Evaluating the Use of Hyperspectral Remote Sensing and Narrowband Spectral Vegetation Indices to Diagnose Onion Pink Root at the Leaf and Canopy Level

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
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December 2019

Authorization to Submit Thesis

This thesis of Kyler D. Beck submitted for the degree of Master of Science with a Major in Plant Science and titled "Evaluating the Use of Hyperspectral Remote Sensing and Narrowband Spectral Vegetation Indices to Diagnose Onion Pink Root at the Leaf and Canopy Level" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

The Treasure Valley of Idaho and Eastern Oregon produces around 30% of the nation's annual summer storage onion crop. To profitably produce an onion crop, growers must attain both high total yields and bulbs of sufficient size. Onion pink root (causal agent *Setophoma terrestris*) is capable of destroying the onion root system during the growing season, resulting in foliar symptoms that imitate those of nutrient or drought stress and bulbs that are underdeveloped and small in size. The aim of this study was to evaluate the usefulness of hyperspectral remote sensing and narrowband spectral vegetation indices (SVIs) as a tool to diagnose onion pink root in the field and discriminate its symptoms from those of nitrogen and water stress. A field experiment was established in Parma, ID in 2018 and 2019 to which 3 moderate and consistent individual stress treatments were applied to the onion cultivars SV4643NT and Vaquero. Pink root, nitrogen, and drought stress were imposed using a combination of natural inoculum, soil fumigation (chloropicrin), fertilizer, and drip irrigation. Onion samples and hand-held, hyperspectral radiometric measurements were collected weekly from the time of bulb initiation to the time of plant maturity to study the effects of stress on onion phenotype and reflectance at the leaf and canopy level. Results from the destructive sampling suggest that the proportion of diseased roots is significantly reduced by soil fumigation with chloropicrin. Furthermore, the total nitrogen content of onion leaves sampled from the nitrogen stressed (non-fertilized) plots were significantly reduced from the content of those taken from optimally fertilized plots. All stress treatments significantly reduced plant biomass, although not at the same growth stage nor to the same extent. Reflectance spectra were analyzed using 40 established SVIs. Preliminary analysis determined a similar relative relationship among treatments for most of the SVIs utilized. Three SVIs: triangular vegetation index (TVI), normalized difference vegetation index (NDVI), and optimized soil-adjusted vegetation index (OSAVI) were selected for detailed analysis. Only small variations in SVI value were observed between treatments at the leaf level. At canopy-level, stress treatments consistently lowered index values, though not always significantly and not always to the same degree. Results from standard regression analysis suggest that SVI value is closely related to the biomass of SV4643NT ($R^2 = 0.67$ to 0.79) and Vaquero ($R^2 = 0.73$ to 0.83) onions, likely because of the direct relationship between plant biomass and parameters which account for the fraction of vegetation that covers the soil (such as leaf area index (LAI)). Overall, we found that SVIs, particularly those which include a band in the near-infrared region (700-1000 nm), are overwhelmingly sensitive to variation in canopy LAI as opposed to other vegetation parameters such as plant pigment composition (i.e. chlorophyll) in scenarios where the crop canopy does not completely cover the soil. This presents a challenge to the practical use of hyperspectral remote sensing and narrowband SVIs as a tool to diagnose onion stress at the canopy level.

Acknowledgements

I extend my sincerest gratitude to Dr. Mike Thornton for this opportunity to train under and to learn from him and for his patience, guidance, and support throughout this process. Additionally, I would like to collectively thank Dr. Brenda Schroeder, Dr. Olga Walsh, and Dr. James Woodhall for serving on my graduate committee and for their generous involvement in the planning and execution of this study. Each of the aforementioned individuals has, in their own way, been instrumental and it has been a great honor to learn from them. Special thanks to Ransey Portenier for allowing me to share his office and for teaching me all aspects related to onion production and research. A huge thank you to Andrew Walker for tending to the onion plots and assisting with data collection and to Oksana Morgan for her assistance with statistical analysis. Lastly, I would like to thank the Idaho-Eastern Oregon Onion Research Committee and the University of Idaho College of Agriculture and Life Sciences for funding this graduate assistantship.

Dedication

I dedicate this work to my wife Sharleen, for her loving encouragement and support over the past two years and to my father Deron who imparted to me a deep appreciation of agriculture and of onions.

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Chapter 1: Introduction

1.1. United States onion production

The United States (U.S.) onion (*Allium cepa*, L.) production averaged 3,255,948 tons per year from 1996 to 2016, making it the 3rd largest onion producer in the world. This is despite the fact that the U.S. ranked only 14th worldwide in harvested hectarage (Hanci 2018). In 2017, the United States Department of Agriculture's National Agriculture Statistics Service (USDA NASS, 2018) estimated the total value of the nation's onion crop to be over 1 billion dollars annually. To provide a more detailed account of the nation's onion production, the USDA separates U.S. onion bulb production into distinct categories based on the season of the year in which harvest occurs and the post-harvest destination of the bulbs. This results in the separation of onions into three distinct categories which are: spring, summer non-storage, and summer storage (USDA NASS, 2018). Since spring harvested onions (produced in the warmer regions of the nation such as California, Texas, and Georgia) are primarily sold immediately to the fresh market, they lack the storage/non-storage distinction. Of the three groups, the production of summer storage onions comprises the majority of U.S. total onion production, representing just over 75% in 2017 (USDA NASS, 2018). These onions are typically grown in temperate climates, planted in the spring and harvested in late summer, stored in bulk after harvest, and gradually released to processors and distributors throughout the winter and spring months.

Within the realm of summer storage onion production, the Pacific Northwest (PNW), comprised of Washington, Oregon, and Idaho, is a standout producer. According to the USDA NASS (2018), the quantity of summer storage onions produced the PNW in 2017 was 2.2 million tons. This means that 54% of all summer storage onions grown and utilized in the U.S. during 2017 originated from the PNW.

1.2. Importance of Treasure Valley onion production

The Treasure Valley encompasses the fertile farmland of Southwestern Idaho and Eastern Oregon's Malheur County. Extensive river and canal networks provide plentiful irrigation throughout the region and, combined with warm summer temperatures, facilitate the successful production of a variety of forage and crop species, including onion. The Treasure Valley makes an important contribution to U.S. dry onion bulb production. Over the past two decades, the Treasure Valley has accounted for 24-30% of the nation's total annual production of summer storage onions (USDA NASS 2001-2018). This translates into the shipment of over 1 billion onion bulbs from the region every year (USA Onions 2019). The vast majority of production in this region consists of long-day, sweet Spanish hybrids. Though yellow cultivars predominate, red and white cultivars are also grown. According to the Idaho-

Eastern Oregon Onion Committee, there are approximately 300 onion growers and 30 onion shippers that reside in the region (USA Onions 2019).

1.3. Marketing requirements

Most onion farms in the Treasure Valley produce between 32 to 160 hectares annually at an estimated cost of approximately $12,400 per hectare (Murray et al. 2018). Onions are a high-value crop and have the potential to justify the substantial cost of production. Two important economic considerations in this respect are high yield and quality. There are multiple components to onion quality including size, appearance, percentage of single-centered bulbs, and susceptibility to sprouting and decay in storage (Sullivan et al. 2001). Prior to reaching the fresh market, onions are graded according to specific size and quality criteria. A crop that fails to meet any of the quality criteria may fail to generate economic return. Bulb size is a particularly important quality component, since onions are packaged and shipped according to size. Markets for onions smaller than 5.7 cm are limited (Sullivan et al. 2001) and bulbs greater than 7.6 cm typically provide the maximum economic return (Geary et al. 2008). Therefore, anything that constrains onion bulb size will also constrain economic return. Plant stress during the growing season is the main reason for reduced bulb size. Stresses most detrimental in this regard can occur from a lack of proper irrigation, a lack of nitrogen fertilizer, or pest and disease pressure.

1.4. Onion diseases

There are many diseases and pests to which onions are susceptible (Brewster 1994). The Treasure Valley offers a suitable environment for the persistence of numerous plant pathogens. Many of these have the ability to reduce yield, size and marketability of onion bulbs. The pathogens most important to the Treasure Valley include Iris yellow spot virus (IYSV) and soilborne fungi. Neck rots caused by *Botrytis allii* and *Fusarium proliferatum* and basal plate rot caused by *Fusarium oxysporum* are examples of pathogens that cause diseases that are particularly detrimental to onion bulbs in the region. Though onions appear healthy at the time of harvest, severe disease symptoms can develop while the onions are in storage. These symptoms are visible, obvious, and quantifiable as bulbs are removed from storage and prepared for shipping. However, not all disease results in physical damage to onion bulbs. Undersized bulbs are less obvious of a disease symptom, yet they are no less economically significant. One disease particularly detrimental to onion production in the Treasure Valley is onion pink root.

1.5. Pink root

Pink root is a fungal root rot of onion caused by the soilborne Ascomycete *Setophoma terrestris*. The causal agent is categorized as a necrotrophic; first destroying root tissue by extracellular lytic enzymes and subsequently colonizing the dead tissue (Zapacosta et al. 2003). It can colonize the roots of most *Allium* spp., however, it is also a weak parasite on the underground parts of a wide variety of other plant hosts (Carrieri et al. 2013). *S. terrestris* has been found to colonize the roots of plants from 76 genera including popular crop species of *Cucurbita*, *Glycine*, *Solanum*, *Sorghum*, *Triticum*, and *Zea* (Kinsey 2002). Onion symptoms caused by *S. terrestris* were first characterized and reported in Texas in 1921 (Taubenhaus and Mally 1921). Since that time, pink root has been identified throughout the tropical and subtropical regions of the world (Wiriyajitsomboon 2015; Carrieri et al. 2013). *S. terrestris* is particularly abundant in warmer climates (Zapacosta 2003) where soil temperatures attain levels that are ideal for pathogen growth and infection (24-28°C according to Brewster 1994). In naturally infested soil, hyphae, pycnidia-bearing conidia, and microsclerotia are the presumed infectious agents of *S. terrestris* (Biles et al. 1992). Microsclerotia are produced as overwintering and dispersion propagules in soil or decaying plant material by many fungal plant pathogens and can remain viable in the soil for up to 10 years (Song 2018). When environmental conditions are suitable, microsclerotia germinate and the resulting conidia will colonize local dead or senescing tissues or infect adjacent plant roots (Figure 1.1).

1.6. Pink root taxonomy

Hansen (1929) was first to classify the pink root causal agent under the genus *Phoma* and to distinguish it from the plethora of other *Fusarium* species that had also been isolated from the pink roots of infected onions. The culmination of Hansen’s research was the identification of a new fungal species which he named *Phoma terrestris* Hansen. Years later, in 1948, it was discovered that the pycnidia of fertile *Phoma terrestris* isolates were consistently found to bear setae (Gorenz et al. 1948). On this basis, the researchers found it fitting to reclassify and rename the pink root fungus as *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, and Larson. Like most taxonomical changes, however, the change from *Phoma* to *Pyrenochaeta* was neither quickly nor fully adopted by the scientific community. Studies from 1948 up to the present day have utilized either of the two taxa to denote the causal agent of onion pink root and they are considered to be synonyms. In more recent years, technological advances in the area of molecular biology have birthed a new genus under which to classify the pathogen, namely *Setophoma* (de Gruyter et al. 2010). Therefore, the taxon *Setophoma terrestris* is utilized here though it is recognized that it is synonymous with both *Phoma terrestris* and *Pyrenochaeta terrestris*.

1.7. Pink root signs and symptoms

The characteristic symptom of this disease is evident from its common name – pink root. As *S. terrestris* infects and colonizes onion roots, infected root tissue initially turns bright pink in color. Disease progression is characterized by the dissemination of the pink hue (and the pathogen) within the infected root, as well as the change from pink to red to purple as infested roots shrivel and die. Uniquely, pink root is confined to the plant roots and the dry outer sheathes of the shoot base (Brewster 1994; Shock and Kimberling 2001) and thus does no direct damage to the bulb. Infection does, however, directly restrict the uptake of water and nutrients to the developing plant. As a result, foliar symptoms of pink root may imitate those of nitrogen and water stress (Lee et al. 2007). This can manifest as leaf tip-burn, a thorough and general leaf chlorosis and wilting, leaf stunting, and (in severe cases) premature plant death (Swett et al. 2019). Yield reduction in cases of severe infection can be extreme, due to the stunted bulb development of affected plants. Visual diagnosis of pink root from foliar symptoms is difficult and thus requires that plants be removed from the soil for root inspection.

It has been found that *S. terrestris* can grow on the outermost scales of white onion bulbs resulting in a red staining of infected areas (Shock and Kimberling 2001). In this instance, however, it is important to clarify that there was no observation of the fungus infecting live onion bulb tissue. Only the dry, outer scales were colonized and therefore concern with this manifestation of the pathogen is primarily aesthetic.

1.8. Management of soilborne onion disease

It is common practice for disease management strategies to be arranged under a few umbrella categories. Utilizing this scheme, management strategies can be categorized into those which seek to *exclude* the pathogen from the environment in which onions are grown, those which actively move to *eradicate* inoculum that is already present in the growing environment or on equipment, and those which seek to *protect* an established crop from existent inoculum via chemical or biological means (Maloy 2005).

One possible way to exclude soil-borne pathogens, like *S. terrestris*, from an onion growing environment is to grow onions on land which has not been previously cropped with *Allium*. This management method is perhaps the most limited in applicability, for a number of reasons. First, onions have been grown in the Treasure Valley for 100 years (Murray et al. 2018). By the present day, it is unlikely that there exists a substantial amount of virgin land that is both conducive and practical to put into onion production. This would also only be an effective method of excluding soil-borne disease method so long as the pathogen one wishes to exclude is not capable of infecting any of the other crops that might have been grown in these locations, making this method even more unlikely in the case of *S. terrestris*. Preliminary results from soil testing at the University of Idaho identified *S. terrestris* in

108 of 139 (78%) samples taken from Southwestern Idaho and analyzed using species specific quantitative polymerase chain reaction (qPCR; Woodhall unpublished). In recent years, the wide-spread adoption of drip irrigation systems has allowed for the utilization of ground which was previously unfit for agriculture due to the slope of the land and the potential for substantial soil erosion if conventional irrigation methods were employed (USA Onions 2019). Therefore, pathogen exclusion may have been a feasible strategy of pink root control under these anomalous circumstances.

A second management strategy for soilborne disease is the active eradication of existing pathogen inoculum. This can be accomplished most effectively by a combination of cultural and chemical control. The first step to effective cultural control of soilborne disease is a structured and thorough planning of crop rotations. A rotation gap of 5-6 years between *Allium* crops has proven effective for controlling pink root in Idaho (Brewster 1994). This gap in time is one way to nonchemically alter the relative quantities of soil inoculum so long as the rotation is composed of nonhost species. In the case of *S. terrestris*, however, rotation to nonhost crops is difficult, since many of the rotation alternatives such as potato (*Solanum tuberosum*), corn (*Zea mays*), and cereals (*Triticum* spp., *Hordeum vulgare*) are also known to be hosts to this pathogen alongside local weedy grasses such as pigweed (*Amaranthus retroflexus*) and crabgrass (*Digitaria sanguinalis*; Wiriyajitsomboon 2015). Therefore, in order to further eradicate existing inoculum, soil treatment must be the primary means. Historically, soil solarization (soil heating) has been explored as a method of controlling soilborne onion disease (Rabinowitch et al. 1981). Although successful, timing of soil solarization in the Treasure Valley is difficult and would need to be scheduled during years in which early maturing crops are grown. By far the most commonly employed soilborne disease management strategy (alongside crop rotation) within the region is soil fumigation (Thornton and Mohan 1996). Fumigation typically occurs in the fall prior to spring planted onions with commercial fumigants like chloropicrin or metam sodium. Although useful in controlling soilborne disease, fumigation is not ideal due to its associated expense, effects on non-target pests, and human health risks (Geary et al. 2008).

The final method for managing soilborne disease is the protection of the crop during the growing season. In endeavoring to protect an onion crop, cultivar selection is the first major step as the use of tolerant cultivars is one of the best nonchemical means of mitigating soilborne disease. Several studies have demonstrated that there are differences in cultivar tolerance to pink root (Thornton and Mohan 1996; Esfahani and Pour 2007), however none have been identified as completely resistant (Murray et al. 2018). Genotypes that display tolerance to *S. terrestris* may have the enhanced ability to put forth new roots later in the growing season (Thornton and Mohan 1996). Daylength also plays a factor in the difference in tolerance between genotypes (Esfahani and Pour 2007). Long-day cultivars bulb and senesce much later in the growing season than their short-day counterparts. This extra time spent in the soil results in more opportunity for interaction between the pathogen and plant roots. Therefore, it is most difficult to attain high levels of disease tolerance in long-day cultivars, such as those grown in the Treasure Valley.

Chemical protection of onions during the growing season is a comparatively new management strategy for soilborne disease. Historically, the soil has been considered too great a barrier to consider chemical protection (Brewster 1994). Water management of onions in the Treasure Valley has made a significant transition over the past two decades from predominantly furrow irrigation to the now widely-used drip irrigation. By placing drip tape near the crop root zone, drip irrigation has opened up a new route of chemical delivery. Several formulations have shown promise in mitigating soilborne pathogens and are currently registered for use in onions. *Bacillus subtilis* subsp. *subtilis* has been observed displaying specific antagonistic activity toward *S. terrestris* under laboratory conditions (Orio et al. 2016) and formulations of this microbe are available for use under the product name Serenade ASO (Bayer Crop Science, Durham, North Carolina, USA). Results suggest, however, that these products do not lead to an overall increase in plant health and marketable yield locally (Reitz et al. 2014). In-season application of penthiopyrad (Fontelis, Dupont de Nemours Inc., Midland, Michigan, USA) may protect onions roots to some degree (Thornton et al. 2017; Murray et al. 2018) but its efficacy is still under investigation.

For effective control of onion pink root, no single management strategy is the answer. Disease can be most effectively mitigated, rather, using a combination of each of the three management strategies. Soil fumigation, nonetheless, remains the most commonly employed chemical control method for soilborne disease.

1.9. Precision agriculture and disease mapping

Precision agriculture is a novel management concept that includes the detailed measurement of and particular response to key intrafield variables. The concept relies on the idea that a field is no longer considered a homogenous entity, but rather a heterogenous one (Ondoua and Walsh 2017). Thereby, farm inputs should be moderated by utilizing the most effective management strategy at the correct location in the field at the right time (Mulla 2012). This means that water, fertilizer and pesticide applications should be tailored to the specific needs of the field in which they are being applied. The first step in precision agriculture is the successful measurement and accurate mapping of in-field variation. In the case of plant disease, traditional disease diagnosis and monitoring methods are time consuming and laborious. Effective scouting requires diligence and perceptiveness on the part of the investigative individual, who must also have some degree of proficiency in symptom identification if the information gained is to be of any use. Yet, even proficient disease scouting generally shows low efficiency (Zhang et al. 2019) and results in only a most general idea of the spatial distribution of disease within a field. The general mapping of disease incidence might be possible using a combination of soil sampling and molecular techniques, which would remove some of the subjectivity of field scouting. However, in order to construct a reasonably representative disease map, the field of interest would need to be rigorously sampled and analyzed. This requires valuable time, especially if protective measures are immediately needed to prevent severe economic losses.

In the case of onion pink root, the mapping of disease pressure within a field might allow for a more structured and direct approach to soil fumigation. At the present time, the mapping of pink root severity could be achieved by manual sampling and would thus require the uprooting and subsequent severity rating of a representative number of plants. Alternatively, the mapping of *S. terrestris* inoculum levels in soil has been accomplished at the University of Idaho. Soil samples from a field in Parma, Idaho were collected and analyzed using species specific qPCR to determine pathogen concentration at different field locations. Preliminary qPCR results suggest that *S. terrestris* inoculum can vary from 0.15 to 4.4 picograms of deoxyribonucleic acid (DNA) per gram of soil (Woodhall unpublished). Although generating pathogen density maps using this method could help direct soil fumigation, density alone may not always directly relate to disease severity. It has been demonstrated that *S. terrestris* isolates vary considerably in aggressiveness (Ferreira et al. 1991) and significant variation can even be seen in isolates obtained from the same field (Kulik and Tims 1960). Therefore, mapping the disease severity of actively growing plants might provide a more accurate idea of where the most virulent propagules reside. This would in fact necessitate the uprooting and rating of numerous onion bulbs if it is to be representative and of any use. On these grounds manual mapping is not a practical undertaking.

1.10. A modern method of disease detection and mapping

Recently, optical sensing has been intensely researched as an innovative alternative to laborious and invasive methods of plant stress and disease detection (Thomas et al. 2018). These optical methods, which measure light, are commonly referred to as remote sensing. Nondestructive remote sensing measurements can occur from multiple platforms, including hand-held sensors, tractor mounted sensors, low-altitude unmanned aerial vehicles (UAVs), and satellites (Thomas et al. 2018). Optical sensors utilized in the remote sensing of plant disease can be either imaging or non-imaging. Imaging sensors result in the collection of vast quantities of data which can be complicated to process but are beneficial since they allow for the creations of detailed disease maps. Hyperspectral remote sensing is the collection of reflectance data over a wide spectral range (typically 350-2500 nanometers (nm) for plant applications) in small spectral increments (typically 1-2 nm; Mulla 2012). It is therefore useful in agriculture applications because it provides the ability to investigate in detail the spectral response of soils and plants over a wide spectral range.

Most remote sensing techniques identify and characterize plant stress through plant response or symptoms (Jones and Vaughan 2010). For this reason, these methods have shown promise for the remote detection and mapping of plant disease. Generally, there are four main symptom categories that are associated with successful remote sensing studies (Zhang et al. 2019). They are, namely, (1) the reduction of biomass and the decrease in leaf area index (LAI), (2) lesions and pustules due to infection, (3) the destruction of plant pigment systems, and (4) wilting. It is important to note that these four categories are not mutually exclusive. Disease can impact several of these categories at the same time, though not necessarily to the same degree. Furthermore, different stages of disease may result in a unique combination of symptoms distinct from those of other disease stages (Zhang et al. 2019).

A main challenge to the practical use of remote sensing as a tool for disease detection is that many different biotic and abiotic stresses can give rise to similar plant responses (Zhang et al. 2012; Yuan et al. 2014). There are several different abiotic stresses which may imitate disease symptoms such as nutrient deficiency, drought, and pesticide damage. If a similar plant response occurs when a crop lacks water and when it is diseased, it is important to make a clear distinction since action taken to manage the stress is dependent on the cause of the stress. Remedial procedures for diseased crops are very different from those of nutrient stressed crops (Zhang et al. 2012). Therefore, there is a need for the research and spectral characterization of specific plant stresses so that unique spectral features, if they occur, may be identified.

1.11. Chapter 1 figures

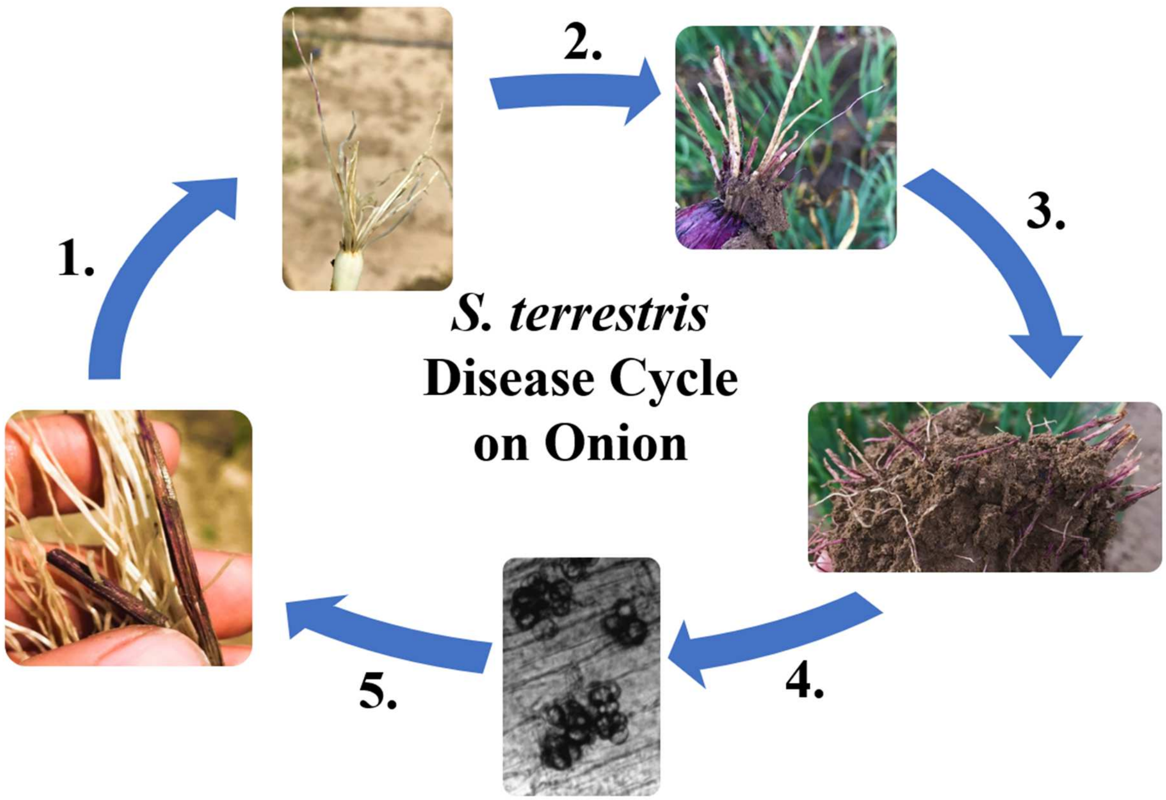

Figure 1.1. Disease cycle of *Setophoma terrestris.* (1) Germinating microsclerotia which have persisted in plant debris or the soil initially infect and colonize onion roots. (2) Fungal colonization spreads throughout the root and adjacent roots are additionally infested. (3) Infested roots slough-off and remain in the soil. (4) Pathogen microsclerotia (picture adapted from Biles et al. 1992) overwinter in plant debris or the soil. (5) In the years a field is rotated away from onion, pathogen propagules can persist in plant debris from most plant species.

Chapter 2: Imposing Crop Stresses and Measuring Their Effect on Onion

2.1. Abstract

Disease pressure from soilborne pathogens like *S. terrestris* continues to be a challenge to local onion growers, since pink root can limit marketable bulb production and thus restrict the overall profitability of a crop. Visually, pink root symptoms are similar to those expressed in onions lacking water and macronutrients. This similarity may confound the practical use of remote sensing in onions, and thus it is important to study the impact of these stresses alongside that of disease. An important component of remote sensing studies is the collection of ground reference data from which meaningful spectral relationships can be derived and validated. In 2018 and 2019, a field experiment was established in Parma, ID with the goal of imposing three primary stresses on an onion crop in order to tangibly measure their impact on plant growth and development and, subsequently (Chapter 3), their impact on plant reflectance. These three stresses were high disease (pink root), nitrogen deficiency, and drought. All three stresses impacted plant biomass development to some extent. The non-fertilized treatment significantly reduced plant biomass from the time of bulb initiation to plant maturity whereas the high disease and reduced water treatment were relatively resistant to biomass reduction until later growth stages. The high disease, non-fertilized, and reduced water treatments, respectively, reduced total yield (relative to the low-disease treatment) 24, 15, and 32% for SV4643NT in 2018, 28, 25, and 22% for SV4643NT in 2019, and 0, 30, and 22% for Vaquero in 2019. In addition, the proportion of bulbs greater than 8.4 cm were significantly decreased in all treatments but the SV4643NT non-fertilized treatment in 2018 and the Vaquero high-disease treatment in 2019. Soil water tension results suggest that a slight drought stress was unintentionally induced in 2018 which may have contributed to the reduced total yield observed in 2018 compared to 2019. Overall, measurable yet sustainable levels of stress were induced in the experimental onion plots, allowing sufficient opportunity for hyperspectral data collection.

2.2. Introduction

Onion production costs in the Treasure Valley are estimated to be around $12,400 per hectare (Murray et al. 2018). If area growers endeavor to profitably produce onions, the total yield of a crop and its quality are important economic considerations. Attaining sufficient bulb size is a particularly important consideration since markets for undersized bulbs are limited (Sullivan et al. 2001). Furthermore, there are incentives to produce bulbs of greater size, as those with diameters greater than 8 cm typically provide the highest economic return (Geary et al. 2008). In respect to bulb size and quality, diseases, pests, and weed competition during the growing season are all challenges to onion production. Feeding damage from onion thrips (*Thrips tabaci*), competition from yellow nutsedge (*Cyperus esculentus*), and soilborne pathogens are of primary importance in the Treasure Valley (Murray et al. 2018). The fungal root rot of onion caused by *Setophoma terrestris* is particularly problematic in places with warm, dry, and temperate climates (Zapacosta et al. 2003), like the PNW. If grown in soil infested with *S. terrestris*, cultivars susceptible to pink root often suffer extensive root loss which reduces bulb size as well as the total quantity of marketable and profitable bulbs. Currently, the most widely practiced methods for disease suppression are crop rotation, cultivar selection and fumigation. Crop rotation is of limited value due to the wide host range of the pathogen (Esfahani and Pour 2008), and fumigation is expensive and subjected to strict regulation due to environmental and human safety concerns (Reitz et al. 2013). There has been recent interest in the use of advanced technologies to support pest management decision-making in the Treasure Valley as well as specific interest in its application for management of onion pink root (Murray et al. 2018). In these respects, optical remote sensing may hold value. The detection and monitoring of disease and pests using remote sensing has shown great potential in some practical applications such as facilitating precision pesticide application in-field for disease and pest control and the support of high-throughput phenotyping of disease tolerant plants (Zhang et al. 2019). In the case of onion pink root, using remote sensing to map disease pressure within a field might allow for a more structured and directed approach to soil fumigation as well as improvement in tolerant cultivar selection. Therefore, there is a need for research that assesses the usefulness of this tool as it relates to onion pink root.

An essential component to a wide range of remote sensing studies is the collection of ground data (Jones and Vaughn 2010). This information, also referred to as ground truth or ground reference data, serves as the point of reference used to derive and validate meaningful relationships between remote sensing data and the variable of interest. Studies on the spectral response of plant disease necessitate the collection of such non-spectral data, usually in the form of visual disease severity ratings (Zhang et al. 2012). In more applied remote sensing studies, in which alternative plant stresses are also considered, additional types of ground reference data become necessary.

Like disease, nutrient and drought stress may also result in the underdevelopment of onion bulbs. If present, these stresses typically induce stunting, yellowing, and leaf tip burn in affected plants, symptoms which are visually similar to those of pink root (Lee et al. 2007). In order for remote sensing to be developed into an effective method of pink root diagnosis and mapping, it must be able to distinguish between these visually similar stresses so that those which may be remedied with fertilizer or irrigation are not mistakenly treated with drip-applied fungicide.

In order to explore the effects of pink root, water, and nitrogen stress on onion, a field experiment was established in 2018 and 2019. The objectives were two-fold. First, to document the relative impact of each stress on plant growth and development, and second to compare two onion cultivars differing in pink root tolerance for response to these stresses. The overall goal was to collect ground reference data on phenotypic response to these three stresses as a first step in developing meaningful relationships with remote sensing data as outlined in Chapter 3.

2.4. Methods and materials

2.4.1. 2018 field trial

Experiment plots: In the fall of 2017, a field location was selected at the University of Idaho Parma Research and Extension Center. The field had been cropped two seasons prior with onions, the season prior with beans, and was known to contain high levels of *S. terrestris*. Soil at this location was a combination of predominantly Greenleaf and Owyhee silt loams at an approximate ratio of 65% and 25%, respectively, with 0 to 1% slopes (USDA NRCS 2019). A composite sample of soil from the upper 30 cm of the profile was submitted to a commercial lab for determination of nutrient content. Based on these sampling results, soil micronutrient and macronutrient levels (with the exception of nitrogen) were amended prior to planting in accordance with University of Idaho guidelines (Sullivan et al. 2001). The red onion cultivar SV4643NT (Seminis Vegetable Seeds, St. Louis, MO, USA) described as large/jumbo in size with a globe shape and red flesh, was utilized in this study. This cultivar is early maturing and exhibits intermediate resistance to *S. terrestris* (Seminis Vegetable Seeds 2019). It was for this reason that the cultivar was selected, to avoid either an overwhelming or underwhelming disease impact (as may have occurred if a highly tolerant or a completely susceptible cultivar was used) to facilitate an array of relative disease severities. Prior to planting, 8 mil Toro Aqua-Traxx drip tape (The Toro Company, Bloomington, MN, USA) was installed in the middle of established beds to an approximate depth of 15.2 cm. On March 29, 2018 pelleted onion seed was directly sown into 2 double rows spaced 55.9 cm apart with an 8.9 cm in-row seed spacing using a Mel Beck 6-row planter (Mel Beck Precision Planters, Nyssa, OR, USA). After planting, four 1.5 m border strips were cleared perpendicular to the onion rows. Following border formation, drip tape lying within the 1.5 m border was unearthed, cut, and removed. This led to the formation of 20 total plots that were 15.24 m long by 3 beds (3.35 m wide). Subsequently, a drip configuration was setup that allowed for varied irrigation and fertigation regimes to be applied to each individual plot. The plants attained 50% emergence on April 18, 2018 and were first irrigated on April 19, 2018 for a duration of 36 hours.

Treatments: The experiment was constructed so that each treatment represented a single, primary stress to the onion crop. The 3 primary stresses investigated in this study were high pink root disease pressure (non-fumigated), nitrogen deficiency (non-fertilized), and water deficiency. A fumigated plot in which onions were grown under standard conditions served as the control. The experiment was arranged as randomized complete block design (see exception for non-fumigated plots outlined below). The treatment combinations used to isolate and draw out the 3 primary stresses are outlined in Table 2.1. It is important to note that the non-fumigated (untreated) check and the fumigated check are hereafter referred to as the high disease and low disease treatments, respectively. It is acknowledged that these treatments could be more appropriately named, since the terms high and low disease might dispose the reader to presume the disease-level of these treatments. Nevertheless, these terms will be utilized for the sake of simplicity. For more information regarding the relative level of pink root symptoms actually observed in these treatments, please refer to the "results" section of the present chapter.

Fumigation: Experimental plots were shank injected with chloropicrin (Strike CP, Trident Ag Products, Woodland, WA, USA) by a commercial applicator during the bedding operation (November 8, 2017). Chloropicrin was applied at a rate of 37.4 L ha^{-1} (61.6 kg a.i. ha^{-1}), with exception of a strip 3 beds-wide (3.35 m) that was not fumigated, and which spanned the entire length of the experimental plot to accommodate commercial application equipment. Therefore, the high disease treatment plots were allocated to the non-fumigated strip in a non-randomized fashion.

Fertilizer applications: In-season fertilizer applications at a rate of 33.6 kg N ha^{-1} occurred bi-weekly beginning at 63 days after planting (DAP) and ending on 105 DAP. This resulted in a total in-season application of 134.4 kg N ha^{-1}. Power-Line 26-0-0-6 liquid fertilizer (Land View Inc., Rupert, ID, USA) was applied via the drip tape on an individual plot basis. To accomplish this, 406 ml of fertilizer was added to 8.9 L of water in an 11.35-L stainless steel beverage tank and mixed. Beverage tanks were fitted with 2 exterior ports on the top of the tank; an "in" port for CO_2 and an "out" port for the liquid contained within the tank. On application days, premixed tanks were transported to the experimental plots and connected to the external injection ports of individual plots via a pressure grade hose. Tanks were then pressurized to approximately 200 Kpa using a 2.3 kg CO_2 tank fitted with an external pressure regulator. When pressurized, liquid fertilizer was capable of overcoming the internal water pressure of the operating drip system (approximately 103 Kpa) and was thus delivered to individual plots through the injection port.

Irrigation scheduling: From the day of planting to 72 DAP, all treatments in the experimental onion plots were irrigated every 5-7 days as needed. In total, all plots received 118 hours of irrigation during this period at a pump flow rate of 40 L min^{-1}. At 72 DAP, Watermark granular matrix sensors (The Irrometer Company Inc., Riverside, CA, USA) were installed at 10 different location within the field. Specific locations were selected to allow for 5 separate comparisons of the soil moisture between reduced water plots and adjacent plots receiving regular water treatments. At each location, sensors were installed in line with an established onion row to 2 different depths, 15.2 cm and 30.5 cm. In total, 20 Watermark sensors were installed in the experimental plots. To automatically log soil moisture data, M. K. Hansen AM500 soil moisture data loggers (M. K. Hansen Company, Wenatchee, WA, USA) were placed between each of the 5 treatment comparisons and were connected to the Watermark sensors using 4-stranded telephone cable. The soil temperature probe was installed directly below the Hansen Data Logger to a depth of 20.3 cm. The Hansen data loggers recorded the soil water tension and soil temperature once every 8 hours. From 72 DAP on, the soil moisture was regularly monitored, and the onions were irrigated when the soil water tension approached a value of 20 centibars (cb) in the regular water treatments. Thus, irrigation was scheduled to maintain an available soil water content above 65-70%. Soil moisture sensors and data loggers were removed from the experimental plots on 135 DAP.

Irrigation reduction: Water duration reductions were accomplished by installing shut-off valves to each of the reduced water treatment drip systems. On days of irrigation, valves were switched to the “closed” position prior to starting the drip system. Then, the drip system was started and the approximate time at which water completely filled the drip system was noted. All irrigations were scheduled for a specific period of time (typically, between 8 and 12 hours) based on the environmental conditions. To reduce the irrigation duration by 33%, the valves were opened after 33% of the scheduled duration had elapsed. Thus, the water-reduced treatment received a 33% duration reduction at each irrigation from 84 DAP to 105 DAP, and a 50% duration reduction at each irrigation from 106 DAP to the final irrigation. On two separate irrigation days (108 and 123 DAP), the treatment 4 valves were unable to be closed prior to watering due to the unexpired reentry interval of an insecticide application. To compensate for the additional 50% water applied to the treatment 4 plots on those days, the shut off valves were closed for the entire duration of the subsequent irrigation. The last irrigation occurred on 156 DAP.

Onion sampling: Onion samples were collected weekly from 93 to 134 DAP to facilitate the collection of ground reference data. On the morning of each sampling date, 25 random plants per plot were removed from the double rows on either side of the 2 data rows (Figure 2.1). Plants were gently unearthed with digging forks to preserve the root mass and remove excess dirt and were then placed into labeled brown paper bags. Bags were transported into an air-conditioned storage to prevent excessive leaf water loss. Collection of ground reference data was immediately initiated once samples were indoors. The various ground data collection procedures utilized in this study are outlined below.

Disease ratings: Pink root severity and incidence was evaluated at each sampling date from 93 to 134 DAP. On each date, 25 random plants per plot were visually classified by the proportion of pink root infected roots per plant on a scale of 0 to 3 where: 0= no pink roots, 1= <10% roots pink, 2= 10-50% roots pink, 3= >50% of roots pink.

Five plants with the highest disease ratings were retained from all high disease plots for hyperspectral analysis at the leaf level and additional plant biophysical data. Likewise, 5 plants with the lowest disease ratings were retained from all additional treatments. For an example of the condition of the retained roots, see Figure 2.2. Prior to statistical analysis, per plot disease rating data was converted to a single value using the disease rating value equation below:

$$\text{Disease Rating Value} = (N_0 \times 0) + (N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3)$$

Where, N_0 = the number of plants rated 0, N_1 = the number of plants rated 1, N_2 = the number of plants rated 2, and N_3 = the number of plants rated 3.

Plant water content: To quantify the effects of water stress, the 5 plants from the low disease and reduced water treatments were further evaluated for leaf water content following hyperspectral analysis. The onion foliage was removed approximately 5 cm up the neck (Figure 2.3), just below the point at which the outermost (oldest) leaves connect. Thereby, all leaves of a given plant were collectively maintained. Subsequently, the foliage of each individual plant was weighed using an Ohaus e400 digital scale (Ohaus Corporation, Parsippany, NJ, USA), recorded, and placed in its own labeled brown paper bag. Bags with samples were transferred to a Napco 630 drying oven (Napco Inc., South Haven, MI, USA). Leaves were dried at 70°C for 72 hours and then reweighed. Plant water content (PWC) for each individual plant was determined using the following equation:

$$\text{PWC} = \frac{\text{Fresh Weight - Dry Weight}}{\text{Fresh Weight}} \times 100\%$$

Leaf nitrogen content: On 92 and 105 DAP, 5 average sized plants per plot were retained from the low disease, non-fertilized, and the reduced water treatments. Their foliage was removed in the same manner as outlined in PWC analysis (Figure 2.3) but was not weighed. The plant samples were placed into a brown paper bag and dried at 70°C for 72 hours. Following desiccation, foliage was ground in a Thomas-Wiley ED-5 Laboratory Mill (Arthur H. Thomas Company Philadelphia, PA, USA). The dry, ground foliage was then transferred to a Thomas-Wiley Mini Laboratory Mill (Arthur H. Thomas Company Philadelphia, PA, USA), reduced even further in size by additional grinding, and passed through a number 40 sieve. Three grams of the finely ground leaf material was placed into a labeled plastic bag, resulting in a single 3 g sample per plot. All ground samples were sent to the University of Idaho Analytical Sciences Laboratory and analyzed for percent total carbon and nitrogen by combustion analysis using a Leco CN 628 (Leco Corporation, St. Joseph, MI, USA).

Yield and bulb size: Onion bulb maturity is attained when the neck of the plant softens and the leaves topple over. In 2018, all experimental plots had at least 50% of the tops fallen over by 126 DAP. The onions in all plots were lifted at 154 DAP. At 173 DAP, the two double rows (data rows) in the middle of each plot (Figure 2.1) were hand harvested and placed into a bulk harvest bag. The onions were transported to the edge of the field and mechanically sized with a Kerian speed sizer (Kerian Machines Inc., Grafton, ND, USA) into the categories of: medium and under (0 to 8.3 cm), large/jumbo (8.4 to 9.4 cm), colossal (9.5 to 11.3 cm), and super colossal (11.4 cm and up).

2.4.2. 2019 field trial

Experiment plots: In the fall of 2018, a field location was selected at the University of Idaho Parma Research and Extension Center. This field, like the one chosen in 2017, had been cropped two seasons prior with onions. The season prior to 2019, the field was cropped with wheat and was known to contain high levels of *S. terrestris*. Soil composition and slope at this location was very similar to that of the field utilized in 2018. Fertilizer applications were made according to University of Idaho guidelines.

In 2019, the experimental plot size was doubled. The yellow onion cultivar Vaquero was utilized in addition to SV4643NT. Vaquero is a long day, yellow medium storage type, globe shaped cultivar (Nunhems USA Inc., Parma, ID, USA). It was chosen as a comparison to SV4643NT because it is readily available, widely grown in the Treasure Valley, and is reported to have medium to high resistance to pink root. Experimental plots of both cultivars were established on March 30, 2019. Pelleted seeds were precision planted using the same equipment and the same planting parameters as in 2018. The same drip tape configuration was utilized as described above. This led to the establishment of 40 total plots that were 15.24 m long by 3 beds (3.35 m wide). The plants attained 50% emergence on April 25, 2018 and were first irrigated on April 26, 2018 for a duration of 36 hours.

Treatments: Treatment parameters were conserved from the 2018 study and applied to both cultivars in separate trials. A randomized complete block experimental design (with 5 replications) was again employed with the exception of the two non-fumigated strips, as outlined for the 2018 study. The treatment combinations are outlined in Table 2.2.

Fumigation: Experimental plots were shank injected with chloropicrin (Strike CP, Trident Ag Products, Woodland, WA, USA) by a commercial applicator during the bedding operation (October 17, 2018). Chloropicrin was applied at a rate of 37.4 L ha^{-1} (61.6 kg a.i. ha^{-1}), the exception being two 3.35 m wide (3 bed) strips that were not fumigated, and which spanned the entire length of the experimental plot to accommodate commercial application equipment. Therefore, all non-fumigated treatments were assigned to these plots in a non-randomized fashion.

Fertilizer applications: In-season fertilizer applications at a rate of 33.6 kg N ha^{-1} occurred every other week beginning at 61 DAP and ending on 103 DAP. This resulted in a total in-season application of 134.5 kg N ha^{-1}. Power-line liquid fertilizer 26-0-0-6 (Land View Inc., Rupert, ID, USA) was applied via the drip tape on an individual plot basis using the same application procedure as in 2018, non-fertilized treatment plots excluded.

Irrigation regime and soil moisture sensors: From the day of planting to 68 DAP, all treatments were irrigated about every 5-7 days. In total, all plots received 82 hours of irrigation during this period at a pump flow rate of 40 L min^{-1}. At 68 DAP, Watermark soil moisture sensors were installed and connected to Hansen data loggers using the same procedure as in 2018. No soil moisture sensors were installed in the Vaquero experimental plots. From 68 DAP on, the soil moisture sensors were regularly monitored, and the onions irrigated when the soil moisture tension approached a value of 20.0 cb in the regular water treatments. The reduced water treatment received a 33% duration reduction at each irrigation from 87 DAP to 100 DAP and a 50% duration reduction at each irrigation from 101 DAP to the end of the season. Water restrictions were successfully applied at each irrigation in 2019.

Onion sampling: Onion samples were collected weekly from 83 to 139 DAP in order to facilitate the collection of ground reference data. On the morning each sampling date, 25 random plants were removed per plot using the same sampling scheme employed in 2018. After all samples were collected, bags were transported into an air-conditioned storage to prevent excessive leaf water loss. Collection of ground reference data was immediately initiated once samples were indoors. The various ground data collection procedures utilized in this study are outlined below.

Disease ratings: Pink root severity and incidence was evaluated weekly from 83 to 139 DAP. On each date, 25 random plants per plot were classified by the proportion of pink root infected roots per plant using the same rating scale employed in 2018. Unlike the 2018 study, however, the 5 plants with the most severe and least severe disease symptoms were not retained and a different hyperspectral leaf-level experiment was conducted (Chapter 3). Additionally, the plant water content analysis was excluded for the 2019 experiment (Chapter 2 results).

Plant sample weights: After the disease rating data was collected, the roots of all 25 of the sampled plants per plot were removed. Plants were then collectively placed onto an Ohaus ES100L scale (Ohaus Corporation Parsippany, NJ, USA) and weighed.

Leaf nitrogen content: On 90 and 111 DAP, 5 average sized plants per plot were retained from the High disease, low disease and non-fertilized treatments of both cultivars. It is important to note that 2019 was the first year that the leaf nitrogen of the high disease plots was analyzed. The foliage was removed in the same manner as outlined in the 2018 plant water content analysis but was not weighed. Five plants from each of the retained plots were placed collectively into a brown paper bag and dried at 70 °C for 72 hours. Samples from each plot were then ground using the same equipment and method utilized in 2018 as described above. All ground samples were sent to the University of Idaho Analytical Sciences Laboratory and analyzed for percent total carbon and nitrogen by combustion analysis using a Leco CN 628 (Leco Corporation, St. Joseph, MI, USA).

Yield and bulb size: In 2019, experimental plots planted with SV4643NT had at least 50% tops fallen by 140 DAP. Plots containing Vaquero reached 50% maturity by 147 DAP. The onions in all plots were lifted at 158 DAP and topped at 171 DAP. At 174 DAP, the middle 4 rows of each plot were hand harvested and mechanically sized utilizing the same equipment and procedure as in 2018.

2.4.3. Statistical analysis

Results from both years were analyzed using the SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA) analysis of variance. In cases when the F-test for treatment was significant, means were separated with Fisher's protected least significant difference (LSD) at the 5% probability level ($P \leq 0.05$).

2.5. Results

2.5.1. 2018 ground reference data

Soil moisture: Soil water tension (SWT), the variable measured by the granular matrix sensors, is inversely related to soil moisture. Thus, the greater the value of soil tension, the dryer the soil and the greater the energy that must be expended by plants to obtain available water. Data acquired using the soil moisture sensors at a 15. 2 cm depth were plotted against time (Figure 2.4). On days when the experimental plots were irrigated, SWT values rapidly plummeted between measurements to a value near the minimum that can be attained (0 cb, the tension of standing water). During the time between irrigations, SWT values steadily increased. This recurring pattern of rapid decrease and steady increase resulted in two distinctive patterns in the resulting soil moisture tension plots.

Regular irrigation treatments ranged from 0 to 40.0 cb during the observational period and attained a maximum SWT of 43 cb at 95 DAP. The mean 15.2 cm SWT for the regular irrigation treatments over the entire measurement period was 12.2 cb. From late June to mid-July (85-111 DAP), the regular irrigation treatments experienced an increase in the soil tension attained on days between irrigation. During this period, the average 15.2 cm SWT increased to 15.2 cb.

Reduced irrigation treatments ranged from 0 to 60.0 cb during the observational period and attained a maximum SWT at 15.2 cm of 67.0 cb at 95 DAP, the same day that the regular irrigation maximum occurred. The overall mean SWT for the reduced irrigation treatments was 20.2 cb during the irrigation reduction period (84 DAP on). The average during the particularly dry period from 85 to 111 DAP was 25.0 cb.

Compared to the 15.2 cm depth, SWT at 30.5 cm exhibited smaller fluctuations (Figure 2.5). In the regular irrigation treatments, SWT ranged from 0 to 20.0 cb and attained a maximum value of 20.2 cb at 95 DAP. Over the entire observational period, the average 30.5 cm SWT was 8.7 cb. SWT at 30.5 cm appeared only slightly impacted by the particularly dry period (85 to 111 DAP) that resulted in larger fluctuations for the regular irrigation treatments at the 15.2 cm depth. The average SWT during this distinct time was 10.5 cb, only a 1.8 cb increase from the season average.

On the other hand, the SWT of the reduced irrigation treatments were markedly increased from 85 to 111 DAP. During this time, plots attained an average SWT of 23.2 cb, a 5.1 cb increase from the total average (18.1 cb) during the irrigation reduction period. The maximum value attained at 30.5 cm was 67.0 cb at 95 DAP.

Soil temperature: Soil temperature at a depth of 20.3 cm ranged from 18 to 29°C in 2018 (Figure 2.6). Diurnal variations in temperature ranged from 1 to 3°C in magnitude and resulted in a plot similar to that observed in SWT. In general, soil temperature increased with time. At 72 DAP, soil temperature was approximately 21°C. At 122 DAP, the soil attained its maximum temperature (27°C) and then began to slowly decrease from 122 DAP to the time at which the sensors were disconnected.

Daytime soil temperatures first entered the ideal range of *S. terrestris* hyphal growth (24-28°C, under laboratory conditions) at 87 DAP. From 102 to 135 DAP, soil temperature at 20.3 cm remained in the 24-28°C range, regardless of the time of day.

Disease rating: Disease rating value is a combined measure of disease incidence and disease severity. Both increased incidence (number of plants showing symptoms) and increased severity (degree to which symptoms have progressed) have the ability to increase the disease rating value, which can range from 0 (all 25 plants rated 0) to 75 (all 25 plants rated 3). In 2018, pink root symptoms were obvious and were observed to breach into the interior of affected roots and spread quickly through individual roots. This resulted in roots that appeared deflated, water-soaked, and fragile which presented a clear distinction between those infected and those uninfected.

Pink root symptoms were present in all treatments beginning at the first sampling date, and then incidence and severity increased over time (Table 2.3). This included onions removed from plots fumigated with chloropicrin. By the first sample date, the high disease plots were rated a value of 45, not far off from a value of 50 (all 25 plants rated 2). In comparison, plants grown in fumigated soil were together rated around 35. On all sampling dates after the first, onions grown in non-fumigated soil (high disease) had significantly higher ratings than onions grown in fumigated soil. Generally, onions from plots treated with chloropicrin were around 10 rating points lower than untreated plots. By the last sampling date, the high disease plot had attained a rating value of 67, whereas, the fumigated treatments attained a value around 57. There was no statistically significant effect of reduced irrigation and lack of fertilizer on the disease rating value. Disease progression in 2018 was best characterized as a fitted polynomial curve with coefficient of determination (R^2) values of 0.9713 and 0.9903 for the non-fumigated and fumigated treatments, respectively (graph not shown).

Plant water content: Overall, it was observed that plant water content varied only slightly over the sampling period, remaining in the range of 89 to 91%, regardless of irrigation treatment (Table 2.4). On the last sampling date, the water content in both treatments began to decrease with plant maturity to values around 87%. No significant difference in plant water content was observed between reduced and full irrigation treatments in 2018.

Leaf fresh weight: Leaves sampled from the regular irrigation treatment weighed more than leaves taken from the reduced treatment at all sampling dates except the first (Table 2.5). In plants obtained from the regular irrigation treatment plots, the foliage of individual plants ranged from 80-167 grams. In plants sampled from reduced irrigation plots, foliage weight ranged from 87-146 grams. The only significant difference between treatments was observed at 120 DAP.

Leaf Nitrogen Content: The total leaf nitrogen content of the plants analyzed in 2018 was observed to be between 2-3% (Table 2.6.). Fertilized plants (low disease and reduced water) sampled at 93 DAP (3 days after the third fertilizer application) had significantly higher leaf nitrogen content compared to plants which were not fertilized. At this date, non-fertilized plants were found to contain 0.56% less total nitrogen. At 105 DAP (1 day after the fourth fertilizer application), there were no statistical differences among the three treatments.

Yield and bulb size: In 2018, the low disease treatment yielded 59 ton ha^{-1} and was statistically higher than all other treatments, except the non-fertilized (Figure 2.7). The non-fertilized treatment attained the second highest yield at 50 ton ha^{-1}, a 15% decrease compared to the low disease treatment. The high disease and reduced water treatments yielded 45 ton and 40 ton ha^{-1}, respectively, and were not significantly different from each other. Overall, the total yield of the high disease treatment was 24% lower than the low disease treatment, whereas reduced water lowered total yield by 32%. In all treatments but the low disease, over 50% of yield consisted of bulbs that were less than 8.3 cm in diameter (Figure 2.7). This treatment also produced the greatest quantity of bulbs greater than 8.4 cm diameter, at 31 ton ha^{-1}.

2.5.2. 2019 ground reference data results

Soil moisture: Values of SWT at a depth of 15.2 cm in 2019 were again dynamic over the observational period treatments receiving both regular and reduced water (Figure 2.8). Regular irrigation treatments ranged in SWT from 0 to 30.0 cb and attained a maximum of 28.6 cb at 128 DAP. This was a narrower range and a lower maximum than in 2018. However, the 2019 mean SWT for the regular irrigation treatments was 15.3 cb, a 3.1 cb increase from 2018. Compared to 2018, the peak SWT attained on days between irrigations in 2019 was more stable over the observational period.

Water duration was cut down in the reduced irrigation treatment beginning at 87 DAP. As a result, the SWT in that treatment ranged from 0 to 60.0 cb during this period and attained a maximum of 56.0 cb at 115 DAP. The overall mean SWT for the reduced irrigation treatment was 21.9 cb during the irrigation reduction period, a 1.7 cb increase over the 2018 average. In 2019, a particularly dry period occurred from 91 to 130 DAP. This period began at a time similar to the 2018 dry period but lasted 13 days longer. The SWT average during the 2019 dry period was 27.1 cb, a 5.2 cb increase from the total average. In comparison, the regular irrigation treatment average increased only 0.4 cb during this time.

In 2019, the SWT at the 30.5 cm depth in the regular irrigation treatments ranged from 10.0 to 25.0 cb and remained stable over time (Figure 2.9). The average SWT in these treatments was 16.9 cb, an 8.2 cb increase over the 2018 average. The maximum SWT attained in the regular irrigation treatments was 25.2 cb on 127 DAP, 5 cb greater than the 2018 maximum.

At the 30.5 cm depth, the reduced irrigation treatment ranged in SWT from 10.0 to 65.0 cb (Figure 2.9). The average SWT over the reduction period was 29.1 cb, an 11.0 cb increase over the 2018 average. From 91 to 115 DAP, SWT steadily increased from 20.0 cb to a maximum of 64.3 cb. During this time, the SWT was only slightly reduced with irrigation. Insensitivity to irrigation can occur if soil sensors lose contact with the soil during dry periods and fail to regain contact when the soil becomes wet again, however this behavior was exhibited by all 5 sensors at the same time and thus it is unlikely that this change was caused by a loss of soil contact. From 115 to 130 DAP, the SWT steadily decreased to a value of 20.0 cb. In total, the water reduced treatment spent 36 days with a SWT at 20.0 cb or greater (often much greater). The average SWT during this dry period was 33.3 cb, a 10.1 cb increase over the average during the dry period of 2018.

Soil temperature: The 2019 observational period for soil temperature at a depth of 20.3 cm began 4 days sooner and ended 10 days later than in 2018. Soil temperature ranged from 13 to 28°C in 2019

(Figure 2.10). Diurnal variations were similar in magnitude to those observed in 2018 and in general, soil temperature increased with time. At 68 DAP, the average soil temperature was approximately 19°C. At 122 DAP, the soil attained its maximum average temperature (24.4°C) and then (similar to 2018) slowly began to decrease from 122 until the time at which the sensors were disconnected. Daytime soil temperatures first entered the ideal range for *S. terrestris* growth at 80 DAP, a week earlier than in 2018. Shortly after this however, at 84 DAP, there was a large drop in soil temperature. In total, soil temperature at 20.3 cm remained within the ideal range (irrespective of diurnal fluctuations) for *S. terrestris* from 106-108 DAP and 126-132 DAP. This was 25 fewer days than in 2018.

Disease ratings: <u>SV4643NT</u> - Similar to 2018, pink root symptoms were present in all treatments beginning at the first sampling date (Table 2.7). A marked increase in rating values was observed between 87 and 91 DAP, during which all treatments increased by 20 rating points. The impact of fumigation in 2019 was significant as onions grown in non-fumigated soil (high disease treatment) had statistically higher pink root ratings compared to the fumigated soil (low disease treatment), except on 125 DAP. Generally, the disease ratings of onions in the high disease treatment were 10 points greater than in the low disease treatment. This, however, was not the case at the last 3 sampling dates, where the gap between high and low disease treatments was closed to about 5 rating points. By 125 DAP, onions in the high disease treatment had nearly attained the largest possible rating value (75) which is the result of all 25 plants having 50% or more of their roots infected (all plants rated 3). On 5 of the 8 sampling dates, no statistical difference was observed between low disease, non-fertilized, and reduced water treatments (all fumigated). On the other three dates, the non-fertilized treatment tended to have lower disease ratings compared to either the low disease or reduced irrigation treatments.

<u>2018 vs. 2019</u> - At 91 DAP, 2019 ratings were about 10 points lower than the ratings observed at 93 DAP in 2018. Thus, *S. terrestris* infection either occurred earlier or more rapidly in 2018 than in 2019. Yet by the end of the 2019 season, onions from fumigated plots had attained values near 70, 10-15 rating points higher than the same treatments at the end of 2018. Overall, pink root was more severe in 2019 for all plots than it was in 2018. It was observed, however, that pink root was established earlier in 2018 than in 2019.

<u>Vaquero</u> - Rating values ranged from 0-50 over the observation period (Table 2.8). Rating values increased appreciably from 87 to 111 DAP, then leveled off. At most sampling dates, plants taken from the high disease treatment had statistically higher pink root ratings compared to those from the low disease treatment. Exceptions to this occurred at 87 and 132 DAP. Overall, there was no statistically significant effect of the reduced water and non-fertilized treatments on disease ratings compared to the low disease treatment.

Cultivar Comparison - Visually, pink root impacted the 2 cultivars in different ways. In Vaquero, symptoms first originated close to the basal plate, did not impact entire individual roots, and appeared to be confined to the exterior of the roots. This resulted in roots that appeared healthy other than a pink exterior sheen. Only late in the season were Vaquero roots observed with symptoms that penetrated the root exterior and which impacted entire roots. On the other hand, in SV4643NT symptoms developed throughout the interior of affected roots and spread quickly through individual roots. This resulted in roots that appeared deflated, water-soaked, and fragile. In general, pink root was more prevalent and severe in SV4643NT than in Vaquero throughout the season. By the last sampling date, rating values around 40 were observed in Vaquero compared to rating values around 70 in SV4643NT. Interestingly, Vaquero disease rating values only slightly increased after 111 DAP whereas the rating values SV4643NT continue to increase.

Plant weights: SV4643NT - Since entire plants (except roots) were weighed at each date, total plant weights are a combination of foliage weight and bulb weight. At early sampling dates, foliage is the main contributor to plant weights. After bulb initiation, bulb weight begins to gradually gain influence on total plant weight. At later sampling dates, bulbs are major contributors to overall plant weights, especially when leaves begin senescing.

Generally, plant weight increased over time, and ranged from 0.4 kg at 87 DAP to 7.8 kg at 139 DAP (Table 2.9). On most dates, the non-fertilized treatment had statistically lower plant weight compared to all other treatments. At 87, 91, 104, and 132 DAP, no statistical difference was observed between the high disease, low disease, and reduced water treatments. Interestingly, plant weights declined in all treatments except the low disease between 132 and 139 DAP, likely due to the weight loss of the onion leaves as they senesced. The low disease treatment was the only one that exhibited an increase in plant weight during this time.

Vaquero - Plant weight increased over time and ranged from 0.35 kg at 87 DAP to 10.3 kg at 139 DAP (Table 2.10). At all sample dates except 87 DAP, the non-fertilized treatment had significantly lower plant weight compared all other treatments. In contrast, there were no differences among the high disease, low disease and reduced water treatments from 87 to 111 DAP. On 125 DAP, however, the reduced water treatment had significantly lower plant weights compared to the low and high disease treatments. The low disease and high disease treatments had similar plant weights on all dates except 132 DAP.

Cultivar comparison - From 87 to 104 DAP plant weights were similar between the two cultivars. At 111 DAP it was observed that Vaquero sample weights had surpassed those of SV4643NT, and they remained that way for the rest of the season.

Leaf nitrogen content: SV4643NT - At both 91 DAP and 111 DAP significant differences in leaf nitrogen content were observed among all treatments (Table 2.11). The most noticeable of these differences was the 0.4 to 0.6% reduction in total nitrogen content observed in the non-fertilized treatment compared to the low disease and high disease treatments. There were no significant differences in leaf nitrogen content between low and high disease treatments on either date.

2018 vs. 2019 - The total nitrogen content of SV4643NT was lower at both sampling dates in 2018 compared to 2019, despite the fact that sampling occurred at similar dates. Unlike 2018, plants from the non-fertilized treatment in 2019 were statistically lower compared to their fertilized counterparts on both sampling dates.

Vaquero - At both sampling dates it was observed that the non-fertilized treatment exhibited a significant reduction in total leaf nitrogen content compared to both the low disease and high disease treatments (Table 2.11). There were no differences in leaf nitrogen content between low and high disease treatments on either date.

Cultivar Comparison - The nitrogen content of the 2 cultivars was found to be similar at both sampling dates, particularly the second at which the average values were the same in plants from the fertilized treatments.

Yield and bulb size: SV4643NT - The low disease treatment produced an average of 71 ton ha^{-1} and was significantly higher compared to the high disease, non-fertilized and reduced water treatments (Figure 2.11). The reduced water treatment resulted in a 21% decrease in total yield compared to the control, while the non-fertilized and high disease treatment resulted in a 24% and 28% yield decrease, respectively. In the low disease treatment 48 ton ha^{-1} of the total yield was greater than 8.4 cm in diameter (68%), while all the other treatments significantly decreased the percentage of large diameter bulbs.

2018 vs. 2019 - Total yield of SV4643NT increased appreciably in 2019 compared to 2018, especially in the low disease, high disease and reduced water treatments, which were all increased by over 10 ton ha^{-1}. The yield of the non-fertilized treatment was reduced more compared to the low disease treatment in 2019 than in 2018 (24% and 15% decrease, respectively). Additionally, the yields of bulbs greater than 8.4 cm in diameter increased for all treatments in 2019. In both years, the high levels of pink root associated with a lack of fumigation resulted in a large adverse effect on yield.

Vaquero - Both the low disease and high disease treatments resulted in total yields around 109 ton ha^{-1} (Figure 2.12). The reduced water treatment produced an average of 84 ton ha^{-1}, significantly less than the low or high disease treatments. The non-fertilized treatment yielded the lowest of all at 76 ton ha^{-1}, a 30 % yield decrease compared to the low and high disease treatments.

The yield of bulbs under 8.3 cm in diameter increased by 10 ton ha^{-1} in the reduced irrigation and non-fertilized treatments compared to the low and high disease treatments (Figure 2.12). This increase in small-diameter bulbs was accompanied by a reduction in the proportion of bulbs greater than 8.4 cm in diameter.

Cultivar comparison - All Vaquero treatments yielded greater than the corresponding SV4643NT treatments. Additionally, the quantity of bulbs greater than 8.4 cm in diameter was higher in Vaquero compared to SV4643NT. The relative yield reduction that resulted from the non-fertilized treatment was slightly greater in Vaquero compared to SV4643NT. In contrast, the effect of pink root disease level on onion yield was very different between the two cultivars. In the case of SV4643NT, yield was significantly reduced in the high disease treatment compared to the low disease treatment (28% reduction), whereas in Vaquero the total yields were not significantly different.

2.6. Discussion

2.6.1. Impact of stress on plant development and yield

S. terrestris has been found to vary both in quantity and pathogenicity among different field locations and even within a single field (Ferreira et al. 1991; Kulik and Tims 1960). Quantitative PCR results from soil samples taken at Parma, ID suggest that *S. terrestris* inoculum varies spatially (Woodhall unpublished). Nevertheless, the natural inoculum relied upon in this study was sufficient to manifest disease throughout the experimental plots in both years of the study. The impact of pink root disease on onion growth and development was very apparent in the red onion cultivar SV4643NT that is described as intermediate in resistance to *S. terrestris*. The ratings of the high disease onions (grown in non-fumigated soil) were on most sampling occasions significantly higher than those of the low disease treatment. This reduction in pink root severity with fumigation is consistent with previous reports (Geary et al. 2008; Hartz et al. 1989). Fumigation increased total bulb yield of SV4643NT by over 25% compared to the non-fumigated treatment in both 2018 and 2019, and also increased the proportion of bulbs greater than 8.4 cm in diameter (Table 2.12). Though total yield was decreased significantly in the SV4643NT high disease treatment, plant biomass (an indicator of stunting) was not significantly influenced by fumigation until 125 DAP in 2019 (Figure 2.13A). These results appear consistent with previous reports of pink root causing a reduction in the number and length of onion leaves (Ferreira et al. 1991) and bulb size (Reitz et al. 2013).

Onions are a shallow-rooted crop. Ninety percent of onion root length resides in the top 18 cm of the soil throughout the growing season (Brewster 1994). In addition, onion roots are sparsely branched and less effective than most other crop plants in extracting nutrients like phosphorus and nitrogen (Sullivan et al. 2001). Non-fertilized onions grown in Parma, ID in 2018 and 2019 were visibly stunted and lighter-green color (mild chlorosis) compared to those that were fertilized. A positive correlation between applied nitrogen fertilizer quantity and onion plant height and leaf number has been observed elsewhere (Ncayiyana et al. 2018). In addition, nitrogen fertilizer applied at 138 kg ha^{-1} (134 kg ha^{-1} was applied at Parma) was adequate to significantly increase plant height, leaves per plant, and leaf length from that of a non-fertilized check (Abdissa et al. 2011). These results agree with those observed at Parma, where samples with reduced total nitrogen content showed significant stunting compared to fertilized (low disease) onions as evidenced by reduced plant weight throughout the season (Figures 2.13B and 2.14B). Plant biomass was significantly impacted by nitrogen deficiency beginning at 91 DAP. This reduction in plant growth would be expected to reduce total yield and bulb size by reducing the leaf area converting sunlight into biomass. The yield results from 2019 in particular agree with another study which found that the total yield of non-fertilized onions was reduced 29% from that of onions fertilized with 150 kg N ha^{-1} and that marketable yield was reduced by 40% (Tsegaye et al. 2016). A similar reduction in proportion of bulbs greater than 8.4 cm was observed at Parma in 2019 (Table 2.12).

Studies on drip-irrigated onion grown in the silt loam of the Treasure Valley suggest that irrigation should occur when SWT at the 20 cm depth reaches 20.0 cb (Shock et al. 2013). In order to maintain available water within the shallow root zone, frequent and sufficient irrigation is essential. Water deficit, even for a short time, can negatively impact onion yield and bulb size. In one study a single stress of 60.0 cb, which occurred late in the growing season, reduced total onion yield by 10% (Pinto et al. 2013). At Parma, total water deficits of 43% and 45% (around the time of bulb initiation) relative to the recommended amount were imposed in the reduced water treatments in 2018 and 2019, respectively. As a result, soil moisture tension increased to levels above the 20.0 cb threshold at both 15 and 30 cm depths, and total yield was lowered by 20-30% (Table 2.12.). These yield results seem to agree with those of other studies, which have found that a 40% irrigation deficit at all growth stages reduces yield 30% (Patel and Rajput 2013; Zheng et al. 2013). In regard to physiological response to water stress, onions have been found to decrease leaf length and leaf area, but only after a prolonged period (30 days) without water (Ghodke et al. 2018). Results from this study point out that for most of the growing season plant biomass in the reduced water treatments was similar to that in the low disease treatment (Figure 2.13C and 2.14C). Overall, reduced water treatments lowered total yields and resulted in a smaller proportion of large diameter bulbs (≥8.4 cm) compared to the control treatment (Table 2.12), despite the fact that they did not appear to be reduced in plant biomass for most of the growing season.

2.6.2. Observed differences in cultivar response to stress

Onion cultivars are known to vary in their susceptibility to *S. terrestris* infection (Esfahani and Pour 2007). In 2019, Vaquero was observed to be more tolerant to pink root than SV4643NT. This was first noted when visually rating plant roots. Infected SV4643NT roots were severely impacted by the disease, which was apparent from roots that were colonized throughout and appeared water soaked, thin, and flattened. Infected Vaquero roots, on the other hand, appeared much less impacted by pink root. Disease symptoms first originated close to the basal plate, did not impact entire individual roots, and appeared to be confined to the exterior of the roots. This resulted in roots that appeared dense and healthy other than a pink exterior sheen. Only late in the season were Vaquero roots observed with symptoms that penetrated the root exterior and which impacted entire roots.

The increased disease tolerance of Vaquero compared to SV4643NT was also observed in the disease rating results, where Vaquero had lower ratings at every sampling date. Whereas the ratings of SV4643NT increased throughout the observational period, the ratings of Vaquero remained stable on the last few sampling dates. This response from Vaquero is similar to that observed elsewhere, in which the pink root severity of Vaquero grown in plots treated with chloropicrin did not increase between two sampling dates late in the growing season (Reitz et al. 2013). This same study also found that chloropicrin at a rate of 37.4 L ha^{-1} (the same rate utilized in 2019) did not significantly increase the yield of Vaquero nor its proportion of marketable bulbs. This result agrees with that observed in 2019 for Vaquero, but not SV4643NT where the reduction in total yield in high compared to low disease treatments was over 30% (Table 2.12). A study on the response of 20 yellow sweet Spanish cultivars and numbered hybrids to pink root in the Treasure Valley found that, in the presence of high pink root pressure, total yield ranged from 47.0 ton ha^{-1} to 94.1 ton ha^{-1} and bulb size ranged from 54% to 92% of yield that was greater than 7.5 cm in diameter (Thornton and Mohan 1996).

Variation in root symptom manifestation among cultivars may have contributed to the stabilization of Vaquero disease ratings mid-season. Since the employed disease rating method accounts only for the approximate percentage of symptomatic roots, an increase in disease severity (penetration of disease symptoms into the root interior) but not an increase in the quantity of symptomatic roots would have resulted in such a stabilization phenomenon. However, it is also possible that Vaquero began to generate new roots during the season and, during the leveling-off of the disease rating value, the rate of root infection and root generation was nearly the same. This mechanism of onion tolerance to pink root has been proposed elsewhere (Thornton and Mohan 1996). Alternatively, hundreds of unique steroidal saponins have been identified in Allium spp. and display varying levels of antifungal activity (Sobolewska et al. 2016). Thus, the observed tolerance of some onion cultivars to pink root (such as in the case of Vaquero in 2019) may relate to the localization of these biologically active saponins in onion roots, which has been observed in the epidermal cell layer in roots of other crop species (Morrissey and Osbourn, 1999). Additionally, increased levels of saponin in tolerant cultivars may explain why disease symptoms were confined to the root exterior in Vaquero but not in SV4643NT. Resistance mechanisms involving lytic enzymes have also been suggested. *Allium fistulosum* roots contain high constitutive levels of glucanases and chitinases and are expressly resistant to *S. terrestris* (Zapacosta et al. 2003). This mechanism may also play a role in tolerance to pink root.

A discrepancy was observed in the yield and bulb size distribution of SV4643NT between 2018 and 2019. All treatments yielded appreciable lower in 2018, suggesting that growing conditions were in some way less favorable that particular season. Climatic conditions were slightly warmer in 2018 which was apparent in the differences between the soil temperature plots (Figure 2.8, Figure 2.10). The beginning of the 2019 season was much wetter and cooler than in 2018 but by the end of the growing season a similar number of growing degree days (approximately 2000) had been accumulated from the time of emergence (outlined in Chapter 3). Based on the temperature results alone, it is reasonable to expect that 2018 would have been more conducive to pink root than 2019. On the contrary, disease ratings were much more severe by the end of the season in 2019 than in 2018, suggesting that 2019 presented superior conditions for pathogen infection and growth. This contradiction might be caused by the depth at which soil temperature was measured (20.3 cm). Since this depth is near the extremity at which onion roots reside, it is likely unfitting to extrapolate these temperature readings to the entirety of the onion root mass, which primarily resides much higher in the soil (Brewster 1994). Soil temperature at a depth of 10 cm may be a more accurate estimator of *S. terrestris* infection, since pink root symptoms on Vaquero were first observed to originate near the basal plate as opposed to root ends.

Therefore, based on the disease rating results, the level of pink root symptoms alone is unable to explain the yield reduction observed in 2018. The timing of pink root infection, on the other hand, may have contributed to this decrease. At the first sampling date in 2018, disease rating values were around 10 points higher than the rating values at nearly the same date in 2019. Wiriyajitsomboon et at. (2015)

found that onion plant height and fresh weight were significantly reduced when plants were infected with *S. terrestris* at an early age (3 weeks) but not when they were infected at later dates (5, 7, and 9 weeks). This was true even though onion roots became more susceptible to pink root as the plant aged. Based on these findings and the findings of the present study, it is possible that the earlier infection in 2018 is in part responsible for the observed decrease in SV4643NT yield. In addition, the singularly detrimental impact of *S. terrestris* infection early in the season may explain the substantial increase in SV4643NT total yield amidst an increased manifestation of pink root in 2019, since extreme disease rating values were not observed until later in the season.

Another factor that may have had an impact is soil moisture. In 2018, SWT increased rapidly between time at which irrigation was scheduled and the time at which the irrigation event occurred (typically a single day difference). As a result, there were several occasions in 2018 when, just prior to irrigation events, the SWT in regular irrigation treatments had attained values near or above 30.0 cb. This unintentional stress was most pronounced at 95 DAP when a SWT of 45.0 cb was recorded. Since a single, late-season water stress of 60.0 cb which lasted 12 days was sufficient to reduce total yield by 10% (Pinto et al. 2013), it is likely that these unintentional water stresses (although less significant) also contributed to the SV4643NT yield discrepancy between 2018 and 2019.

Interestingly, the relative impact of the reduced water treatment on total yield was similar in both cultivars (Table 2.12). Total yield was reduced by around 22% relative to the control in both SV4643NT and Vaquero in 2019 as a result of prolonged water reduction beginning around the time of bulb initiation. For comparison, a single water stress of 70.0 cb stress which was imposed at 118 DAP and lasted for 8 days decreased the total yield of Vaquero by 5 % relative to the control (Pinto et al. 2015). In 2019, impact upon bulb size was less similar between the two cultivars. The proportion of bulbs greater than 8.4 cm was more significantly lowered in SV4643NT than in Vaquero (25% and 19% decrease relative to the control, respectively).

In the non-fertilized treatments, the total yield of Vaquero was more significantly decreased than that of SV4643NT (30% and 25% reduction relative to the control, respectively). Furthermore, the decrease in percentage of bulbs greater than 8.4 cm due to nitrogen stress was less in SV4643NT (12% decrease relative to the control) than in Vaquero (20% decrease relative to the control). These results suggest that SV4643NT was less affected by a lack of fertilizer than Vaquero. It is possible that a lack of fertilizer was more influential upon Vaquero because it has a higher yield potential than SV4643NT. Results from our study suggest that under non-limiting conditions Vaquero can achieve approximately 50% greater yield than SV4643NT. Another Treasure Valley study in which both cultivars were grown found that SV4643NT produced less (45.5 ton ha^{-1}) and Vaquero produced more (131 ton ha^{-1}) than what was observed at Parma in 2019 (Shock et al. 2017). However, SV4643NT produced total yield similar to that observed in Parma in 2019 (75.6 ton ha^{-1}) was attained when the study was repeated and Vaquero yield was unchanged (Shock et al. 2017). Therefore, there is substantial evidence that suggests that Vaquero has a significantly greater yield potential than SV4643NT.

Nitrogen stress impacted biomass earlier than the other stresses (Figure 2.13, Figure 2.14), however it was also the only stress that was imposed all season long. In 2019, the effect of pink root and water stress on the yield and bulb size of the susceptible cultivar (SV4643NT) were not statistically significant from one another. Furthermore, the high disease and reduced water treatments both impacted plant biomass to a similar degree beginning at a similar time (Figure 2.13, Figure 2.14). These results demonstrate the similarity between the relative impact of these two stresses, which may present a challenge to their ability to be distinguished using remote sensing technology.

2.6.3. Summary

In summary, the purpose of these in-field experiments was to generate a field environment suitable to study the impact of pink root, nitrogen and water stress on onion leaf reflectance. The treatments utilized were designed to produce reasonably severe stress on the plants, and thus facilitate season long measurement of leaf reflectance. The ground reference results from both years suggest that measurable levels of disease, nitrogen and water stress were induced in the field plots. Moving forward, it is important to note that non-fertilized onions were significantly stunted throughout the growing season compared to the fertilized, but disease and water stress only stunted plant development in the final few weeks, if at all. In addition, results suggest that a slight drought stress was unintentionally induced in 2018 which may have contributed to the reduced total yield of all SV4643NT compared to 2019. Finally, it is important to note that the comparison between low and high disease treatments is not the comparison of onions with no disease symptoms to onions with severe symptoms. Rather the proportion of plants with moderate to severe symptoms in the high disease treatment was consistently increased compared to the low disease treatment, which had more onions in the low to moderate disease categories.

2.7. Chapter 2 figures

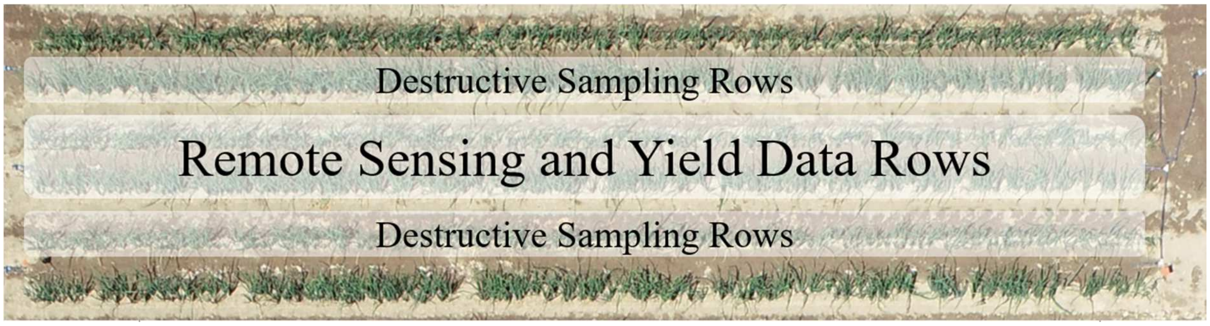

Figure 2.1. Outline of a 15.24 by 3.35 meter experimental plot from above superimposed with descriptions of how each portion was utilized. The middle bed was undisturbed throughout the growing season and was measured with a nondestructive, hyperspectral sensor. The double rows on either side of the middle data bed were destructively sampled for the collection of ground reference data. Unlabeled rows served as borders and were not sampled nor analyzed.

Figure 2.2. Pink root symptoms on SV4643NT onion plants retained after disease ratings for further analysis. (A) Five onion plants with the lowest disease ratings, taken from a fumigated plot. (B) Five onion plants with the highest disease ratings, taken from a non-fumigated plot.

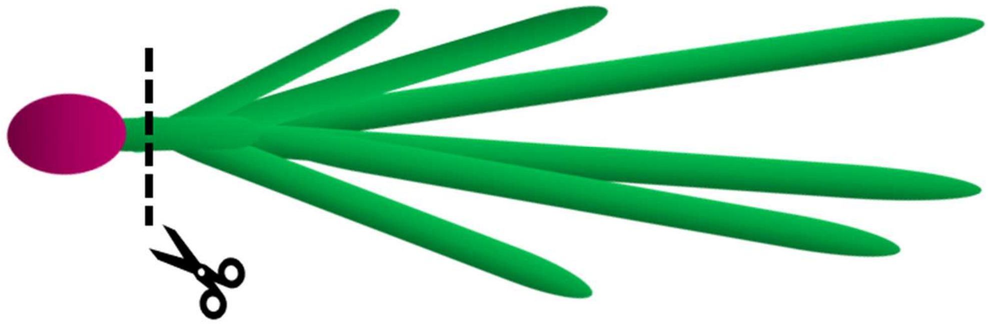

Figure 2.3. Diagram outlining the location at which onion leaves were cut and removed from bulbs prior to plant water content analysis.

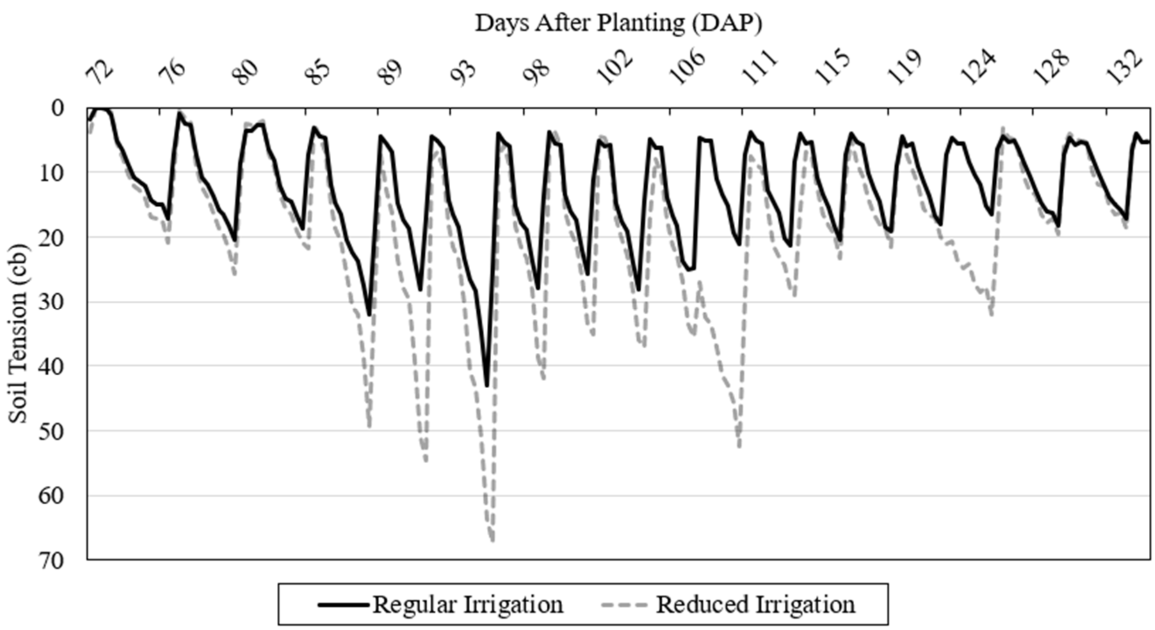

Figure 2.4. Soil water tension of regular and reduced irrigation treatments in at Parma, ID in 2018. Values are means of 5 replications of soil tension at a depth of 15.2 cm.

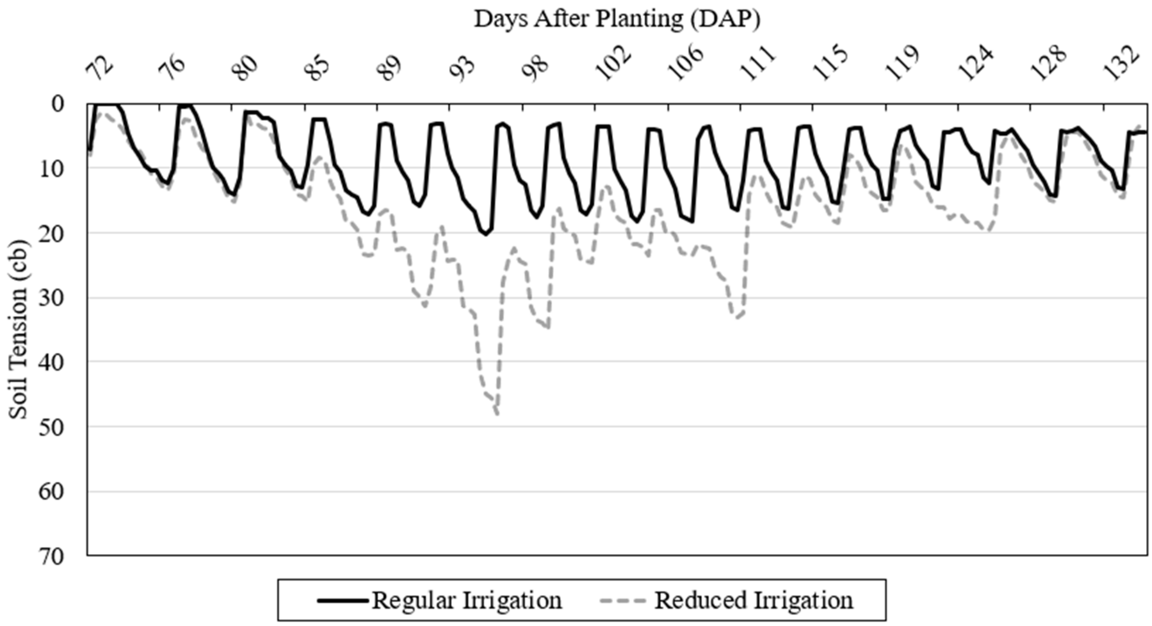

Figure 2.5. Soil water tension of regular and reduced irrigation treatments at Parma, ID in 2018. Values are means of 5 replications of soil moisture tension at a depth of 30.5 cm.

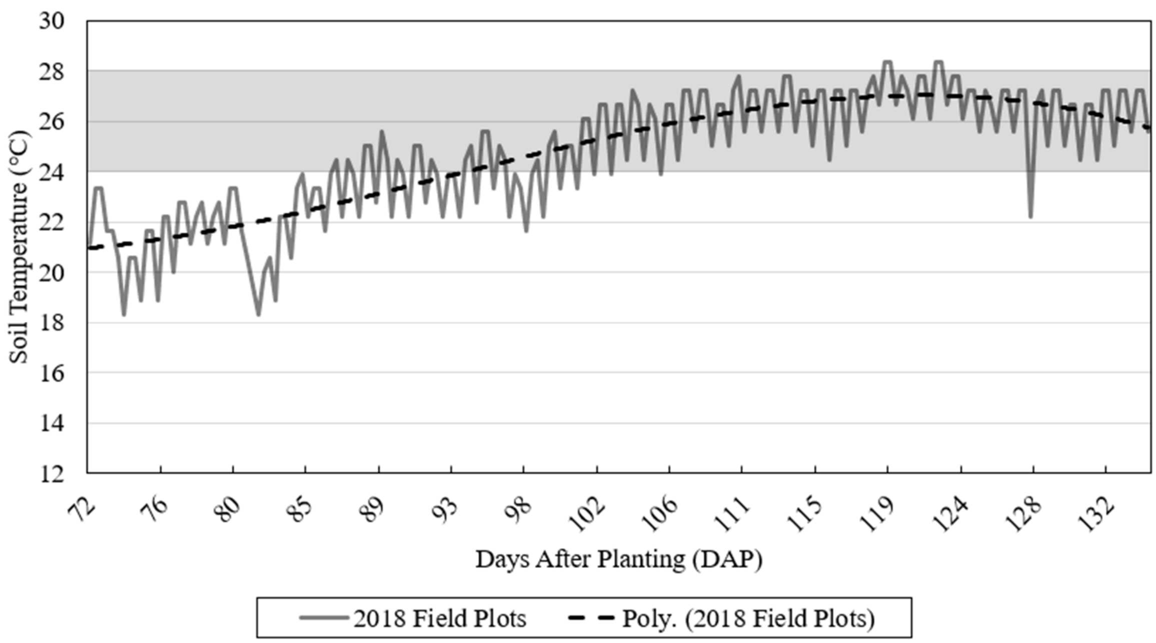

Figure 2.6. Soil temperature at Parma, ID in 2018. The solid gray line is the mean of 5 replications taken at a depth of 20.3 cm. The dotted black line represents the general soil temperature trend. The shaded area encompasses the ideal temperature range for *S. terrestris* growth.

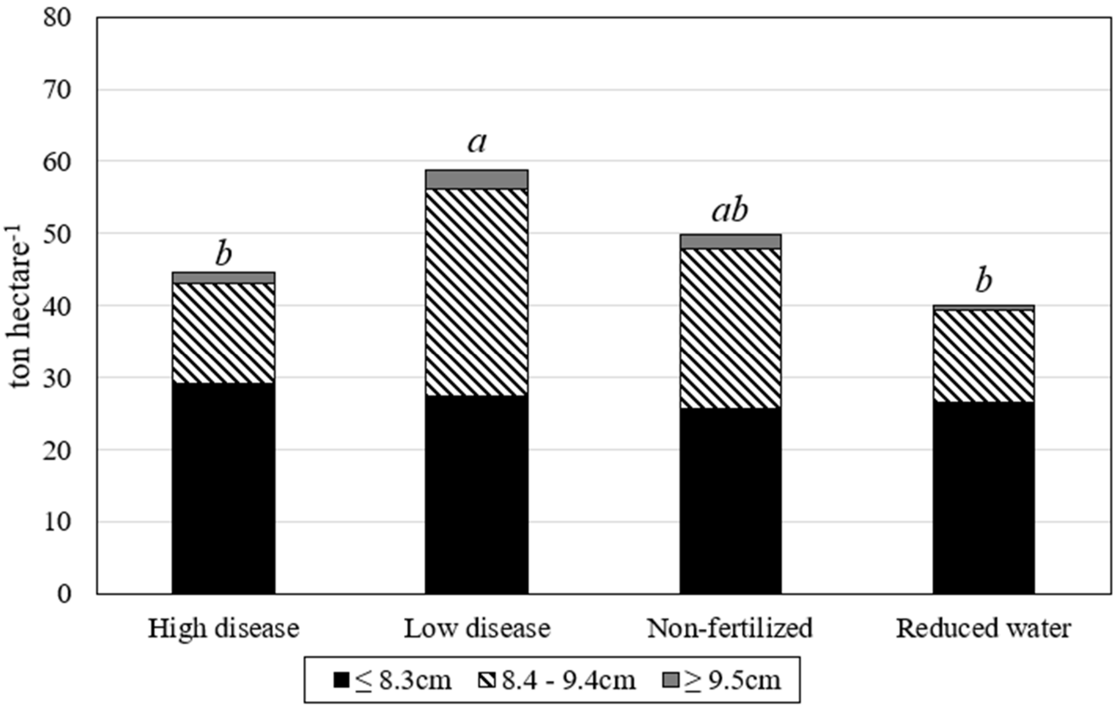

Figure 2.7. Effect of disease, nitrogen, and water stress on total bulb yield and size of SV4643NT onions grown at Parma, ID in 2018. Values are the mean of 5 replicates. Treatments characterized by the same letter are not statistically different (Fisher's protected LSD, $P \leq 0.05$).

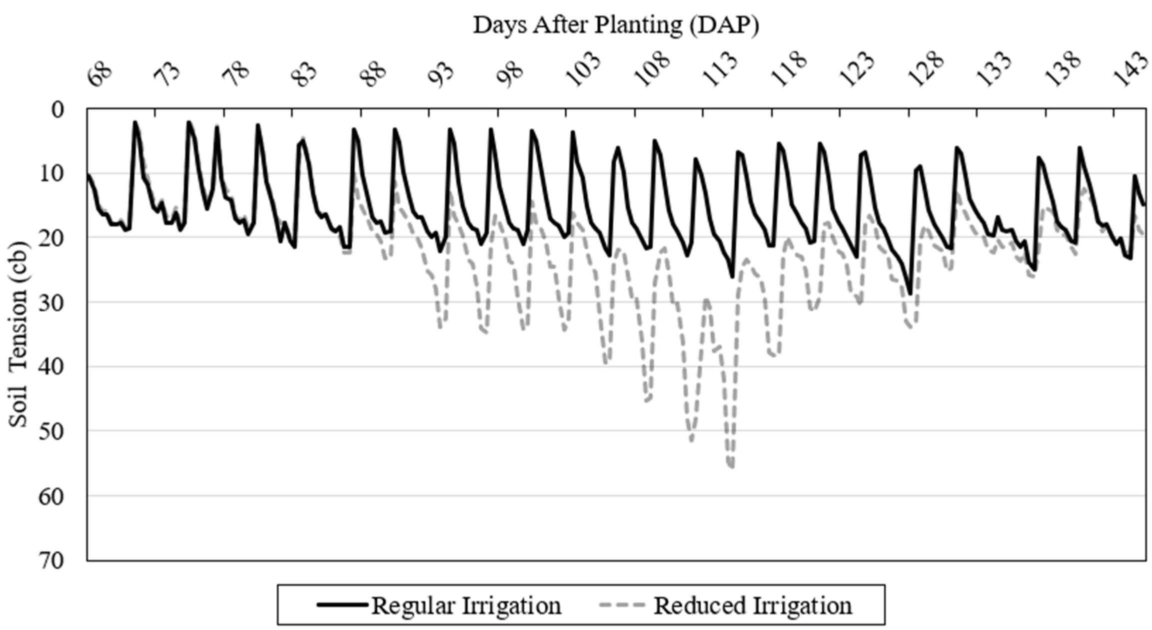

Figure 2.8. Soil moisture tension of regular and reduced irrigation treatments at Parma, ID in 2019. Values are means of 5 replications of soil tension at a depth of 15.2 cm.

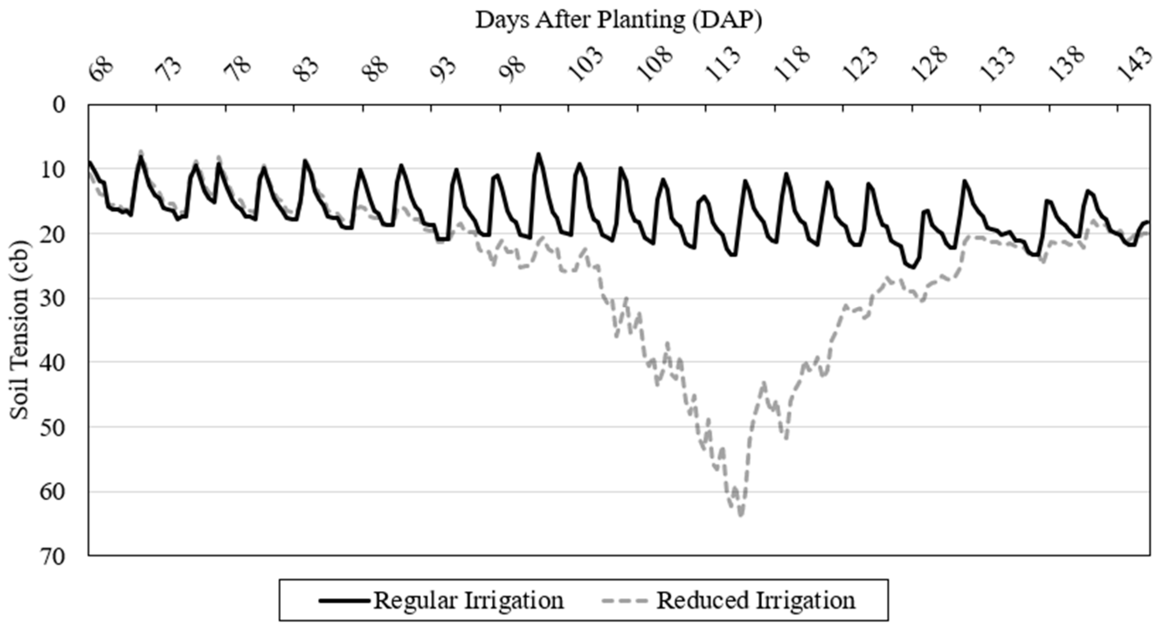

Figure 2.9. Soil moisture tension of regular and reduced irrigation treatments at Parma, ID in 2019. Values are means of 5 replications of soil tension at a depth of 30.5 cm.

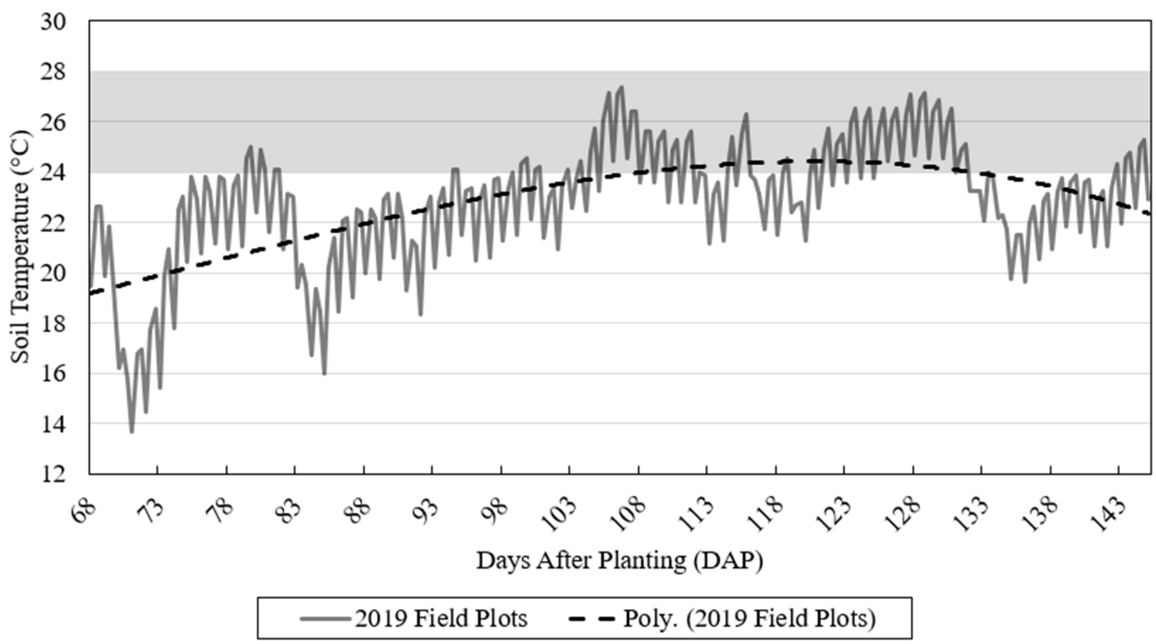

Figure 2.10. Soil temperature at Parma, ID in 2019. The solid gray line is the mean of 5 replications taken at a depth of 20.3 cm. The dotted black line represents the general soil temperature trend in 2019. The shaded area encompasses the ideal temperature range for *S. terrestris* growth.

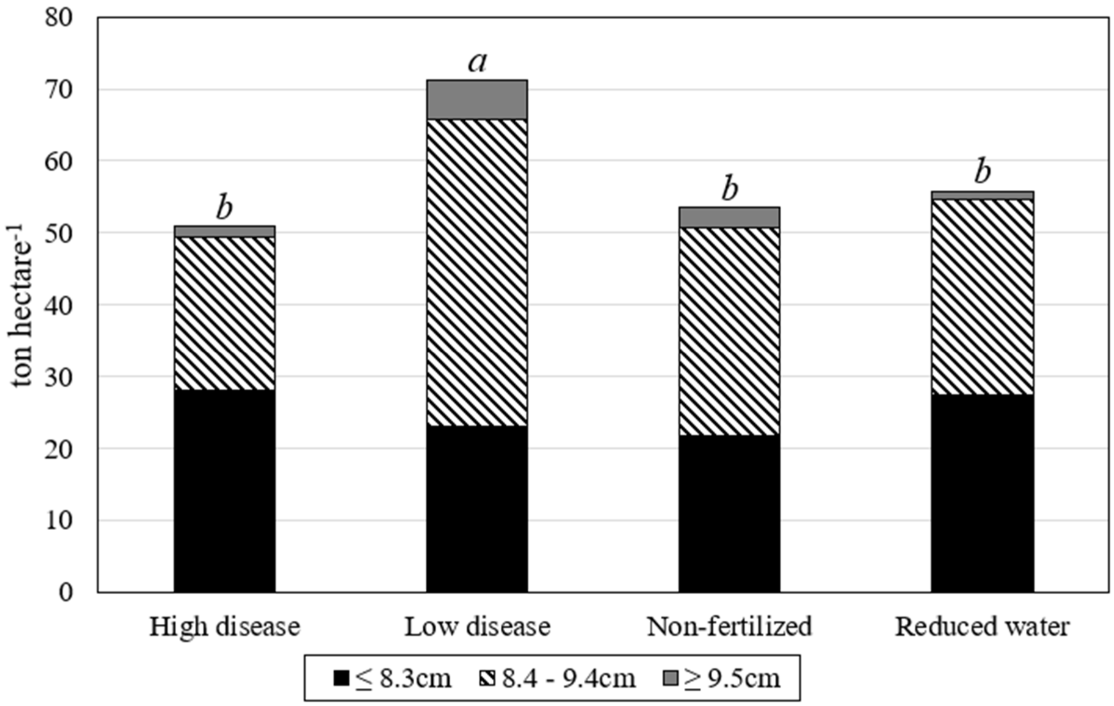

Figure 2.12. Effect of disease, nitrogen and water stress on total bulb yield and size of Vaquero onions grown at Parma, ID in 2019. Values are the mean of 5 replicates. Treatments characterized by the same letter are not statistically different (Fisher's protected LSD, $P \leq 0.05$).

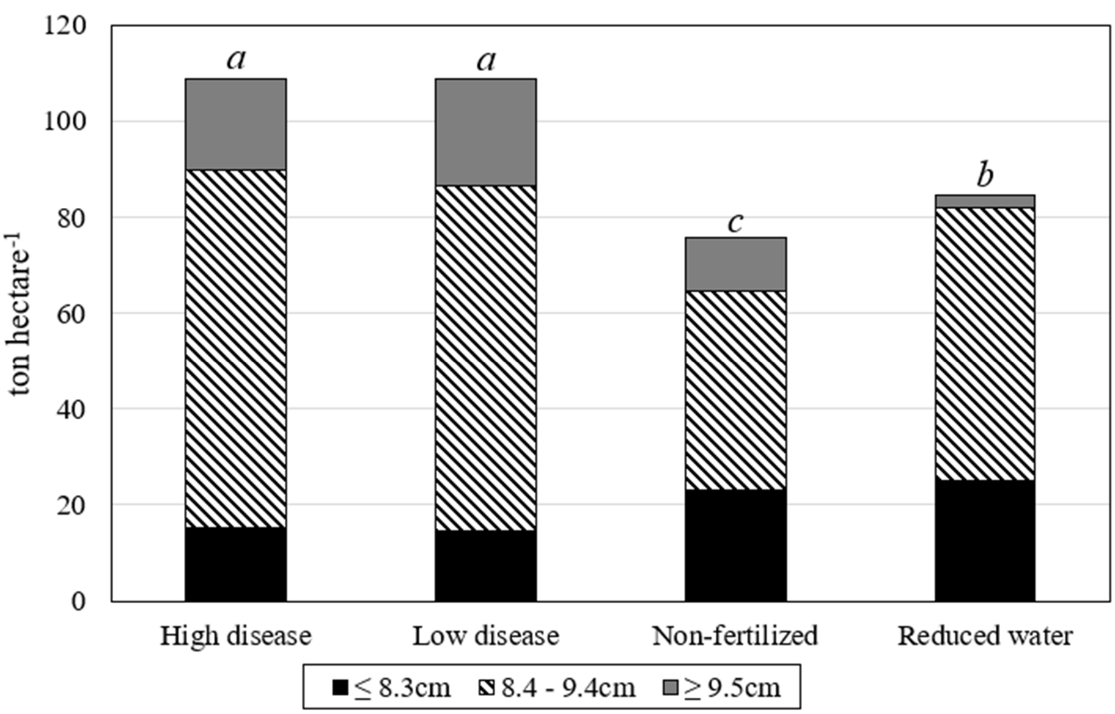

Figure 2.11. Effect of disease, nitrogen, and water stress on total bulb yield and size of SV4643NT onions grown at Parma, ID in 2019. Values are the mean of 5 replicates. Treatments characterized by the same letter are not statistically different (Fisher's protected LSD, $P \leq 0.05$).

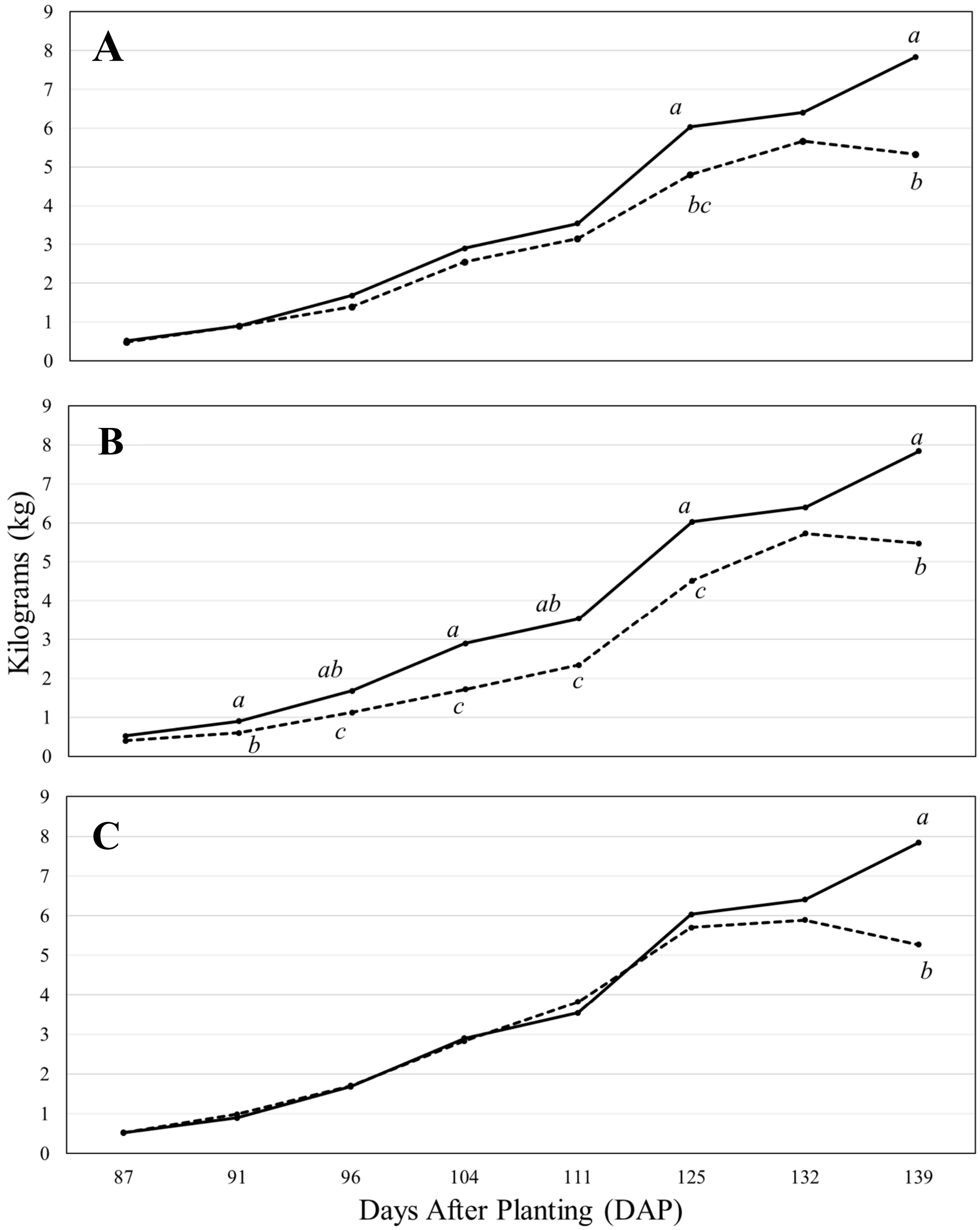

Figure 2.13. Effect of disease, nitrogen and water stress on total plant biomass development over time for SV4643NT onions grown at Parma, ID in 2019. Values are the mean weight (kg) of 5 replicates of 25 plants. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

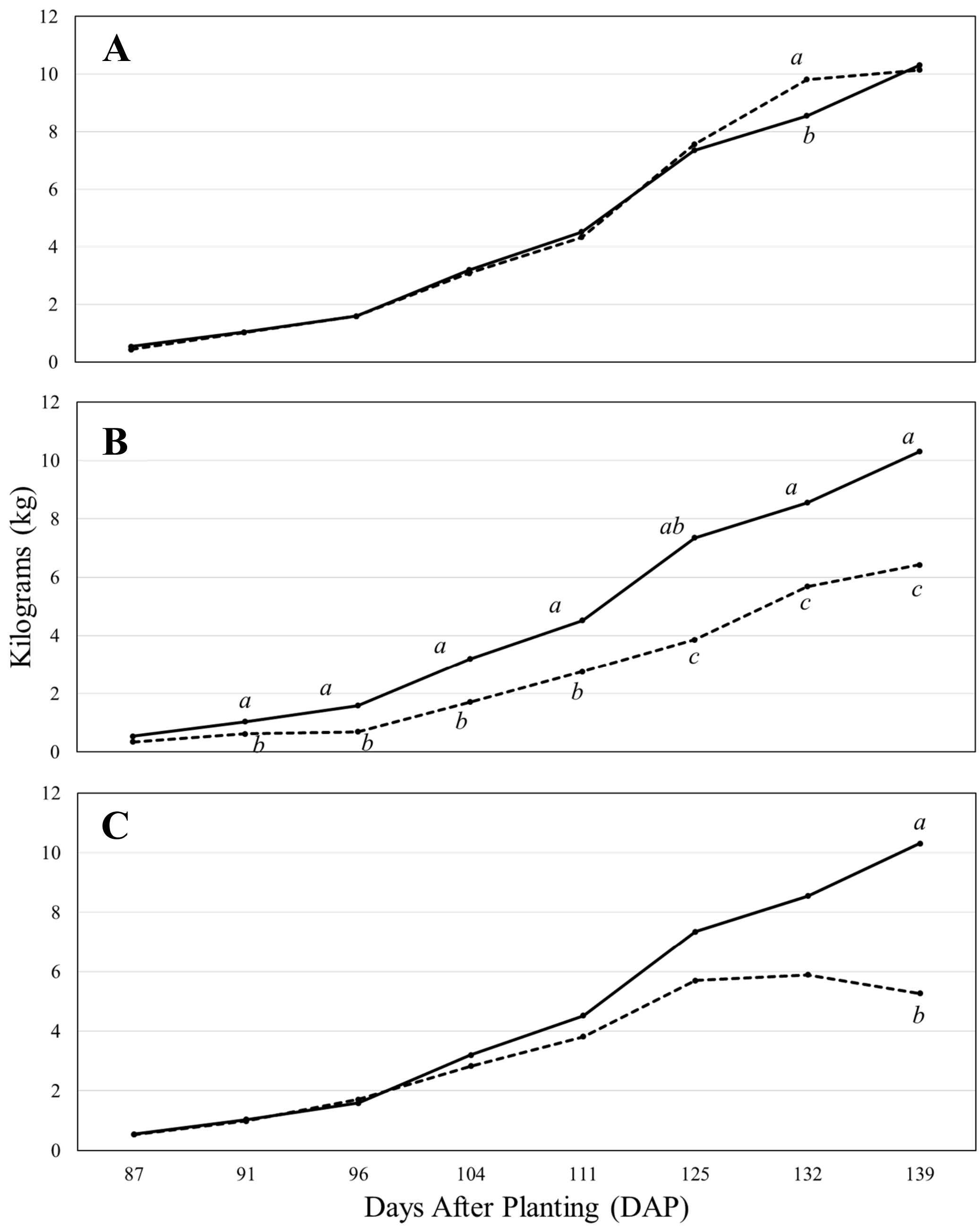

Figure 2.14. Effect of disease, nitrogen, and water stress on total plant biomass development for Vaquero onions grown at Parma, ID in 2019. Values are the mean weight (kg) of 5 replicates of 25 plants. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

2.8. Chapter 2 tables

Table 2.1. Description of 2018 field treatments applied to onion cultivar SV4643NT grown at Parma, ID.

Treatment	Cultivar	Fumigation	Nitrogen fertilizer	Irrigation
1. High disease	SV4643NT	None	134.5 kg ha^{-1}	Normal
2. Low disease	SV4643NT	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Normal
3. Non-fertilized	SV4643NT	Chloropicrin 37.4 L ha^{-1}	None	Normal
4. Reduced water	SV4643NT	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Reduced

Table 2.2. Description of 2019 field treatments applied to onion cultivars SV4643NT and Vaquero grown at Parma, ID.

Treatment	Cultivar	Fumigation	Nitrogen Fertilizer	Irrigation
1. High disease	SV4643NT	None	134.5 kg ha^{-1}	Normal
2. Low disease	SV4643NT	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Normal
3. Non-fertilized	SV4643NT	Chloropicrin 37.4 L ha^{-1}	None	Normal
4. Reduced water	SV4643NT	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Reduced
5. High disease	Vaquero	None	134.5 kg ha^{-1}	Normal
6. Low disease	Vaquero	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Normal
7. Non-fertilized	Vaquero	Chloropicrin 37.4 L ha^{-1}	None	Normal
8. Reduced water	Vaquero	Chloropicrin 37.4 L ha^{-1}	134.5 kg ha^{-1}	Reduced

Table 2.3. Effect of disease, nitrogen, and water stress on pink root disease rating values (unitless) for SV4643NT onions grown at Parma, ID in 2018. Values are the mean of 5 replicates of 25 plants. Values followed by the same letter within a given sample date are not statistically different. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, $P \leq 0.05$).

	Sampling Date (DAP)													
Treatment	**93**		**99**		**105**		**113**		**120**		**128**		**135**	
High disease	45.5	-	54.5	*a*	54.6	*a*	58.1	*a*	63.4	*a*	66.9	*a*	66.8	*a*
Low disease	36.5	-	42.6	*b*	44.4	*b*	49.2	*b*	50.6	*b*	54.4	*b*	57.4	*b*
Non-fertilized	33.0	-	41.0	*b*	48.0	*b*	47.0	*b*	52.0	*b*	52.2	*b*	54.8	*b*
Reduced water	39.5	-	40.0	*b*	44.8	*b*	51.2	*b*	56.8	*b*	55.0	*b*	58.4	*b*
LSD	**ns**		**6.7**		**5.5**		**5.7**		**5.6**		**5.1**		**6.8**	
F-Test	**0.0690**		**0.0009**		**0.0039**		**0.0045**		**0.0008**		**<0.0001**		**0.0101**	

Table 2.4. Effect of low disease and water stress on plant water content (in % water) for SV4643NT onions grown at Parma, ID in 2018. Values are the mean of 5 replications of 5 individual plants. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, $P \leq 0.05$).

	Sampling Date (DAP)													
Treatment	**93**		**99**		**105**		**113**		**120**		**128**		**135**	
Low disease	89.8	**-**	91.3	-	91.2	-	91.3	-	91.2	-	89.9	-	87.8	-
Reduced water	90.0	**-**	91.2	-	90.6	-	91.0	-	90.6	-	89.2	-	87.0	-
LSD	**ns**		**ns**		**ns**		**ns**		**ns**		**ns**		**ns**	
F-Test	**0.6696**		**0.7414**		**0.0711**		**0.2337**		**0.0706**		**0.2877**		**0.6347**	

Table 2.5. Effect of low disease and water stress on total leaf fresh weight (in grams) for SV4643NT onions grown at Parma, ID in 2018. Values are the mean of 5 replications of 5 individual plants. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)													
Treatment	**93**		**99**		**105**		**113**		**120**		**128**		**135**	
Low disease	79.5	-	153.2	-	158.4	-	150.1	-	166.6	*a*	110.6	-	105	-
Reduced water	87.3	-	145.6	-	124.5	-	129.2	-	115.5	*b*	94.2	-	74.7	-
LSD	**ns**		**ns**		**ns**		**ns**		**40.6**		**ns**		**ns**	
F-Test	**0.4969**		**0.5804**		**0.1225**		**0.3153**		**0.0196**		**0.2041**		**0.1237**	

Table 2.6. Effect of low disease, water, and nitrogen stress on percent total nitrogen content of SV4643NT onion foliage grown at Parma, ID in 2018. Values are the mean of 5 replications of foliage from 5 different plants. Values classified with the same letter within a given sample date are not statistically different. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)			
Treatment	**93**		**105**	
Low disease	2.78%	*a*	2.36%	-
Non-fertilized	2.26%	*b*	2.42%	-
Reduced water	2.78%	*a*	2.52%	-
LSD	**0.21**		**ns**	
F-Test	**0.0002**		**0.3363**	

Table 2.7. Effect of disease, nitrogen, and water stress on pink root disease rating values (unitless) for SV4643NT onions grown at Parma, ID in 2019. Values are the mean of 5 replicates of 25 plants. Values classified with the same letter within a given date are not statistically different (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)															
Treatment	**87**		**91**		**96**		**104**		**111**		**125**		**132**		**139**	
High disease	17.0	*a*	38.8	*a*	47.0	*a*	50.2	*a*	63.6	*a*	71.6	*a*	73.6	*a*	74.2	*a*
Low disease	8.8	*b*	22.6	*b*	33.4	*c*	38.2	*b*	54.2	*b*	64.0	*ab*	68.2	*b*	68.8	*b*
Non-fertilized	6.8	*b*	25.4	*b*	36.2	*bc*	39.6	*b*	51.0	*b*	56.0	*b*	66.6	*b*	67.0	*c*
Reduced water	6.8	*b*	26.4	*b*	41.0	*b*	40.8	*b*	51.6	*b*	66.4	*a*	69.0	*b*	69.6	*b*
LSD	**5.8**		**4.4**		**5.1**		**5.6**		**6.1**		**10.0**		**3.6**		**1.4**	
F-Test	**0.0048**		**<0.0001**		**0.0001**		**0.0013**		**0.0016**		**0.0320**		**0.0048**		**<0.0001**	

Table 2.8. Effect of disease, nitrogen, and water stress on pink root disease rating values (unitless) for Vaquero onions grown at Parma, ID in 2019. Values are the mean of 5 replicates of 25 plants. Values classified with the same letter within a given date are not statistically different. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)															
Treatment	**87**		**91**		**96**		**104**		**111**		**125**		**132**		**139**	
High disease	1.8	-	17.6	*a*	32.2	*a*	40.2	*a*	46.8	*a*	42.8	*a*	46.2	-	48.4	*a*
Low disease	0.4	-	10.8	*b*	23.2	*b*	24.8	*c*	35.6	*b*	36.8	*c*	43.0	-	41.6	*b*
Non-fertilized	0.2	-	9.8	*b*	24.8	*b*	26.2	*c*	34.0	*b*	41.2	*ab*	43.4	-	42.2	*b*
Reduced water	0.2	-	10.2	*b*	23.0	*b*	33.4	*b*	37.6	*b*	38.0	*bc*	43.4	-	44.2	*b*
LSD	**ns**		**4.7**		**5.5**		**5.2**		**4.5**		**3.7**		**ns**		**2.9**	
F-Test	**0.1763**		**0.0082**		**0.0079**		**<0.0001**		**<0.0001**		**0.0112**		**0.2985**		**0.0005**	

Table 2.9. Effect of disease, nitrogen, and water stress on total plant biomass development for SV4643NT grown at Parma, ID in 2019. Values are the mean weight (kg) of 5 replicates of 25 plants. Values classified with the same letter within a given date are not statistically different. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)															
Treatment	**87**		**91**		**96**		**104**		**111**		**125**		**132**		**139**	
High disease	0.48	-	0.90	*a*	1.39	*bc*	2.55	*a*	3.15	*b*	4.80	*bc*	5.66	-	5.32	*b*
Low disease	0.52	-	0.90	*a*	1.68	*ab*	2.90	*a*	3.54	*ab*	6.03	*a*	6.40	-	7.84	*a*
Non-fertilized	0.40	-	0.60	*b*	1.12	*c*	1.72	*b*	2.34	*c*	4.52	*c*	5.73	-	5.47	*b*
Reduced water	0.53	-	0.99	*a*	1.71	*a*	2.83	*a*	3.82	*a*	5.70	*ab*	5.89	-	5.27	*b*
LSD	**ns**		**0.18**		**0.29**		**0.43**		**0.57**		**1.02**		**ns**		**0.74**	
F-Test	**0.1906**		**0.0022**		**0.0019**		**<0.0001**		**0.0003**		**0.0181**		**0.2116**		**<0.0001**	

Table 2.10. Effect of disease, nitrogen, and water stress on total plant biomass development for Vaquero onions grown at Parma, ID in 2019. Values are the mean weight (kg) of 5 replicates of 25 plants. Values classified with the same letter within a given date are not statistically different. Sampling dates not classified with letters do not contain statistical differences (Fisher's protected LSD, P ≤ 0.05).

	Sampling Date (DAP)															
Treatment	**87**		**91**		**96**		**104**		**111**		**125**		**132**		**139**	
High disease	0.44	-	1.02	*a*	1.59	*a*	3.08	*a*	4.33	*a*	7.56	*a*	9.81	*a*	10.14	*a*
Low disease	0.54	-	1.04	*a*	1.59	*a*	3.20	*a*	4.52	*a*	7.35	*ab*	8.55	*b*	10.31	*a*
Non-fertilized	0.35	-	0.62	*b*	0.69	*b*	1.71	*b*	2.76	*b*	3.86	*c*	5.68	*c*	6.43	*c*
Reduced water	0.54	-	0.98	*a*	1.66	*a*	2.94	*a*	4.05	*a*	6.33	*b*	7.99	*b*	8.41	*b*
LSD	**ns**		**0.20**		**0.34**		**0.59**		**0.89**		**1.23**		**1.09**		**1.17**	
F-Test	**0.0721**		**0.0013**		**<0.0001**		**0.0002**		**0.0028**		**<0.0001**		**<0.0001**		**<0.0001**	

Table 2.11. Effect of disease and nitrogen stress on percent total leaf nitrogen content of SV4643NT and Vaquero onion foliage grown at Parma, ID in 2019. Values are the mean of 5 replications of foliage from 5 different plants. Values classified with the same letter within a given date are not statistically different (Fisher's protected LSD, P ≤ 0.05).

	SV4643NT				Vaquero			
Treatment	**91 DAP**		**111 DAP**		**91 DAP**		**111 DAP**	
High disease	3.16%	*a*	2.58%	*a*	3.16%	*a*	2.58%	*a*
Low disease	3.00%	*b*	2.60%	*a*	3.24%	*a*	2.60%	*a*
Non-fertilized	2.50%	*c*	2.22%	*b*	2.58%	*b*	2.32%	*b*
LSD	**0.13**		**0.18**		**0.17**		**0.18**	
F-Test	**<0.0001**		**0.0007**		**<0.0001**		**0.0107**	

Table 2.12. Effect of disease, nitrogen, and water stress on total yield and proportion of yield greater than 8.4 cm for SV4643NT (SV) and Vaquero (Vaq) onions grown at Parma, ID. Values are the mean of 5 replications. Treatments characterized by the same letter within a cultivar category are not statistically different (Fisher's protected LSD, P ≤ 0.05).

	Total Yield						Percent Yield ≥ 8.4 cm					
Treatment	**SV 2018**		**SV 2019**		**Vaq 2019**		**SV 2018**		**SV 2019**		**Vaq 2019**	
High disease	44.7	*b*	50.9	*b*	108.6	*a*	34.6	*b*	44.8	*c*	86.0	*a*
Low disease	58.9	*a*	71.2	*a*	108.7	*a*	53.0	*a*	67.6	*a*	86.5	*a*
Non-fertilized	49.8	*ab*	53.6	*b*	75.8	*c*	49.0	*a*	59.2	*b*	69.0	*b*
Reduced water	40.0	*b*	55.7	*b*	84.4	*b*	30.5	*b*	50.7	*c*	69.8	*b*
LSD	**10.7**		**6.0**		**8.3**		**11.2**		**7.6**		**7.0**	
F-Test	**0.0118**		**<0.0001**		**<0.0001**		**0.0002**		**<0.0001**		**<0.0001**	

Chapter 3: Evaluating Crop Stress in Onion with Hyperspectral Radiometric Measurements and Narrowband Spectral Vegetation Indices

3.1. Abstract

There has been recent interest in exploring the capability of remote sensing technology to support pest management decision-making in onion production, especially as it relates to onion pink root. Visually, pink root symptoms are similar to those expressed in onions lacking water and macronutrients. This similarity may confound the practical use of remote sensing in onions, and thus it is important to study the impact of these stresses alongside that of disease. The objective of this study was to evaluate the usefulness of hyperspectral remote sensing as a tool to detect onion pink root in the field and discriminate its symptoms from those of nitrogen and water stress. A field experiment with these three crop stress treatments and a control were established in 2018 and 2019 at Parma, ID to study the impacts of stress on onion reflectance at the leaf and canopy level. Spectral measurements were collected weekly from the time of bulb initiation to plant maturity (tops down) and subsequently analyzed using 40 established spectral vegetation indices (SVIs). Preliminary results indicated that a similar relative relationship in SVI value was observed among stress treatments at the canopy level. From that point forward, three SVIs (the triangular vegetation index (TVI), normalized difference vegetation index (NDVI), and the optimized soil-adjusted vegetation index (OSAVI)) were selected for detailed analysis. Only small variations in SVI value were observed between treatments at the leaf-level. At the canopy-level, stress treatments consistently lowered index values, though not always significantly and not always to the same degree. Results from standard regression analysis suggest that SVI value is related to plant biomass as opposed to any particular stress. This was evidenced by a close relationship between SVI value and the biomass of SV4643NT (R^2 = 0.67 to 0.79) and Vaquero (R^2 = 0.73 to 0.83), which likely arose because of the direct relationship between plant biomass and parameters which account for the fraction of vegetation that covers the soil (such as leaf area index (LAI)). Overall, we found that SVIs, particularly those which include a band in the near-infrared region (700-1000 nm), are overwhelmingly sensitive to variation in canopy LAI as opposed to other vegetation parameters such as plant pigment composition (i.e. chlorophyll) in scenarios where the crop canopy does not completely cover the soil.

3.2. Introduction

Successful management of crop inputs (i.e. fertilizer and water) and pest/disease pressure is essential to profitable onion production. Failure in this area may result in bulbs that do not meet market quality standards and are thus unmarketable. Bulb size is a category of particular importance, since markets for undersized bulbs are limited (Sullivan et al. 2001) and the highest economic return is typically attained from bulbs greater than 8 cm (Geary et al. 2008). One disease important in this respect is onion pink root. It is caused by a soilborne fungus (*S. terrestris*), which destroys and colonizes onion root tissue during crop growth, thereby limiting the plants ability to uptake water and nutrients. Cultivars vary in their relative susceptibility to root colonization (Esfahani and Pour 2007) and the use of tolerant genotypes can help mitigate the negative impacts of pink root. However, no one cultivar is completely resistant (Murray et al. 2018). Though drip applied fungicide during crop growth may suppress the disease (Thornton et al. 2017), soil fumigation with products such as chloropicrin is currently the most widely employed control method. Soil fumigation, however, is expensive and not ideal due to impact on nontarget organisms and potential adverse effects on human health and the environment (Geary et al. 2008). Identification and mapping of pathogen "hot spots" throughout a field may facilitate the precision application of soil fumigants, and thus reduce their use. Manual mapping of pathogen density using molecular techniques is tedious since *S. terrestris* varies widely in its spatial distribution (Woodhall unpublished). There has been recent interest in the use of advanced technologies to support pest management decision-making in the Treasure Valley as well as specific interest in its application for management of pink root (Murray et al. 2018). In this regard, remote sensing may present a useful tool for the management of soilborne disease.

Visible-near infrared (VIS-NIR) spectroscopy is one remote sensing technology that has been found suitable for non-destructive diagnosis of disease in different crops (Sankaran et al. 2012). This technique utilizes VIS-NIR spectral features, of which band reflectance is the most common (Zhang et al. 2019). It is well established that changes in leaf reflectance within the 400-2500 nanometer (nm) wavelength range (most of the incident solar spectrum) may occur when a plant is under stress (Carter 1993). In the visible wavelengths (400-700 nm) reflectance is predominantly impacted by leaf pigment composition whereas in the near infrared region (700-1100 nm) reflectance is influenced by leaf structure, density, and water content (Mahlein et al. 2013; Mulla 2013). Plant disease may impact a number of these leaf characteristics. Therefore, the general hypothesis is that healthy plants interact with and reflect electromagnetic radiation differently from infected plants (Moshou et al. 2011), so it may be possible to distinguish between the two. However, not all plant diseases are suitable for detection using VIS-NIR spectroscopy, only those which result in a detectable spectra alteration. Thus, the first step in determining if a disease can be sensed remotely is the discovery of a unique spectral response to plant disease symptoms.

Visible-near infrared spectroscopy can be accomplished at varying spectral resolutions and is categorized accordingly as either hyperspectral or multispectral. Hyperspectral spectroscopy (the more detailed of the two) differs from multispectral spectroscopy in the continuity, wavelength range, and the spectral resolution (or width) of the measured bands (Mulla 2013). With hyperspectral sensors, reflectance can be resolved in relatively narrow wavelength ranges or “wave-bands,” down to 2 nm or less and is typically continuous over the measured range (Jones and Vaughan 2010). The high spectral resolution of hyperspectral sensors is advantageous since it may facilitate the detection of weak or inconspicuous spectral changes necessary to diagnose plant diseases at an early stage or in complicated scenarios (Zhang et al. 2019).

Additionally, VIS-NIR remote sensing can be accomplished with either imaging or non-imaging sensors. With imaging sensors, the resulting “picture” is composed of multiple pixels, each of which are associated with a unique reflectance spectra. Non-imaging sensors, on the other hand, provide the mean reflectance value (of a given band) over the measured area and, thus, function like a single pixel. Handheld non-imaging sensors are commonly used to study plant-pathogen interaction in scientific field studies, since they allow high throughput measurements and are simplified from other remote sensing platforms (Thomas et al. 2018). Remote sensing studies are initiated at a defined proximity to the object of interest. Thus, studies on plant disease can occur at the leaf-level, where reflectance of single leaves is measured, the plant-level, where the reflectance of the leaves of a single plant are measured, or the canopy-level, where the reflectance of the leaves of multiple plants is measured.

After reflectance data is collected, spectra must be evaluated to determine if there are any wave-bands sensitive to disease. This can be accomplished using algorithms or machine learning techniques which are efficient (Zhang et al. 2019), yet complex and require technical computer skills. An alternative method is the use spectral vegetation indices (SVIs). By calculating ratios from several bands at different wavelengths, SVIs can be used to reduce data dimensionality and thus simplify plant disease detection (Mahlein et al. 2013; Hillnhutter et al. 2011). There are many SVIs that have been independently developed for plant disease diagnosis (Asherloo et al. 2016), leaf pigment concentration (Carter and Miller 1994), and leaf water composition (Pasqualotto et al. 2018). Since there exists such an array of SVIs that have already been developed, one approach to evaluate the usefulness of remote sensing in a particular disease scenario is to test established SVIs.

Several common SVIs have been used previously to study onions at the canopy-level (Bosch Serra and Cassanova 2000; Marino et al. 2013; Marino and Alvino 2015.), however, in all cases plants were grown under optimal conditions. Hyperspectral remote sensing has been successfully applied in the detection of sour skin in post-harvest onion bulbs (Wang et al. 2012) and a subsequent study further explored the optical properties of healthy and diseased onion bulb tissue (Wang et al. 2014). To our knowledge, however, there is almost no evidence in the literature of the influence of disease and stress on onion leaf tissue reflectance characteristics.

In order to explore the impact of abiotic and biotic stress and onion canopy reflectance, a field experiment was established in 2018 and 2019. Study objectives were first, to explore the spectral response of onion to pink root, nitrogen stress, and drought using established SVIs. The second objective was to determine if any SVIs were suitable to distinguish between healthy and stressed onions or distinguish between the different stresses.

3.3. Methods and materials

3.3.1. 2018 remote sensing data collection

Canopy spectral measurements: To study the effect of onion stress on remote sensing data a field experiment was established in 2018. The area of interest was the previously described field trial (Chapter 2), in which three stress treatments were present. On 93 DAP, weekly spectral reflectance measurements at the canopy level were initiated using an ASD FieldSpec 4 High-Resolution Spectroradiometer (Malvern Panalytical Ltd., Westborough, MA, USA) with a pistol grip foreoptic held 1 meter above the soil surface. The foreoptic field-of-view (FOV) was fixed at 25° and all measurements were taken directly above the canopy at a 90° angle to the soil surface (nadir), thus the area measured was determined by the following equation:

$$A = \pi \times (\tan \frac{\alpha}{2} \times d)^2$$

Where, A = the area measured, α = the foreoptic FOV (fixed at 25°), d = the distance between the foreoptic and the target (Figure 3.1.). Pertinent instrument specifications are outlined in Table 3.1.

Prior to spectra collection, the instrument was warmed up for a minimum of 30 minutes and then optimized and calibrated to the current sunlight conditions. An average of 100 dark current scans were calibrated to an average of 50 scans of a Spectralon 99 % white reference standard (Labsphere Inc., North Sutton, NH, USA). Subsequent target reflectance measurements represented an average of 20 scans at an optimized integration time. Parameters were set and measurements collected using RS^3 software version 6.4 (Malvern Panalytical Ltd., Westborough, MA, USA). The spectral sampling interval was automatically interpolated from 1.4 nm to 1 nm at the time of each measurement by RS^3, resulting in a single value for each wavelength from 350-2500 nm. Measurements were confined to the window of 11:00 to 13:00 (solar time) on days in which cloud cover was spare enough to allow for spectra to be collected under clear sky conditions. Weekly measurements continued until 134 DAP.

Canopy spectral sampling pattern: On each measurement date, 10 spectral measurements were acquired in each experimental plot at flag-marked locations. Flags were arranged so that 5 locations were marked on either side of the middle data bed in the center of the onion double row (Figure 3.2).

On collection days, single flags were removed, a measurement was taken, and the flag was replaced. Utilizing this procedure allowed for the measurement of the same group of plants each week. This process was repeated until all 10 measurements within a single plot were complete. Prior to measuring an additional plot, the instrument was recalibrated with the white reference standard. At the 2018 sampling rate, recalibration occurred approximately every 3.5 minutes. On each sampling date, a total of 50 measurements were collected for each treatment, which resulted in the collection of 200 spectra in total within the 2-hour sampling window.

Leaf-level spectral measurements: To measure reflectance at the leaf level, the FieldSpec 4 pistol grip attachment was removed from the foreoptic and replaced with the ASD plant probe fitted with a leaf clip attachment (Malvern Panalytical Ltd., Westborough, MA, USA). Instrument parameters were the same as those described for measurements at the canopy level. Instead of utilizing sun as a light source, the plant probe attachment supplies its own light via an internal, full-spectrum halogen lamp (Welch Allyn, Skaneateles Falls, NY, USA). When a leaf is placed and clamped between the leaf clip and the plant probe measuring window, external light is excluded. Additionally, only leaf material contributes to the resulting spectra. The instrument was optimized and calibrated using the same procedure outlined for the canopy measurements. On onion destructive sampling dates, 5 plants with the most diseased roots and 5 plants with the least diseased roots were retained after disease rating from the non-fumigated and fumigated treatments, respectively. Prior to percent water content analysis (Chapter 2), the dorsal surface of the newest, fully growth leaf of each plant was selected. This offered a relatively flat location to collect reflectance measurements. At an undamaged location 10 to 15 cm up from where the leaf connects to the neck, the plant probe was secured to the leaf and a measurement was taken (Figure 3.3). This process was repeated with all retained plants and white reference calibration was performed approximately every 3.5 minutes.

3.3.2. 2019 remote sensing data collection

Canopy spectral measurements: In 2019, the onion field plots established at Parma, ID (Chapter 2) again served as the area of interest for canopy spectral measurements. One difference was the inclusion of an additional cultivar (Vaquero) for comparison. The first field measurement occurred on 87 DAP using the ASD FieldSpec 4 fitted with a pistol grip attachment, held approximately 1 meter directly above (at nadir) the soil surface. All instrument parameters and calibration procedures were unchanged from the previous year. Canopy measurements were again confined to the widow of 11:00 to 13:00 (solar time) on days with clear sky conditions.

Canopy sampling pattern: Within the experimental onion plots, measurements were taken in the same quantity and same conformation as in 2018 (Figure 3.2). To accommodate the collection of an additional 200 spectra for the second cultivar included in 2019, a new method of marking measurement locations was utilized. Flags, which had to be removed and replaced during spectra collection, were exchanged for colored golf tees placed in the destructive sampling rows directly across from where weekly measurements were taken. Prior to each measurement, the instrument foreoptic was centered in the double row of onions directly across from where the colored golf tee was placed. Thereby, the same group of plants were measured each week. Although the golf tees were offset one row, a sampling pattern remained similar to the one used in 2018 (Figure 3.2). The adoption of this new alignment method reduced the time between white reference calibrations from 3.5 minutes to 2.5 minutes, thus facilitating the collection of 400 spectra within the measurement window.

3.3.3. Spectrum export

Spectrum files were converted to a text file list using the ASD ViewSpec Pro software version 6.2 (Malvern Panalytical Ltd., Westborough, MA, USA). Subsequent spectra analysis was performed in Microsoft Excel version 16.0 (Microsoft Inc., Redmond, WA, USA).

3.3.4. Spectral vegetation index selection

The automatic interpolation of spectral data to an interval of 1 nm allowed for subsequent analysis with any established, narrow-band SVI. In total, 40 established SVIs were calculated for the average spectra of each treatment at each measurement date. Vegetation indices were evaluated by week for their ability to 1) distinguish the low disease spectrum from the high disease spectrum and 2) distinguish the high disease spectrum from the non-fertilized and reduced water spectra.

3.3.5. Spectral vegetation index calculation

To comply with the replication established in the field experiment, the 10 canopy spectra from each plot were averaged by week. Prior to averaging, chosen SVIs were calculated for each of the 10 spectra. The resulting 10 values were then tested for statistical outliers using Tukey's method on a per SVI basis (Tukey 1977). Outliers were removed from the data set and did not contribute to the average. In the same way, statistical outliers were removed from the leaf-level spectra. Since leaf-level measurements only resulted in 5 spectra per plot, a maximum of 5 values contributed to the plot average.

3.3.6. Choosing indices for further analysis

Preliminarily evaluation of the 40 tested SVIs (Appendix A) revealed that though each utilized a unique wavelength ratio, the relative relationship between treatment values were consistently similar for each SVI at the canopy level (Appendix B). In other words, for those SVIs which are directly related to plant health, the low disease attained the highest value, the non-fertilized attained the lowest value, and the high disease and reduced water treatment fell somewhere between the value of these two extremes. For those SVIs which are indirectly related to plant health, the opposite was the case. Therefore, three indices which displayed a capacity for distinguishing between treatments were chosen for further analysis.

The triangular vegetation index (TVI) was the first to appear suitable for differentiating between treatments (Broge and Leblanc 2001). TVI was developed to study changes in plant chlorophyll density. It is unique from other indices in that it is calculated as the area of the triangle defined by the green peak (550 nm), the chlorophyll absorption minimum (670 nm), and the near-infrared (NIR) shoulder (750 nm) that lies in spectral space. It is, therefore, calculated with the following equation:

$$TVI = 0.5 \times (120(\rho750 - \rho550) - 200(\rho670 - \rho550))$$

Where, $\rho550$ = reflectance at 550nm (green), $\rho670$ = reflectance at 670 nm (red), and $\rho750$ = reflectance at 750 nm (NIR).

The sensitivity of TVI to chlorophyll is based on the fact that both chlorophyll absorption which decreases reflectance at 670 nm and leaf tissue abundance which increases reflectance at 750nm will increase the value of the index (Broge and Leblanc 2001).

The normalized difference vegetation index (NDVI) also displayed sensitivity to onion stress in 2018. NDVI remains a popular and widely-used index even though it was the first developed vegetation index and is nearly 50 years old (Rouse et al. 1974). It serves as a general indicator of plant health by utilizing the chlorophyll absorption minimum (670 nm) and reflectance in the near infrared (860 nm). It was calculated with the following equation:

$$NDVI = \frac{(\rho800 - \rho670)}{(\rho800 + \rho670)}$$

Where, $\rho670$ = reflectance at 670nm (Red) and $\rho800$ = reflectance at 800nm (NIR).

The final SVI assessed in detail in this study was the optimized soil-adjusted vegetation index (OSAVI). The OSAVI was developed by Rondeaux et al. (1996) and is essentially the NDVI optimally adjusted to minimize soil background effects. It was calculated with the following equation:

$$OSAVI = (1.16)\frac{(\rho800 - \rho670)}{(\rho800 + \rho670 + 0.16)}$$

Where, $\rho670$ = reflectance at 670nm (red) and $\rho800$ = reflectance at 800nm (NIR).

3.3.7. Statistical Analysis

Weekly treatment averages from 2018 and 2019 were analyzed using the SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA) analysis of variance. In cases when the F-test for treatment was significant, means were separated with Fisher's protected least significant difference (LSD) at the 5% probability level ($P \leq 0.05$). Standard regression analyses were also carried out to assess the correlation between each index, biomass, and disease.

3.4. Results

3.4.1. 2018 results

In 2018, there were no significant differences among the stress treatments at the leaf-level (Figure 3.4A-C). Differences were observed, however, at the canopy-level where leaf tissue abundance is also a factor (Figure 3.4D-F). The TVI values of the high disease treatment was lower than that of the low disease at all measurement dates, but only significantly at two early dates (Figure 3.4D). Overall, these two treatments progressed over time in a manner almost identical. TVI values in the non-fertilized treatment were significantly lower compared to the low disease treatment on all measurement dates but the last (Figure 3.4E). TVI values of the reduced water treatment were mostly similar to those of the low disease treatment, especially early on. The two were significantly different only at 127 DAP.

At the leaf-level, significantly lower NDVI values were observed on 105 DAP in all stress treatments compared to the low disease treatment (non-stressed) and 128 DAP in the non-fertilized and reduced water treatments, specifically (Figure 3.5A-C). At the canopy level, the high disease treatment and reduced water treatment were found to have significantly lower NDVI values on the first 3 measurements and last 3 measurement dates, respectively (Figure 3.5D, E). NDVI values were decreased to a greater extent in the non-fertilized treatment and were significantly different compared to the low disease treatment at all measurements except the last (Figure 3.5F).

OSAVI was unable to distinguish between stress treatments at leaf-level, the exception being the non-fertilized treatment at 128 DAP (Figure 3.6A-C). At canopy-level, results were the same as those observed in the NDVI for the high disease and non-fertilized treatments (Figure 3.6D, E). This was not the case for the reduced water treatment which, unlike NDVI, was only significantly different from the low disease treatment at the 127 DAP measurement (Figure 3.6F).

3.4.2. 2018 Regression

At the leaf-level, TVI appeared to be moderately related ($R^2 = 0.57$) to leaf fresh weight, but this was likely because the index was relatively stable over time (Figure 3.7A). Likewise, a weak relationship was observed between NDVI and OSAVI values and the average leaf biomass (leaf fresh weight) of the onions from the low-disease and reduced water treatments (Figure 3.7B and Figure 3.7C). At the canopy level, all three indices were moderately related to leaf biomass (R^2 = 0.45 to 0.58), but NDVI had the largest coefficient of determination (Figure 3.7D, Figure 3.7E, Figure 3.7F).

At the leaf-level both NDVI and OSAVI were moderately related (R^2 = 0.61 to 0.64) to the disease rating value of all treatments at all sampling dates (Figure 3.8B and Figure 3.8C). At the canopy level, SVI and disease ratings were not closely related (R^2 = 0.13 to 0.26; Figure 3.8E and Figure 3.8F). TVI displayed a weak relationship at both the leaf and canopy level (Figure 3.8A and Figure 3.8D).

3.4.3. 2019 SV4643NT results

TVI values at the canopy level of the high disease treatment were significantly lower than those of the low disease treatment. This primarily occurred on the last four measurements but was also observed at the first measurement (Figure 3.9A). TVI values of the non-fertilized treatment were significantly lower than those of the low disease treatment at all measurements (Figure 3.9B). In the reduced water treatment, TVI index values were nearly identical to those observed in the low disease treatment, until 112 DAP (Figure 3.9C). After this, slightly lower index values were observed, but were only significant at 129 and 147 DAP. The increase at 140 DAP was very similar in both the water reduced and low disease treatments.

NDVI values at the canopy level of the high disease treatment were significantly lower compared to the low disease treatment at the last four measurements and the first measurement (Figure 3.10 A). The non-fertilized treatment attained NDVI values significantly lower than those of the low disease treatment at all measurements but the final (Figure 3.10B). Lastly, index values in the water reduced treatment were also decreased but only significantly so on the second and last 5 measurements (Figure 3.10C).

Values of the OSAVI at the canopy-level were again similar to those of NDVI in 2019. The two differed in that the high disease and non-fertilized treatments were significantly different at one additional measurement with OSAVI (Figure 3.11A, B). This occurred at 112 and 147 DAP in the high disease and non-fertilized treatment, respectively. On the other hand, the reduced water treatment was significantly different at one measurement less (105 DAP) than what was observed with NDVI (Figure 3.11C).

3.4.4. 2019 Vaquero results

In contrast to SV4643NT, significant differences in canopy-level TVI among stress treatments occurred less frequently in Vaquero. Also, in some cases TVI values were increased by stress in Vaquero, while they were always decreased by stress in SV4643NT. For example, the high disease treatment exhibited significantly increased TVI compared to the low disease treatment on two dates, 112 and 123 DAP (Figure 3.12A). Likewise, the reduced water treatment increased TVI compared to the low disease treatment at 104 and 140 DAP (Figure 3.12C). However, TVI values were found to be significantly lower in the non-fertilized treatment compared to the low disease treatment at all measurement dates except the first (Figure 3.12B).

Differences in canopy level NDVI among treatments were similar for Vaquero and SV4643NT. In the high disease treatment NDVI was slightly higher than in the low disease treatment at later measurement dates, but these differences were not significant (Figure 3.13A). The non-fertilized treatment resulted in significantly lower NDVI values than those observed for the low disease treatment at all measurement dates (Figure 3.13B). NDVI of the reduced water treatment were significantly lower than the low disease treatment on the last three measurement dates (Figure 3.13C).

Values of OSAVI at the canopy level for Vaquero were similar to those observed for NDVI. One difference, however, was the fact that the increase in index value of the high disease over the low disease treatment was significant from 112 to 140 DAP (Figure 3.14A). The other difference occurred at 140 DAP in the water reduced treatment. At this measurement, the OSAVI of the reduced water was found to be slightly higher (but not significantly different) than that of the low disease treatment (Figure 3.14C). In this way, the reduced water OSAVI results corresponded with that observed for TVI at the same measurement date. OSAVI was significantly lower in the non-fertilized treatment compared to the low disease treatment on all evaluation dates (Figure 3.14B).

3.4.5. 2019 Regression results

At the canopy level TVI, NDVI, OSAVI were all moderately or closely related (R^2 = 0.67 to 0.79) to SV4643NT plant biomass. (Figure 3.15A, Figure 3.15B, Figure 3.15C) This was also the case with all 3 SVIs and the plant biomass of Vaquero, among which similarly close relationships were determined (R^2 = 0.73 to 0.83; Figure 3.15D, Figure 3.15E, Figure 3.15F). A moderate or close relationship was observed between all 3 SVIs and the disease ratings of both cultivars in 2019 (Figure 3.16), however the relationship was opposite to that observed the year prior.

3.5. Discussion

3.5.1. Impact of treatment on SVI value at the leaf-level

Together the three stress treatments had almost no significant impact on the value of both TVI and OSAVI at the leaf-level. This held despite the fact that plants were intentionally chosen to minimize the overlap of disease symptoms and the symptoms of other stresses. NDVI was most sensitive to onion stress at the leaf-level, but the average NDVI value of the stress treatments was only found to be significantly lower compared to the low disease treatment at 105 and 128 DAP. It appears that on these dates the NDVI values of the low disease treatment increased as opposed to a decrease in the high disease, non-fertilized, and water stress treatments. There was no clear indication of what caused these isolated NDVI increases in the low-disease treatment plots at the leaf-level. To our knowledge this is the first report of vegetative indices being used to study onion at the leaf-level.

Not all diseases are suitable for remote sensing diagnosis. Although symptoms of diseases affecting the plant root system tend to produce less obvious symptoms in the plant foliage compared to foliar diseases (i.e. wheat leaf rust; Zhang et al. 2012) they may be diagnosable if they have systemic physiological effects (Zhang et al. 2019). Pink root, nitrogen, and drought stress were mostly undetectable by TVI, NDVI, and OSAVI at the leaf-level. The foliage of the non-fertilized onion plots could be visually distinguished from the other plots by their mild chlorosis and stunting, which was apparent from the time of bulb initiation to plant maturity. Yet, the SVI values of the non-fertilized treatment was indistinguishable from those of the high disease and reduced water treatment. It is acknowledged that the 3 chosen indices are not ideal for studies at the leaf-level. The insensitivity of OSAVI compared to NDVI to detect the changes in reflectance at the leaf-level may be because OSAVI was developed for use at larger scales, where soil background is a contributing factor (Rondeaux et al. 1996). NDVI is positively correlated with the greenness associated with plant health but is not necessarily closely related to chlorophyll content (Lichtenthaler et al. 1996). On the other hand, TVI is positively related to leaf tissue abundance and the chlorophyll density of the leaf tissue (Broge and Leblanc 2001). At the leaf-level, or in cases where the field of view is entirely composed of leaf material, TVI becomes solely a measure of chlorophyll density. Therefore, the insensitivity of TVI to different treatments at the leaf-level suggest that leaf pigment composition (chlorophyll in particular) may not be significantly altered by the levels of stress imposed in this study. However, leaf chlorophyll was not directly measured in the present study and more research is needed to characterize the effect of onion stress on leaf pigment composition.

3.5.2. Impact of treatment on SVI value at canopy-level

The most obvious change in canopy reflectance was observed in the non-fertilized treatment, which produced significantly lower SVI values compared to the low disease treatment at nearly every measurement date. This was observed in 2018 and 2019 and in both cultivars. Additionally, all three indices were sensitive to nitrogen stress, which caused both an increase in the reflectance of the green and red bands in the visible wavelengths and a decrease in the reflectance of NIR bands (Appendix D). These results differ from those observed in spinach (*Spinacia oleracea*), where non-fertilized plants increased canopy reflectance in NIR bands compared to optimally fertilized plants (Corti et al. 2017).

The high disease and reduced water treatments did not impact canopy reflectance as consistently nor as obviously as the non-fertilized treatment. Furthermore, the SVI response of plants severely infected with pink root and plants under drought conditions was similar in timing and in magnitude, especially in 2019. An exception to this was the Vaquero high disease treatment, which at most measurement dates produced SVI values that were not significantly different from those produced by the low disease treatment. When differences did occur, the high disease (stressed) treatment was found to have a higher SVI value than the low disease treatment. Neither the biomass nor the SVI value of Vaquero was significantly decreased by the high disease treatment. Thereby, the Vaquero disease rating and yield results outlined in Chapter 2 seem to agree with the Vaquero SVI results. Unique behavior in SVI value was observed in the SV4643NT high disease treatment in 2018, where SVI values were significantly lower compared to the low disease treatment at early measurement dates (93-105 DAP) but not at later dates (as was observed in 2019). To our knowledge this is the first characterization of onion stress using hyperspectral remote sensing and SVIs.

TVI, NDVI, and OSAVI were chosen specifically for their aptitude to distinguish treatments at the canopy-level. In addition, NDVI and OSAVI have been used to study onion leaf reflectance elsewhere (Marino and Alvino 2015; Marino et al. 2013), although only under optimal growing conditions. Standard regression analyses were carried out to gain a better understanding of the relationship between SVI and field variables and to determine the cause of SVI variation among treatments. In 2018, leaf biomass was moderately related (R^2 = 4.5 to 5.8) to all three SVIs at the canopy level, but not at the leaf level. Canopy-level relationships between biomass and SVI were improved in 2019, when total plant biomass was measured instead of leaf biomass and all four treatments were included in the analysis. The 2019 regression analyses resulted in coefficients of determination (R^2) which ranged from 0.67 to 0.80 for SV4643NT and from 0.73 to 0.83 for Vaquero.

3.5.3. Factors which influence SVIs

Crop reflectance spectra are determined by several factors including: leaf orientation, leaf pigment composition, shadows within the canopy, leaf area index (LAI), and background soil reflectance (Haboudane et al. 2002, Jones and Vaughan 2010). Lausch et al. (2013) used spectra collected from spring barley at the canopy scale to calculate more than 50 SVIs. They determined that none of the SVIs they studied displayed an explicit sensitivity to a single vegetation parameter but were sensitive to an array of parameters (Leaf area index (LAI), canopy chlorophyll content, gravimetric water content) with overlapping influence. While there is an array of factors that influence crop reflectance, influence is not equally distributed. Rather, vegetation parameters that are closely tied to the fraction of vegetation cover primarily determine SVI value (Jones and Vaughan 2010). One such parameter is leaf area index (LAI), defined as the one-sided leaf area per unit ground area (Ballesteros et al. 2014). According to Baret and Guyot (1991), most commonly used SVIs utilize bands in the red (around 670 nm) and NIR (700-1000 nm) region. In studying maize damage from insect feeding Zhang et al. (2016) found that SVIs which contained a NIR band were sensitive to the LAI reduction of damaged plants. Likewise, Lausch et al. (2013) determined that the NIR region was the most sensitive to changes in plant biomass and LAI. In our preliminary analysis of 40 published SVIs, a similar relative relationship among treatments was observed in the majority of SVIs at the canopy level (Appendix B), suggesting the presence of a dominating factor, whereas few differences were observed at the leaf level (Appendix C). Out of the 40 SVIs, 33 of them (including TVI, NDVI, and OSAVI) contain a band in the NIR region. Therefore, the conserved relationship among treatments as seen in the majority of the 40 analyzed SVIs value was likely caused by the dominating influence of LAI. This might explain why the non-fertilized treatment reduced reflectance in the NIR, the opposite of that observed in a spinach canopy under nitrogen stress (Corti et al. 2017). Other studies have reported a high correlation between OSAVI and NDVI and onion yield (R^2=0.66 and R^2=0.62, respectively) at the canopy level (Marino and Alvino 2015) and a high correlation between onion biomass and yield (R^2=0.79 and 0.87 for two different cultivars; Marino et al. 2013). Therefore, results from the present study and elsewhere indicate that although SVI value is predominantly determined by the fraction of vegetation cover, onion yield, biomass, and LAI are all directly related to SVI value (Ballesteros et al. 2014).

Though all three indices displayed a positive relationship with plant biomass, OSAVI was the most closely related to SV4643NT biomass, whereas TVI was most closely related to Vaquero biomass. There are additional results that suggest that the suitability of an SVI to measure onion biomass varies between cultivars (Marino et al. 2013). Regression results from the present study also suggest that while SVI and plant biomass are closely related, the relationship diminishes as biomass increases. This is evidenced by the parabolic nature of the data points for NDVI and OSAVI vs. plant biomass, especially in the case of Vaquero (Figure 3.15E and Figure 3.15F). A similar relationship between NDVI and leaf area index (LAI) was found by Gupta et al. (2000). Another study determined that this SVI saturation occurs at LAI values of 2.0 in onion (Bosch Serra and Cassanova 2000). Therefore, although LAI was not included in this study, it is reasonable to believe that LAI values in excess of 2.0 were attained within the field plots, as evidenced by the parabolic correlation between SVI and biomass.

It is important to note that a moderate to close relationship between SVI and disease rating value was observed with NDVI and OSAVI at the leaf level in 2018. However, this relationship is likely coincidental, and only occurred because NDVI and OSAVI steadily decreased over time (Figure 3.5 and Figure 3.6), whereas disease rating values steadily increased over time (Table 2.3). Further evidence for the inaccuracy of this relationship was the direct relationship between SVI and disease rating value observed in 2019 (Figure 3.16). Since all three indices are positively related to plant health, it is unreasonable to believe that they are also positively related to the manifestation of disease. Rather, these false relationships likely arose because all measurements (taken at different times and thus different biomasses) were included in the regression analyses.

3.5.4 Evaluating the usefulness of SVIs to diagnose onion pink root

Remote sensing been successfully used to diagnose soilborne disease in other canopy-level studies. Hillnhutter et al. (2011) identified 5 narrowband SVIs that they used to distinguish between sugar beet (*Beta vulgaris*) root damage caused by *Heterodera schachtii* and *Rhizoctonia solani* with a classification accuracy of 64 and 72% for two different sources of hyperspectral data. Yang et al. (2010) used hyperspectral imaging to map incidence of cotton root rot (causal agent *Phymatotrichum onmivorum*) in a commercial cotton field with and accuracy greater than 90%. There are two characteristics of these studies that may have contributed to their success compared to the present study. First, both crops are capable of full canopy closure and were measured at growth stages at which crop foliage was fully grown (the month before cotton harvest and sugar beet growth stage 31). Second, disease symptoms develop rapidly in infected plants, and thus significantly affect crop reflectance. In the case of onion pink root, remote sensing is a challenge because the crop canopy does not completely cover the soil, even when grown under optimal conditions. Therefore, reflectance spectra (and SVI value) are predominantly determined by LAI. Another challenge is the fact that disease symptoms are relatively gradual in their progression. For example, results from Chapter 2 suggest that severe levels of pink root affect the plant biomass of a moderately tolerant cultivar (SV4643NT) in a manner similar to that of a moderate and sustained irrigation reduction. The use of SVIs to diagnose plant disease offers several advantages, including a simplified analysis of hyperspectral data and a reduction in data dimensionality, but the disadvantages of this method (overwhelming sensitivity to LAI as opposed to other vegetation parameters such as foliar pigment composition) are realized in cases where the crop canopy (even under optimal conditions) does not completely cover the soil.

Onion pink root significantly decreased the plant biomass of SV4643NT onions in 2019 compared to a fumigated control. As a result, TVI, NDVI, and OSAVI were also significantly reduced, likely because of the close relationship between plant biomass and LAI. A reduction in plant biomass is a general response to plant stress, as evidenced by the observed biomass reduction in all stress treatments in 2019 (except Vaquero high disease). As referenced by Dashti et al. (2019) in regard to the challenges associated with remote sensing studies at the plot and canopy scale, “a relation, obtained by statistically correlating remote sensing measurements and field observations, is useful only for those locations and times other than those used to establish the correlation (Verstraete et al. 1996).” Remote sensing relationships developed in scientific studies are only useful if they can be successfully applied to real life field scenarios. A similar warning against an overdependence on SVIs is issued by Jones and Vaughan (2010) in stating that changes in particular SVIs, which are primarily related to changes in vegetation fraction, have been used to quantify plant stresses as different as drought, salt damage, pollutant damage, disease, and insect infestation. It is possible for remote sensing relationships developed under a limited range of environmental conditions to successfully distinguish plant stress and yet be largely inapplicable to real life scenarios. In order to map pink root “hot spots” within a commercial onion field in which an array of abiotic or biotic stressors may be present, a unique spectral symptom must first be identified.

3.5.5. Accounting for the influence of LAI on reflectance spectra and SVI

The similar relative response of the 40 analyzed SVIs to the imposed stress treatments suggests that the majority of them are predominantly influenced by LAI. However, each SVI represents a unique wavelength ratio, and is not necessarily influenced by LAI to the same degree. One way to account for the influence of a single vegetation parameter on an SVI is to use mathematical modeling. By employing such an approach, one study suggests that soil, LAI, leaf angle, and soil-LAI interactions, respectively, account for 0.97, 96.33, 0.89, and 0.78% of NDVI variation whereas these parameters account for 0.06, 97.37, 1.74, an 0.15% of OSAVI variation in scenarios where clay and sandy soil is present (Rondeaux et al. 1996). Therefore, so called “soil-adjusted” vegetation indices (i.e. OSAVI) are advantageous because they reduce the influence of soil brightness parameters relative to non-adjusted SVIs (i.e. NDVI). Likewise, Daughtry et al. (2000) determined that LAI, chlorophyll, and chlorophyll-LAI interactions accounted, respectively, for 60, 27, and 13% of variation in the modified chlorophyll absorption reflectance index (MCARI). Unfortunately, outside of these examples, the influence of individual vegetation parameters has not been widely researched and there remains ambiguity when interpreting SVI data.

The present study utilized a non-imaging hyperspectral sensor which measures the mean reflectance of the area determined by the field of view and target distance (Thomas et al. 2018). As a result, the acquired spectra (essentially a single pixel) is a linear combination of the spectra of plant and soil material (Jones and Vaughan 2010). One way to better understand this relationship is to utilize the pure spectrum of a plant and the pure spectrum of the soil as “end members” in what is called a linear mixing model, which outlines the influence of the fraction of plant and soil upon the resulting spectra. A linear mixing model of an onion foliage spectrum (acquired at the leaf-level) and a dry soil spectrum was generated to assist in understanding this relationship (Figure 3.17).

3.6. Conclusion

The aim of this study was to evaluate the usefulness of hyperspectral remote sensing as a tool to detect onion pink root in the field and discriminate its symptoms from those of nitrogen and water stress. In the field, crop stresses were successfully imposed and resulted in a decrease in plant biomass, total yield, and bulbs $\geq$ 8.4 cm, with the exception of the Vaquero high disease treatment. Although varying levels of pink root, nitrogen, and water stress symptoms were observed in the onion plots, results suggest that SVI variation was primarily the result of biomass (as it relates to LAI) differences among treatments.

3.7. Chapter 3 figures

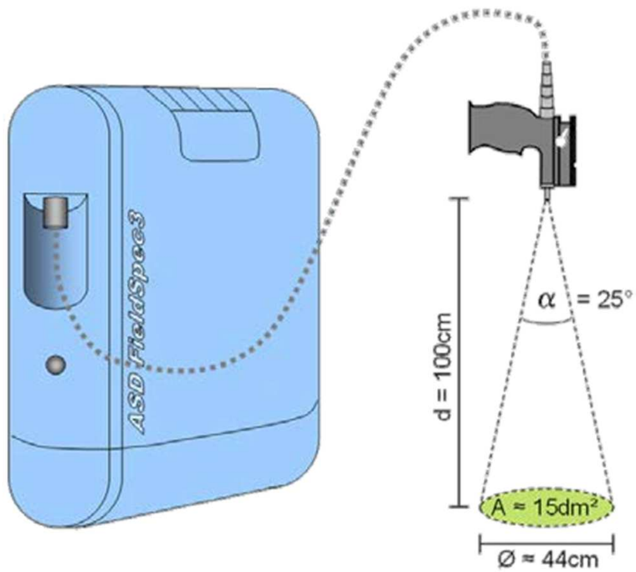

Figure 3.1. Diagrammatic outline of the field of view (FOV) geometry and technical arrangement of the ASD FieldSpec 4 (Adapted from Danner et al. 2015) utilized to collect in-field spectra at Parma, ID.

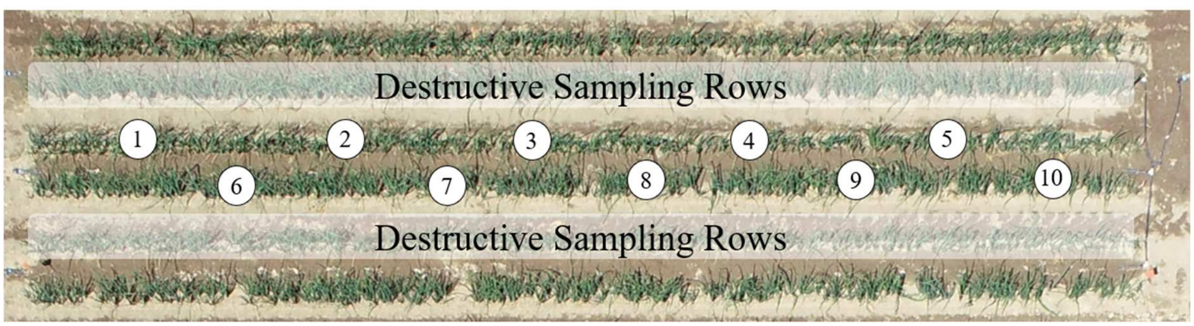

Figure 3.2. Outline of a 15.24 m by 3.35 m experimental plot from above superimposed with descriptions of how each portion was utilized. The middle bed was undisturbed throughout the growing season and was measured with a spectroradiometer at ten locations similar to those outlined in the numbered white circles. The double rows on either side of the middle data bed were destructively sampled for the collection of ground reference data. Unlabeled rows served as borders and were not sampled nor analyzed.

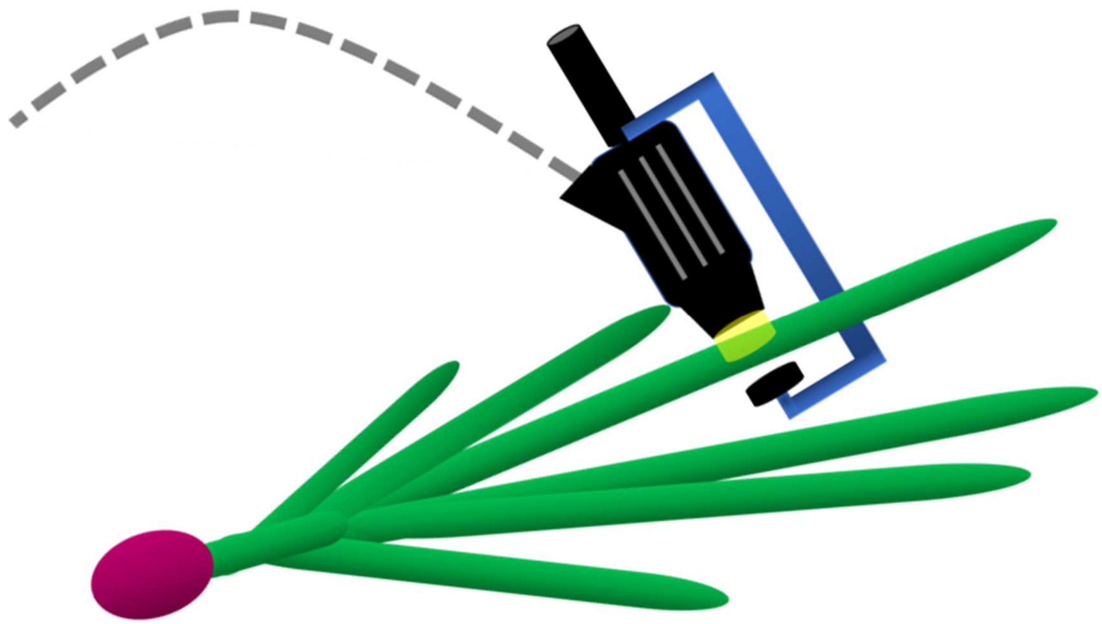

Figure 3.3. Diagrammatic outline of a leaf-level measurement collected on the dorsal surface of the newest fully-grown leaf using an ASD plant probe with a leaf clip attachment.

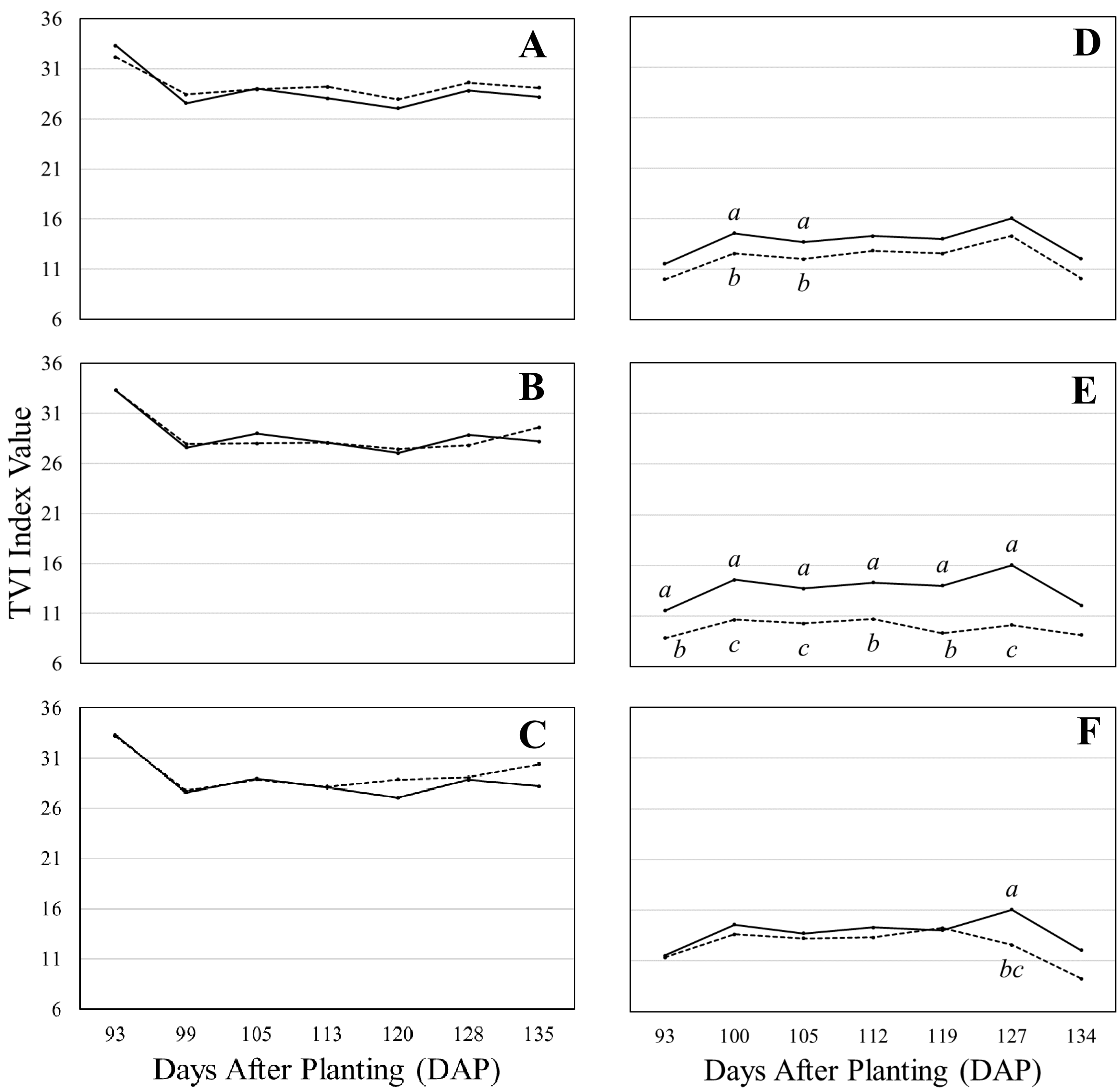

Figure 3.4. Effect of disease, nitrogen and water stress on TVI value over time for SV4643NT onions measured at the leaf and canopy-level in 2018. Leaf-level values are the mean of 5 replicates of 5 plants. Canopy-level values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments at leaf-level. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at leaf-level. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments at leaf-level. (**D**) Comparison of low disease (solid) high disease (dashed) treatments at canopy-level. (**E**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at canopy-level. (**F**) Comparison of low disease (solid) and reduced water (dashed) treatments at canopy-level. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

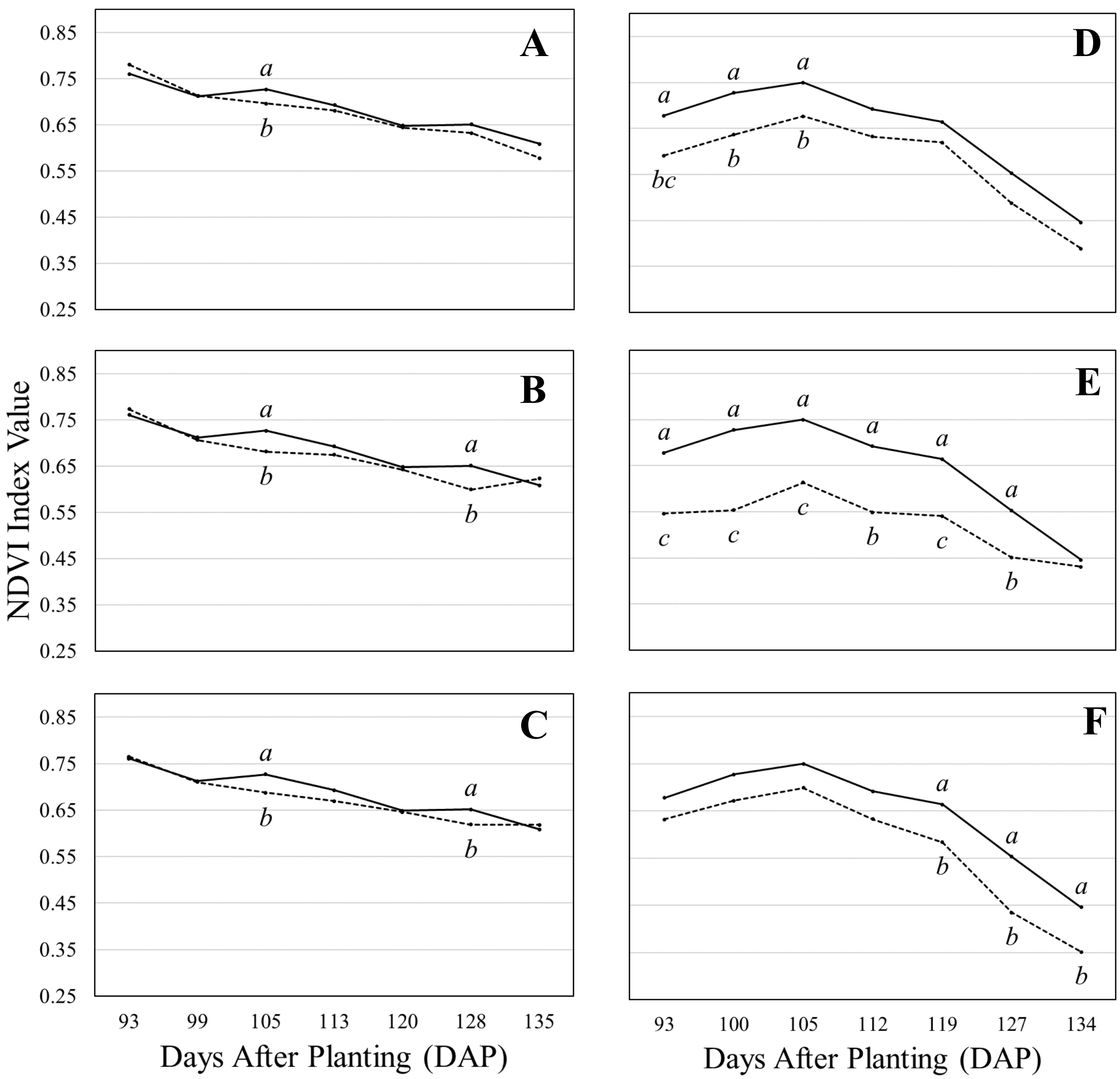

Figure 3.5. Effect of disease, nitrogen and water stress on NDVI value over time for SV4643NT onions measured at the leaf and canopy-level in 2018. Leaf-level values are the mean of 5 replicates of 5 plants. Canopy-level values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments at leaf-level. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at leaf-level. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments at leaf-level. (**D**) Comparison of low disease (solid) high disease (dashed) treatments at canopy-level. (**E**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at canopy-level. (**F**) Comparison of low disease (solid) and reduced water (dashed) treatments at canopy-level. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

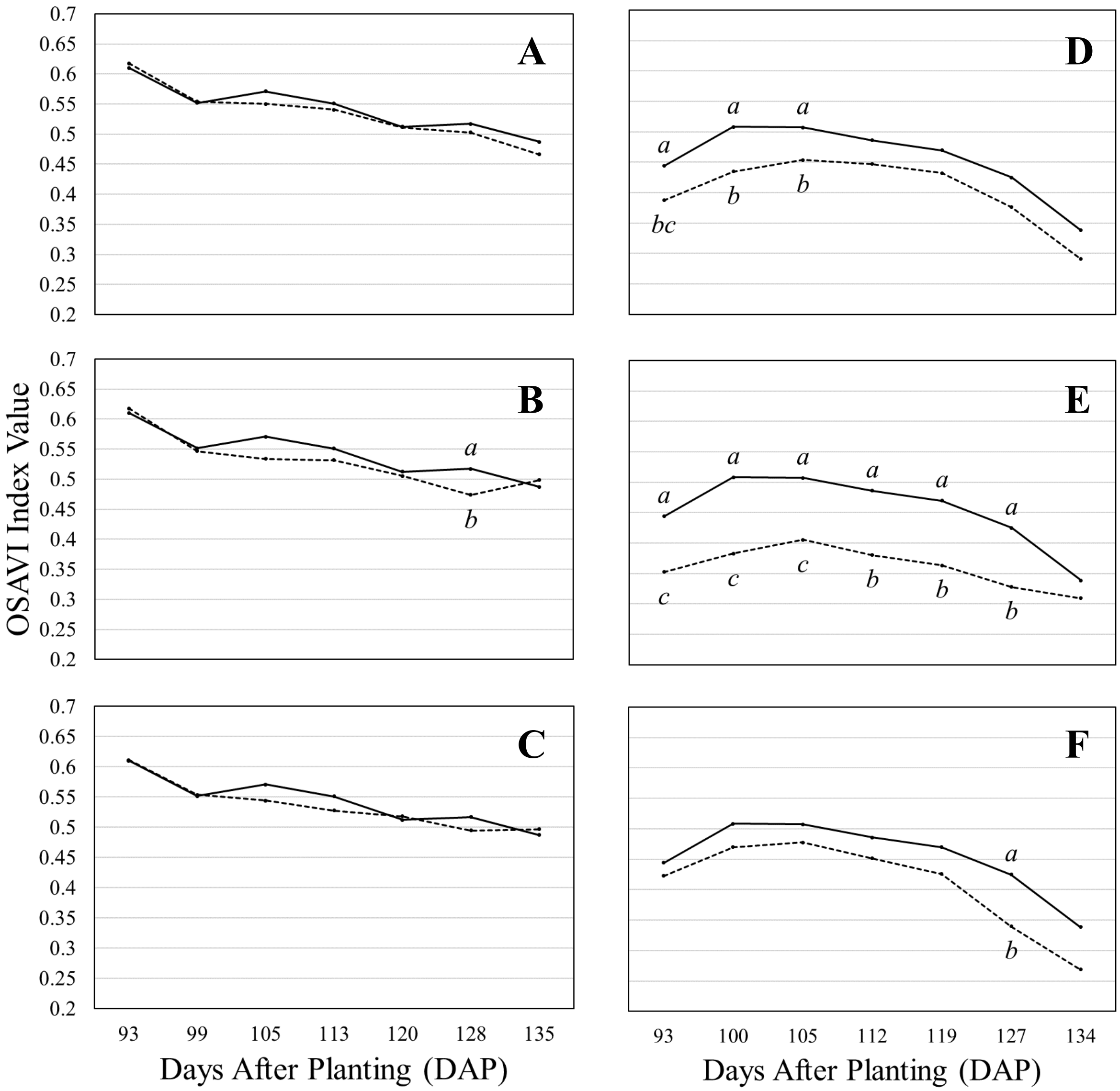

Figure 3.6. Effect of disease, nitrogen and water stress on OSAVI value over time for SV4643NT onions measured at the leaf and canopy-level in 2018. Leaf-level values are the mean of 5 replicates of 5 plants. Canopy-level values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments at leaf-level. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at leaf-level. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments at leaf-level. (**D**) Comparison of low disease (solid) high disease (dashed) treatments at canopy-level. (**E**) Comparison of low disease (solid) and non-fertilized (dashed) treatments at canopy-level. (**F**) Comparison of low disease (solid) and reduced water (dashed) treatments at canopy-level. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

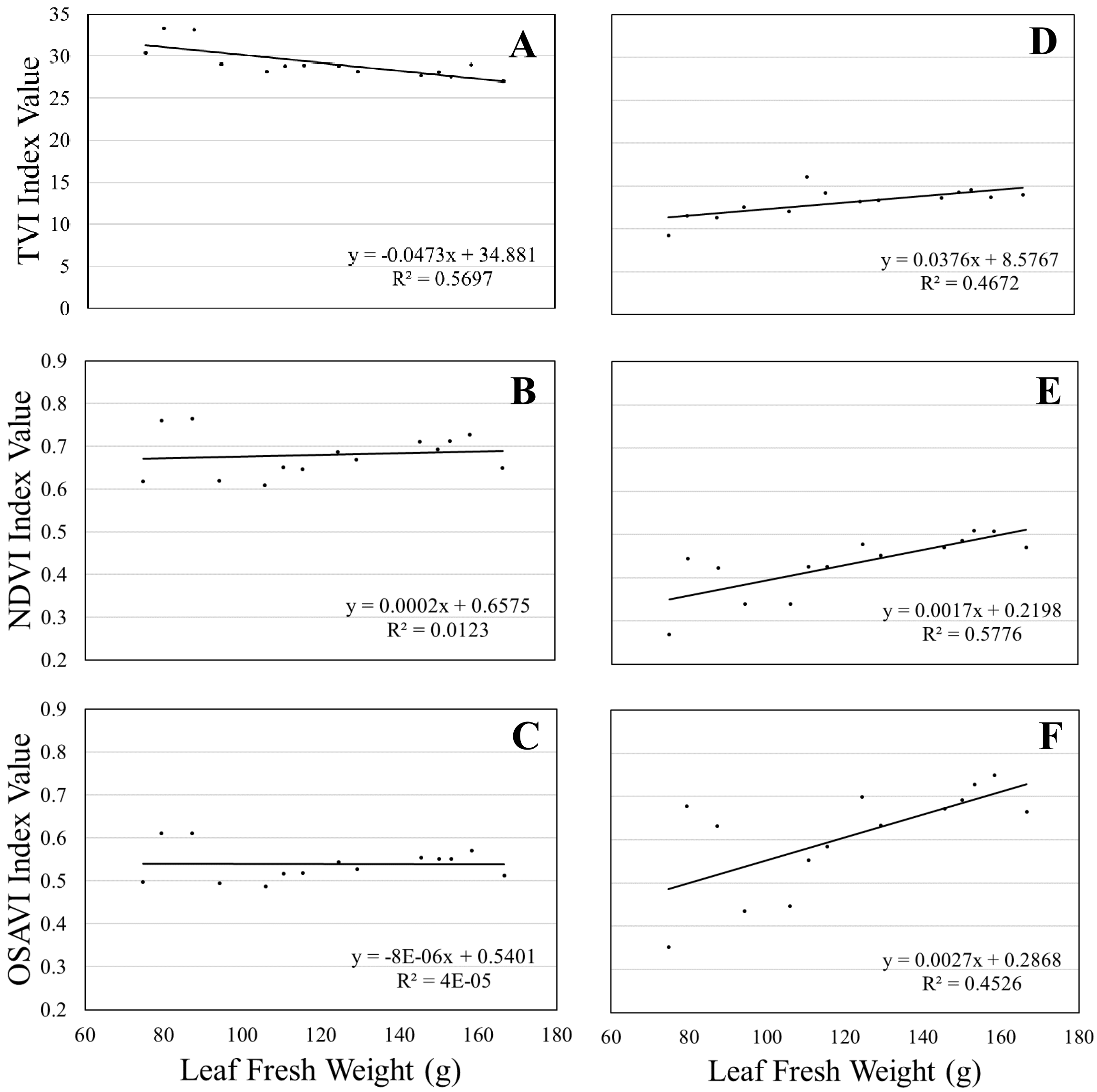

Figure 3.7. Regression analysis of TVI, NDVI, and OSAVI and leaf biomass (g) of only the low disease and reduced water treatment SV4643NT onions measured at the leaf and canopy-level in 2018. (**A**) Leaf-level TVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14. (**B**) Leaf-level NDVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14. (**C**) Leaf-level OSAVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14. (**D**) Canopy-level TVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14. (**E**) Canopy-level NDVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14. (**F**) Canopy-level OSAVI values vs. leaf biomass of low disease and reduced water treatment at all measurements, n=14.

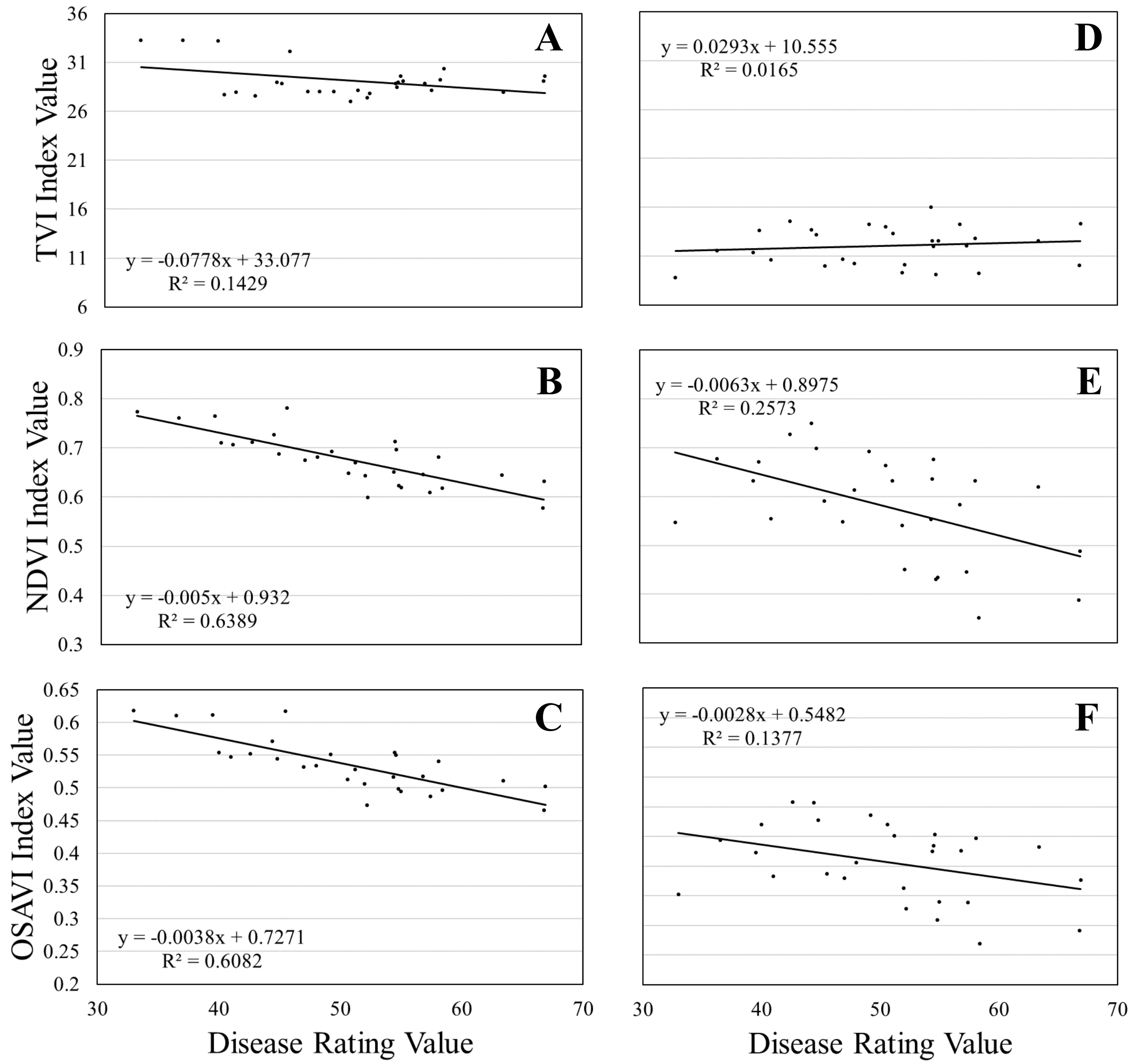

Figure 3.8. Regression analysis of TVI, NDVI, and OSAVI values and disease rating values (unitless) of all treatments of SV4643NT onions measured at the leaf and canopy-level in 2018. (**A**) Leaf-level TVI values vs. disease rating value of all treatments at all measurements, n=32. (**B**) Leaf-level NDVI values vs. disease rating value of all treatments at all measurements, n=32. (**C**) Leaf-level OSAVI values vs. disease rating value of all treatments at all measurements, n=32. (**D**) Canopy-level TVI values vs. disease rating value of all treatments at all measurements, n=24. (**E**) Canopy-level NDVI values vs. disease rating value of all treatments at all measurements, n=24. (**F**) Canopy-level OSAVI values vs. disease rating value of all treatments at all measurements, n=24.

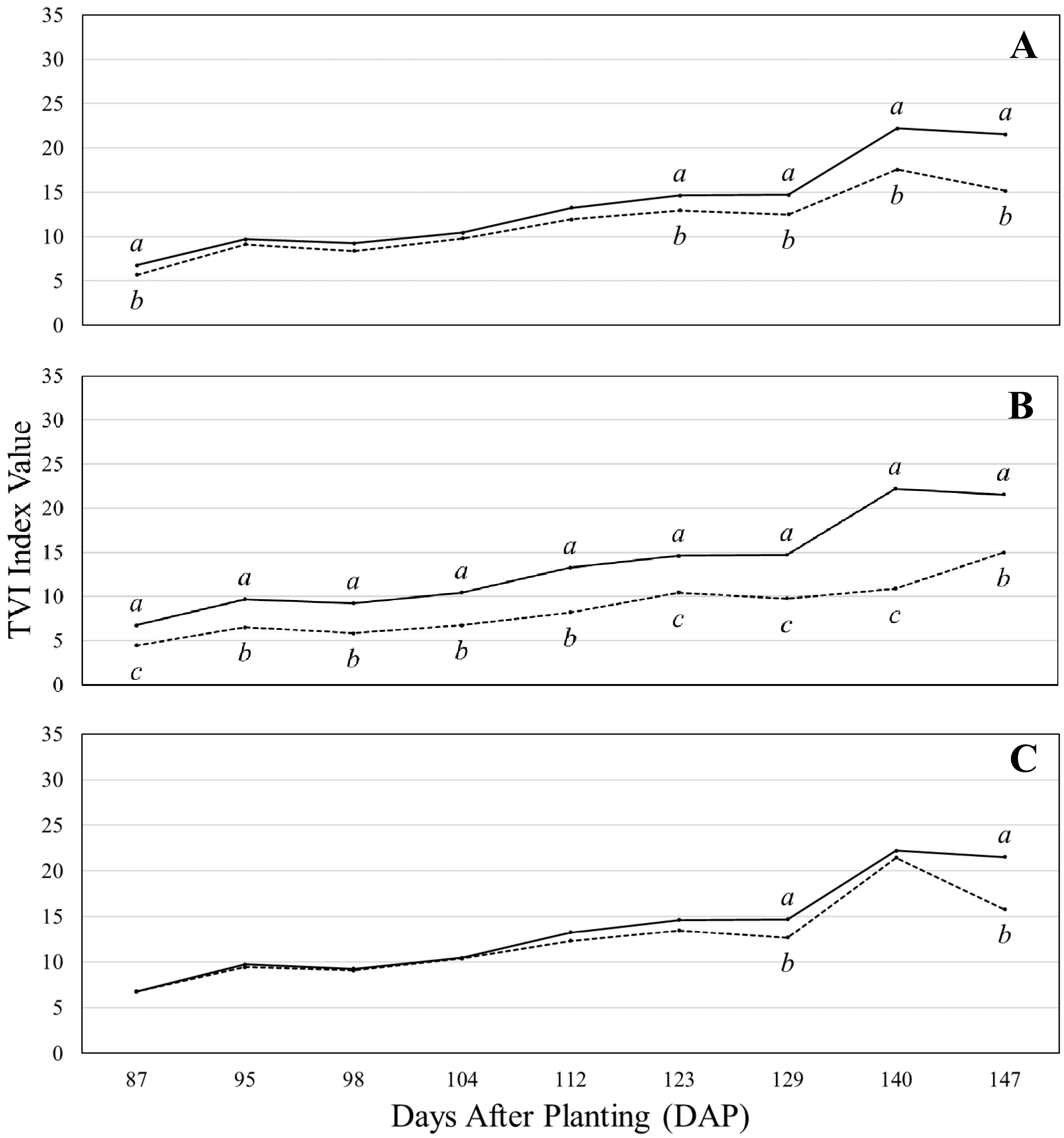

Figure 3.9. Effect of disease, nitrogen and water stress on TVI value over time for SV4643NT onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, P ≤ 0.05).

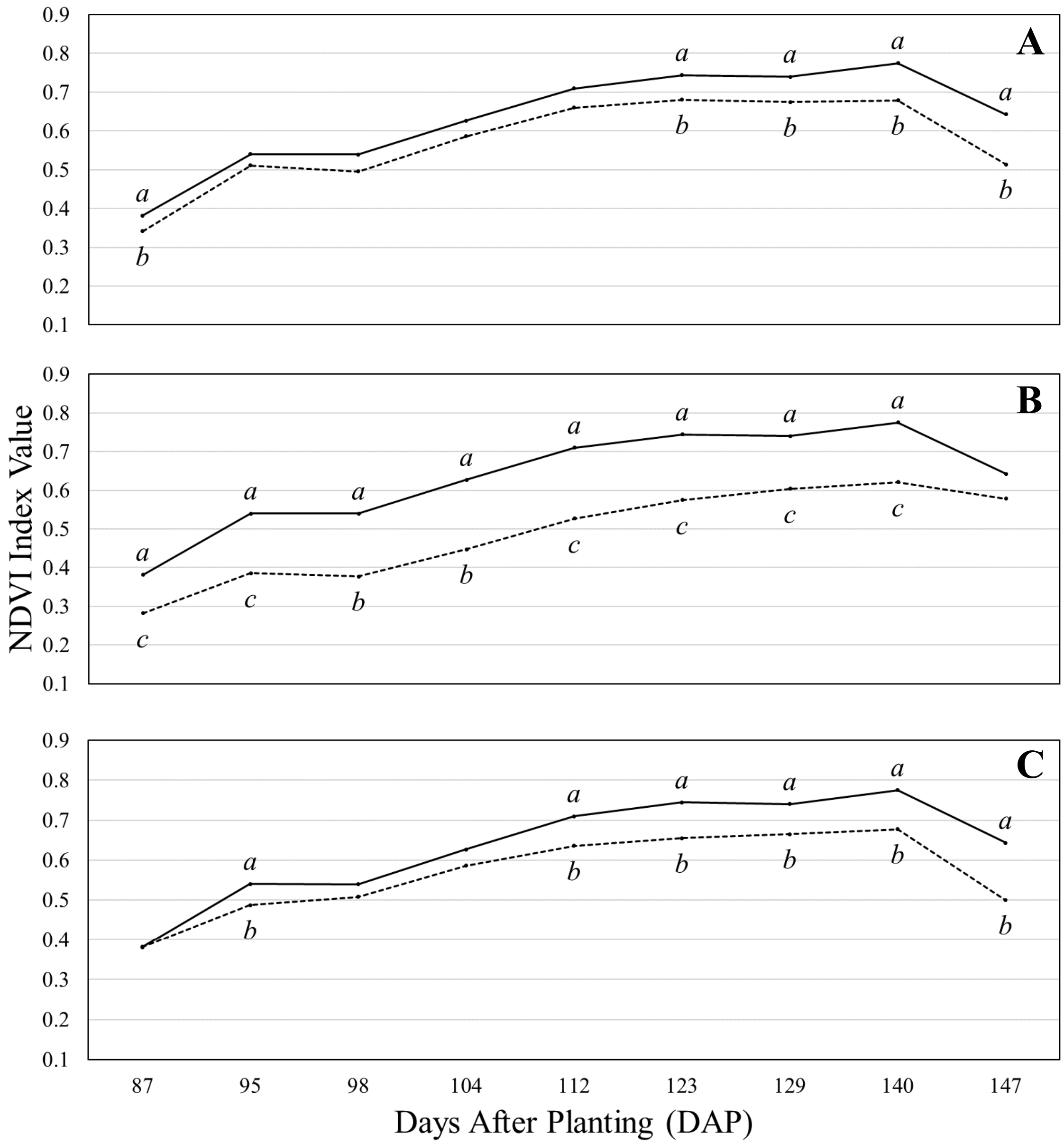

Figure 3.10. Effect of disease, nitrogen and water stress on NDVI value over time for SV4643NT onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

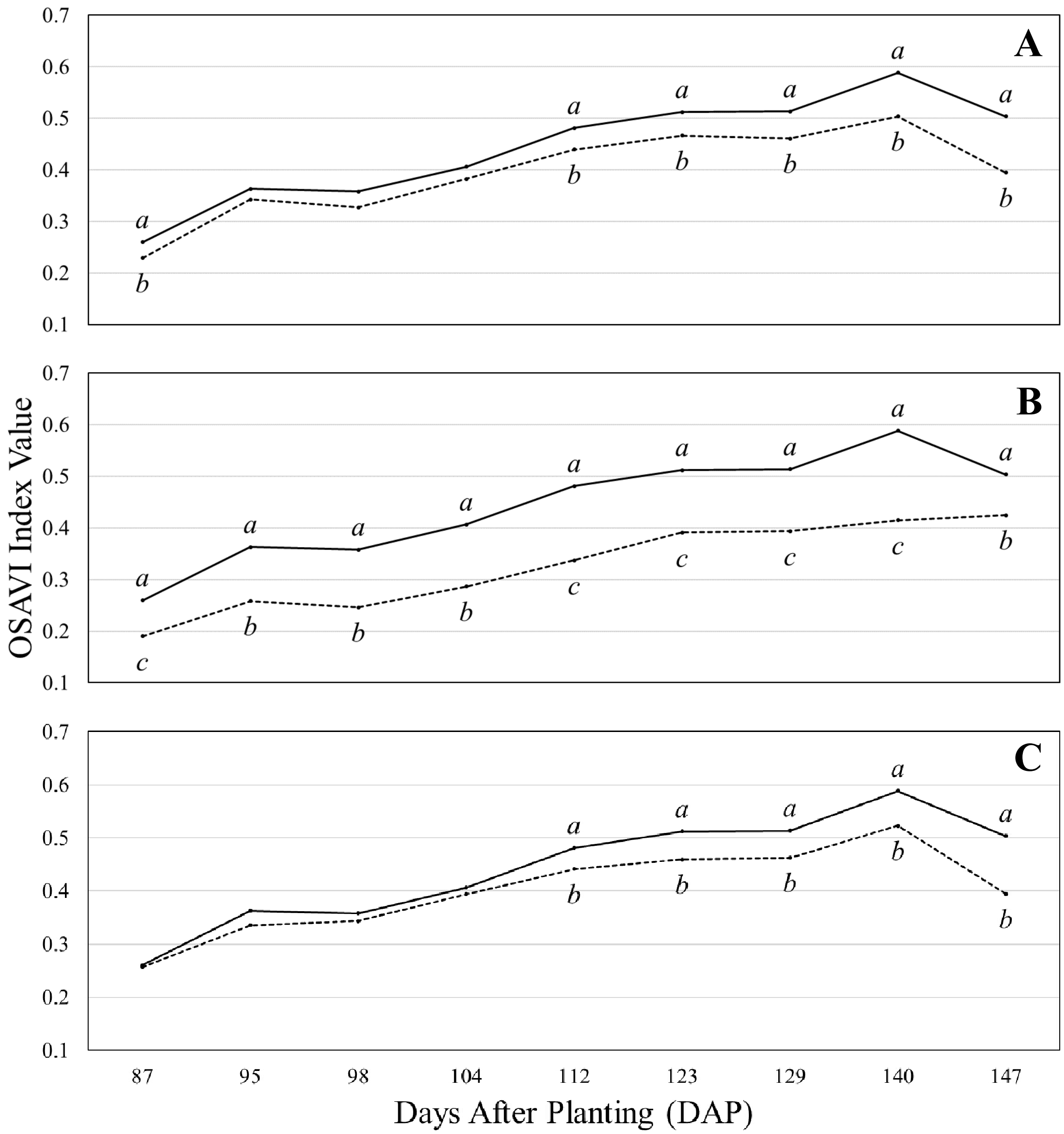

Figure 3.11. Effect of disease, nitrogen and water stress on OSAVI value over time for SV4643NT onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, P ≤ 0.05).

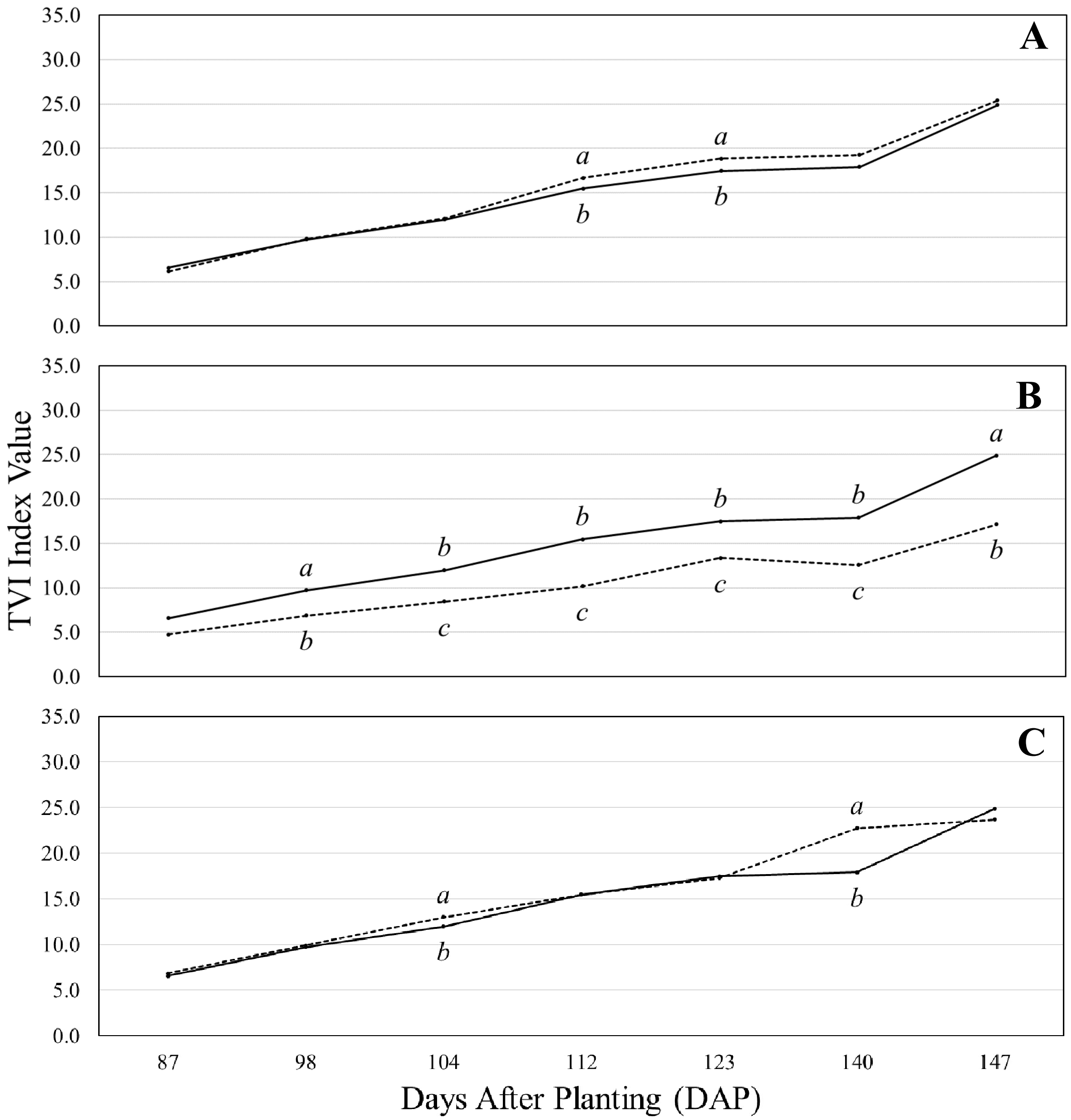

Figure 3.12. Effect of disease, nitrogen and water stress on TVI value over time for Vaquero onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

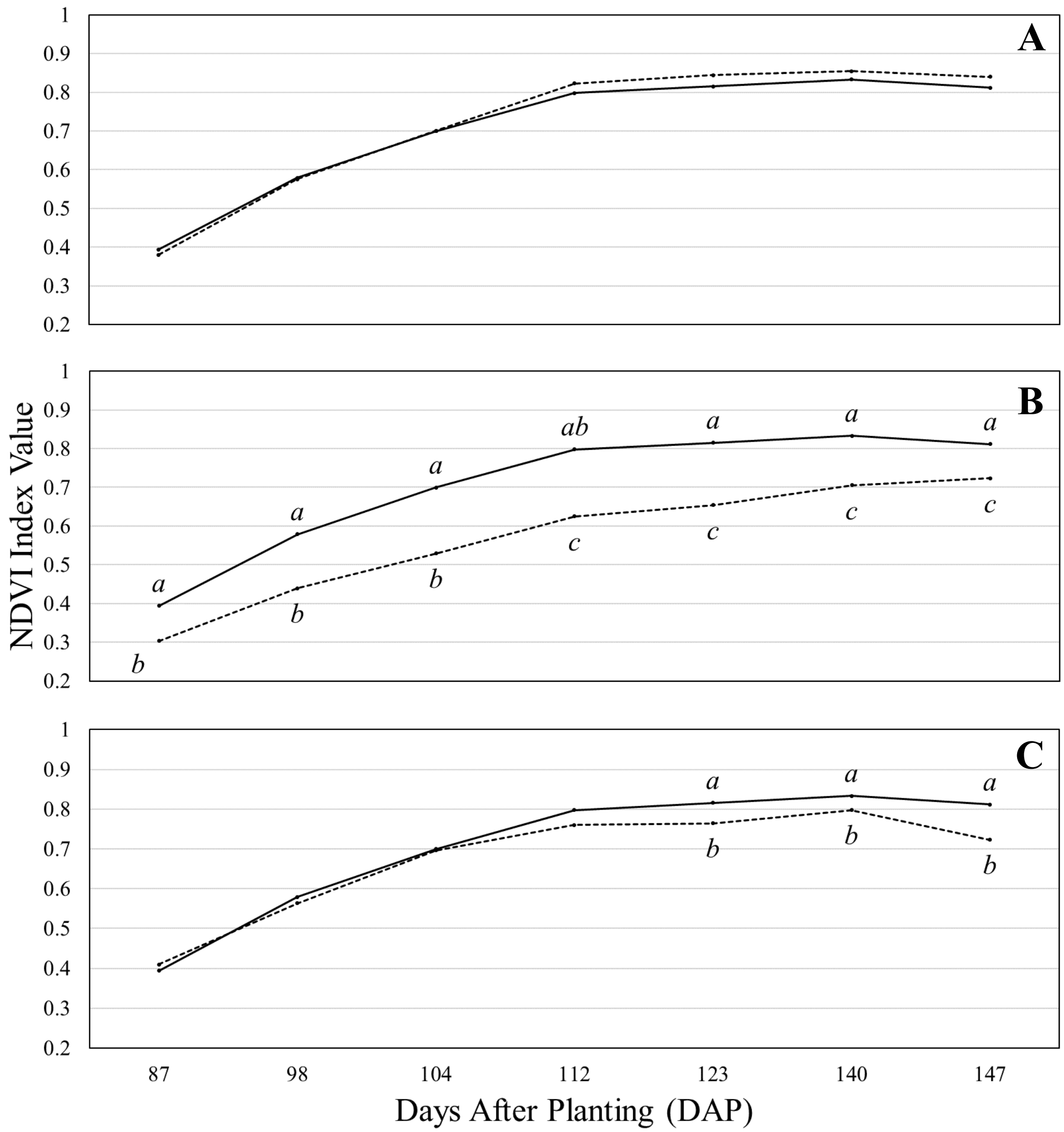

Figure 3.13. Effect of disease, nitrogen and water stress on NDVI value over time for Vaquero onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, $P \leq 0.05$).

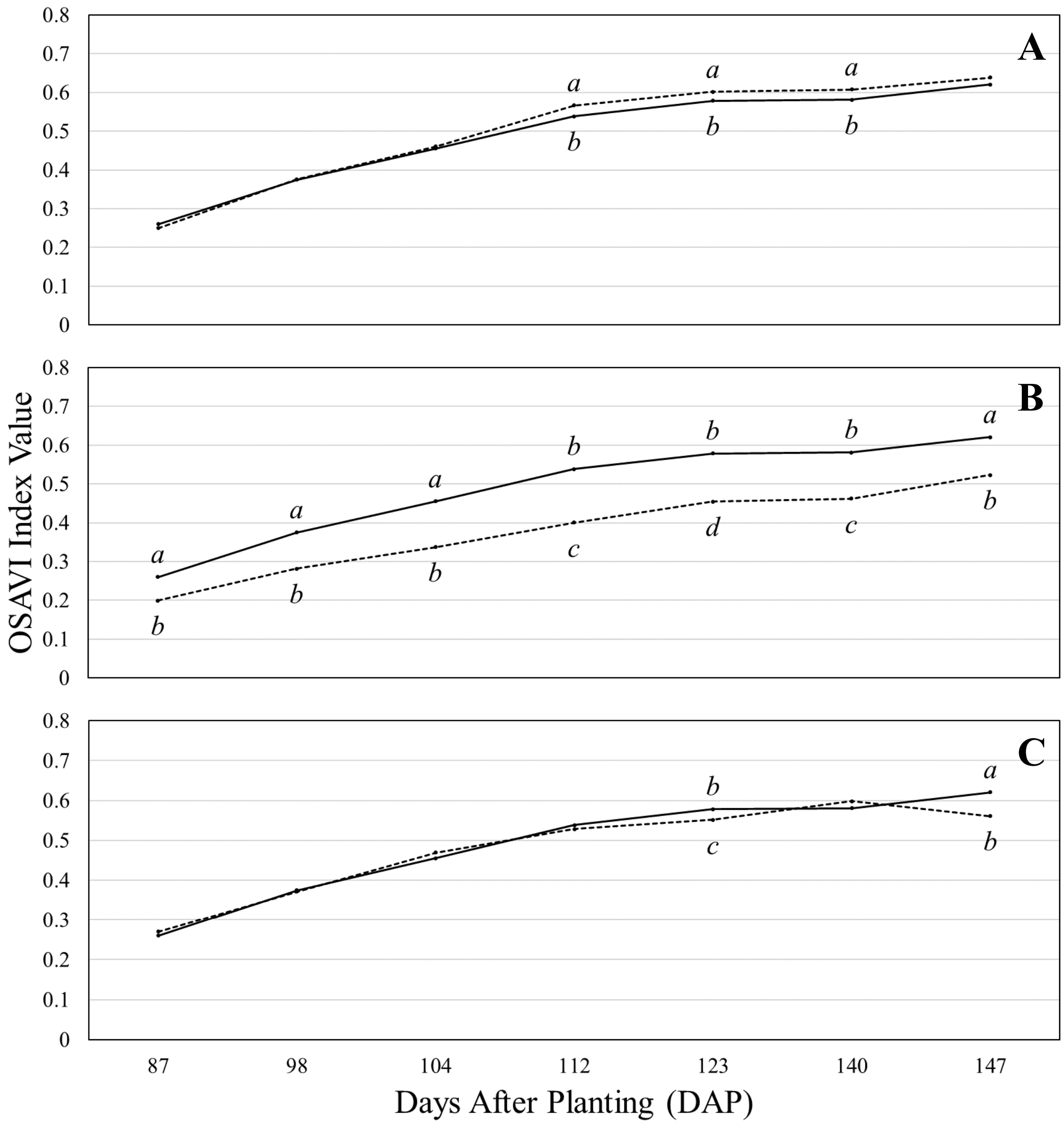

Figure 3.14. Effect of disease, nitrogen and water stress on OSAVI value over time for Vaquero onions measured at the canopy-level in 2019. Values are the mean of 5 replicates of 10 measurements. (**A**) Comparison of low disease (solid) high disease (dashed) treatments. (**B**) Comparison of low disease (solid) and non-fertilized (dashed) treatments. (**C**) Comparison of low disease (solid) and reduced water (dashed) treatments. Data points associated with different letters are statistically different (Fisher's protected LSD, P ≤ 0.05).

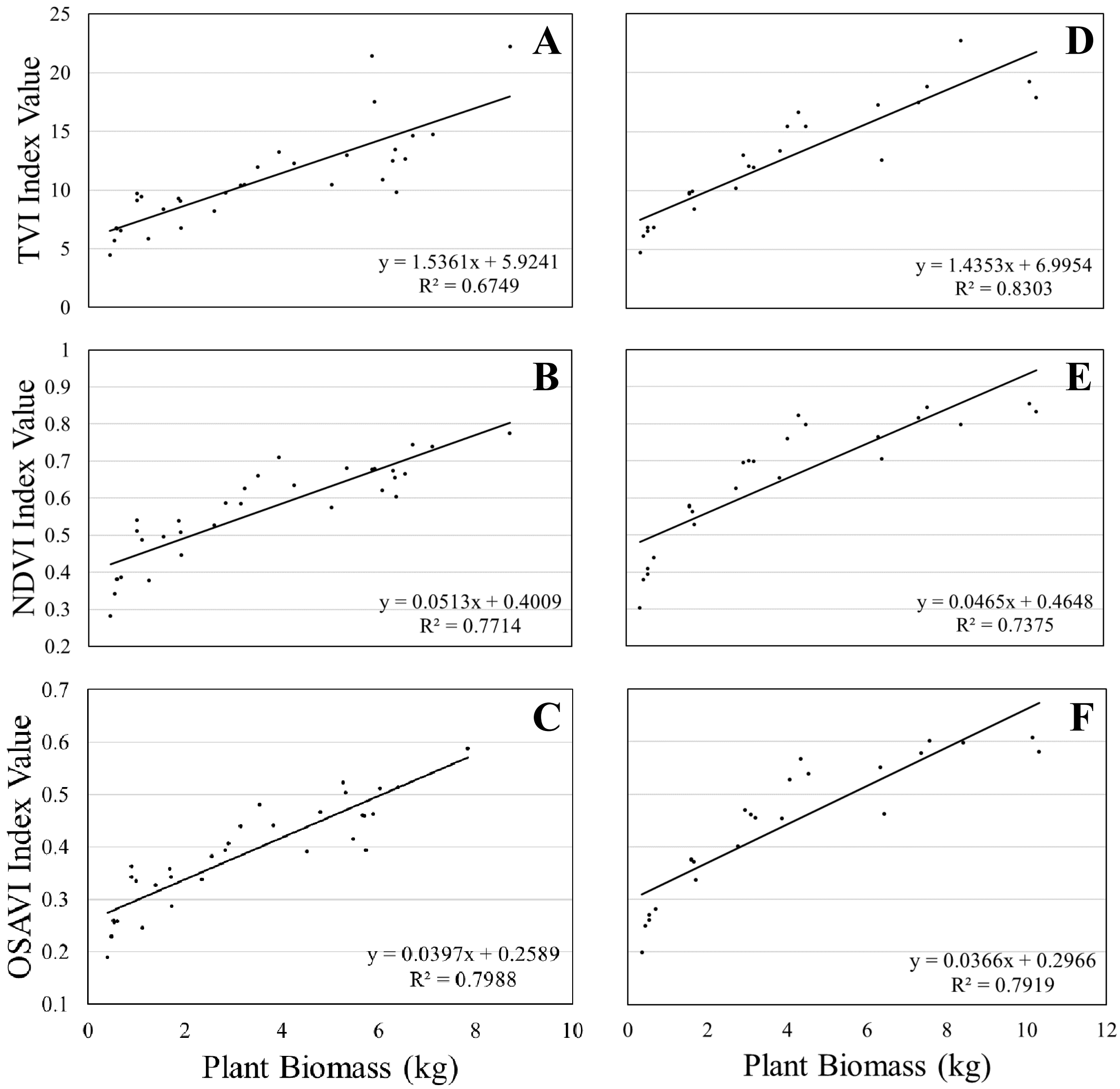

Figure 3.15. Regression analysis of TVI, NDVI, and OSAVI values and plant biomass (g) of all treatments of SV4643NT and Vaquero onions measured at canopy-level in 2019. (**A**) SV4643NT TVI values vs. plant biomass at all measurements, n=32. (**B**) SV4643NT NDVI values vs. plant biomass at all measurements, n=32. (**C**) SV4643NT OSAVI values vs. plant biomass at all measurements, n=32. (**D**) Vaquero TVI values vs. plant biomass at all measurements, n=24. (**E**) Vaquero NDVI values vs. plant biomass at all measurements, n=24. (**F**) Vaquero OSAVI values vs. plant biomass at all measurements, n=24.

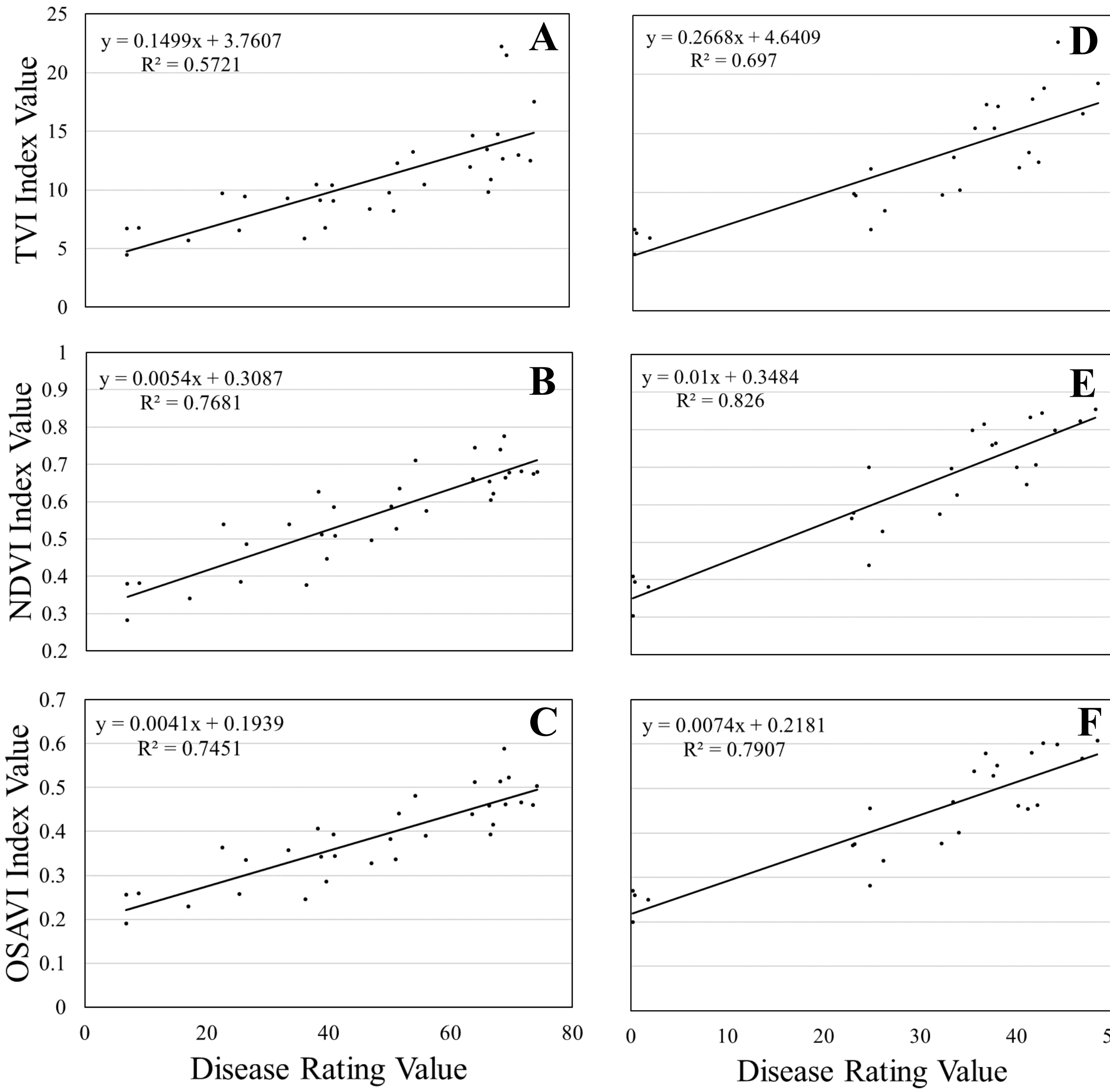

Figure 3.16. Regression analysis of TVI, NDVI, and OSAVI values and disease rating values (unitless) of all treatments of SV4643NT and Vaquero onions measured at the canopy-level in 2019. (**A**) TVI values vs. disease rating value of all SV4643NT treatments at all measurements, n=32. (**B**) NDVI values vs. disease rating value of all SV4643NT treatments at all measurements, n=32. (**C**) OSAVI values vs. disease rating value of all SV4643NT treatments at all measurements, n=32. (**D**) TVI values vs. disease rating value of all Vaquero treatments at all measurements, n=24. (**E**) NDVI values vs. disease rating value of all Vaquero treatments at all measurements, n=24. (**F**) OSAVI values vs. disease rating value of all Vaquero treatments at all measurements, n=24.

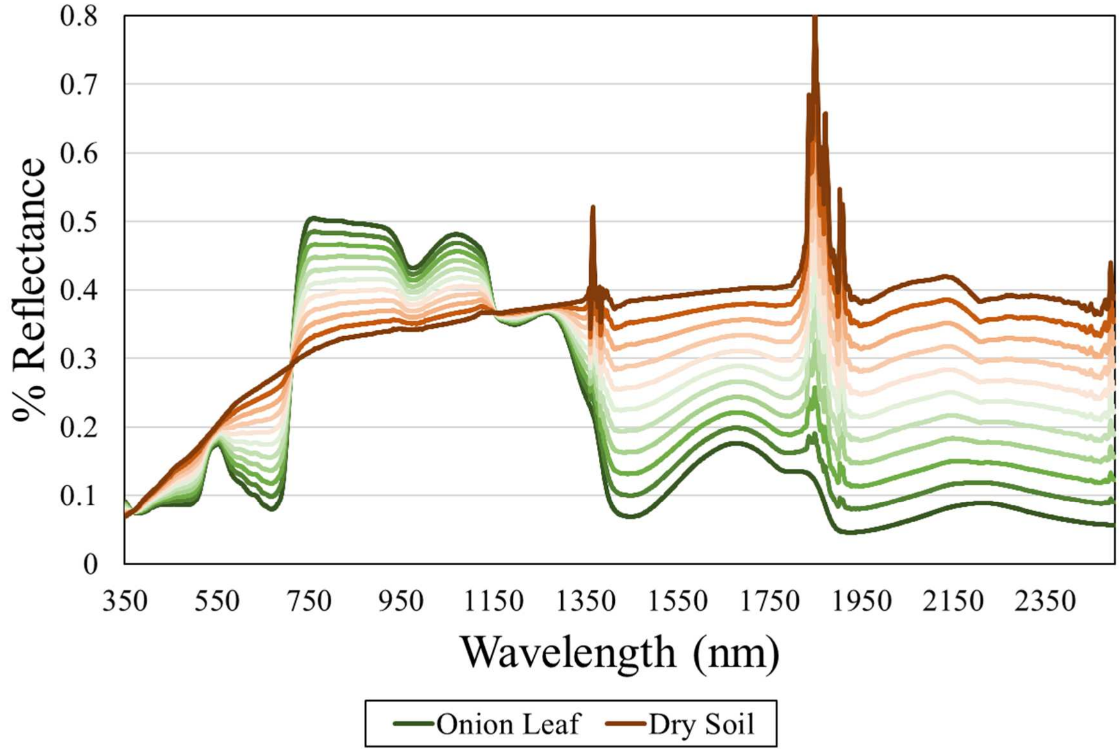

Figure 3.17. Linear mixing model of a pure SV4643NT onion spectrum and a pure spectrum of dry soil collected at Parma, ID in 2019. The dark green and dark brown spectra represent plant and soil, respectively. Lighter shades of color represent the mixing of plant and soil spectra in 10% increments. Green spectra are plant dominated (over 50% plant) whereas brown/tan spectra are soil dominated (over 50% soil). Noise in the pure soil spectra at 1350, 1800, and 2500 nm results from atmospheric water absorption and is an artifact of reflectance measurements collected under natural illumination.

3.8. Chapter 3 tables

Table 3.1. Specification for the ASD FieldSpec 4 Hi-Res Spectroradiometer used to collect reflectance data at Parma, ID.

Instrument Specifications	
Spectral Range	350-2500 nm
Spectral Resolution	3 nm at 700 nm
	8 nm at 1400 nm
	8 nm at 2100 nm
Sampling Interval	1.4 nm at 350-1000 nm
	2 nm at 1000-2500 nm
Wavelength Accuracy	± 1.0 nm for any one wavelength feature
	±0.5 nm across wavelength range

Table 3.2. Calendar dates, days after planting (DAP), and accumulated growing degree days (base 50 °F) since plant emergence (GDD_{50}) at the time of reflectance measurements of SV4643NT onions grown at Parma, ID in 2018.

Canopy-Level			
Measurement	**Date**	**DAP**	**GDD_{50}**
Canopy 1	6/29/2018	93	1108
Canopy 2	7/7/2018	100	1259
Canopy 3	7/12/2018	105	1371
Canopy 4	7/19/2018	112	1533
Canopy 5	7/26/2018	119	1688
Canopy 6	8/3/2018	127	1879
Canopy 7	8/9/2018	134	2007

Leaf-Level			
Measurement	**Date**	**DAP**	**GDD_{50}**
Leaf 1	6/29/2018	93	1108
Leaf 2	7/6/2018	99	1237
Leaf 3	7/12/2018	105	1371
Leaf 4	7/20/2018	113	1555
Leaf 5	7/27/2018	120	1712
Leaf 6	8/2/2018	128	1855
Leaf 7	8/10/2018	135	2030

Table 3.3. Calendar dates, days after planting (DAP), and accumulated growing degree days (base 50 °F) since plant emergence (GDD_{50}) at the time of reflectance measurements of SV4643NT and Vaquero onions grown at Parma, ID in 2019.

Canopy-Level			
Measurement	**Date**	**DAP**	**GDD_{50}**
Canopy 1	6/24/2019	87	846
Canopy 2	7/2/2019	95	991
Canopy 3	7/5/2019	98	1046
Canopy 4	7/11/2019	104	1166
Canopy 5	7/19/2019	112	1354
Canopy 6	7/30/2019	123	1585
Canopy 7	8/5/2019	129	1729
Canopy 8	8/16/2019	140	1962
Canopy 9	8/23/2019	147	2113

Leaf-Level			
Measurement	**Date**	**DAP**	**GDD_{50}**
Leaf 1	5/30/2019	62	418
Leaf 2	6/20/2019	83	801
Leaf 3	7/11/2019	104	1166
Leaf 4	8/8/2019	132	1801
Leaf 5	8/22/2019	146	2090

Chapter 4: Summary and Future Directions

4.1. Experiment summary

The ground reference results from both years suggest that measurable levels of disease, nitrogen and water stress were induced in the field plots. The most obvious of the three stresses was the non-fertilized treatment in which onions were significantly stunted throughout the growing season compared to those fertilized and were determined to have a significantly lower leaf nitrogen content. The high disease and reduced water treatment, on the other hand, only stunted plant development in the final weeks leading up to plant maturity, despite the fact that disease was present and soil water content was reduced much earlier in the season. The high disease, non-fertilized, and reduced water treatments, respectively, reduced total yield (relative to the low-disease treatment) 24, 15, and 32% for SV4643NT in 2018, 28, 25, and 22% for SV4643NT in 2019, and 0, 30, and 22% for Vaquero in 2019. In addition, the proportion of bulbs greater than 8.4 cm were significantly decreased in all treatments but the SV4643NT non-fertilized treatment in 2018 and the Vaquero high-disease treatment in 2019. Soil water tension results suggest that a slight drought stress was unintentionally induced in 2018 which may have contributed to the reduced total yield observed in 2018 compared to 2019.

Preliminary analysis of 40 published SVIs indicated that the relative relationship among treatments was conserved in nearly all of the analyzed SVIs. Three specific SVIs (TVI, NDVI, and OSAVI) were chosen for further analysis. Results suggest that plant biomass and SVI value are closely related, as evidenced by the high coefficient of determination values for SV4643NT (R^2 = 0.67 to 0.79) and Vaquero (R^2 = 0.73 to 0.83) in 2019. Other remote sensing studies suggest that although SVIs are influenced by several factors, vegetation parameters that account for the fraction of vegetation cover, such as LAI and biomass, are the primary influencer of SVI value. Therefore, each stress treatment yielded significantly lower SVI values relative to the low disease treatment (though not necessarily at the same time nor to the same degree) likely because of the reduction in biomass (and LAI) characterized in Chapter 2. Although there is an array of SVIs that each utilize a unique wavelength combination, many of them (particularly those which include a band in the NIR) are predominantly sensitive to changes in LAI, in scenarios where the crop canopy does not completely cover the soil. The inherent sensitivity of SVIs to a single vegetation parameter (LAI) overwhelms their potential response to other parameters, such as plant pigment composition (i.e. chlorophyll), except in scenarios where the crop canopy completely covers the soil. This characteristic of SVIs may render them useful for other purposes, like yield prediction (Jones and Vaughan 2010), but it also remains a challenge to their usefulness in onion stress diagnosis.

4.2. Future directions

The relatively low canopy cover of an onion crop is responsible for the overwhelming sensitivity of SVIs to changes in LAI. Although biomass (LAI) reduction from pink root did correspond to a decrease in TVI, NDVI, and OSAVI, a spectral change unique to onion pink root remains to be found. As a next step, alternative methods of spectra analysis should be employed to identify a unique spectral change that results from *S. terrestris* infection, if such a change exists. Potential methods include the RELIEF-F algorithm as described by Mahlein et al. (2013) or principal component analysis (PCA). Due to the gradual progression of pink root symptoms, it is reasonable to expect that a spectral disease indication will be subtle, and thus the leaf-level spectra (from which the influence of vegetation fraction and LAI is excluded) may present the best opportunity for further investigation. In addition, the canopy level experiment should be repeated in the future, since it is more applicable to production agriculture than leaf-level studies. Although biomass measurements did help indicate the source of SVI variation, they were not ideal to account for the fraction of vegetation present in each measurement. A better measure might be green canopy cover, which can be extracted from overhead red-green-blue (RGB) images of experimental plots (Ballesteros et al. 2014). If vegetation fraction can be accurately accounted for, some of the more subtle effects of stress upon the onion canopy reflectance might become measurable and assist in future efforts to diagnose and map onion pink root.

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Appendix A: Description and source of analyzed spectral vegetative indices used to evaluate onion response to crop stress

#	Index Name	Equation	Source
1	ARI	$(R_{550})^{-1} - (R_{700})^{-1}$	Gitelson et al. 2001
2	CTR2	R_{695} / R_{760}	Carter and Miller 1994
3	Curvature	$(R_{683})^2 / (R_{675} \text{ x } R_{691})$	Zarco-Tejada et al. 2001; Pontius et al. 2005
4	DSWI	$(R_{802} + R_{547}) / (R_{1657} + R_{682})$	Galvao et al. 2005
5	GI	R_{554} / R_{677}	Zarco-Tejada et al. 2005
6	GM 1	R_{750} / R_{550}	Gitelson and Merzlyak 1994; Gitelson et al. 1996
7	GM 2	R_{750} / R_{700}	Gitelson and Merzlyak 1994; Gitelson et al. 1996
8	GNDVI	$(R_{780} - R_{550}) / (R_{780} + R_{550})$	Gittelson et al. 1996; Babar et al. 2006
9	MCARI	$((R_{701} - R_{671}) - 0.2(R_{701} - R_{549})) / (R_{701} / R_{671})$	Daughtry et al. 2000
10	mNDVI_705	$(R_{750} - R_{705}) / (R_{750} + R_{705} - 2(R_{445}))$	Datt 1999; Sims and Gamon 2002
11	MSI	R_{1600} / R_{819}	Hunt and Rock 1989; Ceccato et al. 2002
12	MSR	$((R_{800} / R_{670}) - 1) / ((R_{800} / R_{670}) + 1)^{1/2}$	Chen 1996; Haboudane et al. 2004
13	MTVI	$1.2(1.2(R_{800} - R_{550}) - 2.5(R_{670} - R_{550}))$	Haboudane et al. 2004
14	NBNDVI	$(R_{850} - R_{680}) / (R_{850} + R_{680})$	Thenkabail et al. 2000
15	NDVI	$(R_{800} - R_{670}) / (R_{800} + R_{670})$	Rouse et al. 1974; Lichtenthaler e al. 1996
16	NPCI	$(R_{680} - R_{430}) / (R_{680} + R_{430})$	Penuelas et al. 1994; Devadas et al. 2009
17	NRI	$(R_{550} - R_{670}) / (R_{550} + R_{670})$	Filella et al. 1995
18	NSRI	R_{890} / R_{780}	Liu et al. 2014
19	NWI_1	$(R_{970} - R_{900}) / (R_{970} + R_{900})$	Babar et al. 2006
20	NWI_2	$(R_{970} - R_{850}) / (R_{970} + R_{850})$	Babar et al. 2006
21	ROSAVI	$(R_{800} - R_{670}) / (R_{800} + R_{670} + 0.16)$	Rondeaux et al. 1996
22	PhRI	$(R_{550} - R_{531}) / (R_{550} + R_{531})$	Gamon et al. 1992
23	PMI	$((R_{520} - R_{584}) / (R_{520} + R_{584})) + R_{724}$	Mahlein et al. 2013
24	PRI	$(R_{531} - R_{570}) / (R_{531} + R_{570})$	Gammon et al. 1992; Penuelas et al. 1994
25	PSRI	$(R_{680} - R_{500}) / R_{750}$	Merzlyak et al. 1999
26	RVI (800,450)	R_{800} / R_{450}	Yang et al. 2009
27	RVI (800,670)	R_{800} / R_{670}	Baret and Guyot 1991
28	RVI (950,450)	R_{950} / R_{450}	Yang et al. 2009
29	RVSI	$(R_{714} + R_{752}) / (2 - R_{733})$	Merton and Huntington 1999
30	SAVI	$1.7((R_{800} - R_{670}) / (R_{800} + R_{670} + 0.7)$	Huete 1988
31	SIPI	$(R_{800} - R_{445}) / (R_{800} + R_{680})$	Penuelas et al. 1995; Devadas et al. 2009
32	SIWSI	$(R_{860} - R_{1640}) / (R_{860} + R_{1640})$	Fensholt and Sandholt 2003
33	TCARI	$3(R_{700} - R_{670}) - 0.2(R_{700} - R_{550}) \text{ x } (R_{700} / R_{670})$	Haboudane et al. 2002
34	TVI	$0.5(120(R_{750} - R_{550}) - 200(R_{670} - R_{550}))$	Broge and Leblanc 2000
35	VARI	$(R_{550} - R_{670}) / (R_{550} + R_{670} - R_{480})$	Gitelson et al. 2002

36	Vogelmann 1	R_{740} / R_{720}	Vogelmann et al. 1993; Zarco-Tejada et al. 1999
37	Vogelmann 2	(R_{734} – R_{747}) / (R_{715} – R_{726})	Vogelmann et al. 1993; Zarco-Tejada et al. 1999
38	Vogelmann 3	(R_{734} – R_{747}) / (R_{715} – R_{720})	Vogelmann et al. 1993; Zarco-Tejada et al. 1999
39	WBI	R_{970} / R_{900}	Penuelas et al. 1995
40	WI	R_{900} / R_{970}	Penuelas et al. 1995

Appendix B: Average spectral vegetation index (SVI) value of SV4643NT treatments at the canopy level in 2018 (105 DAP)

Index	High Disease	Low Disease	Non-fertilized	Reduced Water
ARI	3.81	3.36	3.72	3.34
CTR2	0.25	0.20	0.31	0.24
Curvature	0.96	0.95	0.96	0.96
DSWI	2.22	2.78	1.89	2.33
GI	1.12	1.28	1.07	1.15
GM 1	4.19	4.91	3.56	4.32
GM 2	3.24	4.03	2.70	3.42
GNDVI	0.64	0.69	0.59	0.65
MCARI	0.01	0.01	0.02	0.02
mNDVI_705	0.58	0.64	0.51	0.60
MSI	0.32	0.25	0.38	0.30
MSR	1.68	2.11	1.38	1.76
MTVI	0.33	0.38	0.28	0.36
NBNDVI	0.67	0.75	0.61	0.69
NDVI	0.68	0.75	0.61	0.69
NPCI	0.30	0.24	0.34	0.28
NRI	0.05	0.10	0.03	0.06
NSRI	1.02	1.01	1.03	1.02
NWI_1	-0.13	-0.15	-0.11	-0.14
NWI_2	-0.13	-0.16	-0.11	-0.14
OSAVI	0.45	0.51	0.40	0.47
PhRI	0.06	0.06	0.06	0.06
PMI	0.06	0.07	0.05	0.07
PRI	0.05	0.04	0.06	0.05
PSRI	0.07	0.04	0.09	0.06
RVI (800,450)	8.40	10.08	7.17	8.61
RVI (800,670)	5.17	6.97	4.12	5.50
RVI (950,450)	7.13	8.19	6.24	7.22
RVSI	-0.01	-0.01	-0.02	-0.01
SAVI	0.37	0.41	0.32	0.39
SIPI	1.10	1.06	1.15	1.09
SIWSI	0.49	0.57	0.42	0.51
TCARI	0.06	0.06	0.06	0.06
TVI	12.03	13.63	10.42	13.09

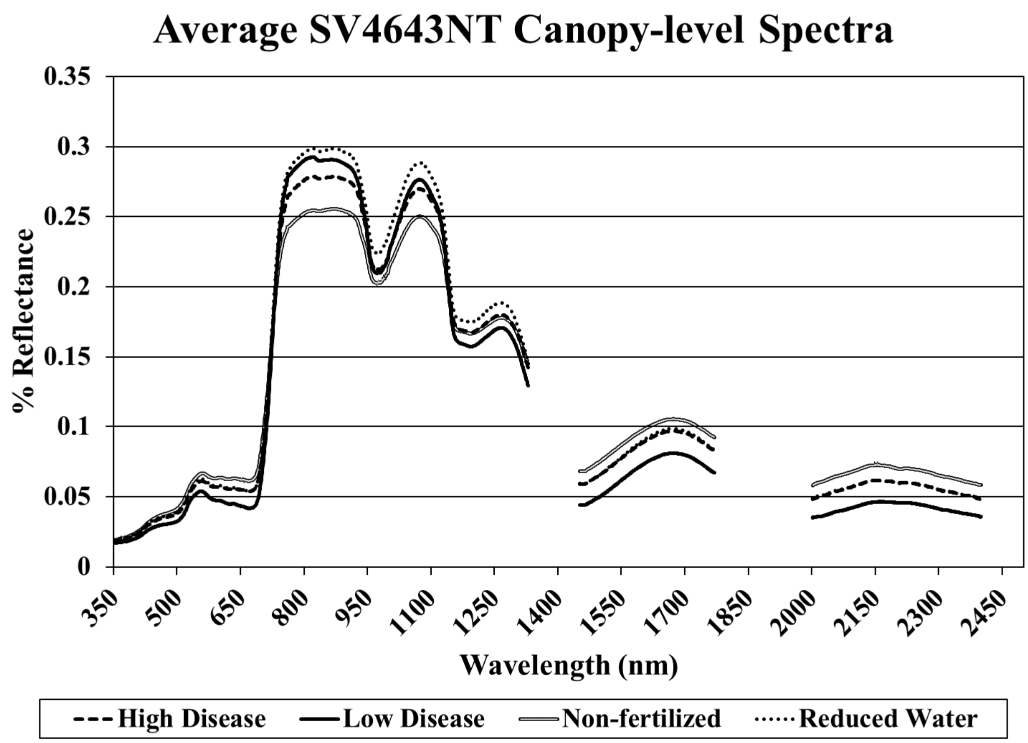
VARI	0.08	0.18	0.05	0.10
Vogelmann 1	1.57	1.70	1.45	1.61
Vogelmann 2	-0.13	-0.17	-0.10	-0.15
Volgelmann 3	-0.15	-0.19	-0.11	-0.16
WBI	0.77	0.73	0.80	0.76
WI	1.30	1.37	1.25	1.32

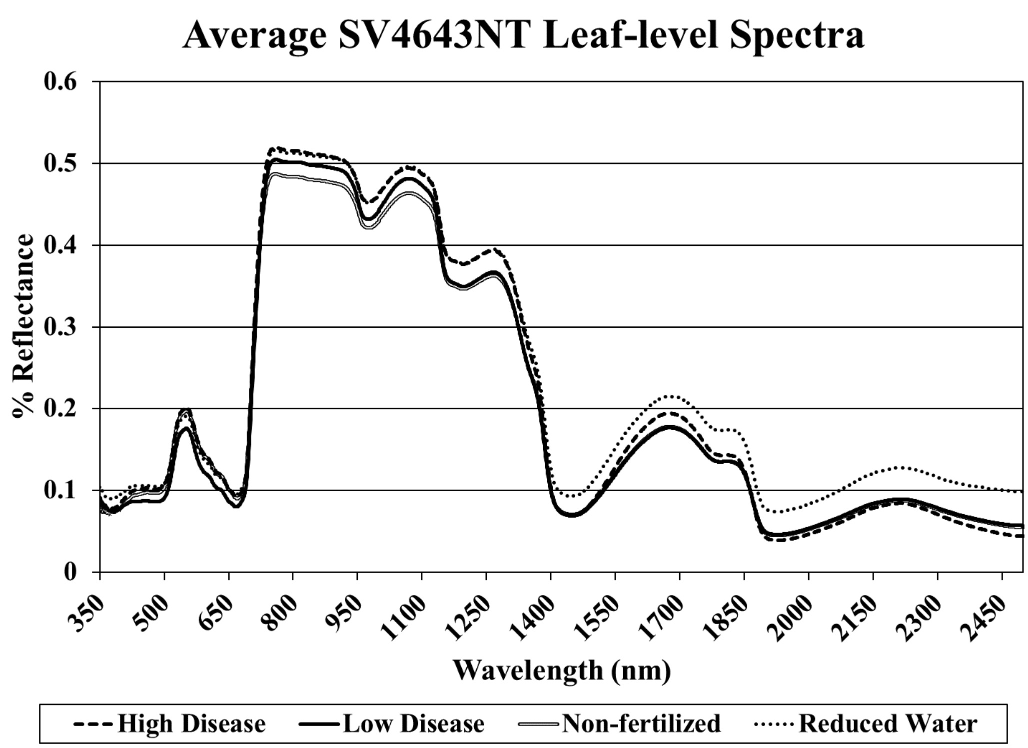
Appendix C: Average spectral vegetation index (SVI) value of SV4643NT treatments at the leaf level in 2018 (105 DAP)

Index	High Disease	Low Disease	Non-fertilized	Reduced Water
ARI	0.05	0.04	-0.02	0.15
CTR2	0.29	0.26	0.31	0.29
Curvature	0.94	0.96	0.93	0.96
DSWI	2.40	2.58	2.49	2.22
GI	2.08	2.10	2.13	1.93
GM 1	2.58	2.86	2.47	2.70
GM 2	2.55	2.84	2.48	2.62
GNDVI	0.44	0.48	0.42	0.46
MCARI	0.05	0.04	0.05	0.05
mNDVI_705	0.45	0.49	0.44	0.48
MSI	0.33	0.30	0.32	0.37
MSR	1.77	1.96	1.73	1.74
MTVI	0.76	0.76	0.73	0.75
NBNDVI	0.68	0.71	0.67	0.67
NDVI	0.69	0.73	0.69	0.69
NPCI	0.00	0.00	0.01	-0.02
NRI	0.31	0.31	0.32	0.28
NSRI	0.98	0.98	0.98	0.99
NWI_1	-0.05	-0.07	-0.06	-0.06
NWI_2	-0.06	-0.07	-0.06	-0.06
OSAVI	0.55	0.57	0.54	0.54
PhRI	0.04	0.04	0.04	0.04
PMI	0.41	0.41	0.40	0.42
PRI	-0.02	-0.03	-0.02	-0.03
PSRI	-0.02	-0.01	-0.02	-0.01
RVI (800,450)	5.10	5.79	5.02	4.85
RVI (800,670)	5.51	6.28	5.38	5.39
RVI (950,450)	4.72	5.28	4.61	4.49
RVSI	-0.05	-0.06	-0.05	-0.05
SAVI	0.54	0.56	0.53	0.54
SIPI	1.00	1.00	1.00	0.99
SIWSI	0.45	0.49	0.47	0.42
TCARI	0.32	0.29	0.32	0.30
TVI	29.05	29.15	28.01	28.98

VARI	0.55	0.56	0.56	0.53
Vogelmann 1	1.26	1.30	1.25	1.28
Vogelmann 2	-0.04	-0.04	-0.04	-0.04
Volgelmann 3	-0.04	-0.05	-0.04	-0.04
WBI	0.90	0.88	0.89	0.90
WI	1.11	1.14	1.13	1.12

Appendix D: Average SV4643NT treatment spectra from 105 DAP in 2018





Appendix E: 2018 remote sensing plot map

Plot Designation (i.e. R101):

Trial Letter (R= remote sensing), Replicate number, Treatment Number

Treatments

01) No fumigant + Regular nitrogen + Regular irrigation (high disease)

02) Chloropicrin @ 37.4 L ha^{-1} + Regular nitrogen + Regular irrigation (low disease)

03) Chloropicrin @ 37.4 L ha^{-1} + No in-season nitrogen + Regular irrigation (non-fertilized)

04) Chloropicrin @ 37.4 L ha^{-1} + Regular nitrogen + Regular irrigation (reduced water)

Appendix F: 2019 remote sensing plot map

Plot Designation (i.e. R101):

Trial Letter (R= red cultivar, Y= yellow cultivar), Replicate number, Treatment Number

<u>Treatments</u>

01) No fumigant + Regular nitrogen + Regular irrigation (high disease)

02) Chloropicrin @ 37.4 L ha^{-1} + Regular nitrogen + Regular irrigation (low disease)

03) Chloropicrin @ 37.4 L ha^{-1} + No in-season nitrogen + Regular irrigation (non-fertilized)

04) Chloropicrin @ 37.4 L ha^{-1} + Regular nitrogen + Regular irrigation (reduced water)