 2 received no foliar insecticide applications, while in treatments 3, 4 and 5 thrips and IYSV were controlled with a foliar program consisting of sequential applications of Movento (365 ml/ha), Radiant (585 ml/ha) and Agri-Mek (256 ml/ha). Weekly foliar insecticide applications began when thrips populations reached the threshold of 1 to 3 per plant, and there were a total of 6 applications between May 23 and July 2.

Fungicide application: A set of sequential foliar applications of the fungicide/bactericide products Tanos (585 ml/ha) and Kocide (1.12 kg/ha) were applied to treatment 5 only. All foliar treatments were applied with a CO2 powered backpack sprayer operated at 2.8 kg/cm^2 and applying a total volume of 375 liters/hectare. Non-ionic surfactant was added to the spray tank at mixing (0.25% v/v).

Table 2: List of insecticides and fungicide/bactericides applied in the 2014 trial. Commercial name, kind of pesticide active ingredient and concentration are shown.

Commercial name	Kind of pesticide	Active ingredient	Concentration of active ingredient
Movento	**Insecticide**	**Spirotetramat**	**15%**
Radiant	**Insecticide**	**Spinetorant**	**11.70%**
Agri-mek	**Insecticide**	**Abamectin**	**8%**
Tanos	**Fungicide**	**Famoxadone**	**25%**
		Cimoxanil	**25%**
Kocide	**Fungicide/ Bactericide**	**Copper Hydroxide**	**53.8% of Cu**

Simulated rainfall: Irrigation application was used to simulate conditions that occurred during the 2013 production season when rain near lifting was associated with a high incidence of bacterial and fungal decay during storage (M. Thornton, *personal communication*). A sprinkler irrigation system was set up in the field to simulate rainfall events prior to harvest. On 3 September, 19 millimeters of water was applied to all treatments, except 2 and 4, which were covered by tarps. The onions were lifted to initiate field drying on 9 September.

Thrips population: The number of onion thrips (both adult and larval stages) per plant was determined every two weeks from 22 May to 11 August (Table 3). At each sample date, leaves from five plants were destructively harvested from each plot. The leaves were cut from the area around the onion "neck" to 10 centimeter above the neck, and then placed in a jar which contained a soapy water solution. The water in each jar was strained through filter paper, and the leaves were rinsed with water to dislodge any thrips attached to the leaves, and the rinsate was filtered. The resulting sample was placed under a stereoscope, and the total number of nymphs and adults were counted where the number obtained represented the population of the 5 plants analyzed. In this way, the analysis of the data was done with the mean of the number of thrips counted in the 5 plants of the different field treatment through the seventh sample dates. In addition, the data was analyzed as the mean of thrips counted from 5 plants at different dates through the different field treatments. In this trial only field treatments 1 through 4 were measured.

Table 3: Dates of sampling for thrips number evaluation trial 2014.

Sample number	Date
1	**05/22/2014**
2	**06/2/2014**
3	**06/18/2014**
4	**07/1/2014**
5	**07/14/2014**
6	**07/28/2014**
7	**08/11/2014**

IYSV incidence: The incidence of IYSV was determined visually on 15 July and 4 August by evaluating all plants in the center bed of each plot for presence of typical virus lesions (white to straw colored areas that elongate at the leaf grows) (Schwartz and Mohan 2008). The total

number of plants exhibiting IYSV lesions was divided by the total number of plants per plot to calculate % infection.

Plant maturity: Plant maturity, indicated by visually estimating the proportion of plants with necks that had softened and fallen over, was rated on 25 August.

Harvest: Onion bulb samples for storage evaluations were harvested by hand on 26 September. Replication of the field treatments was maintained during collection of the storage samples. Onions were first graded according to diameter, and only bulbs with diameters between 7.5 to 11.25 centimeters were included in the samples. The bulbs were put into 22.7 kg bags, and the bags were labeled by field and curing treatment (see below). The tag color represented the different storage's evaluation times, and the codes were the different field and post-harvest curing treatments.

Curing treatment: There were 5 different post-harvest curing treatments. All the treatments were done in the Parma Research Center storage facilities where there were different rooms that could be set to different curing temperatures. The curing treatments were the following: 1.Curing at ambient temperatures for 2 weeks, 2. Curing at 35ºC 2 weeks, 3. Curing at 30ºC for 2 weeks, 4. Curing at ambient temperatures for 2 weeks, followed by 30ºC for two weeks, and 5. Curing at ambient temperatures for 2 weeks, followed by 35ºC for two weeks.

Storage evaluation: After the respective curing treatments, the samples were placed in a single storage room, and held between 2ºC and 8ºC (the beginning of the storage season was characterized by a period of higher temperatures). The samples were held between 50 and 90% relative humidity, with a period of lower values at the beginning (Appendix). The onions were evaluated at 2 different dates to determine the incidence of decay. The dates of evaluation were: 3 months after harvest (18 December, 2014), and 6 months after harvest (18 March, 2015).

Evaluations: First, the total number of onion bulbs in each sample was determined. Second, each bulb was cut in half, lengthwise through the neck. Each bulb was evaluated visually for presence of decay, and the diseased onions were inspected to determine if the primary causal agent was bacterial or fungal. Random bulbs exhibiting typical rot symptoms were collected and for causal agent isolation.

The results of the storage evaluation were expressed as the proportion of decayed onions. The analysis was complete for each curing parameter by each field treatment. In this way, analysis was completed to detect differences among curing treatments and between field treatments separately and their interaction. Also, the evaluation was done on two different dates, 3 months and 6 months postharvest. Lastly, this data was analyzed to determine if there were differences in the observed percentages of bacterial rot. For those reasons the analyses were divided in all the curing and field treatments 1 to 5 for the two different evaluation dates, 3 and 6 months, and the evaluation was also divided in total decay analysis and bacterial decay analysis.

The media used for pathogen isolation were nutrient broth yeast extract agar (NBY) with addition of 50 µg/ml cyclohexamide (Sigma-Aldrich®, Saint Louis, MO) for bacteria isolation, and potato dextrose agar with 100 µg/ml streptomycin (Sigma-Aldrich®, Saint Louis, MO) for fungal isolation (Appendix)

2.8.2. Trial 2015

Field treatments: The field plots were direct sowed on 18 March, 2015 at the Parma Research Station and Extension Center. The planting arrangement, irrigation and other cultural practices were similar to 2014. There were five field treatments arranged in a randomized completely block factorial design with 1 replication per each of the four blocks. The design was mixed, where field and curing were already fixed treatment and blocks were randomized. The five fields treatments were the same as in 2014 (Table 1).

Thrips control: Thrips management programs varied from the first trial, and the insecticides were selected to either promote or minimize insect feeding damage and IYSV infection. Treatments 1 and 2 received no foliar insecticide applications, while in treatments 3, 4 and 5 thrips and IYSV were controlled with a foliar program consisting of sequential applications of Exirel (986 ml/ha), Lannate (3.5 liters/ha) and Radiant (585 ml/ha). Weekly foliar insecticide applications began when thrips populations reached the threshold of 1 to 3 per plant, and there were a total of 9 applications between May 26 and July 21.

Fungicide application: The fungicide/bactericide program for field treatment 5 was the same as 2014 trial.

Table 4: List of insecticides and fungicide/bactericides applied in the 2015 trial. Commercial name, kind of pesticide active ingredient and concentration are shown.

Commercial name	Kind of pesticide	Active ingredient	Concentration of active ingredient
Exirel	**Insecticide**	**Cyantraniliprole**	**10%**
Radiant	**Insecticide**	**Spinetorant**	**11,70%**
Lannate	**Insecticide**	**Methomyl**	**29%**
Tanos	**Fungicide**	**Famoxadone**	**25%**
		Cimoxanil	**25%**
Kocide	**Fungicide Bactericide**	**Copper Hydroxide**	**53,8% of Cu**

Simulated rainfall: Irrigation was applied to treatment 1, 3 and 5 on September 2. A total of 19 millimeters of water was applied, and the onions lifted on 8 September.

Thrips populations: The same procedure was used as in the first year, with population counts done on 5 dates between 3 June and 29 July (Table 5).

Table 5: Dates of sampling for thrips number evaluation trial 2015.

Sample number	Date
1	**06/3/2015**
2	**06/17/15**
3	**06/30/2015**
4	**07/15/2015**
5	**07/29/15**

IYSV incidence: This analysis was done in the same way as the first year. The evaluation dates were the 14 and 27 July.

Plant maturity: The proportion of mature plants as indicated by necks that had fallen over was determined on 17 August.

Harvest: The harvest was done by hand on 1 October, and the procedure was the same as in 2014.

Curing treatment and storage evaluation: The 5 curing treatments were the same as the first year described above. Following the curing treatments, the samples were stored between 5ºC

and 10ºC, and the relative humidity was maintained between 50% and 80%. The temperature was higher at the start of the storing period, and lower at the end of the storage season. Similarly relative humidity started at lower values and finished at higher values (Appendix). The storage evaluation was done at 3 and 6 months after the harvest on 1 February, 2016 and 7 April, 2016, respectively.

Random bulbs exhibiting typical rot symptoms were collected from the samples and causal agents were isolated on using ½ V8 media with 50 µg/ml tetracycline (Sigma-Aldrich®, Saint Louis, MO) and 50 µg/ml streptomycin (Sigma-Aldrich®, Saint Louis, MO) and NBY (Appendix).

2.8.3. Statistical analysis

Observing the distribution of the data obtained from the different measures, it was observed that the different data fitted in different distribution patterns. In this way, the thrips population data is represented by a Poison distribution, and the decayed onion proportion is represented by a binomial distribution. Hence, with some data distributed as binomial and Poison the ANOVA loses power because this analysis has as assumption that the data is normal distributed. To meet this assumption the non-normally distributed data was transformed using arcsine, square root or other kind of transformations which fit de data in normal distributions. Although, these transformations can adjust the data to a normal distribution, the ANOVA is demonstrated to lose accuracy when data is transformed (Stroup 2014). Therefore, the analysis of the data was completed using the Generalized Linear Mixed Model (GLMM) because it has the feature of adapting to all data distributions, and it gives more accurate results in non-normal distributions (Stroup 2014). Using SAS (SAS Institute Inc., Cary, NC) the data was analyzed using the glimmix procedure.

Trial 2015 was analyzed differently than trial 2014, as the block variable was used as random effect which decreased the error variable, giving accuracy to the analysis.

2.9. Results

2.9.1. First year's results

IYSV incidence: On the first evaluation date there were significant differences among the four field treatments (Figure 6). Treatments 1 and 2 (without thrips control) had IYSV incidence of 2.5 % and 2.3 % which as significantly higher than treatments 3 and 4 (with thrips control) which were 0.67 % and 0.26 %, respectfully. Similar results were obtained on the second evaluation date, but there was larger differences among treatments due to the fact that for field treatments 1 and 2 all the plots (8 plots) presented 100 % infected plants (Figure7). This fact made the data more extreme and the analysis not as consistent (dispersion). However, the graphs (Figure 6 and 7) show very consistent differences among field treatments for both dates.

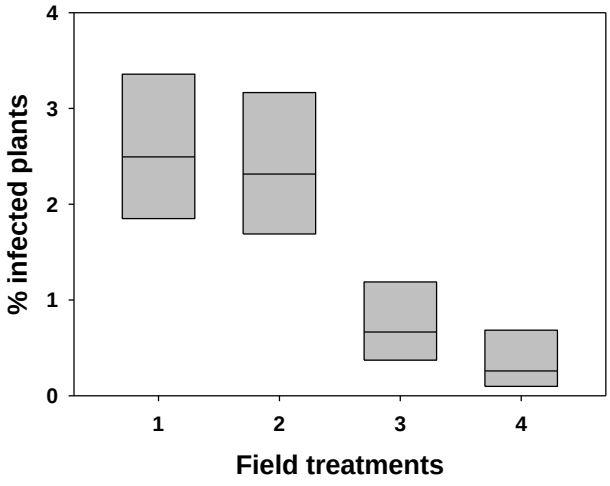


Figure 6: The effect of insecticide treatment on IYSV infected plant proportions on 15 July, 2014 for Vaquero onions grown at Parma, ID. Values are means of 4 replications with 95% confidence limits.

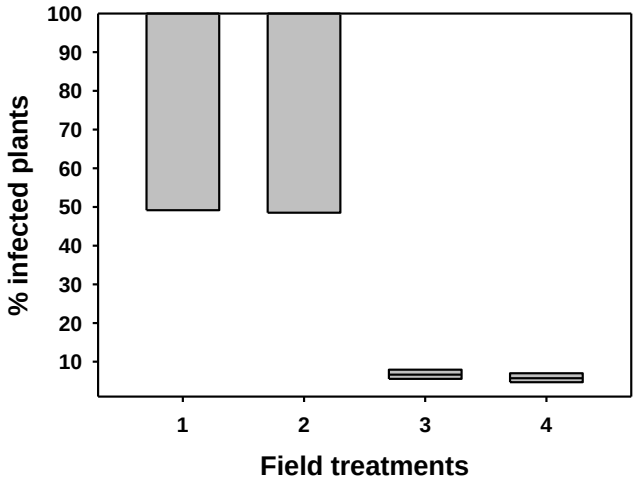


Figure 7: The effect of insecticide treatment on IYSV infected plant proportions on 4 August, 2014 for Vaquero onions grown at Parma, ID. Values are means of 4 replications with 95% confidence limits.

Thrips population: The data obtained during the seven evaluation dates showed that there were significant differences among the field treatments, and between the different dates, but this analysis showed that there were interaction between field treatments and date. Therefore, because of this interaction it is more difficult to interpret the main effect of the field treatments or dates. However, the analysis of data showed that in field treatments1 and 2 (without thrips control) more thrips were present compared to the other treatments (Figure 8), and also thirps populations were higher on the 4^{th} and 5^{th} sample dates compared to earlier or later in the season (Figure 9). Also it is remarkable that the interaction plot (Figure 10) shows a considerable peak in date 4 and 5 for the field treatment 1 and 2 in the means of the number of thrips counted, whereas the other field treatments also show peaks, but at considerably lower values.

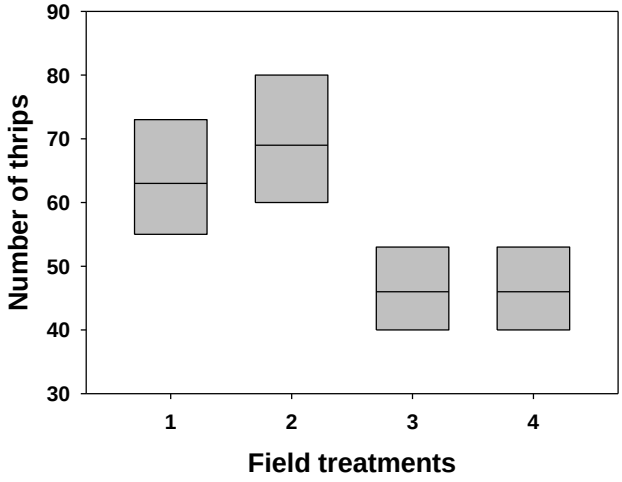


Figure 8: Main effect of insecticide treatment on mean thrips populations (No. per 5 plants). Values are means of the 4 field treatments and 4 reps with their 95% confidence limits where treatments 1 and 2 did not have insecticide applications.

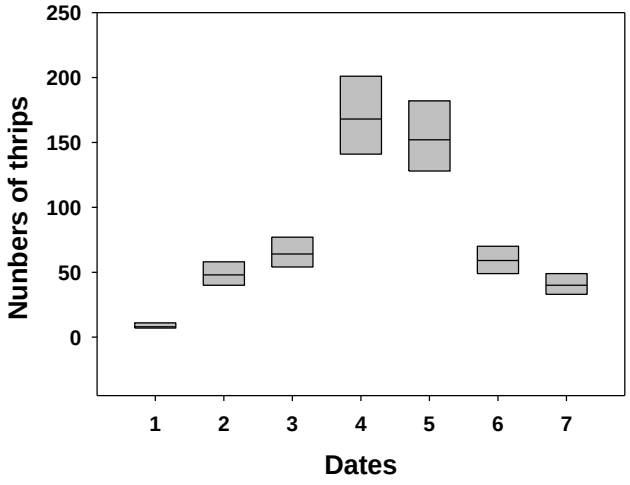


Figure 9: Main effect of sample date on mean thrips populations (No. per 5 plants). Values are means of 4 field treatments and 4 reps with their 95% confidence limits.

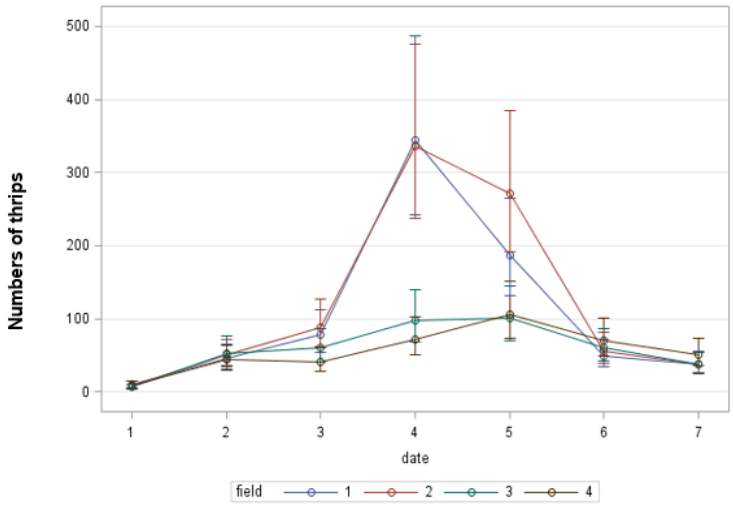


Figure 10: Interaction between sampling date and insecticide treatment on mean thrips population (No. per 5 plants) Values are means of 4 reps and their 95% confidence limits.

Plant maturity: The evaluation was done close to crop maturity of almost all the treatments, and the values showed a skewed distribution of the data towards 100% maturity, principally in field treatments 3, 4 and 5 (94 %, 82 %, 85%, respectively). Therefore, the analysis showed significant differences (p-value < 0.0001) between field treatments 1 and 2, and the other 3 field treatments (Figure 11), but it also showed a large variability that makes the analysis less confident. Nevertheless, there were important differences, with treatments 1 and 2 (without thrips control), having significantly lower proportion of mature plants than the other treatments (with thrips control).

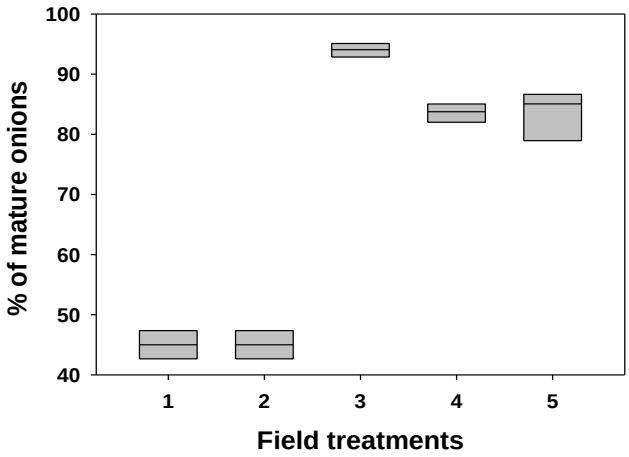


Figure 11: Effect of field treatment on proportion of mature plants (top down) with 95% confidence limits. Values are means of 4 reps.

Storage evaluation:

Total decay after 3 months: In this analysis the variables evaluated included field treatments, curing treatments and the interaction between them. At this evaluation date the interaction between field and curing treatments were not significant. Thus, the analysis focused on the main effect of field treatment and curing treatment (Table 6).

Table 6: Summary of statistical analysis of decayed onion proportions trial 2014.

Date evaluation	Evaluation	Effect	Num DF	Den DF	F value	Pr > F
3 months	**Total decay**	**Field**	**4**	**75**	**18.31**	**< 0.0001**
		Curing	**4**	**75**	**40.47**	**< 0.0001**
		Field*Curing	**16**	**75**	**1.13**	**0.3475**
	Bacterial decay	**Field**	**4**	**75**	**0.34**	**0.8509**
		Curing	**4**	**75**	**1.15**	**0.3382**
		Field*Curing	**16**	**75**	**0.93**	**0.5372**
6 months	**Total decay**	**Field**	**4**	**71**	**26.33**	**< 0.0001**
		Curing	**4**	**71**	**22.20**	**< 0.0001**
		Field*Curing	**16**	**71**	**2.33**	**0.0079**
	Bacterial decay	**Field**	**4**	**71**	**4.17**	**0.0043**
		Curing	**4**	**71**	**20.36**	**< 0.0001**
		Field*Curing	**16**	**71**	**1.50**	**0.1255**

Field treatments 1 and 2 had the highest proportion of decayed onions, with 25.66 % and 24.88% respectively and these were significantly different than treatments 3, 4 and 5 (Figure 12). A single degree of freedom contrast that compared treatments 1 and 2 (non thrips control) to treatments 3, 4 and 5 (thrips control) showed significant differences (p-value < 0.0001). Non differences were observed from the effects of rainfall simulation, or fungicide-bactericide application. The curing treatments also had significant effects on the total proportion of decay after 3 months of storage (Figure 13). Curing treatment 1 (ambient air for 2 weeks) exhibited the lowest proportion of decayed onions, and this curing treatment was significant different from the other four treatments. On the other hand, curing treatment 5 (two weeks ambient followed by two weeks at 35ºC) had the highest proportion of decayed onions, being significantly different compared to the other treatments.

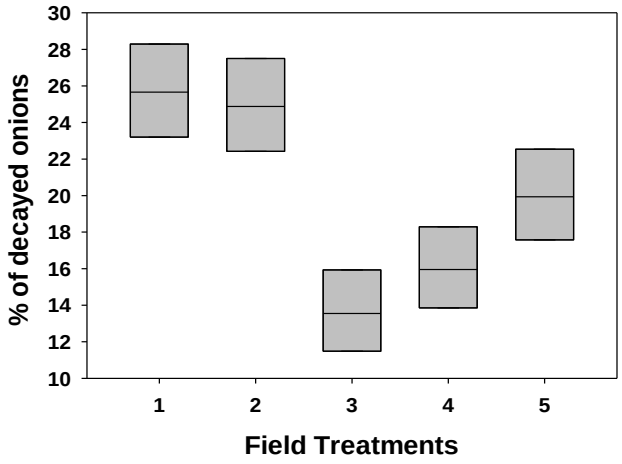


Figure 12: Effect of field treatment (thrips control, rain simulation and fungicide application (see Table 1) on proportion of decayed onions after 3 months storage. Values are means of 4 replications and 5 post-harvest curing treatments, with 95% confidence limits.

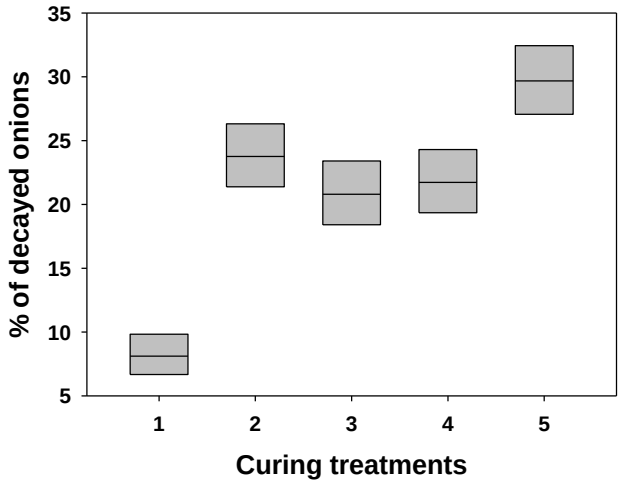


Figure 13: Effect of post-harvest curing treatment on proportion of decayed onions after 3 months storage. Values are means of 4 replications and 5 field treatments with 95% confidence intervals.

Bacterial decay after 3 months: No significant differences were found in the proportion of bacterial decay observed among field or curing treatments, and there was not interaction between them (Table 6).

Total decay after 6 months: In this case, there was a significant interaction between field and curing treatments (Table 6).

Nevertheless, the main effects of field treatment and curing treatment were similar to the 3 months evaluation (Figure 14 and 15). Field treatments 1 and 2 (without thrips control) had the highest incidence of decay after 6 months of storage, while curing treatment 1 (ambient air) was again lowest in decay incidence, with curing treatment 5 (ambient for 2 weeks followed by 35oC for 2 weeks) exhibiting the highest level of decay. The significant interaction was due to the different response of the treatments 4 and 5 in the curing 2 (35ºC) where the incidence of decay was not increased as in the other three field treatments (Figure 16).

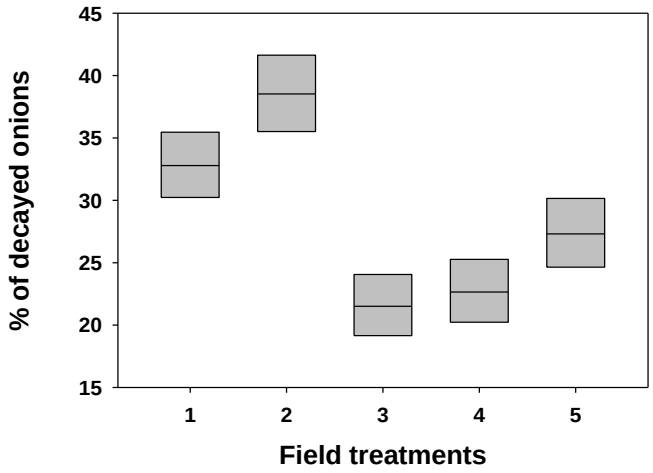


Figure 14: Effect of field treatment (thrips control, rain simulation and fungicide application (see Table 1) on proportion of decayed onions after 6 months storage. Values are means of 4 replications and 5 post-harvest curing treatments, with 95% confidence limits.

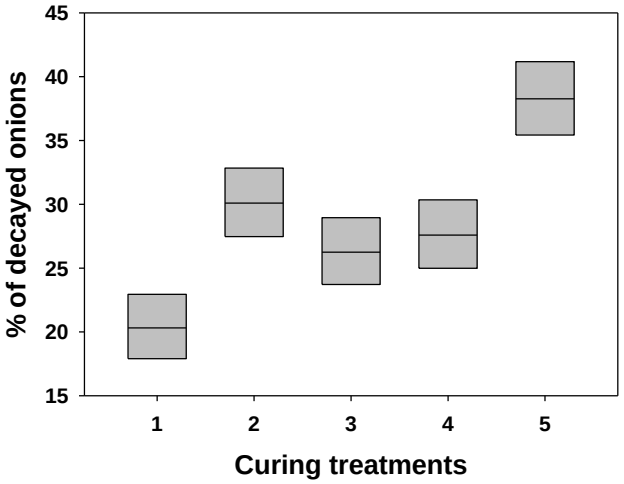


Figure 15: Effect of post-harvest curing treatment on proportion of decayed onions after 6 months storage. Values are means of 4 replications and 5 field treatments with 95% confidence intervals.

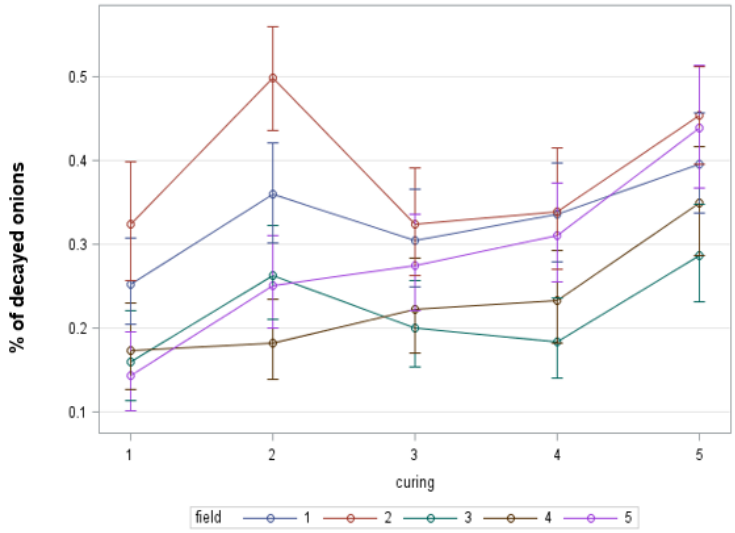


Figure 16: Interaction plot between field and curing treatment on means of decayed onion proportions and their 95% confidence limits.

Bacterial decay after 6 months: There were significant differences in proportion of bacterial decay at 6 months due to both field and curing treatments, and the interaction between them was not significant (Table 6).

Field treatment 1 and 2 exhibited significantly less bacterial decay in comparison with treatments 3, 4 and 5 (Figure 17). This fact is supported by the single degree of freedom contrast, that grouped treatment 1 and 2 against 3, 4 5, which was significant.

Curing treatment1 (ambient air) had a higher proportion of bacterial decay compared to all other curing treatments (Figure 18).

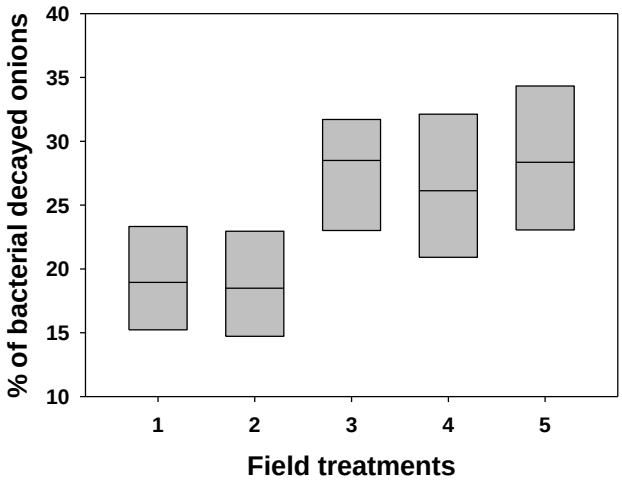


Figure 17: Effect of field treatment (thrips control, rain simulation and fungicide application (see Table 1) on proportion of bacterial decayed onions after 6 months storage. Values are means of 4 replications and 5 post-harvest curing treatments, with 95% confidence limits.

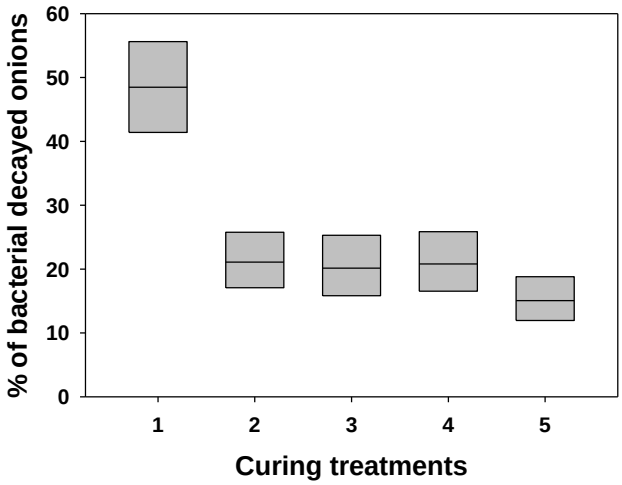


Figure 18: Effect of post-harvest curing treatment on proportion of bacterial decayed onions after 6 months storage. Values are means of 4 replications and 5 field treatments with 95% confidence intervals.

2.9.2. Second year's results

IYSV evaluation: Similar to the 2014 trial, on the first date field treatments 1 and 2, without thrips control, significantly higher IYSV infection compared with field treatments 3 and 4 with thrips control (Figure 19). Similar results were obtained in the second evaluation date where again treatments 1 and 2, without thrips control had higher IYSV infection than treatments 3 and 4, with insecticide application (Figure 20). It is important to mention that the % of

infected plants values of the 2015 trial in both dates were lower than the values obtained in the 2014 trial.

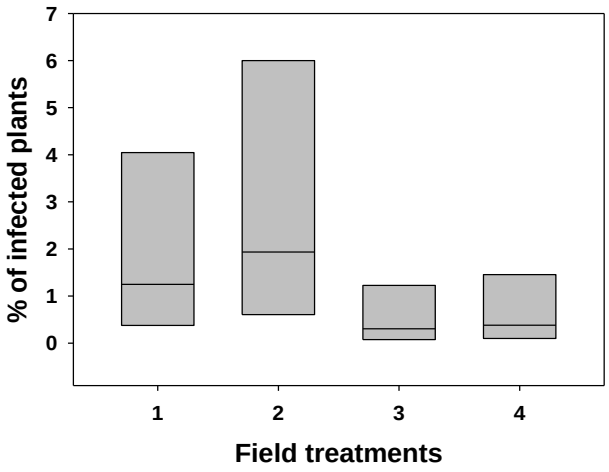


Figure 19: The effect of insecticide treatment on IYSV infected plants proportion on 14 July, 2015 for Vaquero onions grown at Parma, ID. Values are means of 4 blocks with 95% confidence limits.

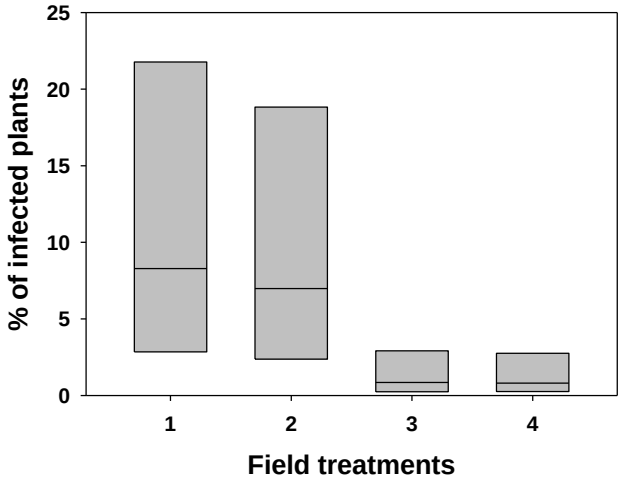


Figure 20: The effect of insecticide treatment on IYSV infected plants proportion on 27 July, 2015 for Vaquero onions grown at Parma, ID. Values are means of 4 blocks with 95% confidence limits.

Thrips population: In 2015, thrips population started to increase later in the season, so there were 2 fewer evaluation dates than in 2014. There were significant differences among the field treatments in thrips populations, and also among sample dates. There was not significant interaction between field treatments and sample date. For that reason, it could be inferred that field treatments 1 and 2, (without thrips control), had higher thrips populations than the field

treatments 3 and 4, (with thrips control) (Figure 21). Also, sample date 3 (30 June) had the highest thrips population (Figure 22). In addition, fewer thrips were present through the different sample dates in the 2015 trial in comparison with 2014.

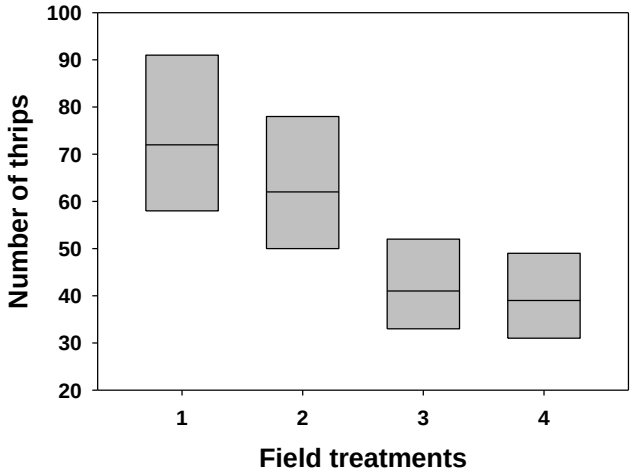


Figure 21: Main effect of insecticide treatment on mean thrips populations (No per 5 plants). Values are means of the 4 field treatments and 4 blocks with their 95% confidence limits where treatments 1 and 2 did not have insecticide applications.

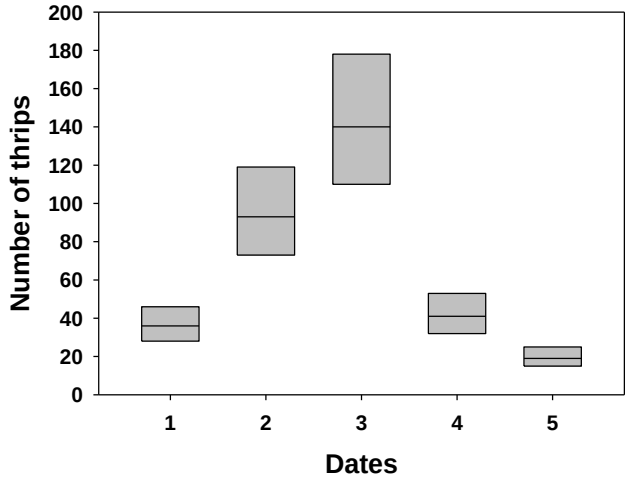


Figure 22: Main effect of sample date on mean thrips populations (No. per 5 plants). Values are means of 4 field treatments and 4 blocks with their 95% confidence limits.

Plant maturity: Similar to the 2014 trial, this evaluation was done close to final maturity in field treatments 3, 4 and 5. There were significant lower proportion of mature plants (top

down) in the field treatments 1 and 2, (without thrips control), compared to field treatments 3, 4 and 5, (with thrips control) (Figure 23).

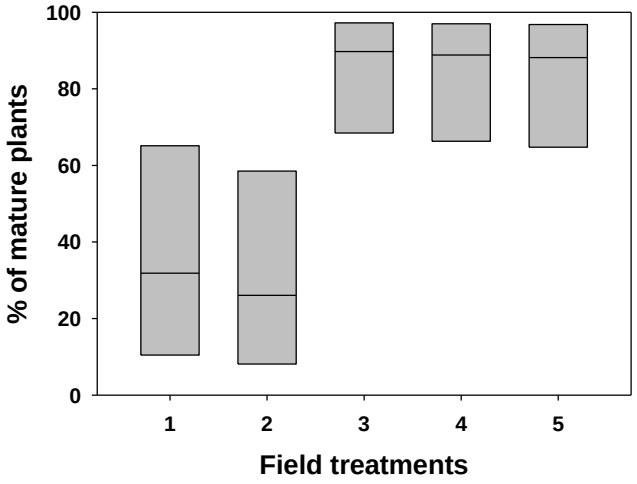


Figure 23: Effect of field treatment on proportion of mature plants (top down). Values are means of 4 blocks with 95% confidence limits.

Storage evaluation:

Total decay after 3 months: There were significant differences in proportion of decayed onions due field and curing treatments, and non-significant interaction among them. Therefore, the variables were analyzed separately by field treatment and by curing treatments.

Table 7: Summary statistical analysis of decayed onion proportions trial 2015

Date evaluation	Evaluation	Effect	Num DF	Den DF	F value	Pr > F
3 months	Total decay	Field	4	71	4.25	0.0039
		Curing	4	71	30.52	< 0.0001
		Field*Curing	16	71	0.43	0.9695
	Bacterial decay	Field	4	72	0.04	0.9964
		Curing	4	72	1.18	0.3281
		Field*Curing	16	72	0.45	0.9611
6 months	Total decay	Field	4	72	3.08	0.0212
		Curing	4	72	62.79	< 0.0001
		Field*Curing	16	72	1.70	0.0652
	Bacterial decay	Field	4	72	0.82	0.5170
		Curing	4	72	8.43	< 0.0001
		Field*Curing	16	72	1.24	0.2576

Field treatment 1 (no thrips control) had significantly higher proportion of decayed onions compared to the other treatments (Figure 24). Although field treatment 2 was not significantly different than 1, it was not significantly different than 3 too. Also, a single degree of freedom comparison between field treatments without insecticide application (1 and 2) and with insecticide application (3, 4 and 5) showed significant differences (*p*-value 0.0002), where treatments with no insecticide had the highest proportion of decayed onion. No differences were observed from the effects of rainfall simulation, or fungicide-bactericide application. The curing treatment 2 (35ºC for 2 weeks) was the treatment with the significantly highest percentage of decayed onions, on the other side curing treatment 1 (ambient curing) was the one with the least percentage of decayed onions (Figure 25).

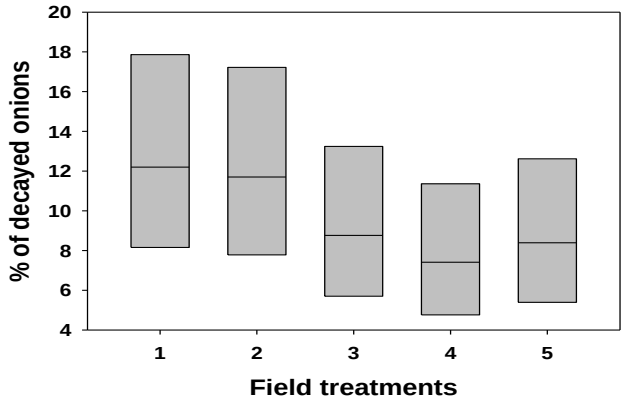


Figure 24: Effect of field treatment (thrips control, rain simulation and fungicide application (see Table 1) on proportion of decayed onions after 3 months storage. Values are means of 4 blocks and 5 post-harvest curing treatments, with 95% confidence limits.

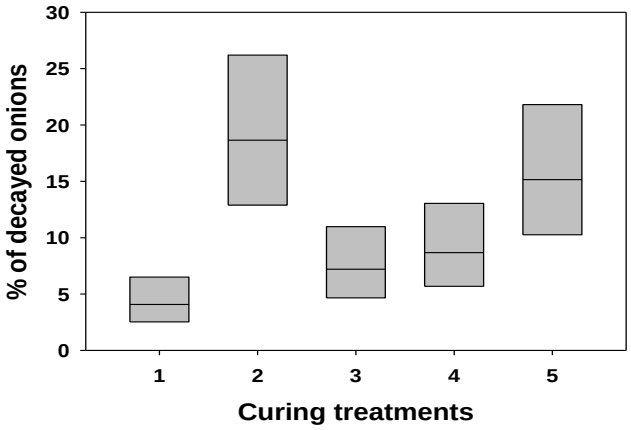


Figure 25: Effect of post-harvest curing treatment on proportion of decayed onions after 3 months storage. Values are means of 4 blocks and 5 field treatments with 95% confidence intervals.

Bacterial decay after 3 months: There were not significant differences in the proportion of bacterial decay observed among field or curing treatments. (Table 7).

Total decay after 6 months: In the 6 month evaluation, the analysis showed that there were significant differences of percentage of decayed onions in the field treatments, in the curing treatments, and non-significant interactions between the mentioned variables was found (Table 7).

Therefore, field treatments 1 and 2, and this time also treatment 5 were the ones with significant more rot onion proportion (Figure26). However, a one degree contrast between field treatments without thrips control (1 and 2) and with thrips control (3, 4 and 5) showed significant differences (*p*-value 0.0031) where again the first ones presented higher percentage of decayed onions than the treatments with insecticide application. Again, non-differences were observed from the effects of rainfall simulation, either fungicide-bactericide application. Similarly of three months evaluation, the curing treatment 1 (ambient curing) presented the lowest significant decayed onion proportion, and on the other side, curing 2 was the one with the significant highest proportion of decayed onion (Figure 27). It is important to mention that the percentage of decayed onions at 3 and 6 months evaluation on the 2015 trial were lesser than the 2014 trial in all the variables evaluated.

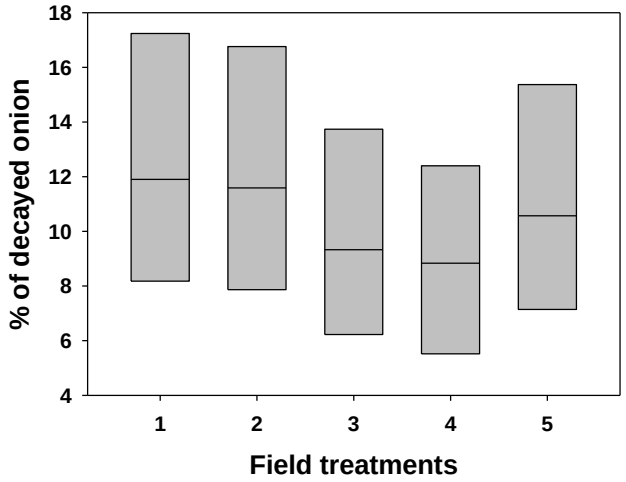


Figure 26: Effect of field treatment (thrips control, rain simulation and fungicide application (see Table 1) on proportion of decayed onions after 6 months storage. Values are means of 4 blocks and 5 post-harvest curing treatments, with 95% confidence limits.

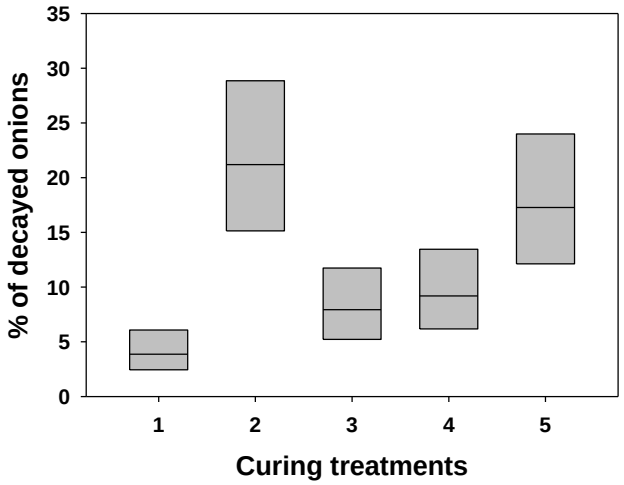


Figure 27: Effect of post-harvest curing treatment on proportion of decayed onions after 6 months storage. Values are means of 4 blocks and 5 field treatments with 95% confidence intervals.

Bacterial decay after 6 months: There were not significant differences among field treatments, but there were significant differences between curing treatments (Table 7).

Therefore, the curing treatment 5 (2weeks of ambient curing, and 2 weeks at 35ºC) presented the highest percentage of decayed onion (Figure 28).

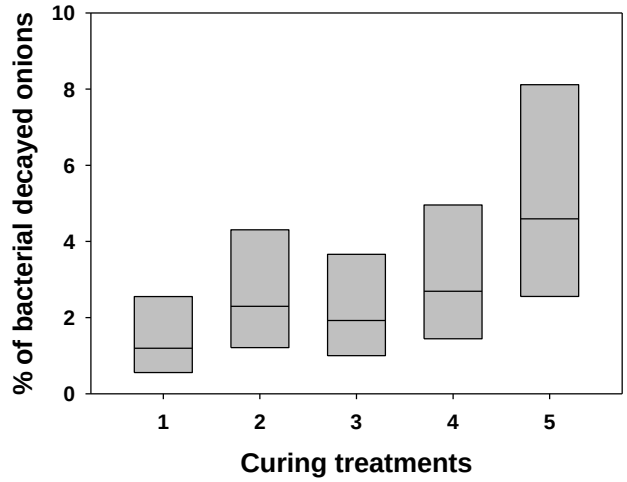


Figure 28: Effect of post-harvest curing treatment on proportion of bacterial decayed onions after 6 months storage. Values are means of 4 replications and 5 field treatments with 95% confidence intervals.

2.9.3 Pathogen identification

The onion bulbs were sampled based on the expression of typical bacterial rot and/or fungal symptoms. In this way, fungi and bacteria were cultured separately on PDA, ½ V8 or NBY media, respectively. Afterwards, fungi were identified visually by microscope observation, and the bacterial growth were saved for further DNA sequencing identification. Growth on ½ V8 promoted more fungal sporulation making it easier to identify the specific fungal pathogens.

Table 8 and 9 shows a summary of the fungi identification.

Table 8: Pathogen identification from decayed onion samples collected from the 2014 storage trial on two sample dates.

	Time in storage	
Pathogen	**3 months**	**6 months**
***Botrytis* sp.**	**9***	**1**
***Fusarium* sp.**	**43**	**30**
***Aspergillus* sp.**	**2**	**8**
***Penicillium* sp.**	**0**	**2**

*Probably mis-identified

Table 9: Pathogen identification from decayed onion samples collected from the 2015 trial on two sample dates.

	Time in storage	
Pathogen	**3 months**	**6 months**
***Botrytis* sp.**	**1**	**0**
***Fusarium* sp.**	**32**	**20**
***Aspergillus* sp.**	**25**	**23**
***Penicillium* sp.**	**8**	**11**
Bacteria	**1**	**18**
Yeast	**1**	**0**

2.10. Discussion

Results from both years demonstrate an almost direct relationship between thrips population and IYSV infestation because the field treatment without thrips control showed higher IYSV infection levels and higher thrips populations (Figure 6, 7, 19, 20 and 8, 21). This relationship agrees with reports from other production areas (Gent et al. 2006). Also analyzing the thrips

population by sample date (Figure 9 and 22) showed that the peak of the population was mid-season during both years, as others have reported (Reitz 2014a). This fact is a very important consideration in commercial thrips control recommendations. Another observation was that IYSV infection levels increased during the latter part of the season because in both years the second IYSV (Figure 7 and 20) evaluations determined a higher incidence of infected plants compared to the first evaluation date (Figure 6 and 19). This observation agrees with previous reports (Gent et al. 2006). This fact may sound obvious, but it is important to know because early IYSV scouting could infer lower infection levels than are present in the crop at harvest.

The relationship between thrips populations, IYSV incidence and proportion of mature plants at the end of the season has been observed by growers, but there are no previously published reports on this aspect of the disease symptoms. This delay or change in plant maturity could presumably increase decay in storage by providing an entry point to the pathogens that cause decay in stored onions (Figure 11 and 23).

It is important to mention that there were differences between the 2 years in thrips population and IYSV infection. The 2014 trial had higher values in both measures. The 2014 trial showed a peak which was represented by 2 dates (Figure 9). Those dates corresponded to the samples taken on the 1st of July and the 14th of July where the mean number of thrips per 5 plants averaged 168 and 152, respectively. On the other hand, the 2015 trial had the thrips population peak at similar dates (Figure 22), 30th of June and 15th of July, but the populations were considerable fewer than in 2014. There are two possible explanation for the differences observed in thrips populations between years. First, the 2015 population started to increase later than in 2014 (Table 3 and 5). This fact is a little difficult to explain because winter and spring were similar in both seasons, with 2015 being warmer than 2014. The second explanation relates to the fact than in 2015 the decrease in thrips populations between the end of June and mid-July was very drastic, almost 3.5 times (Figure 22), in comparison with the same period in 2014 when the population did not decline (Figure 9). Looking closer at this time period in 2015, it was observed that between the 26th of June and the 4th of July there were 9 days where the maximum temperatures were always over the 38ºC (Table 10). Recent research reported that *Frankliniella occidentalis* (western flower thrips), exhibited decreased longevity and fecundity of females following heat expose, leading to a decrease in population

(Wang et al. 2014). Thus, a similar effect could have caused the big decline in the thrips population in the 2015 trial. This fact could be important because it is possible that it might affect the rest of the 2015 trial results.

Table 10: Maximum temperatures during 9 days of 2015 from Parma weather station.

Date	Maximum Temperature ºC
06/26/2015	**38.1**
06/27/2015	**38.3**
06/28/2015	**39.5**
06/29/2015	**39.3**
06/30/2015	**39**
07/01/2015	**38**
07/02/2015	**39.7**
07/03/2015	**39.4**
07/04/2015	**38.1**

Field treatments without thrips control exhibited the highest incidence of decay in storage in both years (Figure 12, 14, 24 and 26). There were again differences between the 2 years, with the 2014 trial showing clearer differences among treatments than the 2015 trial. This fact supports the argument that thrips and the corresponding IYSV infection are important factors that increase decay in storage because there were higher thrips population and more IYSV infection in 2014 compared to 2015. Therefore, these results suggest a connection between thrips-IYSV and decay in storage. This relationship has been proposed before (du Toit 2014), but this is the first report documenting increased decay in IYSV infected bulbs. Also, in the last few seasons there have been concerns about more dry scale symptoms and more *Fusarium proliferatun* and bacterial rot in storage associated with very warm growing seasons (M. Thornton, *personal communication*). As warm seasons tend to increase thrips population and IYSV infection, which could increase the incidence of dry scale and decay in storage by *Fusarium proliferatum* or bacteria. It is interesting to note that isolations of decayed onions from both years of this study found *Fusarium* spp. The highest proportion of *Fusarium* isolation was during 2014 (Tables 8 and 9). This observation was confirmed by the macroarray results (See Chapter 3). This observation may be related to *Fusarium proliferatum* ability to live as a saprophyte (Stankovic et al. 2007) in dead or dying tissue and grow under

the low moisture (Marin et al. 1995) conditions found in onion with IYSV infected leaves on the dry scales on the interior of the bulbs.

There were no differences found between treatments with and without simulated rainfall (Figures 12, 14, 24 and 26). These results are similar to those obtained by other research in the same production area (Beaver and Devoy 1988) where topped onions were sprinkler irrigated. The results of both studies suggest than a single rainfall event close to harvest or during field curing would not affect the incidence of decay in storage. It appears that the overall weather conditions are most likely to influence the decay incidence in storage. An example is given in the Figure 29. The rainfall events and high relative humidity at the end of the season in 2013 was associated with an unusually high incidence of bacterial decay in storage, and this situation might have been caused by the overall climatic conditions and not a single rainfall event.

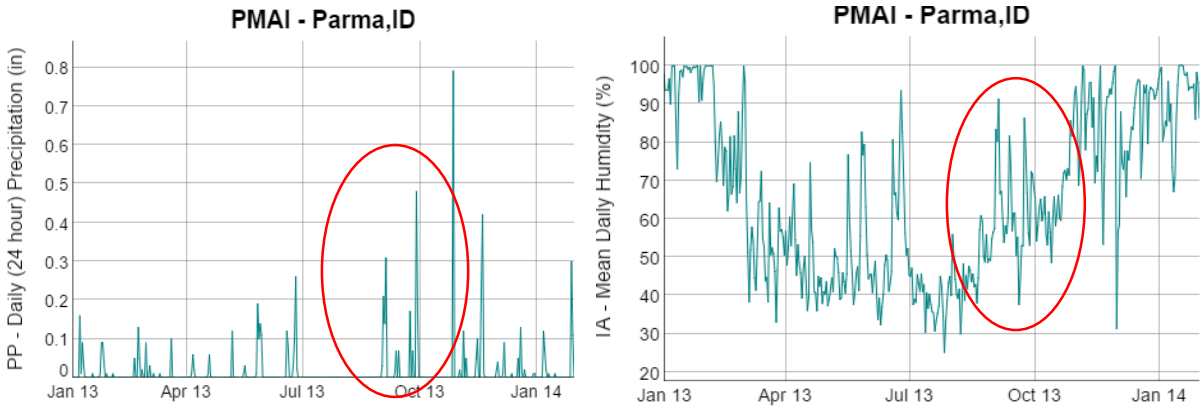


Figure 29: Daily precipitation and daily average of relative humidity in the 2013 in Parma Idaho. Extracted from http://www.usbr.gov/pn/agrimet/graphs.html.

Also, non-significant differences were observed among field curing with and without fungicide-bactericide application (Figure 12, 14, 24 and 26). These observations coincided with other studies (Beaver and Devoy 1986, Beaver and Devoy 1988), and these could be explained by a few factors. First, when the "neck" of the onion is the target area of the application, it is difficult for the products to penetrate in this area because, in general, the leaves of the onion neck are together in a tight fashion. Another possible reason is the application timing, as some studies indicate that the precise time of product application determines its effect (Schwartz et al. 2002). Finally and related to the last possible reason, the

variability in the efficacy of these products could be due to the weather conditions during the season.

There were significant differences in decay incidence due to curing treatment in both years (Figures 12, 14, 24 and 26). Curing at higher temperatures resulted in more decay compared to ambient curing. First, it is important to note that in 2014 there was a higher proportion of decay than in 2015, suggesting a logical interaction between field conditions and curing conditions. So, when field conditions result in more decay in storage, curing conditions can also result in a higher proportion of decayed bulbs. As other studies have reported (Vaughan et al. 1964, Stanger et al. 1987-88, Schroeder and du Toit 2010), exposure to high temperature for 2 weeks increases decay in storage. However, most other reports have found that much higher temperatures and extended exposure are required for heat curing to increase decay (Vaughan et al. 1964, Stanger et al. 1987-88). This could suggest that the problem would be an interaction between temperatures and time (see appendix aging experiment). Also, in this study there were some differences among curing treatments that are difficult to explain. In particular the differences between curing at 35ºC after previous ambient curing, compared to heat curing at 35ºC immediately after harvest (Figure 13, 15, 25 and 27). These treatments resulted in conflicting results in 2014 compared to 2015. These results would suggest that there could be some interaction with the relative humidity conditions during the curing process because it is known that the loss of water from the onions depends on the relative humidity (Vaughan et al. 1964). In this study the relative humidity was partially controlled, however, the curing treatments were done in different storage facilities in both years. Therefore, this suggests that in future research, it would be necessary to evaluate the role that relatively humidity could play in the curing process and incidence of decay.

Lastly, no inferences could be made about the relative effect of field or curing practices on bacterial decay because the results were not clear. Therefore, it was not possible to find a pattern that explained the proportion of bacterial decay in this study.

CHAPTER 3: DNA MACROARRAY AS AN EARLY DETECTION TOOL

3.1 Abstract

Greater than 60% of the storage onion bulbs are produced in the Pacific Northwest. These bulbs are cured and placed in storage in the fall and stored for 1-6 months. They are then sold throughout the season when no fresh onions are available. It is during the long term storage that onion decay can develop and incur significant financial losses to stakeholders. At harvest onions are cured in the field and when they are placed in storage the curing process can be continued with ambient or heated forced air. The decision to use to the different curing treatments depends on the presence of different pathogens that are able to cause decay of the onion in storage. If it was possible to know which pathogens were present prior to curing, this would guide the stakeholder's decisions as to how to manage onion crops in storage. Different methods of pathogen detection and identification have been used, but recently the focus has been on the available molecular techniques because they are faster, accurate, and pathogen taxonomy knowledge is not required to utilize the methods. One of these techniques is a DNA macroarray which has the feature of detecting multiple pathogens in a single assay in an accurate and rapid fashion. Thus, with 14 fungi, 12 bacteria and 1 yeast that could cause decay of onion bulbs in storage, this technique could be used prior to placing the onions in storage in order to determine which pathogens are present. This information would provide critical information to help stakeholders to make storage decisions to optimize the storage duration for each onion crop.

3.2. Importance of pathogen identification

Onion storage is an important activity in the Pacific Northwest where approximately 63% of the USA summer dry onions are held in storage for one to six months (NASS 2016). These onions are held in storage in order to supply the market when onion are not actively being harvested. Stored onions are sold at a higher price compared to onions sold at harvest, however, long term storage of onions does have a risk of loss to developing storage rots. With production and storage costs greater than $1000/hectare this can represent a significant financial impact to the stakeholder (http://www.ipmcenters.org/CropProfiles/docs/WAonions.pdf).

Before harvest, onions can become latently infected by pathogens that cause decay to develop in storage (Schwartz and Mohan 2008, Kritzman et al. 1981). Since the onions are not symptomatic at harvest and the incidence of infection and the causal agents are unknown, storage managers are forced to make decisions based on conditions during the growing season, and/or their own experience. Therefore, onions placed in storage are either ambient or heat cured, depending on the storage manager's decisions. As already mentioned, heat curing could induce other issues. For that reason, knowing if certain pathogens are present would provide critical information and would allow more informed decisions to be made relative to the curing and storage of each onion field.

3.3. Pathogen identification techniques

The identification of plant pathogens requires significant expertise and is a time consuming process. Fungal and bacterial pathogens of onion have typically been identified using culture-based methods. These methods are limited by the fact that some pathogens are difficult to culture, and identifications require extensive knowledge of both the fungal and bacterial plant pathogens of onion (Lievens and Thomma 2005). With the adoption of molecular techniques, principally the use of polymerase chain reaction (PCR), new techniques have been developed for the identification of plant pathogens which require less specialized expertise and provide greater sensitivity (Lievens and Thomma 2005). In addition, the application of real-time PCR enables the quantitation of the pathogens (Mckay al. 2002). The development of a multiplex assay enables the detection of multiple pathogens, but this is limited to six or less at a time because of the available technology (Mckay al. 2002). With 14 fungi and 12 bacteria that are able to cause onion bulb decay, the application of PCR detection methods does not provide a simple straightforward method for the identification of all the onion bulb rot pathogens using a simple detection tool.

3.4. DNA Macroarray

DNA macroarray technology is a good detection technique that enables the detection of a large number of pathogens in a single test (Lievens et al. 2005, Sholberg et al. 2005, Tambong et al. 2006). This tool has been applied to the detection of viruses, fungi and bacteria. In general, DNA macroarrays consist of pathogen-specific oligonucleotides that are bound to a solid support such as a membrane (Lievens et al. 2012). The pathogen specific oligonucleotide

sequences are identified from DNA sequences located within regions of conservation such as ITS, IGS or 16S rRNA gene regions. These sequences will have regions of conservation enabling conserved primers to be used to indiscriminately amplify DNA regions which contain sequences unique to the target pathogens. This PCR product is labeled and hybridized to the membrane with pathogen specific oligonucleotides. Only complementary DNA present in a PCR product will hybridize with the oligonucleotides and can then be visualized in a colorimetric or fluorescent manner (Lievens et al. 2012). An important feature of this technology is that theoretically the number and types of pathogens that could be identified in one test is unlimited (fungi, bacteria, nematodes and viruses) (Lievens et al. 2012 Agindotan and Perry 2007), and the method can give some measure of quantity of pathogen present with the intensity of the hybridization reaction (Lievens et al. 2005). Therefore, a DNA macroarray is an effective method for the specific detection of a large number of plant pathogens.

Molecular tools require only very small quantities of pathogen DNA for detection. Unfortunately, included with this high sensitivity comes the problem of false positives (Lievens and Thomma 2005). This problem refers to the possibility that DNA amplified from a non-target organisms bind the oligonucleotides printed on the membrane. In this way false positives could come from closely related organisms or another strain of the same organism that is nonpathogenic and not of importance. The quantities for detection are really low, recent research (Urbez-Torres et al. 2015) demonstrated that a DNA macroarray was able to detect the presence of pathogen even when no band was observed after visualization of the putative PCR product on an agarose gel. Also, it was demonstrated that detection with a DNA macroarray can occur when DNA from the target pathogen is as low as 42 to 72 femtograms in the sample. This detection limit is similar to the detection limit previously reported for real time assays (Urbez-Torres et al. 2015).

The ability to quantify the amount of pathogen present can be critical knowledge for management of certain diseases. Real time PCR is the most reliable method for detection and quantification of plant pathogens (Lievens and Thomma 2005). However, with the proper design, it is possible to use a DNA macroarray to detect and quantify plant pathogens. Using a DNA macroarray, Lievens et al. (2005), found a direct relationship ($R = 0.97$) between the hybridization strength and the concentration of *Verticillium dahliae* spores present.

There are several aspects that need to be considered when choosing to develop and use a DNA macroarray technique over other diagnostic techniques. First, the DNA macroarray technique can be completed in as little as 2 to 3 days, which is a considerable time savings compared to traditional techniques (Lievens and Thomma 2005). Second, this method doesn't require personnel with a high level of fungal or bacterial expertise, such as in traditional identification where taxonomic knowledge is required. Third, the DNA macroarray can detect numerous different pathogens from the same sample, saving time and reducing cost (Lievens and Thomma 2005). Finally, the equipment needed for running the DNA macroarray is found in many traditional laboratories (Lievens and Thomma 2005). All these features make the DNA macroarray an attractive pathogen detection tool.

Despite the fact that the DNA macroarray has many advantages, there are some limitations that almost all molecular diagnostic techniques share. First, there are potential problems with the specificity, either because the initial pathogen classifications of the organisms used to develop the technique are such that the morphological and biological features do not always match phylogenies shown by nucleic acid-based techniques (Lievens and Thomma 2005), or because the causal agents do not match the natural clades (Vincelli and Tisserat 2008). Also, other specificity problems arise for pathogens such as *Fusarium oxysporum,* which have pathogenic and non-pathogenic strains (Recobet et al. 2003) which will both be detected despite the fact that only the pathogenic strains are of critical importance. Another possible issue can be the selection of the tissue to be analyzed which is fundamental considering that only 1 to 2 µl of sample extract is ultimately assayed in this kind of technique (Urbez-Torres et al. 2015, Lievens and Thomma 2005). Detection assays using PCR techniques, where the amplification rate is exponential, are at risk for contamination issues (Urbez-Torres et al. 2015, Lievens and Thomma 2005). In addition, there are some compounds, such as phenolics, that could negatively affect the PCR procedure. Many DNA extraction kits have been developed to address this issue and provide high quality purified DNA (Urbez-Torres et al. 2015, Lievens and Thomma 2005). Lastly, the PCR procedure will amplify DNA from both living and dead cells, hence this could cause false positives (Urbez-Torres et al. 2015, Lievens and Thomma 2005). Nevertheless, there are some available techniques that can be used to prevent this last problem (Urbez-Torres et al. 2015, Lievens and Thomma 2005).

Given the advantages and relatively few limitations compared to other diagnostic techniques, it seems that the DNA macroarray is a promising method to help onion producers identify latent infections of decay organisms prior to storage. This tool would provide a risk assessment step to aid in postharvest management of onion bulbs increasing the financial returns for the stakeholder.

3.5. Research approach

Therefore, the objective of the research in this chapter, is to analyze the performance of the DNA macroarray technique in onion targeting bacteria, fungi and yeast that cause onion bulb rot in storage, and determine if there are correlations among pathogens detected and rot in storage. The DNA macroarray was developed to detect the 14 fungi and 12 bacteria able to cause onion bulb rot in storage (Arif et al. 2013). It is hypothesized that the DNA macroarray could detect latently infected bulb rot pathogens present in onions prior to symptom development at harvest. It is also hypothesized that the pathogens detected are the ones causing onion bulb rot in storage.

3.6. Materials and methods

Onion sampling for the DNA macroarray: Two bags of twenty onion bulbs each were removed from each of five field treatments (Table 1), in year one and two. One bag from each treatment was stored at 5ºC until the onion bulbs were processed. The second bag from each field treatment was cured at 35ºC for 2 weeks and then stored at 5ºC until the onion bulbs were processed. To process onion bulbs, they were cut transversally near the neck of the bulb using a sterile knife. The dry outer scales were removed and the onion slice, approximately 5 mm in width, was placed in an extraction bag of 15 cm by 28 cm (BIOREBA, Eurofins STA Laboratories Inc., Longmont, CO), and stored at -20ºC. The onion bulb slice was allowed to thaw and macerated using a rubber mallet. The resulting onion extract was removed and stored in 15 ml sterile centrifuge tube (GeneMate, ISC Bioexpress) at -20ºC. From the twenty onion bulbs from each field treatment processed four bulbs were used for DNA extraction and DNA macroarray testing. The onion extract from each of the remaining sixteen bulbs were stored at -20ºC. In this way, four onion bulbs per each of five field treatments, per post-harvest and post curing were evaluated using the DNA macroarray as described below for the year 1 and year 2 trials.

DNA extraction and PCR amplification: A 1 ml aliquot of macerated onion was used for DNA extraction using the Power Soil® DNA Isolation extraction kit (MO BIO laboratories, Inc., Carlsbad, CA), using the manufactures' recommended procedures. The quality of DNA was evaluated using gel electrophoresis and stored -20ºC for further analysis. PCR amplification using fungal primers mix (4 different primers, 0.2 µM of each one, Table 11) included a 2 µl DNA aliquot with Promega GoTaq Polymerase (Promega, Madison, WI) under standard conditions (5 µl of 5X Promega GoTaq® buffer, 0.2 mM dNTPs, 0.2 µM of each primer, 100 ng of template, 0.5 µl of Promega GoTaq® Polymerase (5 U/ µl) and 15.5 µl of sdH_2O in a 25 µl reaction volume). PCR conditions included a 3 min hot start at 95°C followed by 35 cycles of 30 seconds denaturation at 95°C, 1 min annealing temperature (57ºC) as listed for each respective primer set, and 2 min extension at 72°C, followed by a final extension period of 3 min at 72°C, followed by a 15ºC hold. PCR amplicons were visualized on a 1% agarose (Gene Pure LE, Cat. No. E-3120-500, ISC BioExpress, Kaysville, UT) gel with ethidium bromide (EtBr) (0.0001 mg/ml) after electrophoresis in 0.5 X TBE buffer (0.312 M Tris, 0.312 M boric acid, 0.007 M EDTA). PCR amplification using bacterial primers (4 different primers, 0.2 µM of each one, Table 11) included a 1 µl DNA aliquot with Promega GoTaq® Polymerase under standard conditions (5 µl of 5X Promega GoTaq® buffer, 0.2 mM dNTPs, 0.2 µM of each primer, 100 ng of template, 0.25 µl of Promega GoTaq® polymerase (5U/ µl) and 14 µl of sdH_2O in a 25 µl reaction volume). PCR conditions included a 2 min hot start at 95°C followed by 35 cycles of 1 min denaturation at 95°C, 1 min annealing temperature (50ºC) as listed for each respective primer set, and 1 min extension at 72°C, followed by a final extension period of 5 min at 72°C, and a 15ºC hold using an Eppendorf AG 22331 thermocycler (Eppendorf, Hamburg, Germany). PCR amplicons were visualized on a 1% agarose (Gene Pure LE, Cat. No. E-3120-500, ISC BioExpress, Kaysville, UT) gel with ethidium bromide (EtBr) (0.0001 mg/ml) after electrophoresis in 0.5X TBE buffer (0.312 M Tris, 0.312 M boric acid, 0.007 M EDTA). The PCR product was cleaned with the Ultra Clean® PCR Clean-Up kit (MO BIO laboratories, Inc., Carlsbad, CA), or the QIAquick® PCR Purification Kit (QIAGEN®, Valencia, CA). The PCR product was used as template for the hybridization probe for DNA macroarray hybridization. This step removes the primers from the probe eliminating their ability to produce false positives.

Table 11: Primers used in the fungal and bacteria PCR amplification.

	Bacteria		Fungi	
Primers	53 mix		Fungal primers mix	
	53F	GGGAAACAGGTTAATATTCC	BA2F	GTGGGGGTAGGATGAGATGATG
	53[a]	GGACAACAGGTTAATATTCC	BA1R	TGAGTGCTGGCGGAAACAAA
	62 mix		ITS4-A1	ATGCTTAAGTTCAGCCGGGTA
	62R	GGTACCATTTTGCCTAGTTC	ITS5	GGAAGTAAAAGTCGTAACAAGG
	62ar	GGGGCCATTTTGCCGAGTTC		

Array design: The DNA macroarray consists of oligonucleotide sequences designed to be specific for the 14 fungi and 12 bacteria able to cause bulb decay (Arif et al. 2013, Schroeder, *unpublished*). All the oligonucleotides (Appendix) were synthesized commercially by Sigma (Sigma-Aldrich®, Saint Louis, MO) as desalted primers. The bacterial oligonucleotides 2 µl (100 µM) were printed to Nytran® N (GE Healthcare Life Science Whatman TM), and the fungal oligonucleotides 2 µl (40 µM) were printed to Amersham Hybond (TM –N+, location). Oligonucleotide sequences equivalent to the primers used to amplify the PCR fragments were included as hybridization controls. Each oligo was printed in duplicate, and the oligonucleotides were crosslinked using Spectro Linker™ XL-1000 UV Crosslinker (Spectroline®, Westbury, NY). The oligonucleotide sequences and order on the blots are listed in the appendix. The design of the bacterial blot is such that it was divided into two separate blots with controls because of melting temperature differences in the oligonucleotides that require different post hybridization washes listed below.

Hybridization procedure: PCR product is labeled and hybridized using the DIG High Prime DNA Labeling and Detection Kit II (Roche, Indianapolis, IN) according to manufacturer's instructions. Using the labeled amplicon resulting from the fungal primers, 4 µl of cleaned PCR product was placed in a PCR tube and denatured at 100ºC for 10 min. The tube was immediately placed on ice. A 1 µl aliquot of digoxigenin molecule (DIG Vial 1 of the Roche kit) was added to incorporate dUTP into the DNA probe as DIG-11-dUTP via the Klenow DNA polymerase in a method known as random-primed labeling method (Eisel et al. 2008). Therefore, the mix of DIG and PCR product was incubated at 37ºC for at least 20 hours in the PROBLOT 12S hybridization oven (Labnet International Inc. Edison, NJ). After incubation, the reaction was stopped by placing the reaction mix at 65ºC for 10 min and then denatured at

100ºC for 5 min and immediately placed in ice. The membrane with oligonucleotides already printed and crosslinked, was pre-hybridized, in a small plastic box (7.5 cm X 10.5 cm X 3 cm) covered with 6 ml of the DIG Easy Hyb buffer provided by the Roche kit (Appendix) for at least 30 min at 42ºC in the hybridization oven with agitation. A 2 µl aliquot of the labeled probe was placed in 5 ml of fresh DIG Easy Hyb buffer in the hybridization box maintained at 42ºC with agitation for at least 16 hours.

To remove nonspecifically bound probe from the blot, 25 ml of 2X SSC and 0.1% SDS was added to the membrane at room temperature with the maximum possible agitation (85 rpm) for 5 min. This step was repeated once. The membrane was then washed twice with 25 ml of 0.1X SSC and 0.1 % SDS (pre-warmed to wash temperature) at 45ºC with agitation (85 rpm) for 15 min. This was followed with the detection step including a brief wash, 1-5 min in 20 ml washing buffer (Appendix) to remove the previous solution. This was followed by incubation for 30 min in 60 ml of blocking solution (Appendix) and a 30 min incubation in 15 ml of antibody solution (Roche kit). The membrane was washed twice for 15 min in 60 ml of washing buffer to remove the extra antibody and other compounds. The membrane was equilibrated for 2-5 min in 15 ml of detection buffer (pH 9.5). In the final step, the membrane was placed on a developer folder in a plastic sheet and 1 ml of CSPD ready to use (vial 5 of the Roche detection kit) was added and the membrane was covered and incubated for 5 min at room temperature. The excess liquid was squeezed out and the edges of the plastic sheet were sealed and incubated at 37°C for 10 min. The membrane was exposed to F-BX810X-ray film (Phenix Inc., Candler, NC) for 6 min. Finally, the film was developed using the developer machine Kodak X-OMAT 1000A PROCESSOR (Kodak, Rochester, NY)

Using the labeled amplicon resulting from the bacterial primers the procedure was almost the same except for the first washing step which included two initial washes with 15 ml of 2X SSC, 0.1 % SDS for 10 min at 42°C. Then, the second set of washes were different depending on the bacterial membrane used (large or small) as listed in the appendix. The large blot was washed twice for 25 min at 48°C with 15 ml of 0.1X SSC, 0.1% SDS. The small blot was washed twice for 25 min with 15 ml of 0.1X SSC, 0.1% SDS.at 56°C, and it was rinsed also in 15 ml with 0.1X SSC, 0.1% SDS. The development steps were the same for the fungal and bacterial blots.

3.6. Results

3.6.1. DNA extraction and PCR amplification

Genomic DNA extractions from onion slices resulted in an adequate quantity of DNA in good quality for use in PCR (Figure 30). Even when the DNA was not the best quality it was still sufficient for PCR amplification resulting in the presence of the appropriate size amplicon (Figure 31).

Figure 30: Electrophoresis on 1 % agarose gel with Ethidium Bromide of DNA extraction visualized with UV light.

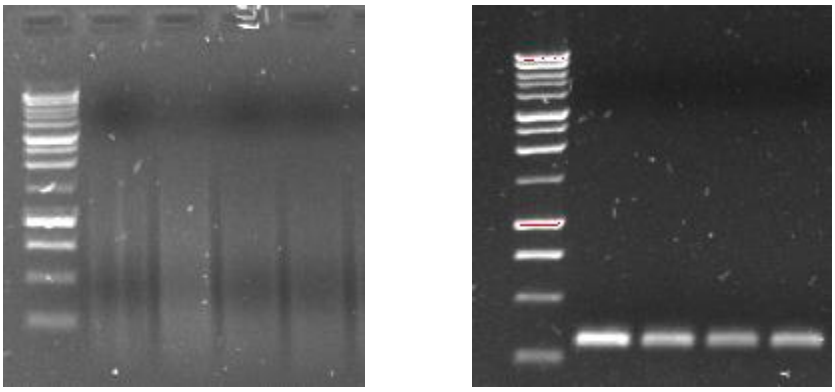


Figure 31: Fungal PCR product with low yield visualized on agarose gel. On the right a good yield bacterial PCR product visualized on agarose gel with EtBr.

3.6.2. DNA Macroarray assays results

2014 postharvest sampling: Pathogens were detected by the DNA macroarray in 16% of the 76 bulbs assayed from the 2014 postharvest samples. Only 4% of the assayed bulbs presented visual decay symptoms when sampled, and from these symptomatic bulbs the pathogens *Fusarium proliferatum* and *Aspergillus niger* (fungi) and *Pantoea agglomerans*, *P. ananatis*, *Pseudomonas marginalis pv. marginalis*, *P. aeruginosa* and *Enterobacter cloacae* (bacteria) were detected. *F. proliferatum*, *F. oxysporum*, *A. niger*, *Botrytis cinerea* and *Penicillium polonium* (fungi) and *P. agglomerans*, *P. ananatis*, *Burkholderia cepacia*, *Pseudomonas marginalis pv. marginalis*, *P. aeruginosa*, *Rahnella* sp. and *Enterobacter cloacae* (bacteria)

were detected from asymptomatic bulbs, representing 12% of the asymptomatic bulbs assayed (Table 12).

When the profile of the detected pathogens was further evaluated, 85% of the fungal detections corresponded to *Fusarium proliferatum* (Table 13). In the bacterial profile, the list of detected species was broader and only 25% of the detected bacteria corresponded to a single pathogen (*Pantoea agglomerans)* (Table 14).

Table 12: Summary of the assays conducted on the bulbs sampled after the harvest of the 2014 trial.

Fungal-Bacterial assays – postharvest Bulb Trial 2014

Bulb Assayed	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Asymptomatic bulbs	11	15	16	16	15	73	96 Total Bulbs
Pathogens detected	1	2	0	4	2	9	12 Asymptomatic
Symptomatic bulbs	1	1	0	0	1	3	4 Total Bulbs
Pathogens detected	1	1	0	0	1	3	100 Symptomatic
Total bulbs	12	16	16	16	16	76	

Table 13: Fungal profile detected by the DNA macroarray on onion bulbs from the post-harvest sampling trial 2014.

Fungi – postharvest Bulb Trial 2014

Fungi Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Fusarium oxysporum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Fusarium proliferatum	**4**	**2**	**0**	**3**	**2**	**11**	**84.6**
Aspergillus niger	**1**	**0**	**0**	**0**	**0**	**1**	**7.7**
Aspergillus flavus	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium breviconbactum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium expansum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium polonium	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium digitatum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis aclada	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis byssoidea	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis allii	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis porri	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis cinerea	**0**	**0**	**0**	**0**	**1**	**1**	**7.7**
Kluveromyces	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Fungi Detected	**5**	**2**	**0**	**3**	**3**	**13**	**100**

Table 14: Bacterial profile detected by the DNA macroarray on bulbs from the postharvest sampling trial 2014

Bacteria- postharvest Bulb Trial 2014

Bacteria Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Enterobacter cloacae	**0**	**0**	**0**	**1**	**1**	**2**	**17**
Burkholderia gladioli pv. Alliicola	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Burkholderia cepacia	**0**	**0**	**0**	**0**	**1**	**1**	**8**
Pantoea agglomerans	**0**	**1**	**0**	**1**	**1**	**3**	**25**
Pantoea ananatis	**0**	**1**	**0**	**1**	**0**	**2**	**17**
Pseudomonas marginalis pv. marginalis	**0**	**1**	**0**	**0**	**0**	**1**	**8**
Pectobacterium carotovorum subsp. carotovorum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Dickeya chrysanthemi	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudomonas aeruginosa	**0**	**1**	**0**	**0**	**0**	**1**	**8**
Erwinia rhapontici	**0**	**0**	**0**	**0**	**0**	**0**	**0**
***Rahnella* sp.**	**0**	**1**	**0**	**1**	**0**	**2**	**17**
Pseudomonas viridiflava	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Bacteria Detected	**0**	**5**	**0**	**4**	**3**	**12**	**100**

2014 post curing sampling: The DNA macroarray analysis conducted with onion bulbs post curing in 2014 showed that pathogens were detected in 22% of the onion sampled. Asymptomatic bulbs represented 87% of the sample set, and only 14% of those bulbs were

positive for pathogens. In contrast, from the onion bulbs that showed symptoms (13% of total) pathogen were detected from 80% of symptomatic bulb (Table 15). It is important to mention that samples, where only pathogen genera were detected, were not counted, otherwise the result of the symptomatic bulbs detected would be close to 100%.

In the fungal profile, 67% of the bulbs were positive for *Fusarium proliferatum*, and 8% were positive for *Aspergillus niger* (Table 16). In the bacterial profile, *Pantoea agglomerans* was the most detected bacterial pathogen, with 38% of the samples (Table 17). Almost equal number of bacteria were detected on post-harvest and post-curing samples, however it is important to clarify that in the post-curing samples fewer assays were done.

Table 15: Summary of the assays conducted on the bulbs sampled after the 35ºC curing in the 2014 trial.

Fungal-Bacterial assays – post curing Bulb Trial 2014

Bulb Assayed	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Asymptomatic bulbs	**11**	**9**	**16**	**15**	**15**	**66**	**87 Total Bulbs**
Pathogens detected	**1**	**1**	**3**	**3**	**1**	**9**	**14 Asymptomatic**
Symptomatic bulbs	**1**	**7**	**0**	**1**	**1**	**10**	**13 Total Bulbs**
Pathogens detected	**1**	**5**	**0**	**1**	**1**	**8**	**80 Symptomatic**
Total bulbs	**12**	**16**	**16**	**16**	**16**	**76**	

Table 16: Fungal profile detected by the DNA macroarray on bulbs sampled after the 35ºC curing in the 2014 trial.

Fungi – post curing Bulb Trial 2014

Fungi Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Fusarium oxysporum	**0**	**0**	**0**	**1**	**0**	**1**	**6.7**
Fusarium proliferatum	**1**	**4**	**3**	**2**	**0**	**10**	**66.7**
Aspergillus niger	**1**	**1**	**0**	**0**	**2**	**4**	**26.7**
Aspergillus flavus	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium breviconbactum	**0**	**0**	**0**	**0**		**0**	**0**
Penicillium expansum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium polonium	**0**	**0**	**0**	**0**		**0**	**0**
Penicillium digitatum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis aclada	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis byssoidea	**0**	**0**	**0**		**0**	**0**	**0**
Botrytis allii	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis porri	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis cinerea	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Kluveromyces	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Fungi Detected	**2**	**5**	**3**	**3**	**2**	**15**	**100**

Table 17: Bacterial profile detected by the DNA macroarray on bulbs sampled after the 35ºC curing in the trial 2014.

Bacteria- post curing Bulb Trial 2014

Bacteria Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Enterobacter cloacae	**0**	**1**	**0**	**2**	**0**	**3**	**23.5**
Burkholderia gladioli pv. alliicola	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Burkholderia cepacia	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pantoea agglomerans	**1**	**2**	**0**	**2**	**0**	**5**	**38**
Pantoea ananatis	**1**	**2**	**0**	**0**	**0**	**3**	**23.5**
Pseudomonas marginalis pv. marginalis	**0**	**1**	**0**	**0**	**0**	**1**	**7.5**
Pectobacterium carotovorum subsp. carotovorum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Dickeya chrysanthemi	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudomonas aeruginosa	**0**	**1**	**0**	**0**	**0**	**1**	**7.5**
Erwinia rhapontici	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pseudomonas viridiflava	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Bacteria Detected	**2**	**7**	**0**	**4**	**0**	**13**	**100**

2015 postharvest sampling: The fungal profile of the 2015 trial at post-harvest sampling presented a broad distribution profile of different pathogens detected where *Fusarium oxysporum* was pathogen that showed most detection with a 29 % of the total fungi detected (Table 18).

Table 18: Fungal profile detected by the DNA macroarray on bulbs coming from the postharvest sampling trial 2015.

Fungi – postharvest Bulb Trial 2015

Fungi Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Fusarium oxysporum	**0**	**0**	**0**	**1**	**1**	**2**	**29**
Fusarium proliferatum	**0**	**0**	**0**	**0**	**1**	**1**	**14**
Aspergillus niger	**0**	**0**	**0**	**0**	**1**	**1**	**14**
Aspergillus flavus	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium breviconbactum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium expansum	**0**	**0**	**0**	**0**	**1**	**1**	**14**
Penicillium polonium	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium digitatum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis aclada	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis byssoidea	**0**	**0**	**0**	**0**	**1**	**1**	**14**
Botrytis allii	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis porri	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis cinerea	**0**	**0**	**0**	**0**	**1**	**1**	**14**
Kluveromyces	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Fungi Detected	**0**	**0**	**0**	**1**	**6**	**7**	**100**

2015 post curing sampling: The detection profile in this sampling set showed a short number of samples with detection where *Aspergillus niger* was the most detected pathogen with the 80% of the total detected fungi (Table 19).

Table 19: Fungal profile detected by the DNA macroarray on bulbs sampled after the 35ºC curing in the trial 2015.

Fungi – post curing Bulb Trial 2015

Fungi Detected	Field Trt. 1	Field Trt. 2	Field Trt. 3	Field Trt. 4	Field Trt. 5	Total	%
Fusarium oxysporum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Fusarium proliferatum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Aspergillus niger	**0**	**1**	**2**	**0**	**1**	**4**	**80**
Aspergillus flavus	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium breviconbactum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium expansum	**0**	**0**	**0**	**0**	**1**	**1**	**20**
Penicillium polonium	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Penicillium digitatum	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis aclada	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis byssoidea	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis allii	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis porri	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Botrytis cinerea	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Kluveromyces	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Total Fungi Detected	**0**	**1**	**2**	**0**	**2**	**5**	**100**

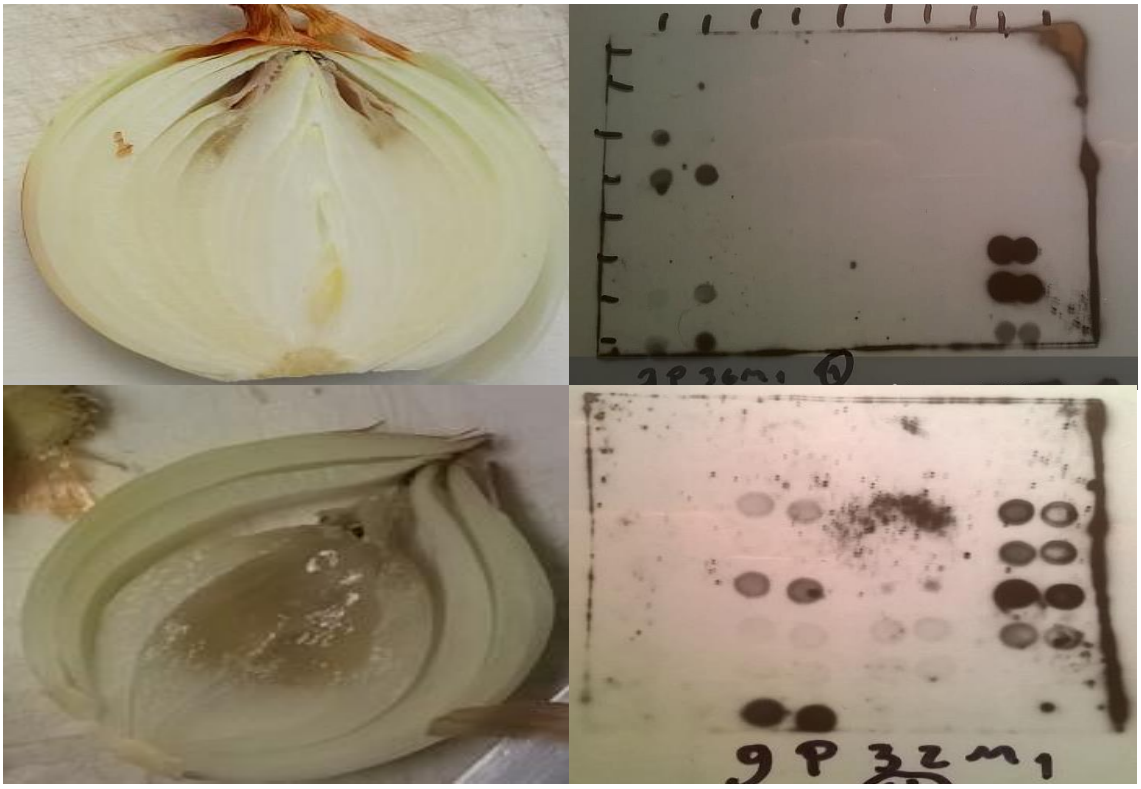


Figure 32: Pictures from onion slices used in the experiment which presented fungal and bacterial symptoms, and blots detecting fungi and bacteria.

3.8. Discussion

The results obtained in this research highlighted some features of DNA macroarray technique. When comparing the data obtained in chapter 3 with chapter 2 in a broad sense, it could be observed that the predominate fungal species in the 2014 trial was *Fusarium proliferatum* (Table 11 and 13), and in the 2015 trial was *Aspergillus niger* (Table 16 and 17). Comparing these result with the isolation done from decayed onions (Table 6 and 7) shown in chapter 2, some similarities could be observed where in the 2014 trial the 77% of the isolations were *Fusarium sp.*, and in the trial 2015 again *Fusarium sp.* was the most observed, but only with 37% of the isolations followed closely by *Aspergillus sp.* which was isolated from34% of the samples. Therefore, the DNA macroarray showed similar results compared to the isolations obtained from decayed onions, demonstrating that this molecular technique could be a powerful detection technique. Similarly, the results obtained in chapter 2 and the results obtained in the DNA macroarray, showed that a greater percentage of decayed onion were obtained in the trial 2014 than in the trial 2015 (Figure 12, 13 and 16 versus 23 and 26), as

similar in the trial 2014 pathogen detection was completed using the DNA macroarray for more samples than in the 2015 trial (Table 10 to 17). In this way, the DNA macroarray technique gave an overview of the species profile and the percentage of decay.

Comparing the DNA macroarray results for the postharvest and post curing samples, some observations were made. First, comparing the fungal profile it could be observed that there was an increase in the detection of *Aspergillus niger* in the post curing samples in both years (Table 12 and 15), principally in the 2015 trial. This result coincides with some observation already mentioned from other sources (Crowe 2000, Suberizer 2012) which suggest that heat curing could increase the incidence of *Aspergillus niger*. It was expected that the DNA macroarry would detect more bacteria in the post-curing samples, as suggested by other researchers (Schroeder and du Toit 2010, Schroeder et al. 2012). This was not apparent in this research, but it is important to mention that less post-curing bulbs were assayed in comparison with post-harvest bulbs assayed. For that reason, it is not possible to make a strong conclusion about the potential to detect bacteria from bulbs after heat curing.

Analyzing the procedures used in this research for the DNA macroarray technique, the first step was the DNA extraction. Therefore, it is known than DNA extraction and the following PCR are affected by polysaccharides, polyphenolic compounds and some soil compounds (Vojkovska et al. 2014). All of these compounds can be found in onions (Mitra et al. 2011), and they could affect the yield of DNA and PCR products. In this research we used the PowerSoil extraction kit (MO BIO laboratories, Inc., Carlsbad, CA) which is recommended for DNA extraction on vegetables for pathogens (Vojkovska et al. 2014). However, better results were obtained when the DNA extraction was done under cold conditions. Another issue that could have affected the DNA extraction in onions is that bulbs have a high water content, about 87% (Mitra et al. 2011). Because of this when the targets are pathogen DNA in asymptomatic bulbs, the dilution factor could affect the final results. Thus, in this research we worked with transversal onion slices of around 5 mm width. Nevertheless, differences were observed in the final results (on the blots) when small differences in the DNA extraction procedure were evaluated, suggesting that it might be it is necessary to adjust the target sampling area from the onion. This could be achieved by taking only the tissue portion from

the neck and from the root plate, which are the areas where pathogens most often initiate decay.

Following the PCR process, 5-10 ng of DNA are needed for EtBr observation (Sambrook and Russel 2006). Although, generally in the fungi PCR amplification bands on the agarose gel could not be observed, the following results on the DNA macroarray, where control dots were pretty strong, suggest that PCR step was not an issue in amplification of both fungi and bacteria. It is important to mention that DNA macroarrays are a lot more sensitive than observation in agarose gel where the DNA amount needed is about 5 ng and in the DNA macroarray could be as low as 40 femtograms (Urbez-Torres et al. 2015).

The results of this study allow us to make some inferences about the DNA macroarray technique. First, that the DNA macroarray method is a very sensitive detection tool as has been reported by previous research (Urbez-Torres et al. 2015). This observation is supported by the positive pathogen detection in many asymptomatic bulbs. Another interesting observation was that many positive pathogen detections corresponded to *Fusarium proliferatum* in both symptomatic or asymptomatic bulbs, and this pathogen was also observed in many of the post-harvest and post-curing samples from the trial outlined in chapter 2 as it was mentioned. Another positive aspect of the DNA macroarry technique was that symptomatic onion bulbs always gave a positive pathogen detection, and in many cases the identified pathogen corresponded to the typical symptoms observed. This fact demonstrated that the DNA macoarray could work well for identification. However, in this last point it would be necessary to confirm that the pathogen isolated from the bulb were the ones identified by the assay, and in this way validate the technique (Urbez-Torres et al. 2015) (already validated with pathogens cultures).

On the other hand, one of the points that warrants further research is the relatively low detection of *Botrytis spp.* This pathogen, causing neck rot, has been previously reported to be commonly found in onions produced in western ID (Vaughan et al 1964). However, it is important to mention that in both 2014 and 2015 environmental conditions may not have been conducive for *Botrytis spp.* infections due to extremely warm temperatures during the growing season (see chapter 2). Second and lastly, some differences in the detection result were

observed in the two different season where in the 2015 trial there were a decrease of the percentage of symptomatic bulbs with detection.

4. References

Anonymous. 2012. Successful Onion Storage Management in the Columbia Basin. [http://www.suberizer.com/successfull-onion-storage-management-in-the-columbia-basin/].

Agindotan, B. Perry, K. L. 2007.Macroarray Detection of Eleven Potato-Infecting Viruses and Potato spindle tuber viroid. Plant Disease. 92:730-740.

Arif, M. Vahling-Armstrong, C. M. Knerr, J. Lupien, S. Dugan, F. du Toit, L. Schroeder, B. K. 2013. Modification of Oligo Design for Enhanced Sensitivity of a DNA Macroarray for Detection of Fungal Onion Bulb Rot Pathogens. Poster Session: Detection and Diagnosis – Fungi and Oomycetes. The American Phytopathological Society.385-P.

Beaver, R. G. and Devoy, M.L. 1986. The Effect of Seasonal Application of Fungicide on the Incidence of Neck Rot in Onions. Idaho, USA: University of Idaho Southwest Idaho Research and Extension Center Parma: Progress Report.

Beaver, R. G. and Devoy, M.L. 1988. The Effect of Seasonal Application of Fungicide on the Incidence of Neck Rot in Onions. Idaho, USA: University of Idaho Southwest Idaho Research and Extension Center Parma: Progress Report.

Beer, S. Asselin, J. A. Bonasera J. 2013. Update on Bacterial Diseases of Onion: Detecting Bacterial Pathogens, Bacteria in Soil and Water, Suppressive Soil, Varietal Susceptibility and the Effect of Actigard® on Bacterial Decay. New York, USA: Plant Pathology and Plant-Microbe Biology, Cornell University and Christy Hoepting, Vegetable Program.

Bishop, A. L. 1990. Internal Decay of Onion Caused by *Enterobacter cloacae*. Plant Disease. 74:9:692-694.

Brewster, J. L. 2006. Onions and Other Vegetable Alliums. Crop Production Science in Horticulture Series No. 3. CAB International, Wallingford, UK.

Carrieri, R. Raimo, F. Pentangelo, A. Lahoz, E. 2013. *Fusarium proliferatum* and *Fusarium tricicnctum* as Causal Agents of Pink Rot of Onion Bulbs and the Effect of Soil Solarization Combined with Compost Amendment in Controlling Their Infection in Field. Crop Protection. 43: 31-37.

Ceponis, M. J. Capellini, R. A. Lightner, G. W. 1986. Special Report Disorders in Onion Shipment to the New York Market, 1972-1984. Plant Disease. 70:988-991.

Cilia, V. Lafay, B. and Christen, R. 1996. Sequence Heterogeneities among 16S Ribosomal RNA Sequences, and Their Effect on Phylogenetic Analyses at the Specie Level. Molecular, Biology and Evolution. 13:451461.

Cortes, I. Livieratos, I. C. Derks, A. Peters, D. Kormelink, R. 1998. Virology: Molecular and Serological Characterization of *Iris Yellow Spot Virus*, a New and Distinct Tospovirus Species. Phytopathology. 88:1276-1282.

Crowe, F. 2000. Management of Fungal Diseases in Onion and Garlic. Oregon, USA: Oregon State University Extension Service: IPM in Oregon: Achieve and Future Directions. Special Report. 1020. 172-177.

Crowe, F. J. and Pappu, H. R. 2005. Outbreak of *Iris Yellow Spot Virus* in Onion Seed in Central Oregon. Plant Disease. 89:105.

du Toit, L. J. and Derie, M. L. and Pelter, G. Q. 2004. Prevalence of *Botrytis spp.* in Onion Seed Crops in the Columbia Basin of Washington. Plant Disease. 88:10.

du Toit, L. 2014. *Iris Yellow Spot Virus* Management Considerations. In: 2014 PNVA Convention & Trade Show. [http://www.pnva.org/files/files/duToit2014PNVA-IYSVMgmt.pdf]. Accesses on 8 June 2015.

Dutta, B. Barman, A. K. Srinivasan, R. Avci, U. Ullman, D. E. Langston, D. B. Gitaitis R. D. 2014. Transmission of *Pantoea ananatis* and *P. agglomerans*, Causal Agents of Center Rot of Onion (*Allium cepa*), by Onion Thrips (*Thrips tabaci*) Through Feces. Phytopathology. 104:812-819.

Eisel, D. Seth, O. Grünewald-Janho, S. Kruchen, B. Rüger, B. 2008. DIG Aplication Manual for Filter Hybridization. ACTIVE ARTWARE Gruppe Saarbrücken. Roche Diagnostics GmbH.

Fessehaie, A. De Boer, S. H. Lévesque, C. A. 2002.Molecular Characterization of DNA Encoding 16S– 23S rRNA Intergenic Spacer Regions and 16S rRNA of Pectolytic Erwinia Species. Canadian Microbiology Journal. 48: 387–398.

Food and Agriculture Organization of the United Nations. 2015. Statistic Division. [http://faostat3.fao.org/browse/Q/QC/E].

Gent, D. H. du Toit, L. J. Fichtner, S. F. Krishna Mohan, S.Hanu R. Pappu, H. Schwartz, H. F. *Iris yellow spot virus*: An Emerging Threat to Onion Bulb and Seed Production. Plant Disease. 90:1468-1480.

Gitatis, R. Walcott, R. Culpepper, S. Sanders, H. Zolobowska, L. Langston, D. 2002a. Recovery of *Pantoea ananatis*, Causal Agent of Center Rot of Onion, from Weeds and Crops in Georgia, USA. Crop Protection. 21:983-989.

Gitatis, R. D. Wells, M. L. Sanders, Riley, D.G. Walcott, R. R. 2002b. Association of the Bacterium *Pantoea ananatis* with Tobacco Thrips, *Frankiniella fusca*. Phytopatology. 92:S149

Gitaitis, R. D. Walcott, R. R. Sanders, H. F. Zolowbska, L. Diaz-Perez, J. C. 2004. Effects of Mulch and Irrigation System on Sweet Onion: II. The Epidemiology of Center Rot. Journal American Society for Horticultural Science. 129:225-230.

Hall, M. J. Mohan, K. Knott, E. A. Moyer J. W. 1993. Tospovirus Associated with Scape Blight of Onion (*Allium cepa*) Seed Crop in Idaho. Plant Disease. 77:956.

Harrow, K.M. and Harris, S. 1969. Artificial Curing of Onions for Control of Neck Rot (Botrytis allii Munn), New Zealand Journal of Agricultural Research. 12:592-604.

Hayden, N. J. and Maude, R. B. 1992. The Role of Seed-borne *Aspergillus niger* in Transmission of Black Mold of Onion. Plant Pathology. 40:573-581.

Idaho Eastern-Oregon Onion Committee. 1999. The Marketing Policy for the 1999–2000 Shipping Season. Idaho Easter Oregon Onion Committee, Parma.

Jones, H. A. Mann, L. K. 1963. Onions and their Allies: Botany, Cultivation and Utilization. Interscience Publishers, New York. 286.

Kritzman, G. Chet, I. Gilan, D. 1981. Spore Germination and Penetration of *Botrytis allii* Into *Allium Cepa* Host. Botanical Gazette (Chicago). 142:151-155.

Lievens, B. Brouwer, M. Vanachter, A. C. R. C. Levesque, C. A. Cammue, B. P. A. Thomma, B. P. H. J. 2003. Design and Development of a DNA Array for Rapid Detection and Identification of Multiple Tomato Vascular Wilt Pathogens. FEMS Microbiology Letter. 223:113-122.

Lievens, B. Brouwer, M. Vanachter, A. C. R. C. Levesque, C. A. Cammue, B. P. A. Thomma, B. P. H. J. 2005. Quantitative Assessment of Phytopathogenic Fungi in Various Substrates Using a DNA Macroarray. Environmental Microbiology. 7:1698–1710.

Lievens, B. Thomma, B. P. H. J.Recent 2006. Developments in Pathogen Detection Arrays: Implications for Fungal Plant Pathogens and Use in Practice. Phytopathology. 95:1374-1380.

Lievens, B. Juste, A. Willems, K. A. 2012. Fungal Plant Pathogen Detection in Plant and Soil Samples Using DNA Macroarrays. , Plant Fungal Pathogens: Methods and Protocols, Methods in Molecular Biology. 835:491-507.

Mackay, I. M. Arden, K. E. Nitsche, A. 2002. Survey and Summary: Real-Time PCR in Virology. Nucleic Acids Research. 30:1292-1305.

Marin, S. Sanchis, V. Magan, N. 1995. Water activity, Temperature, and pH Effects on Growth of *Fusarium moniliforme* and *Fusarium proliferatum* Isolates From Maize. Canadian Journal. Microbial. 41: 1063-1070.

Matson, W. E. Mansour, N. S. Richardson, D. G. 1985. Onion Storage Guidelines for Commercial Growers. Oregon, USA: Oregon State University: Pacific Northwest Cooperative Extension Bulletins. 277.

Maude, R.B. and Presly, A. H. 1977. Neck rot (*Botrytis allii*) of Bulb Onions. I. Seed Borne Infection and its Relationship to the Disease in the Onion Crop. Annals of Applied Biology 86 (20):163-180.

Mitra, J. and Shrivastava, S. L. and Rao, P. S. 2011. Onion dehydration: a review. Food Science Technology. 49:267–277.

Maw, B. W. Butts, C. L. Purvis, A. C. Seebold, K. Mullinix, B. G. 2004. High−temperature Continuous−flow Curing of Sweet Onions. Applied Engineering in Agriculture. 20: 657−663.

Nagerabi, S. A. F. Ahmed, A.H.M. 2003. Storability of Onion Bulbs Contaminated by *Aspergillus niger* Mold. Phytoparasitica. 31:515-523.

National Agricultural Statistic Service. United States Department of Agriculture. 2016. Quick Stat. [https://quickstats.nass.usda.gov/].

National Onion Association. 2011. All About Onion. [https://www.onions-usa.org/all-about-onions/history-of-onions].

Pelter, G. Q. Sorensen, E. J. 2004. Crop profile for onions in Washington. USDA National Information System Pest Management Center.

Ransey, G.R. and Lorbeer, J. W. 1985. Ecology and Epidiomiology: The Role of Temperature and Free Moisture in Onion Flower Blight. Phytopathology. 76:612-616.

Reitz, S. 2014a. Onion Thrips (Thysanoptera: Thripidae) and Their Management in the Treasure Valley of the Pacific Northwest. Florida Entomologist. 97:349-354.

Reitz, S. 2014b. Survey: Internal Dry Scale and Associated Bulb Rots. Oregon, USA: Malheur County Extension, Oregon state University. [http://extension.oregonstate.edu/malheur/].

Sambrook, J. and Russel, D. W. 2006. Detection of DNA in Agarose Gels. CSH Protocols. Doi:10.1101/pdb.prot4042.

Schroeder, B. K. du Toit, L. J. 2010. Effects of Postharvest Onion Curing Parameters on Enterobacter Bulb Decay in Storage. Plant disease. 94:1425-1430.

Schroeder, B. K. Humann, J. L. du Toit L. J. 2012. Effects of Postharvest Onion Curing Parameters on the Development of Sour Skin and Slippery Skin in Storage. Plant Disease. 96:1548-1555.

Schwartz, H. F. Otto, K. L. Gent, D. H. 2002. Relation of Temperature and Rainfall to Development of *Xanthomonas* and *Pantoea* Leaf Blights of Onion in Colorado. Plant Disease. 87:11-14.

Schwartz, H. F. and Gent, D. H. 2007 Onion: Bacterial Soft Rot. High Plains IPM Guide, a Cooperative Effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University. Onion, Disease, Bacterial Rot.

Schwartz, H. F. and Mohan, K. 2008. Compendium of Onion and Garlic Diseases and Pests. Minnesota, USA. The American Phytopathological Society.

Schwartz, H.F. and Bartolo, M.E. 2011. Botrytis, Downy Mildew and Purple Blotch of Onion. Colorado, USA: Colorado State University: Fact Sheet No. 2.941 (Crop Series | Diseases.)

Schwartz, H. F. 2012. Pest Management Strategic Plan for Dry Bulb Storage Onions in the United States. Western Integrated Pest Management Center. [http://www.wripmc.org].

Schwartz, H. F. 2013. Onion Health Management and Production. Colorado, USA: Colorado State University Fort Collins.

Shock, C. C. Feibert, E. Jensen, L. Mohan, S. K. Saunders, L. D. 2008. Onion Variety Response to *Iris Yellow Spot Virus*. HortTechnology. 18:539-544.

Sholberg, P. O'Gorman, D. Bedford, K. Lévesque, C. A. 2005. Development of a DNA Macroarray for Detection and Monitoring of Economically Important Apple Diseases. Plant Disease. 89:1143-1150.

Stanger, C. Ishida, J. Shock, C. Stieber, T. 1988. Artificial Drying of Onion to Improve Storage Quality. Oregon, USA: Agricultural Experiment Station Oregon State University Corvallis: Special report 832.

Stanger, C. Ishida, J. Shock, C. Stieber, T. 1989. Artificial Drying of Onion to Improve Storage Quality. Oregon, USA: Agricultural Experiment Station Oregon State University Corvallis: Special report 844.

Stankovic, S. Levic, J. Petrovic, T. Logrieco, A. Moretti, A. 2007. Pathogenicity and Mycotoxin Production by *Fusarium proliferatum* Isolated from Onion and Garlic in Serbia. European Journal Plant Pathology. 118:165–172.

Stroup, W. W. 2014. Rethinking the Analysis of Non-Normal Data in Plant and Soil Science. Agronomy Journal. 106:1-17.

Sullivan, D. M. Brown, B.D. Shock, C.C. Horneck, D.A. Stevens, R.G. Pelter, G.Q. Feibert, E.B.G. 2001. Nutrient Management for Onions in the Pacific Northwest. Pacific Northwest Extension Publication, Oregon State University, Washington State University, University of Idaho. PNW 546.

Tambong, J. T. de Cock, A. W. A. Tinker, M. N. A. Le´vesque, C. A. 2006. Oligonucleotide Array for Identification and Detection of Pythium Species. Applied and Environmental Microbiology. 72: 2691–2706.

Taylor, A. G. Eckenrode, C. J. Straub, R. W. 2000.Seed Coating Technologies and Treatments for Onion: Challenges and Progress. New York, USA: Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva.

Teviotdale, B. L. Davis, R. M., Guerard, J. P. Harper, D. H. 1989. Effect of Irrigation Management on Sour Skin of Onion. Plant Disease. 73:819-822.

Thornton, M. 2012. Management of Onions to Mitigate Storage rot. Presentation at the Pacific Northwest Vegetable Association Meeting [http://www.pnva.org/files/files/]. Accessed 8 January 2015.

Urbez-Torres, J. R. Haag, P. Bowen, P. Lowery, T. O'Gorman, D. T. 2015. Development of DNA Macoarray for the Detection and Identification of Fungal Pathogens Causing Decline of Young Grapevine. Phytopathology. 105:1373-1388.

United State Department of Agriculture. 1997. Fresh Fruit and Vegetable Arrival Totals for 20 Cities. Agricultural Marketing Service FVAS-3 Calendar Year 1997. 72–73.

Vahling-Armstronga, C. Dungb, J. K. S. Humannc j. L. Schroederc, B. K. 2016. Effects of Postharvest Onion Curing Parameters on Bulb Rot Caused by *Pantoea agglomerans*, *Pantoea ananatis* and *Pantoea allii* in storage. Plant Pathology. 65:536–544.

Vincelli, P. Tisserat, N. 2008. Nucleic Acid–Based Pathogen Detection in Applied Plant Pathology. Plant Disease. 92:660-669.

Walcott, R. R. Gitaitis, R. D. Castro, A. C. Sanders, F. H. Diaz-Perez, J. C. 2002. Natural Infestation of Onion Seed by *Pantoea ananatis*, Causal Agent of Center rot. Plant Disease 86:106–11.

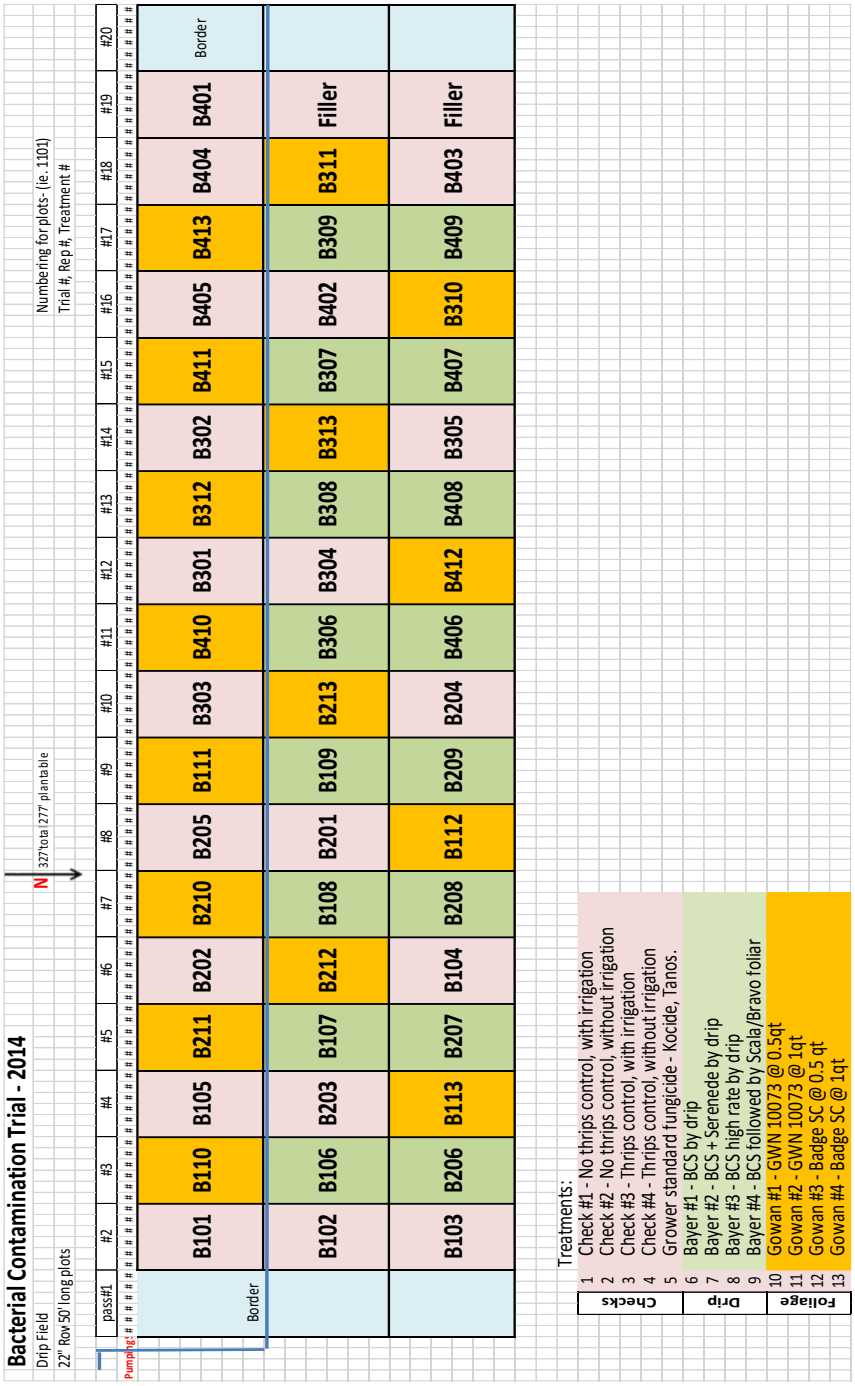
Wang, J. C. Hong, B. Z. Li, G. Wang, J. P. Zheng, C. Y.2014. Effects of Exposure to High Temperature on *Frankliniella occidentalis* (Thysanoptera: Thripidae), under Arrhenotoky and Sexual Reproduction Conditions. Florida Entomologist. 97:504-510.

Waters, T. Wohleb, C. 2014. Onions Thrips. Washington, USA: Washington State University Extension: Fact Sheet. FS126E.

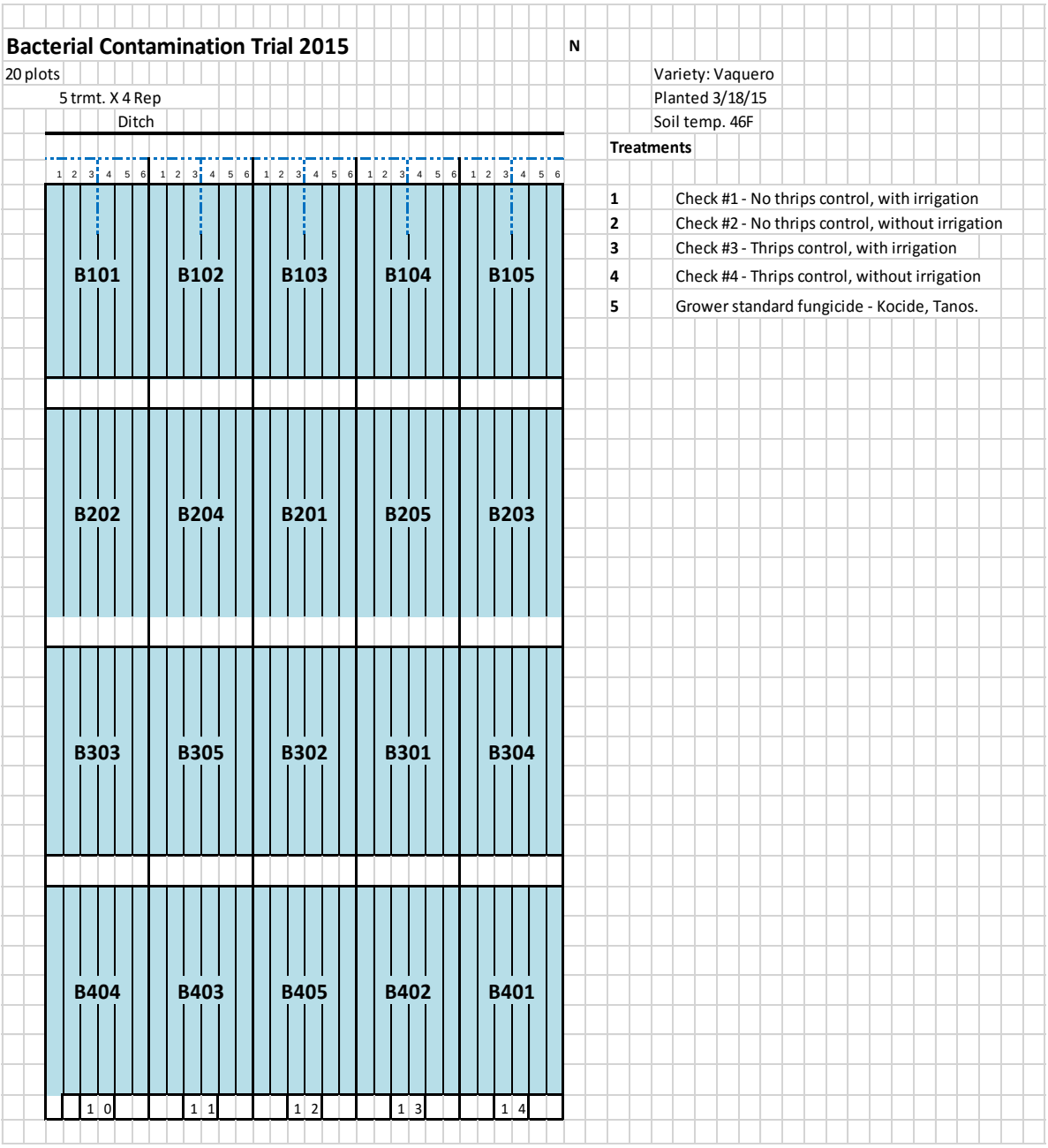
Wright, P. J. Hale, C. N. Fullerton, R. A. 1993. Effect of Husbandry Practices and Water Applications During Field Curing on the Incidence of Bacterial Soft Rot of Onions in Store. New Zealand Journal of Crop and Horticultural Science. 21:161-164.

Appendix

Field plots trial 2014



Field plots trial 2015



Fertilizers and herbicides Trial 2014

FERTILIZER Trial 2014	Total N-P-K (kg/ha)	
	Nitrogen	Phosphorous
	46-0-0	11-52-0
Pre plant		89P (without Avail)
In season	26-0-0-6	
22-May	22	
29-May	22	
5-Jun	22	
12-Jun	33	
19-Jun	33	
27-Jun	22	
3-Jul	22	

HERBICIDES APPLIED Trial 2014		
Application	Date	Material and Rate/ha
spray pre emergene	5-Abr	Round up 2.47 l./ha
spray pre emergene	5-Abr	Prowl 2.32 l./ha
ground sprayed	8-May	Buctril 0.93 l./ha
ground sprayed	27-May	Poast 1.85 l./ha
ground sprayed	3-Jun	Buctril 0.93 l./ha
ground sprayed	3-Jun	Goal 0.15 l./ha

Fertilizers and herbicides Trial 2015

FERTILIZER Trial 2015	Total	N-P-K (kg/ha)
	Nitrogen	Phosphorous
	46-0-0	11-52-0
Pre plant		67P
In season	26-00-6	
22/5/2015	33	
5/6/2015	33	
19/6/2015	33	
3/7/2015	33	

HERBICIDES APPLIED Trial 2015		
Application	Date	Material and Rate/ha
Spray Tractor	03/30/15	Round up 2.47 l./ha
Spray Tractor	03/30/15	Prowl 2.32 l./ha
Spray Tractor	05/07/15	Buctril 0.93 l./ha
Spray Tractor	05/29/15	Buctril 0.93 l./ha
Spray Tractor	05/29/15	Goal 0.15 l./ha
Spray Tractor	06/03/15	Poast 1.85 l./ha
Spray Tractor	08/17/15	MH 30 9.89 l./ha

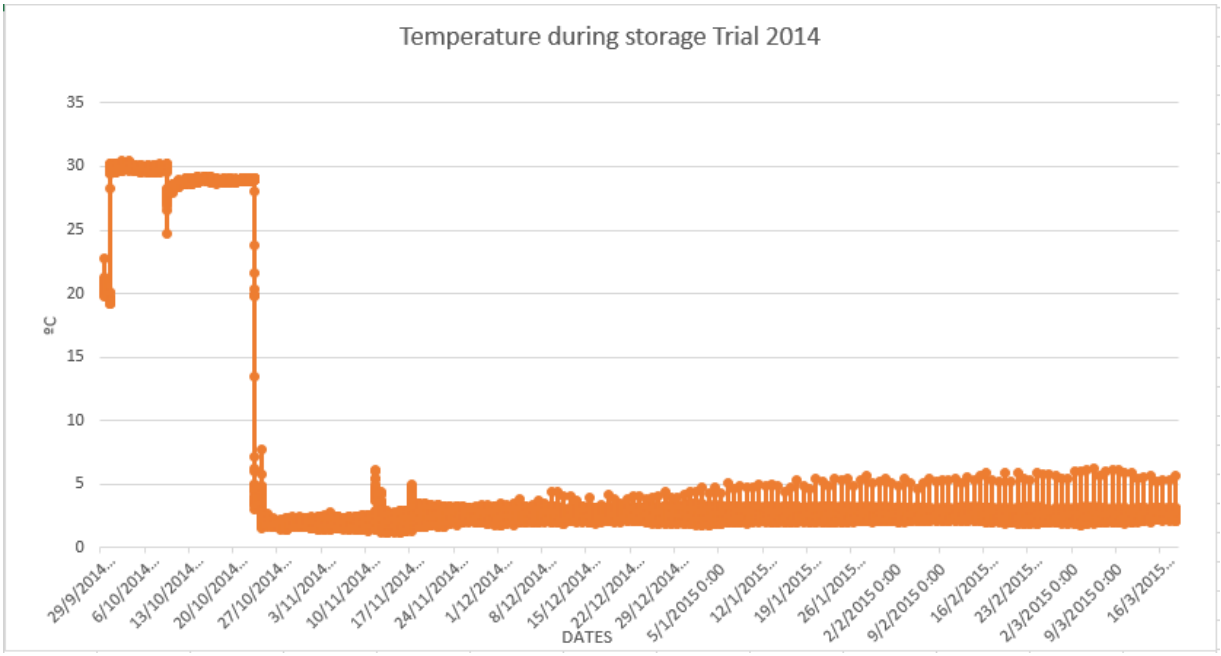
Media used for the isolations

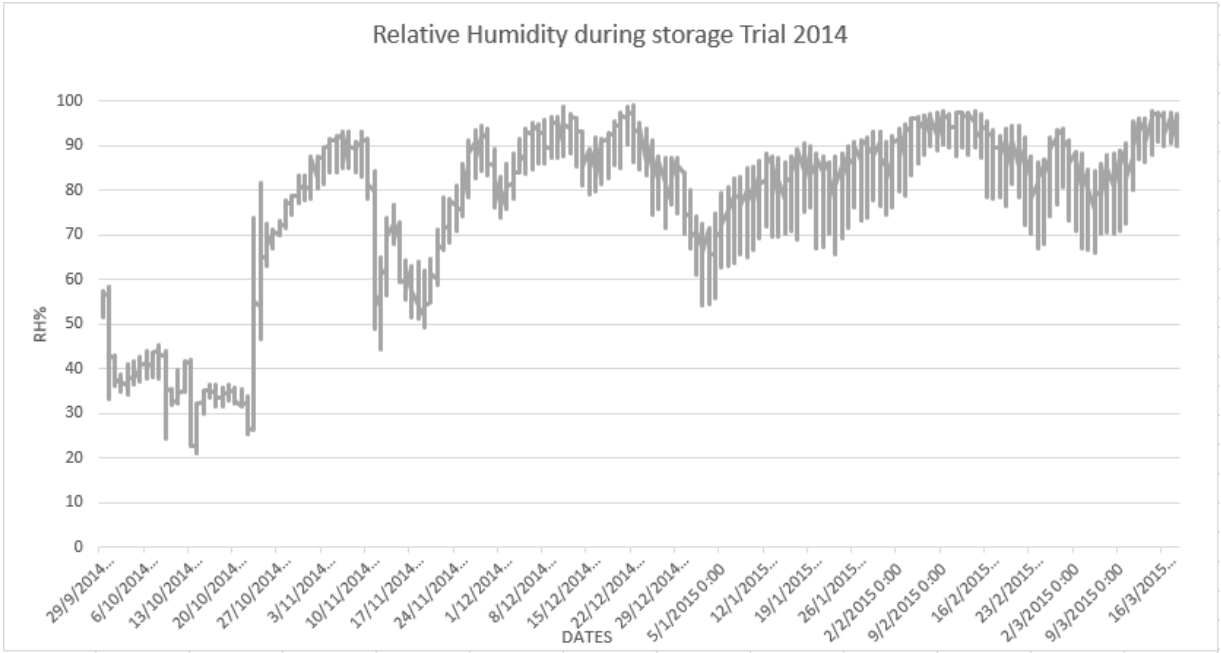
Media used Bacteria growth trial 2014-2015		
Nutrient Broth Yeast- 1 liter		
Component	**Quantity**	**Observation**
Nutrient broth	8 g	-
Yeast Extract	2 g	-
K_2HPO_4 (anhydrous)	2 g	-
KH_2PO_4	0.5 g	-
di H_2O	950ml	-
Agar	15 g	Add at the end before autoclave.
10% glucose	5 ml	Add before pour the plates
1 M $MgSO_4$	0.1 ml	Add before pour the plates
Cyclohexamide	50 mg	Stock 2.5 mg/ml in water filter sterilize. Add before pour plates.

Media used Fungi growth trial 2014		
Potato Dextrose Agar- 1 liter		
Component	**Quantity**	**Observation**
Potato dextrose	1 g	-
Agar	20 g	Add at the end.
Ethanol	6 ml	Addition before pour the plates.
di H_2O	1000 ml	-
Strepomycin	100 mg	Stock 50 mg/ml in water filter sterilize. Add before pour plates.

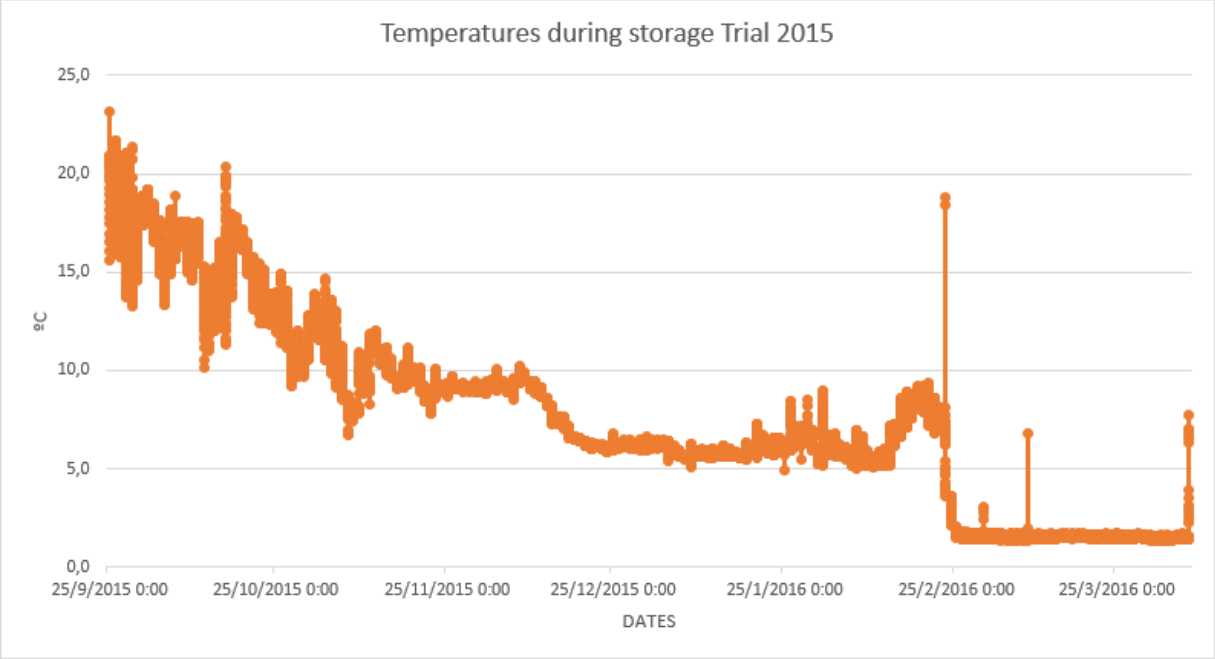
Media used Fungi growth trial 2015		
1/2 V8- 1 liter		
Component	**Quantity**	**Observation**
V8 juice	100 ml	Original recipe.
$CaCO_3$	1.5 g	Not go into solution. Mix when pouring.
Bacto agar	20 g	-
di H_2O	900 ml	-
Tetracycline	50 mg	Fresh 50 µg/ml in 70% EtOH. Add before pour plates.
Streptomycin	50 mg	Stock 50 mg/ml in water filter sterilize. Add before pour the plates

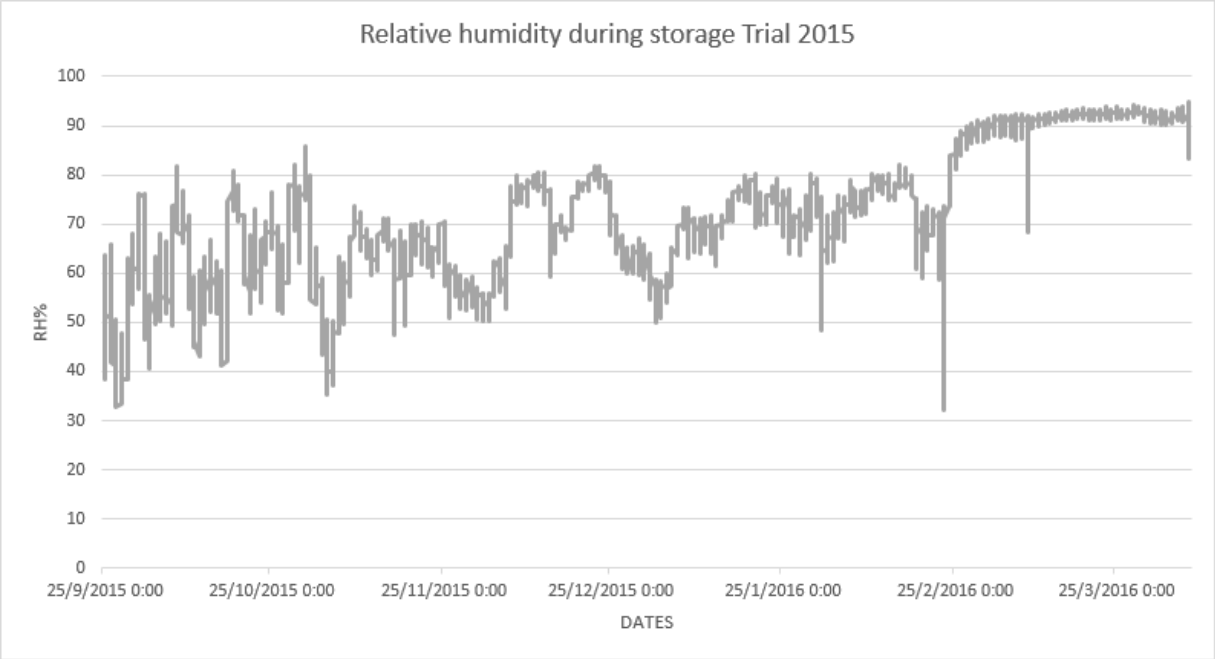
Temperature and relative humidity Trial 2014





Temperature and relative humidity Trial 2015





Fungal oligonucleotides

Yeast	Pen(+)-	TTTCTTTCTTTCTTTTCTTTCTTTCTTTCTTTTGTATTGTGAATTGCAGATTTTCGT
	KluK2V1	TTTTTTCTTTTTCTTTTTTCTTTTTCTTTTTTTTACCTTTGGGTTTGGTAGTGAGTGA
Fusarium	Fus2-M4	TTTTCTTCTTTCTTTTCTTCTTTCTTTTCTTTGAAGTTACATATAGAAACAGAGTTT
	ITS-fu-f-	TTTCTTTTCTTTTCTTTTTTTTTTTTTTCTTTTTTTCTTTTCAACTCCCAAACCCCTGT
	Fus6-M4	TTTTCTTTTTTTTCTTTTTTTTCTTTTTTTCTTTTTTTTCGAATTAACGCGAGTCCCAA
	F.	TTTTTTTTTCTTTTTTTTCTTTTTTCTTTTTTTTTTTTAGCACAGCTTGGTGTTGGGAC
	Fus9-M4	TTTTTTTTTTCTTTTTTTTTCTTTTTTTTTTCTTTTTTTAATGGAAGCTGCAGCGAGA
	Fus10-	TTTTTTTTTTCTTTTTTTTTTTTTTTTTTTCTTTTTTTTAAGGGCTCGCCGATCCCCAA
Aspergillus	AspGn1-	TTTTTTTCTTTCTTTTCTTCTCTTTTTTGAGATCCATTGTTGAAAGTTTTAACTGAT
	AspGn3-	TTTTTTTTTTCTTTTTTTTCTTTTTCTTTTTTCTTTTTTTTCGACCCAACACACAAGC
	AspN3-	TTTTTTCTTTTTTTCTTTTTTTTTTTTCTTTTTTTTCAGACTGCACGCTTTCAGACAGT
	AspN4-	TTTTTTTCTTTTTCTTTTTTTTTTTTTCTTTTTTTTTTTTTACAGTGTTCGTGTTGGGGTCT
	AspF1-	TTTTTTTCTTTTTTTTTCTTTTTTTTCTTTTTTCAGACTTCACTAGATCAGACAGAGT
	AspF3-	TTTTTTTTTTTCTTTTTTCTTTTTCTTTTTTTTTTCAACTAAGGTACAGTAAACACGGG
Penicillium	Pen1-M4	TTTTTTTTTCTTTTTTTTTCTTTTTTCTTTTTCTTTTTTTTCCAACCTCCCACCCGTGT
	Pen2-M4	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACGCGGTGCCGCCGCTGCCTT
	PenD20-	TCTTTTTCTTTTCTTTTCTTTTTTCTGAAGATTGCAGTCTGAGTGAAAACGAAATT
	PenDV1-	TTTTTCTTTCTTTCTTTTCTTTTCTTTTTTTTGAAGATTGCAGTCTGAGTGAAAACG
	PenE17-	TTTTTCTTTTTTTTTTTTTCTTTTTTTTTTAACTCTGCCTGAAGATTGTCGTCTGAGT
	PenE18-	TTTTTTCTTTTTTTTTTTCTTTTCTTTTTTTTTTCGTGTTTATTTACCTCGTTGCTTCG
	PenEV1-	TTTTTTTTTTCTTTTTTTTCTTTTTTCTTTTTTTTCTCTGCCTGAAGATTGTCGTCTGA
	PenEV2-	TTTTTTTTTCTTTTTTTTTCTTTTTTTTCTTTTTTTTTCCCGAACTCTGCCTGAAGATTG
	PenB7-	TTTTTTTTTCTTTTTTTTTTTCTTTTTTTTTTTTTTAGTTCTAAGGTGTCTTCGGCGAG
	PenB8-	TTTTTTTTTTTTTTTTTTCTTTTTTTTTTTTCTTTTTTTACGTCCCCCGGCAGCCAAA
	PenB10-	TTTTTTTTTTTCTTTTTTTTTTTTTTTCTTTTTTTTTTTTTTCGGAAGGAGGACGGAGCC
	PenG5-	TTTTTTTCTTCTTCTTTCTTCTTCTTTTTAACTTATTTAGTTTATGCTCAGACTGCA
	PenG6-	TTTTTTTTTTTCTTTTTTTTTCTTTTTTTTTTTTTTAGTTCAATAGTGTCTCCGGTGCG
	PenGV1-	TTTTTTTTTTTCTTTTTTTTTCTTTTTTTCTTTTTTTGCGCACCGGAGACACTATTG
	PenPv1-	TTTTTTCTTTTTTTCTTTTTTTTTTCTTTTTTTCCGAACTCTGTCTGAAGATTGAAGT
	PenP5-	TTTTTTTTTTTCTTTTTTTTTCTTTTTTTCTTTTTTTGCGCACCGGAGACACTATTG
Botrytis	Asp(+)-	TTTTTCTTTTCTTTTTTCTTTTCTTCTCTTTTGCTTGGTATTGAGTCTATGTCAGTA
	Bt3f-M4	TTTTTCTTTTCTTTTTTTTCTTTTTTTCTTTTTTTAGTAGATGGTCGAAGGAACGCT
	BotC1-	TTTTCTTCTTTCTTTTTTTCTTTTTGGATATGAATTAACATACGAGTCACCAAAAA
	BotC2-	TCTTTTTTCTTTTCTTTTCTTTTTTTTTCCAAAGCTTCTTCTCGAATTCATTAATAA
	B. cin	TTTTTCTTTTCTTTTTTCTTTCTTCTCTTTTCTACCACAAATAGCTACAACATACCT
	B. cin	TTTTTCTTTTTTTTCTTTCTTTTCTTTTTCTTTTTTTTTGCAAGCCCAGTAGCAGAAA
	BotB1-	TTCTTTTTTTTCTTTTTTTTTCTTTTTTTCAAAGCTCCTTCTCGACTTTATTAACAA
	B.bys	TTTTTCTTTTCTTTTTTTTTTTCTTTTCTTCTCTTTTCTCGTGCTGTTTGCGCAGATA
	Btacl 1-	TTTCTTTTCTTTTCTTCTTTTTTTCTTTTTTTCTTTTCGTTTTTCGGTGACTCATATG
	BotA2-	TTTCTTTTTTTTTCTTTTTTTCTTTTTTTCCAAAACTTCTTCTCGACTTCATCAACA
	B.acl up	TTTTTCTTTTCTTTTTTCTTTCTTCTCTTTTCGCATATATTTTAGTAAAATGGACCT
Control	ITS4A1-	TTTTTTCTTTTCTTTTTTTTTTTTCTTTTCTTCTCTTTTCTATGCTTAAGTTCAGCGGG
	BA1r-M4	TTTTTCTTTTCTTCTTTTTTTTTTTTTCTTTTCTTCTCTTTTGAGTGCTGGCGGAAAC
	Fus1-M4	TTTTCTTTTTTTTCTTTTCTTTTTTCTTTTTTTTTGCATTTTGCTGCGTTCTTCATCGA

Fungal oligonucleotides explanation

	Fusarium sp.	*F. oxysporum*	*F. proliferatum*	*Aspergillus sp.*	*A. niger*	*A. flavus*	*Penicillium sp.*	*P. brevicombactum*	*P. expansum*	*P. polonium*	*P. digitatum*	*Botrytis sp.*	*B. aclada*	*B. byssoidea*	*B. allii*	*B. cinérea*	*Kluveromycies*
Pen(+)-M4																	✓
KluK2V1-M4																	✓
Fus2-M4	✓	✓	✓														
ITS-fu-f-M4	✓	✓	✓														
Fus6-M4		✓															
F.provF2V1-M4		✓															
Fus9-M4			✓														
Fus10-M4			✓														
Pen1-M4							✓	✓	✓	✓	✓						
Pen2-M4							✓	✓	✓	✓	✓						
AspGn1-M4				✓	✓	✓											
AspGn3-M4				✓	✓	✓											
AspN3-M4					✓												
AspN4-M4					✓												
AspF1-M4						✓											
AspF3-M4						✓											
PenD20-M4											✓						
PenDV1-M4											✓						
PenE18-M4									✓								
PenEV2-M4									✓								
PenB8-M4								✓									
PenB10-M4							✓	✓	✓	✓	✓						
PenG6-M4							✓	✓	✓	✓	✓						
PenGV1-M4							✓	✓	✓	✓	✓						
PenPv1-M4										✓							
PenP5-6v-M4										✓							
Asp(+)-M4												✓	✓	✓	✓	✓	
Bt3f-M4												✓	✓	✓	✓	✓	
BotC1-M4																✓	

BotC2-M4															✓	
B.cin Up3-M4															✓	
B.cin Up1-M4															✓	
BotB1-M4													✓			
B.bys Up3-M4													✓			
Btacl1-M4												✓		✓		
BotA2-M4												✓		✓		
B.acl up 1 -M4												✓		✓		
ITS4A1-M4																
BA1r-M4																
Fus1-M4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
(at least 1 of the 3)																

Bacterial oligonucleotides

Large Blot	
Burk. OTO	ACGGATCGCGGAAGGTTGTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTACGGATCGCGGAAGGTTGTC
B.gladioli-1 OTO	GGGTGCAAAATTCAAGGGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTGGGTGCAAAATTCAAGGGCGT
B. glad-2 OTO	CCTCTAAGCTTCAGTTTAACAGTGACCGTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTCCTCTAAGCTTCAGTTTAACAGTGACCGT
B. glad-2 ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTCTAAGCTTCAGTTTAACAGTG ACCGT
B. pyrro OTO	CGGGCGCGGAATTCAAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TCGGGCGCGGAATTCAAG
B. pyrro. ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGGGCGCGGAATT CAAG
B. complex OTO	CGGGTGCGTAATTCAAGGGTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTCGGGTGCGTAATTCAAGGGTGT
B. complex ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGGGTGCGTAATTCAAGG GTGT
P. agglom. OTO	GTGTAGGCGGAGTGTCCAGGTAAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTGTGTAGGCGGAGTGTCCAGGTAAA
ALT. P. agglom OTO	GGACGCTTGTTAACGCTGAGGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTGGACGCTTGTTAACGCTGAGGC
P. alli-anan OTO*	GGTTCACTTTACACTGAGGCGTGACGACTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTGGTTCACTTTACACTGAGGCGTGACGAC
P. alli-anan . ALLT*	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTTCACTTTACACTGAGGCGTG ACGAC
P. marg OTO	CTTTTAGTTAACGAAGTGGTTGATGCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTCTTTTAGTTAACGAAGTGGTTGATGCC
P. marg ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTTTTAGTTAACGAAGTGGTTGA TGCC
P. marg M OTO	GCTGATGACGAGTTAACTTTTAGTTAACGTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTGCTGATGACGAGTTAACTTTTAGTTAACG
Pseud. OTO	AGGCCGAGAGCTGATGACGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTAGGCCGAGAGCTGATGACGAGT

P. carotov. OTO	GCGTGAAGGTGGATGACTTTGGTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTGCGTGAAGGTGGATGACTTTGGTA
P. carotov. ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCGTGAAGGTGGATGACTTT GGTA
E. rhap/pers OTO	AGCGTGTAGGCTTGAGTTCCAGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTAGCGTGTAGGCTTGAGTTCCAGG
E .rhap/pers ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGCGTGTAGGCTTGAGTTC CAGG
Rahn/Serr OTO	TTCAAGCGTGTAGGGGGTGTGACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTCAAGCGTGTAGGGGGTGTGAC
Rhan/Serr ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAAGCGTGTAGGGGGTG TGAC
Serr/Rahn OTO	ATCCGGTTACTTGTCAACCCTGAGGCGTGTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTATCCGGTTACTTGTCAACCCTGAGGCGTG
Serr/Rahn. ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATCCGGTTACTTGTCAACCCTGAG GCGTG
Dickeya OTO	AAGCGTGCAGGTGGGTGGACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTAAGCGTGCAGGTGGGTGGAC
Dickeya ALLT	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAGCGTGCAGGTGGGT GGAC
53F OTO	GGACAACAGGTTAATATTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTGGACAACAGGTTAATATTCC
53F Jodi OTO	GGGAAACAGGTTAATATTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTGGGAAACAGGTTAATATTCC
62 R ALLT OTO	GGGGCCATTTTGCCGAGTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTGGGGCCATTTTGCCGAGTTC
62R Jodi OTO	GGTACCATTTTGCCTAGTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTGGTACCATTTTGCCTAGTTC

Small Blot	
E_cloacae A1 SH OTO	TAAGCATGTAGGCGGAGGTTCCAGGTAATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAGCATGTAGGCGGAGGTTCCAGGTAA
E_cloacae A-SH OTO	CTTNTTAACGCTGAGGTGTGATGACGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTTNTTAACGCTGAGGTGTGATGACGAG
E_cloacae-A1 OTO	GTCCCGGTTTAAGCATGTAGGCGGAGGTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTCCCGGTTTAAGCATGTAGGCGGAGGTTC
E. cloacae A	TTAACGCTGAGGTGTGATGACGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAACGCTGAGGTGTGATGACGAG
53F Jodi OTO	GGGAAACAGGTTAATATTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGAAACAGGTTAATATTCC
53F OTO	GGACAACAGGTTAATATTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGACAACAGGTTAATATTCC
62R Jodi OTO	GGTACCATTTTGCCTAGTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGTACCATTTTGCCTAGTTC
62 R ALLT OTO	GGGGCCATTTTGCCGAGTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGGGCCATTTTGCCGAGTTC

Bacterial oligonucleotides explanation

	Burkholderia sp.	*B. ambifaria*	*B. cenocepacia*	*B. cepacia*	*B. gladioli*	*B. pyrrocinia*	*D. dadantii*	*E. cloacae*	*E. periscina* or *E. rhaponitici*	*P. aeurginosa*	*P. agglomerans*	*P. alli* or *P. ananatis*	*P. carotovorum*	*P. marginalis*	*Pseudomonas* sp.	*Rahnella* sp.	*Serratia* sp.	*Klebsiella* sp.
Burk.	✓	✓	✓	✓	✓	✓												
B. complex OTO		✓	✓	✓														
B. complex ALLT		✓	✓	✓														
B.gladioli-1 OTO					✓													
B_glad-2 OTO					✓													
B_gladioli-2 ALLT					✓													
B.pyrro OTO			X			✓												
B_pyrro. ALLT			X			✓												
Dickeya OTO							✓											
Dickeya ALLT							✓											
E.rhap/pers OTO									✓									
E.rhap/pers ALLT									✓									
E_cloacae A1 SH OTO								+/-										
E_cloacae A-SH OTO								+/-										
E_cloacae-A1 OTO								+/-										X
E. cloacae A								+/-										
P. agglom. OTO											✓							
ALT.P.agglom OTO								X			+/-							
P. alli-anan. OTO												✓						X
P_alli-anan. ALLT												✓	X					
P. carotov. OTO													✓					
P. carotov. ALLT													✓					
P. marg OTO										X				✓	X			

P. marg ALLT										**X**				✓	**X**			
P. marg M OTO														✓	**X**			
Pseud. O TO										✓				✓	✓			
Rahn/Serr OTO																✓	✓	
Rhan/Serr ALLT																✓	✓	
Serr/Rahn OTO																✓	✓	
Serr/Rahn. ALLT																✓	✓	
Positive control (at least 1 of the 4)	✓	✓	✓	✓	✓	+/-	✓	✓	✓	✓	✓	✓	✓	✓	✓	+/-	✓	✓

✓= expected 100% target hybridization when probe concentration above detection limit
X= one of the strains tested showed a false positive
+/- = variable hybridization depending on strain tested

Solutions used in the DNA Macroarray

Solutions Required for Macroarray Detection Procedure

Solution/Buffer	Chemical	Stock conc.	Proprtion (ml)
Washing Solutions			
2X SSC & 0.1% SDS	SSC	20X	100
	SDS	10%	10
	Water	-	890
	Total	**-**	**1000**
0.1X SSC & 0.1% SDS	SSC	20X	5.0
	SDS	10%	10
	Water	-	985
	Total	**-**	**1000**
Washing Buffer	Maleic Acid Buffer	10X	100
	Tween20	-	3.00
	Water	-	897
	Total	**-**	**1000**
Blocking Solution (1X)	Maleic Acid Buffer	10X	100
			100
	Blocking solution (**vial 6**)	10X	800
	Water	-	1000
	Total	**-**	
Antibody Solution	Blocking Solution	1X	100
	Anti-Digoxigenin-AP (vial 4)		10 µl
Detection Buffer (1X)	Detection Buffer	5X	100
	Water		400
	Total		**500**

Stock solution DNA Macroarray

-20X SSC pH: 7

NaCl---- 175.3 grams

Sodium Citrate---- 88.2 grams

800 ml di H_2O

Adjust pH with 14 N HCl

Rinse solution to 1 liter and autoclave

-Maleic Buffer 10X pH: 7.5

Maleic acid---- 116.1 grams (1M)

NaCl---- 87.66 grams (0.15M)

800 ml di H_2O

Adjust pH with NaOH

Rinse solution to 1 liter and autoclave

-Detection Buffer 5X pH: 9.5

Tris-HCl----40.57 grams

NaCl---- 29.25 grams

800 milliliters di H_2O

Adjust pH with NaOH

Rinse solution to 1 liter and autoclave

Solution, buffers and reagents of the Roche detection kit II

Bottle/ Cap	Label	Content including function
1	DIG-High Prime	• 50 µl DIG-High Prime • 5 × conc. labeling mixture containing optimal concentrations of random primers, nucleotides, DIG-dUTP (alkali-labile), Klenow enzyme and buffer components • ready-to-use • clear, viscous solution • for efficient random primed labeling of DNA
2	DIG-labeled Control DNA	• 20 µl • [5 µg/ml] pBR328 DNA (linearized with Bam HI) • clear solution • determination of labeling efficiency
3	DNA Dilution Buffer	• 3 vials of 1 ml • [50 µg/ml fish sperm DNA in 10 mM Tris-HCl, 1 mM EDTA; pH 8.0 at 25°C] • clear solution
4	Anti-Digoxigenin-AP Conjugate	• 50 µl • [750 U/ml] • from sheep, Fab-fragments, conjugated to alkaline phosphatase • clear solution
5	CSPD ready-to-use	• 50 ml CSPD • clear solution • Chemiluminescent substrate for alkaline phosphatase
6	Blocking solution	• 4 × 100 ml • 10 × conc. • yellow, viscous solution
7	DIG Easy Hyb granules	4 bottles for 100 ml DIG Easy Hyb buffer each, for the hybridization of DNA

Extra experiments aging

In the trial 2015 an extra experiment was done. The test consisted in harvesting 8 extra bags which were cured at 35ºC only for 2 days. The idea was to cure onion bulbs at high temperature for a short exposure time, and comparing this with the treatment where onion bulbs were cured at 35ºC for 2 weeks.

Therefore, the eight bags after short curing were kept with the rest of the bags, and then they were evaluated at the six months. In this way, after the evaluation, statistical analysis showed that there were significant differences between short curing and two long curing treatments at 35ºC where the mean of the short curing was about the 7% of decayed onion proportion against the 17% and 21% of rot onion proportion in long curing. Also, the extra curing evaluation showed that there was no significant difference among this curing with the ambient curing which always obtained the least proportion. This result suggests that heat curing affects the onion when prolonged exposure. However, curing of 2 days would be a very difficult task under commercial conditions.

The following figure shows the plot of means of proportion of decayed onions with their confidence limits. The extra curing was labeled as curing 6 in the plot.

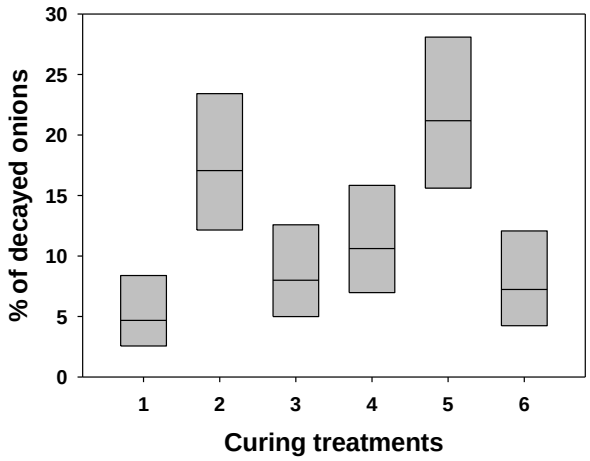


Fig. Nº 40: plot of means % decayed onion proportion and their 95 % confidence limits for the same five curing of the research plus the extra curing number 6 (2 days at 35ºC).

Extra experiment, DNA macroarray of symptomatic bulbs

In some symptomatic decayed bulbs from a grower field, DNA macroarrays were conducted. Here some picture of the bulbs and the results obtained.

Pictures of rot onion with *Fusarium proliferatum* or bacterial rot and the blot showing *F. proliferatum* detection.