Spring 2016

IPM2.0: PRECISION AGRICULTURE FOR SMALL-SCALE CROP PRODUCTION

Matt Wallhead
University of New Hampshire, Durham

IPM2.0: PRECISION AGRICULTURE FOR SMALL-SCALE CROP PRODUCTION

By

Matthew Wallhead

B.S. in Plant Health Management, Ohio State University, 2007

M.S. in Plant Pathology, Ohio State University, 2012

DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

in

Plant Biology

May, 2016

Dissertation Committee

This dissertation has been examined and approved in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Biology by:

Dissertation Director, Dr. Kirk Broders, Assistant Professor, Plant Biology

Dr. Cheryl Smith, Extension Professor, Cooperative Extension

Dr. Rich Smith, Assistant Professor, Natural Resources and the Environment

Dr. Shane Bradt, Extension Associate Professor, Cooperative Extension

Dr. Barry Rock, Professor Emeritus, Natural Resources and the Environment, EOS

On 06-10-2015

Original approval signatures are on file with the University of New Hampshire Graduate School.

Dedication

This dissertation is dedicated to my wife Charity.

Acknowledgments

I would like to express my appreciation and thank my advisor, Dr. Kirk D. Broders, for his time and patience. Thank you for the opportunity to serve as your student. Special thanks to my dissertation committee members, Dr. Rich Smith, Dr. Cheryl Smith, Dr. Shane Bradt and Dr. Barry Rock all for their guidance and insight. Thanks to my mentors Mary Martin and Jon Chappell for sharing their wisdom and expertise and guiding me along the way, for this privilege I am more than grateful. Many thanks for all the lessons learned, it has been an honor to work with all of you. The most special thanks goes to my wife, Charity, for her understanding, support and love throughout this whole experience.

Table of Contents

ABSTRACT

IPM2.0: PRECISION AGRICULTURE FOR SMALL-SCALE CROP PRODUCTION

by

Matthew Wallhead

University of New Hampshire

May, 2016

In order to manage pests impacting New England crop production integrated pest management (IPM) practices should be reevaluated or updated regularly to ensure that effective control of crop pests is being achieved. Three fungal taxa, *Colletotrichum gloeosporioides, C. acutatum*, and *Glomerella cingulata*, are currently associated with bitter-rot of apple (*Malus domestica*), with *C. acutatum* typically being the dominant species found in the northeastern United States. However, a recent phylogenetic study demonstrated that both *C. gloeosporioides* and *C. acutatum* are species complexes with over 10 distinct species being recovered from apple between the two studies. Based on this recent information, the objectives of this study were 1) to complete a phylogenetic analysis to determine species diversity and distribution of *Colletotrichum* isolates associated with bitter-rot and Glomerella leaf spot in the northeastern United States and 2) to evaluate the sensitivity of these isolates to several commercially used fungicides. A multi-gene phylogenetic analysis was completed using ITS, GADPH and BT gene sequences in order to determine which species and how many species of *Colletotrichum* were infecting apples in the northeastern U.S. The results of this study demonstrated that *C. fioriniae* is the primary pathogen causing both bitter rot and Glomerella leaf spot in the northeastern U.S. A second experiment was conducted in order to update management practices for apple scab, caused by the ascomycete *Venturia inaequalis*. The objective of this project was to evaluate the ability of RIMpro, an apple scab warning system, to control apple scab in New England apple orchards in addition to evaluating the performance of potassium bicarbonate + sulfur as a low-cost alternative spray material for the control of apple scab suitable for organic apple production. Use of RIMpro allowed for the reduction in the total number of spray applications made during the primary scab season by two sprays in 2013 and one spray in 2014 (28% and 25% reductions, respectively). Also, the potassium bicarbonate + sulfur treatment was shown to provide the same level of control as Captan. Finally, disease outbreaks, insect infestation, nutrient deficiencies, and weather variation constantly threaten to diminish annual yields and profits in orchard crop production systems. Automated crop inspection with an unmanned aerial vehicle (UAV) can allow growers to regularly survey crops and detect areas affected by disease or stress and lead to more efficient targeted applications of pesticides, water and fertilizer. The overall goal of this project was to develop a low cost aerial imaging platform coupling imaging sensors with UAVs to be used for monitoring crop health. Following completion of this research, we have identified a useful tool for agricultural and ecological applications.

Investigation of the Ecology, Epidemiology, and Genetic Diversity of *Colletotrichum* spp. infecting fruit crops in the Northeastern U. S.

Matt Wallhead[1] and Kirk Broders[1]

[1]Department of Biological Sciences, University of New Hampshire, Durham, NH 03824.

Abstract

Three fungal taxa, *Colletotrichum gloeosporioides*, *C. acutatum*, and *Glomerella cingulata*, are currently associated with bitter-rot of apple (*Malus domestica*), with *C. acutatum* typically being the dominant species found in the northeastern United States. However, a recent phylogenetic study demonstrated that both *C. gloeosporioides* and *C. acutatum* are species complexes with over 10 distinct species being recovered from apple between the two studies. Based on this recent information, the objectives of this study were 1) to complete a phylogenetic analysis to determine species diversity and distribution of *Colletotrichum* isolates associated with bitter-rot and Glomerella leaf spot in the northeastern United States and 2) to evaluate the sensitivity of these isolates to several commercially used fungicides. Isolates were recovered from apple leaves and fruits collected during the 2011-2013 growing seasons from various locations in Massachusetts, New Hampshire and New York. A multi-gene phylogenetic analysis was completed using ITS, GADPH and BT gene sequences in order to determine which species and how many species of *Colletotrichum* were infecting apples in the northeastern U.S. Results indicate that species diversity on apple was limited, with *C. fioriniae* representing the dominant species recovered from both apple leaves and fruit in the northeastern U. S. The results of this study demonstrated that *C. fioriniae* is the primary pathogen causing both bitter rot and Glomerella leaf spot in the northeastern U.S. This finding is significant due to the fact that current management practices have been developed for *C. acutatum*, which calls for the need to revisit the epidemiology of the pathogen and to update management practices accordingly.

Introduction

The genus *Colletotrichum* comprises approximately 600 species of plant pathogens that affect over 3,200 species of plants (O'Connell *et al.* 2012). Usually, the fungus infects more than one part of the plant, which results in multiple cycles of infection throughout the growing season (Waller 1992). The genus has a broad host range, infecting some of the most economically important crops in the country and throughout the world with a wide phenotypic array of diseases. These diseases include bitter rot of apple, which has been associated with up to 50% crop losses and Glomerella Leaf Spot (GLS) of apple, which has been linked to defoliation events with up to 75% loss of leaves (Gonzalez et al. 2006). Historically, chemical control has been used for managing this disease. The appearance of isolates that are resistant to the most commonly used fungicides, however is becoming increasingly widespread (Torres-Calzada 2015).

Chemical control of *Colletotrichum* spp. relies heavily on several classes of fungicide. These classes including the benzimidazole, Sterol Inhibitors (SI or DMI), Succinate Dehydrogenase Inhibitors (SDHI) and Quinone outside Inhibitors (QoI or strobilurin) classes. However, it is increasingly common to find isolates that are less sensitive or even resistant to these fungicides (Hu et al. 2015, Torres-Calzada et al. 2015). Resistance to benzimidazoles has been seen in populations of *C. acutatum* and *C. gleosporioides* (Astua et al 1994, Peres et al. 2004, Reuda-Hernandez, Solano 1995). The strobilurins (QoI) are a different group of fungicides that are used against many diseases, including anthracnose diseases on a variety of hosts (Avila-Adame et al. 2003). Several plant pathogenic *Colletotrichum* spp. have developed resistance to strobilurin fungicides including isolates of *C. siamense* isolated from peach and blueberry and *C. gloeosporioides* isolated from strawberry. (Banno et al. 2009, Farman 2001, Gisi et al. 2002, Hu et al. 2015, Inada et al 2008, Torres-Calzada et al. 2015). Triazole fungicides are the most widely used class of fungicides for the control of fungal diseases of plants (Poole et al. 2014). Triazole resistance is now widely reported in the agricultural sectors including powdery mildew populations from barley growing regions around the world (Tucker et al. 2015, Wyand et al. 2005).

When combating fungi and developing new integrated pest management strategies, proper diagnosis and identification is key. Prior to the development of molecular methods, differentiation among *Colletotrichum* species was difficult and relied primarily on the host infected and visual identification of conidia (Milgroom & Peever 2003). In the past, *C. acutatum* was predominately associated with fruit diseases in the northeastern United States and *C. gloeosporioides* was most frequently associated with apple disease in the south and mid-Atlantic regions of the U. S. (Freeman *et al.* 1998). The diversity encompassed by each of these names was so broad that it conveyed limited information to plant pathologists. *C. acutatum* and *C. gloeosporioides* were recently identified as species complexes calling into question the identity of individual isolates previously classified as members of these species (Damm *et al.* 2012, Weir *et al.* 2012).

Molecular techniques have been developed to identify individual species within the *C. acutatum* species complex (Damm *et al.* 2012), and these techniques have been used to identify *C. fioriniae* as the primary pathogen causing bitter rot and Glomerella leaf spot of apple (Peralta & Broders 2012) in New Hampshire, which had previously been attributed to *C. acutatum* and *Glomerella cingulata*, respectively. Based on the above information the objectives of this project were to: 1) complete a phylogenetic analysis to determine how many species were associated with bitter rot and leaf spot of apple as well as their distribution in the northeastern United States and; 2) evaluate the sensitivity of these isolates to several commercially used fungicides.

Materials and Methods

Fungal Material. A survey of *Colletotrichum* in the northeastern U.S. was done by collecting a variety of infected plant tissues originating from hosts including, apple, apricot, blueberry, cherry, and pepper. Infected plant samples were obtained from growers and from collaborators throughout the region (Table 1). Diseased tissues were excised and surface sterilized in a 10% bleach solution for 2 minutes. Isolates were cultured on potato dextrose agar (PDA) (Fisher Scientific, Pittsburgh, PA) acidified with 750 μl of

50% lactic acid. Cultures were then transferred to cellophane PDA until the mycelium from each sample could be removed and lyophilized.

Phylogenetic Analysis. DNA was isolated using a modified CTAB extraction method, and amplified using polymerase chain reaction (PCR) at the ITS, GADPH, and β-tubulin gene regions using the primer pairs ITS1 (Gardes & Bruns 1993) and ITS4 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), and BT2Fd/BT4R (Woudenberg et al. 2009) respectively. Successful amplification was confirmed using gel electrophoresis. After all regions were amplified, PCR products were purified and sequenced using the same primers at the Hubbard Center for Genomic Sciences (HCGS) at the University of New Hampshire. Once the sequences were received from the HCGS, the forward and reverse complements of the DNA were trimmed and edited using the program Bioedit (Hall 1999). Sequences were analyzed to determine phylogenetic and molecular diversity using MEGA 6.0. Sequence data from studies by Damm et al. (2012) were imported from Genbank and utilized to infer speciation and outgroups by maximum likelihood (ML) and neighbor-joining (NJ) methods. Gaps were treated as missing data; with missing or ambiguous sites ignored for the affected pairwise comparison. All positions were included in the analysis and relative support for the branches were estimated with 1000 bootstrap replications for the NJ and ML analyses.

Field Inoculation Assays. A field trial was conducted to investigate the effects of a pre-harvest application of a systemic induced (SI) elicitor on the development of post-harvest bitter-rot on apple. The trial took place during the 2012 growing season at Woodman Farm located in Durham, NH. The four varieties evaluated were Marshall Mac, Macoun, Cortland and Mutsu. Treatments included an untreated control (UTC), Regalia (A.I. 5% extract of *Reynoutria sachalinensis*) (Marrone Bio Innovations, Davis, CA), Timorex (A.I. 222g/L Tea tree oil) (AgNova Technologies, Box Hill North, Australia) and Captan 50 WP (A.I. 48.9% N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) (Arysta Lifescience, Cary, NC). The experimental design was a randomized complete block design with 3 replicate blocks and 3 trees per block for each of the four cultivars used in this study. Ten fruit per tree were tagged for use in this study. All treatments were applied to trees using a Solo 451 Mist Blower calibrated to deliver 35 gallons/A on August 30, 2012. Fruit were then inoculated 48 hours later at dusk on September 1, 2012 using a mix of four single-spore *Colletotrichum* isolates collected from apples during the 2011 field season. Tagged apples were inoculated with 3 ml of the conidial suspension (1 x 10^4) using a Nalgene spray bottle, Tween 20 was added at a rate of 0.125% v/v. *Colletotrichum* conidia for the inoculum were grown on malt extract agar (MEA) (Fisher Scientific, Pittsburgh, PA) amended with streptomycin sulfate (0.1g/L) to inhibit bacterial growth. Conidia were harvested 7-10 days after plating the cultures by flooding the plate with sterile deionized water, then scrapping the plates gently using a rubber policeman with an angled edge (Fisher Scientific, Pittsburgh, PA), then the resulting suspension was filtered through cheesecloth and the suspension was stored in 1L glass bottles at 20° C until ready for seven days prior to use. Tagged apples were harvested 1 month later on October 5, 2012, and placed in apple crates and put into cold storage (38° F). External disease severity was rated every 2 weeks for 6 total weeks at which point the fruit was cut open and rated for internal rot symptoms. An ANOVA was conducted to determine treatment effects. Means were compared using Student's t test with the statistical software JMP Pro 11 (SAS Institute, Cary, NC).

Fungicide Sensitivity Assay. The response of radial growth for two *C. fioriniae* isolates to different SI elicitors was determined with *in vitro* mycelia growth assays. Two isolates, BH2 and WPA, were plated onto MEA, and were grown at 25° C with a 12-hour photoperiod. A 3mm plug was extracted from the edge of actively growing plates for each isolate and placed onto MEA plates amended with 100, 1,000 or 10,000ppm Regalia (Marrone Bio Innovations, Davis, CA), or MEA plates amended with 100, 1,000 or 10,000ppm Timorex (AgNova Technologies, Box Hill North, Australia). The plates were incubated at 25° C in complete darkness for 8 days. Control plates consisted of a 3mm plug of each isolate placed on non-amended MEA. The experiment was repeated twice with 3 replicates per isolate and concentration. Plates were arranged in a randomized complete block design within the incubator. The diameter of each colony was measured in 2 places then averaged at 2, 4 and 6 days. Mean radial growth was compared between isolates and fungicide concentrations. The ANOVA and mean separation procedure were performed using the statistical software JMP Pro 11 (SAS Institute, Cary, NC).

A separate assay was conducted to observe the effects of Regalia and Timorex on the germination of *C. fioriniae* condia. The same isolates and amended media used in the radial growth experiment were used in this study. To obtain conidia, MEA plates were flooded with a spore suspension containing 1 x 10^{4} sp/ml for each isolate used in the study and the excess liquid was poured off. Plates were then place under lights with a 12-hour photocycle at 22°C. Conidia were harvested 7-10 days after plating by flooding the plate with sterile deionized water and gently dislodging conidia with a rubber policeman. The resulting solution was filtered through cheesecloth then stored in 1L glass bottles at 20° C until ready for use. Prior to plating out spores, conidial solutions were adjusted to 1 x 10^{4} using a hemocytometer (Hausser Scientific, Horsharm, PA). One mL of the conidial solution was pipetted onto each plate and spread using a 90° sterile glass rod. The experiment was repeated twice with 3 replicates per isolate and concentration. Plates were arranged in a randomized complete block design within the incubator. A dissecting microscope was used to count the number of spores germinating out of 100 spores per plate, 48 hours after plating. A one-way analysis of variance was conducted to determine significance of treatment effects. The ANOVA and mean separation procedure were performed using the statistical software JMP Pro 11 (SAS Institute, Cary, NC).

Results

Fungal Material. 38 isolates of *Colletotrichum* from 3 locations throughout Massachusetts, New Hampshire, and New York were evaluated in this study, including isolates from apple, apricot, cherry, pepper and blueberry (Table 1).

Phylogenetic analysis. The combined alignment of *Colletotrichum* species consisted of sequences of ITS, β-tubulin, and GADPH genes from 38 species of *Colletotrichum*. The bootstrap consensus tree from the neighbor-joining analysis was included in Figure 1 with bootstrap values included at each node. The results of the phylogenetic analysis (Fig. 1) demonstrate that, with the exceptions of isolate HV1, the majority of isolates collected from apples throughout Massachusetts, New Hampshire and New York were members of *C. fioriniae*. This included all bitter rot and GLS isolates. *C. fioriniae* is in the *C. acutatum* complex. Samples from hosts including, apricot, blueberry and pepper were also analyzed. In most cases isolates collected from blueberry, pepper, and apricot belonged to the same clade identified as *C. fioriniae.* However, one of the three apricot isolates (HV1) was found to have the greatest homology *to C. nymphaea* based on the multi-gene phylogeny.

Field Inoculation Assay. When variety was averaged together, fungicide treatment did not have a significant effect on post-harvest bitter rot development for the four varieties evaluated (P=0.91). Differences between internal and external rot symptoms were not significantly different (P>0.05) (Fig. 2). Variety had a significant effect on post-harvest bitter rot development with Mutsu showing the lowest levels of disease for both internal and external disease ratings (P=0.00005) (Fig. 3 and 4). There was no interaction between fungicide and cultivar (P>0.05). Arc-transformation of the disease severity ratings yields the same results as analyzing the raw percentage values (Fig. 5).

Fungicide Sensitivity Assay. The response of radial growth and spore germination for two *C. fioriniae* isolates to different SI elicitors was determined with *in vitro* assays. Results of the ANOVA for the radial growth experiment 8 days post inoculation onto the amended media showed no significant differences (P>0.05) in radial growth between the two isolates (BH2 and WPA) or for any product x concentration combination tested (Figure 6). Significant differences in radial growth were observed between isolates grown on unamended media and the fungicide-amended media at various concentrations (P<0.05). Timorex at 100ppm had the largest diameter colonies whereas Timorex at 10,000ppm had significantly smaller diameter colonies than all other treatments ($P<0.0001$) (Figure 6). For the germination assay marginally significant differences ($P=0.06$) were observed between treatments for isolate BH2, with the unamended media having the highest rate of germination and Timorex at 10,000ppm having the lowest rate of germination (Figure 7). Similar trends were observed for the second isolate (WPA) although there were no significant differences ($P>0.05$) in colony diameters between the unamended media and the fungicide amended media (Figure 8).

Discussion

Based on DNA sequence data for three genes, the 38 strains studied were assigned to two species of which 37 strains were classified as *C. fioriniae* and one as *C. nymphaea.* Results clearly show that species diversity on apple was limited with *C. fioriniae* representing the dominant species recovered from both apple leaves and fruit in the northeastern U.S. Additionally, *C. fioriniae* has been shown to be the dominant species in the region for several other important crop species, including blueberry, strawberry, pepper, tomato and apricot. This study demonstrates that *C. fioriniae* is the primary pathogen causing both bitter rot and Glomerella leaf spot on apple in the northeastern U.S. These results are similar to the results reported in a “First Report” by Kou (2014) where postharvest decay on apple fruit was found to be caused by *C. fioriniae* in the United States.

Host resistance to the bitter rot pathogen proved to be the most effective means of reducing disease severity levels as observed in the Post-Harvest Bitter Rot trial where Mutsu was shown to have the lowest levels of disease followed by Macoun, Cortland and Marshall Mac, respectively. Growers concerned with controlling bitter-rot in the northeastern U.S. could consider planting Mutsu as part of an integrated pest management program due to it being less susceptible to bitter-rot than the other three varieties evaluated in this study (Macoun, Cortland and Marshall Mac). These results confirm earlier reports by Peralta et al. (2012) where Pioneer Mac was the most susceptible and cultivars Honey Crisp, Mutsu, and Smoothie were the least susceptible to point-inoculations with *C. acutatum* (Peralta & Broders 2012). A single application of a SI elicitor 2 weeks prior to harvest did not reduce post-harvest bitter-rot levels significantly in this study. Synergistic effects between Regalia and other fungicides have been reported for other crops (Su 2010). Despite the lack of efficacy observed in this study botanical products or biofungicides for protecting apple production hold promise and warrant further investigation. Biofungicides and botanical products (i.e. essential oils) have been shown in a recent study to be effective at controlling apple pathogens other than *Colletotrichum* spp (Ab-El-Latif 2016).

Practical implications of this study indicate that the same spray material could be used for multiple host crops due to the lack of species diversity observed in this study. Since it is the same pathogen infecting multiple host crop species similar management options could be utilized across crops given the product being used was labeled for the crop being treated. Conversely caution should be utilized when choosing spray materials to avoid the development of fungicide resistance (Avila 2003, Griffee 1973, Wong 2007). Multiple *Colletotrichum* species have been shown to be resistant to the MBC, QoI and DMI classes of fungicides (FRAC 2013). According to FRAC (Fungicide Resistance Action Committee) a species closely related to *C. fioriniae*, *C. gleosporioides* is classified as a plant pathogen showing a medium risk of development of resistance to fungicides (FRAC 2014). Biofungicides are defined as pesticides that have fundamentally different modes of action from conventional pesticides, and thus present fewer risks to human health and the environment (EPA 2007). This group of products includes less broadly toxic antimicrobials and chemicals that stimulate changes in plant physiology or metabolism to make hosts more resistant to pathogens (Rosenberger 2003). Biofungicides such as Timorex, Regalia or potassium bicarbonate may help reduce the risk of resistance development to conventional fungicides when incorporated into an IPM program.

Future research should focus on the epidemiology and ecology of the infection process of *C. fioriniae* as all current data available in refereed publications was generated for *C. acutatum*, which is composed of multiple species. While *C. fioriniae* was isolated from multiple hosts in this study, there is currently no experimental data on the ability of individual *C. fioriniae* isolates to infect multiple hosts. However, there are examples of *C. acutatum* and *C. gleosporioides* that can infect multiple hosts. Cross-infection potential of *Colletotrichum* species was documented by Berstein (1995), Freeman and Shabi (1996), Freeman (2001), and Mackenzie (2007). Differences in pathogenicity between strains from different hosts have been observed in several studies (Damm 2012). Some fruit diseases caused by members of the *C. acutatum* complex have been shown to be caused by distinct phylogenetic lineages (Peres 2008).

Like *Colletotrichum acutatum*, *Colletotrichum fioriniae* should be considered as a pathogen of multiple host plant species. There is limited evidence of species diversity within New England/Northeastern U.S with one exception being an isolate from apricot originating from New York, which was found to be infected by *C. nymphaeae*, a species previously unidentified in this region of the U.S. *Colletotrichum nymphaeae* was recently reported to infect apple fruits and foliage in Kentucky and southern Brazil (Munir 2015, Velho et al. 2015). *Colletotrichum nymphaeae* has also been identified as the causal agent of celery stunt in Japan and has be found infecting strawberry in Slovenia (Weber et al. 2015, Yamagishi et al. 2015) This leads to the question of how many other species of *Colletotrichum* exist in the northeastern U.S. as non-dominant species in addition to the questions pertaining to the location of the geographic boundaries for *Colletotrichum fioriniae*. In areas such as the northeastern U.S. many growers, such as CSA's (community supported agriculture) commonly find themselves growing a large variety of crops. This wide array of crop diversity (45) on relatively small acreages (3-27 acres) may present new opportunities for cross-infection from adjacent crops or fields. Growers may need to more closely consider where crops are planted on a farm or in an orchard in order to maximize the effectiveness of their IPM plans (Galt 2012, Lass 2003).

Table 1. Isolates of *Colletotrichum* spp. studied

Isolate	Host	Tissue	Location
AH-01	Cherry	Fruit	Concord, NH
AH-02	Apple	Fruit	Concord, NH
AH-03	Apple	Fruit	Concord, NH
AH-04	Apple	Fruit	Concord, NH
BB2-1-1	Blueberry	Fruit	Durham, NH
BB2-2-1	Blueberry	Fruit	Durham, NH
BB2-3-1	Blueberry	Fruit	Durham, NH
BB2-4-1	Blueberry	Fruit	Durham, NH
BB2-5-1	Blueberry	Fruit	Durham, NH
BB2-6-1	Blueberry	Fruit	Durham, NH
BH-01	Apple	Fruit	Strafford, NH
BH-02	Apple	Fruit	Strafford, NH
BH-03	Apple	Fruit	Strafford, NH
CNY2	Apple	Fruit	Hudson Valley, NY
CNY3	Apple	Leaf	Hudson Valley, NY
CNY4	Apple	Leaf	Hudson Valley, NY
CNY46	Apple	Fruit	Hudson Valley, NY
CNY6	Apple	Leaf	Hudson Valley, NY
CNY6	Apple	Fruit	Hudson Valley, NY
DH-01	Apple	Fruit	Madbury, NH
HV1	Apricot	Fruit	Hudson Valley, NY
HV2	Apricot	Fruit	Hudson Valley, NY
HV3	Apricot	Fruit	Hudson Valley, NY
KF-1	Apple	Fruit	Madbury, NH
Mil2	Apple	Fruit	Milton, NH
Mil3	Apple	Fruit	Milton, NH
Mt. Ples1	Apple	Fruit	Lancaster, NH
Mt. Ples2	Apple	Fruit	Lancaster, NH
Pep1-1	Pepper	Fruit	Durham, NH
Wood-01	Apple	Fruit	Durham, NH
Wood-02	Apple	Fruit	Durham, NH
Wood-03	Apple	Fruit	Durham, NH
Wood-04	Apple	Fruit	Durham, NH
Wood-05	Apple	Fruit	Durham, NH
Wood-06	Apple	Fruit	Durham, NH
Wood-07	Apple	Fruit	Durham, NH
Wood-08	Apple	Fruit	Durham, NH
Wood-09	Apple	Fruit	Durham, NH
Wood-11	Apple	Fruit	Durham, NH

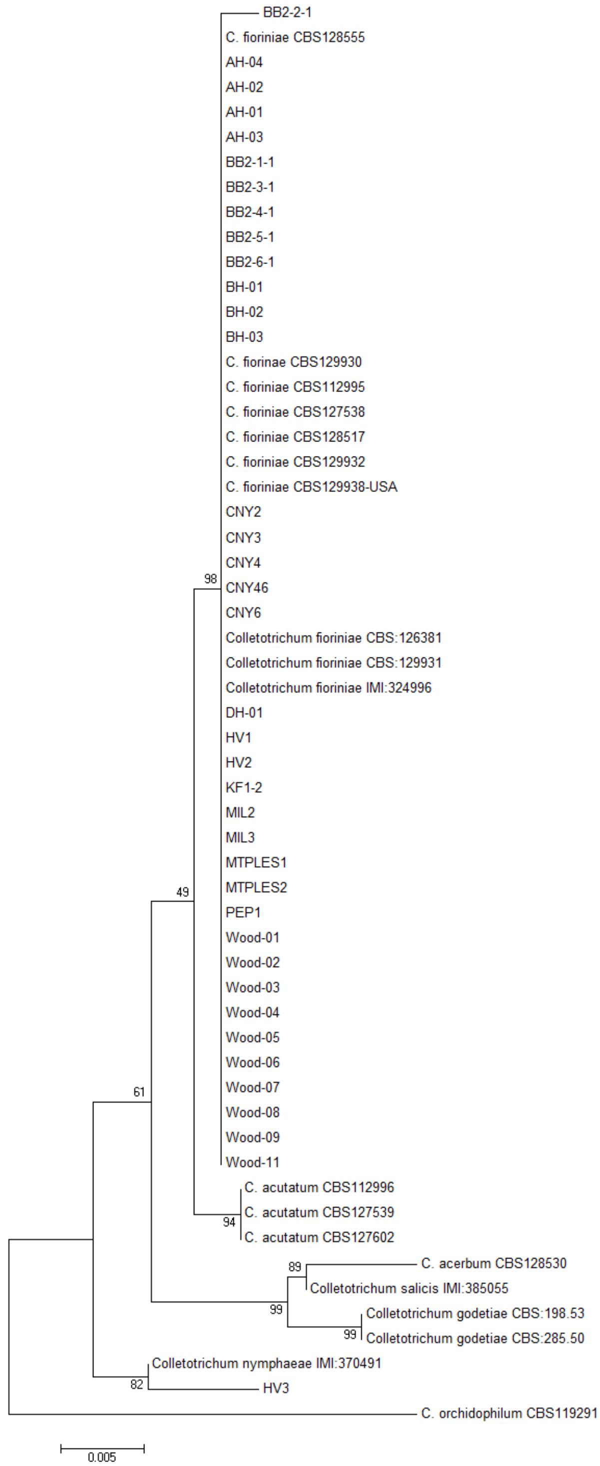

Figure 1. Phylogenetic tree (NJ) displaying results of sequencing 38 isolates collected between 2011 and 2013 from sites across the northeastern U.S. The results of the neighbor-joining test indicate that all isolates collected from apple throughout New England and New York are members of *C. fioriniae* with HV3 from apricot being the one exception, belonging to *C. nymphacea*.

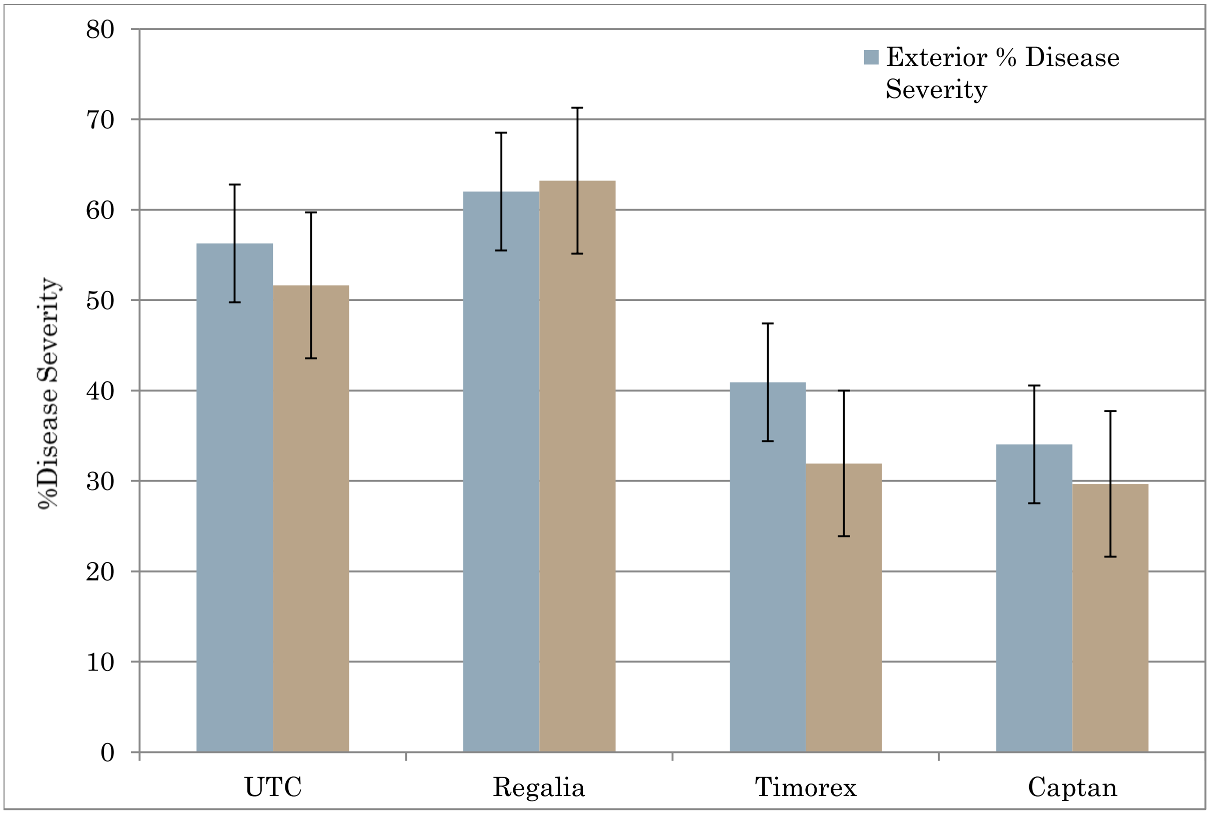

Figure 2. Chart showing percent disease severity with standard error bars for four varieties (Mac, Macoun, Cortland and Mutsu) grouped by treatment for exterior bitter rot symptoms (gray bar) and interior bitter rot symptoms (tan bar) after being kept in cold storage for 6 weeks. Differences between internal and external rot symptoms were not significant (P>0.05) indicating that it is suitable to rate whole-fruit only, thus eliminating the need to cut fruit open to rate internal rot.

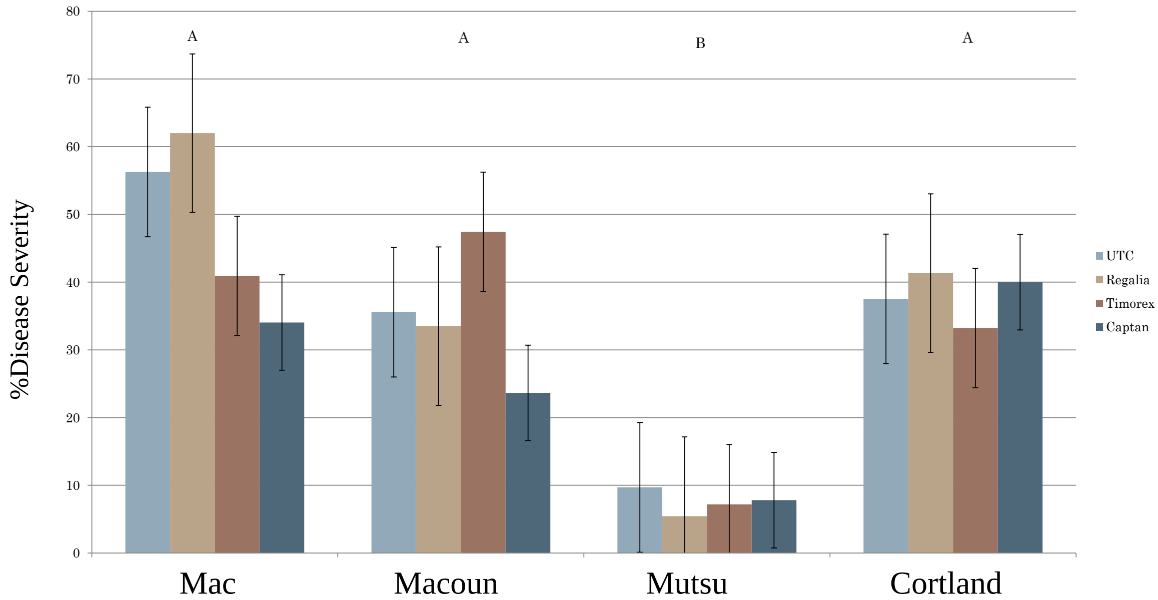

Figure 3 Percent disease severity for external bitter rot symptoms with standard error bars for four varieties (Mac, Macoun, Mutsu and Cortland) grouped by treatment for exterior bitter rot symptoms after being kept in cold storage for 6 weeks. Treatment did not have a significant effect on percent disease severities (P>0.05). While differences were not significant between treatments, significant differences were observed between varieties with Mutsu having the lowest average disease severity (P<0.05).

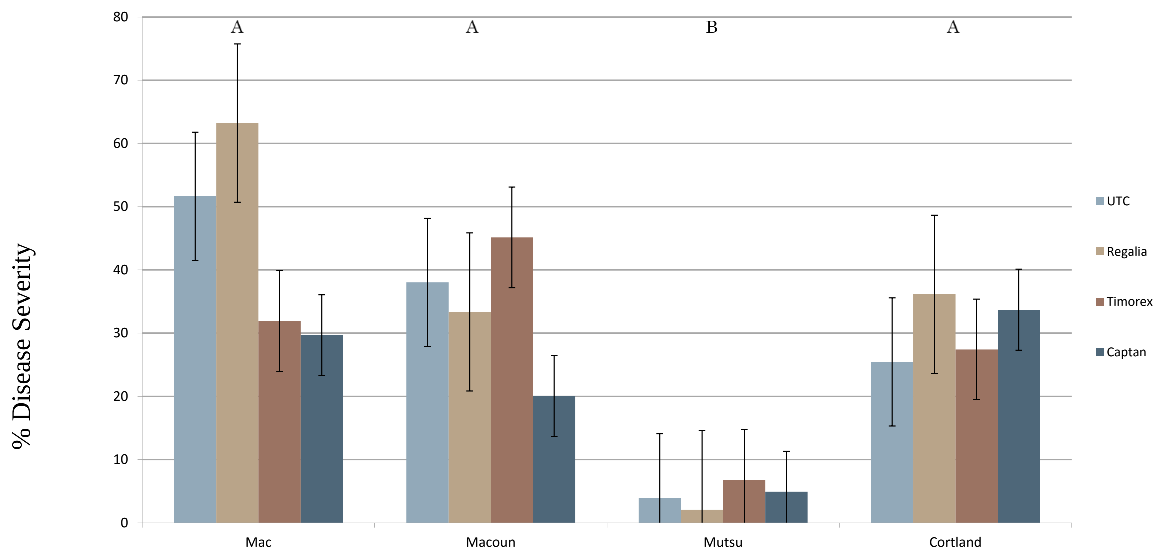

Figure 4. Chart showing percent disease severity for internal bitter rot symptoms with error bars for four varieties (Mac, Macoun, Mutsu and Cortland) grouped by treatment. Differences were not significant between treatment (P>0.05). Significant differences (P<0.05) were observed between varieties with Mutsu having the lowest average disease severity.

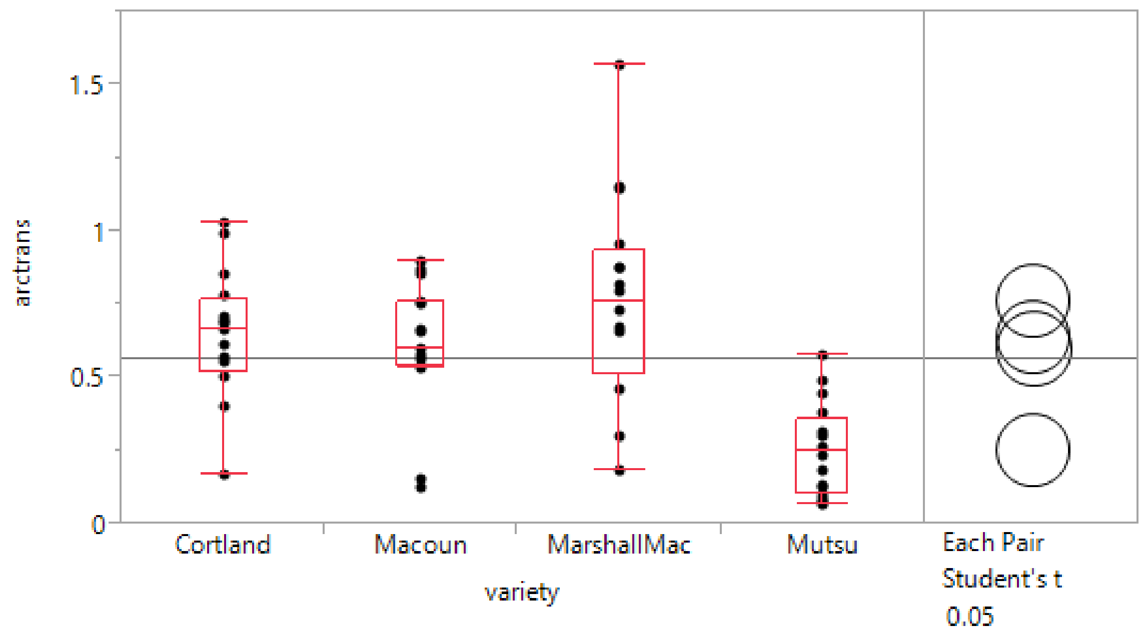

Figure 5. Box-plots and results of Student's t means comparison (P=<0.05) for external Bitter-rot ratings after 6 weeks in cold storage. For the plot of Student's t comparison overlapping circles are not considered to be significantly different from one another. Mutsu had significantly less disease than the other three varieties tested (Cortland, Macoun and Marshall Mac) (P<0.05)

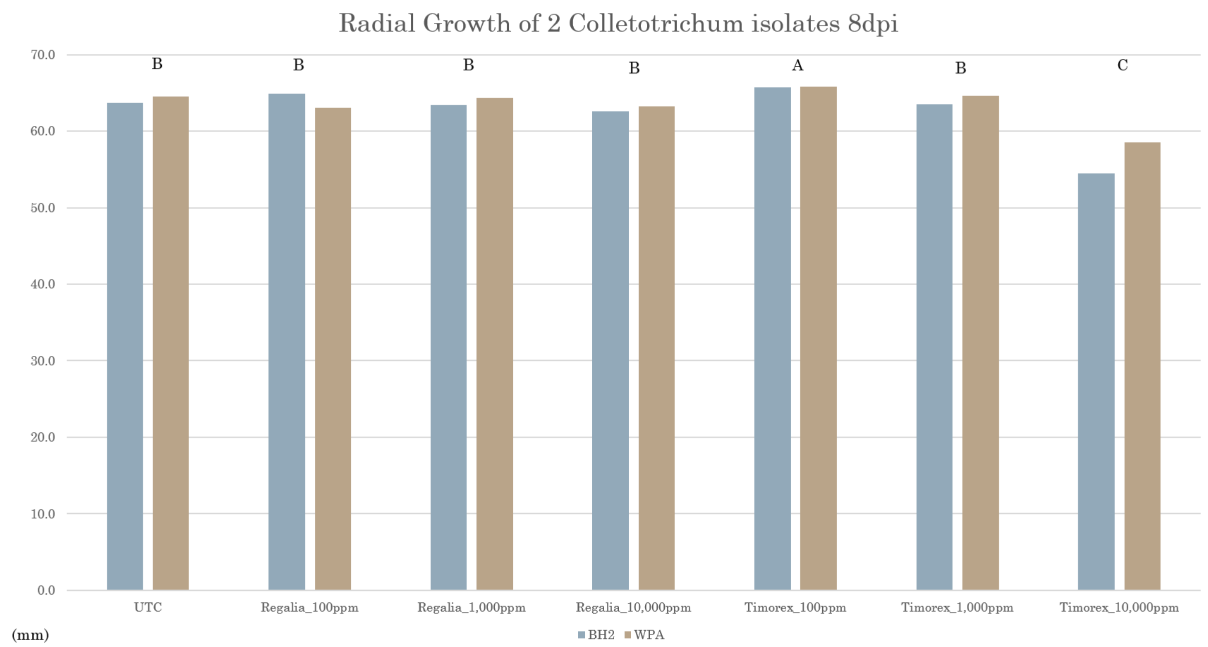

Figure 6. Radial growth for two isolates (BH2 and WPA) grouped by treatment 8 days post inoculation onto amended media. Isolate did not have a significant effect on radial growth (P>0.05). While differences were not significant between isolates, significant differences (P <0.05) were observed between treatments with Timorex at 10,00ppm having the smallest average diameter colony.

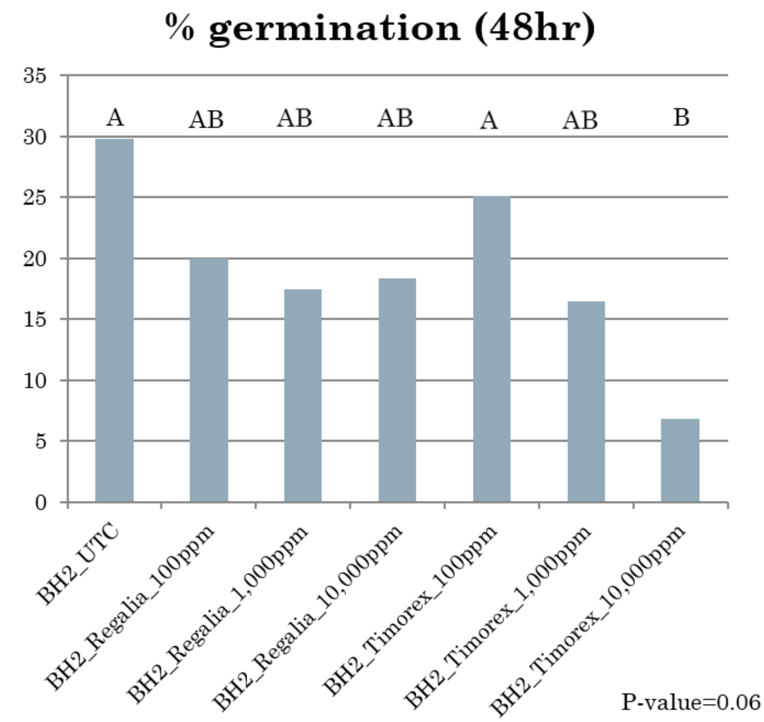

Figure 7. Chart showing average percent germination for isolate BH2 48-hours post inoculation onto amended media. Marginally significant differences (P =0.06) were observed between treatments with 10,000 ppm Timorex having the least amount of spores germinated.

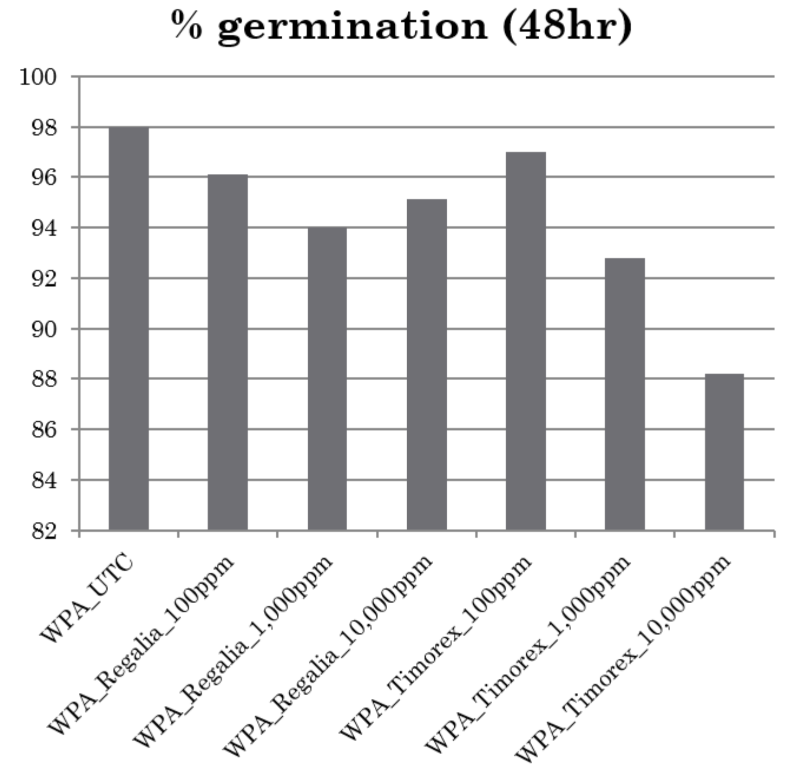

Figure 8. Chart showing average percent germination for isolate WPA 48-hours post inoculation onto amended media. No significant differences (P>0.05) were observed between treatments.

References

Abd-El-Latif, F. 2016. Postharvest Application of Some Essential Oils for Controlling Gray and Blue Moulds of Apple Fruits. Plant Pathology Journal, 15, 5.

Astua, G., Arauz, L. F., and Umana, G. 1994. Reduced sensitivity to thiabendazole in *Colletotrichum gloeosporioides* isolated from papaya. Agronomía Costarricense, 18, 35-39.

Avila-Adame, C., Olaya, G., and Köller, W. 2003. Characterization of *Colletotrichum graminicola* isolates resistant to strobilurin-related QoI fungicides. Plant Disease, 87, 1426-1432.

Banno, S., Yamashita, K., Fukumori, F., Okada, K., Uekusa, H., Takagaki, M., and Fujimura, M. 2009. Characterization of QoI resistance in *Botrytis cinerea* and identification of two types of mitochondrial cytochrome b gene. Plant Pathology, 58, 120-129.

Bernstein, B., Zehr, E., Dean, R. 1995. Characteristics of *Colletotrichum* from peach, apple, pecan and other hosts. Plant Disease, 79, 478–482.

Damm, U., Cannon, P., Woudenberg, J., and Crous, P. 2012. The *Colletotrichum acutatum* species complex. Studies in Mycology, 73, 37-113.

EPA. 2003. What are biopesticides? Published online.

Farman, M. L. 2001. The molecular basis of field resistance to QoI fungicides in *Pyricularia grisea*. Phytopathology, 91, S110.

FRAC. List of Potential Pathogenic Organisms Resistant to Disease Control Agents. Revised January 2013. Published online.

FRAC. 2014. Pathogen Risk List. Published online.

Freeman, S., Katan, .T, and Shabi, E. 1998. Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. Plant Disease, 82, 596-605.

Freeman, S., Shabi, E. 1996. Cross-infection of subtropical and temperate fruits by *Colletotrichum* species from various hosts. Physiological and Molecular Plant Pathology, 49, 395–404.

Freeman, S., Minz, D., Maymon, M., Zveibil, A. 2001. Genetic diversity within *Colletotrichum acutatum sensu* Simmonds. Phytopahology, 91, 586–592.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 41, 95-98.

Galt, R., O'Sullivan, L., Beckett, J., and Hiner, C. 2012. Community supported agriculture is thriving in the Central Valley. California Agriculture, 66, 8-14.

Gonzalez, E., Sutton, T., and Correll, J. 2006. Clarification of the etiology of Glomerella leaf spot and bitter rot of apple caused by *Colletotrichum* spp. based on morphology and genetic, molecular, and pathogenicity tests. Phytopathology, 96, 982-992.

Griffee, P. 1973. Resistance to benomyl and related fungicides in *Colletotrichum musae*. Transactions of the British Mycological Society, 60, 433-439.

Gisi, U., Sierotzki, H., Cook, A., and McCaffery, A. 2002. Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Management Science, 58, 859-867.

Hu, M. J., Grabke, A., Dowling, M. E., Holstein, H. J., and Schnabel, G. 2015. Resistance in *Colletotrichum siamense* from peach and blueberry to thiophanate-methyl and azoxystrobin. Plant Disease, 99, 806-814.

Inada, M., Ishii, H., Chung, W. H., Yamada, T., Yamaguchi, J., and Furuta, A. 2008. Occurrence of strobilurin-resistant strains of *Colletotrichum gloeosporioides* (*Glomerella cingulata*), the causal fungus of strawberry anthracnose. Japanese Journal of Phytopathology, 74, 114-117.

Kou, L., Verneta, G., Luo, Y., and Jurick, W. 2013. First report of *Colletotrichum fioriniae* causing postharvest decay on 'Nittany' apple fruit in the United States. Plant Disease, 98, 993.

Lass, D., Stevenson, G., Hendrickson, J., and Ruhf, K. 2003. CSA Across the Nation: Findings from the 1999 CSA Survey. Center for Integrated Agricultural Systems (CIAS), College of Agricultural and Life Sciences, University of Wisconsin-Madison.

MacKenzie, S., Peres, N., Barquero, M., Arauz, L., and Timmer, L. 2009. Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. Phytopathology, 99, 620–631.

Milgroom, M., and Peever, T. 2003. Population biology of plant pathogens - The synthesis of plant disease epidemiology and population genetics. Plant Disease, 87, 608-617.

O'Connell, R., Thon, M., Hacquard S. 2012. Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. Nature Genetics, 44, 1060-1067.

Peralta, C. and Broders, K. 2012. Characterization of virulence and genotypic diversity of *Colletotrichum acutatum* isolates recovered from apple in New Hampshire. Phytopathology, 102, 91-91.

Peres, N., Souza, N., Peever, T., and Timmer, L. 2004. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. Plant Disease, 88, 125-130.

Peres, N., MacKenzie, S., Peever, T., Timmer, L. 2008. Postbloom fruit drop of citrus and Key lime anthracnose are caused by distinct populations of *Colletotrichum acutatum*. Phytopathology, 98, 345–352.

Poole N., Arnaudin M. 2014. The role of fungicides for effective disease management in cereal crops. Canadian Journal of Plant Pathology, 36, 1–11.

Rosenberger, D. 2003. Factors limiting IPM-compatibility of new disease control tactics for apples in eastern United States. Plant Health Progress, August, 1-10.

Rueda-Hernández, K., Cardona, A., Cadavid-Restrepo, G., Benjumea, C., Gutierrez, G., and Isaza, R. 2013. Differential organ distribution, pathogenicity and benomyl sensitivity of *Colletotrichum* spp. from blackberry plants in Northern Colombia. Journal of Phytopathology, 161, 246-253.

Solano, V., and Arauz, L. F. Control of papaya anthracnose with field fungicide sprays in the Atlantic region of Costa Rica. Agronomía Costarricense, 19, 25-30.

Su, H. (2010. Synergistic effects between Regalia and other fungicides in controlling cucumber powdery mildew and lettuce downy mildew. Phytopathology, 100, S123-S124.

Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I., Martin-Mex, R., Nexticapan-Garcez, A., and Perez-Brito, D. 2015. Sensitivity of *Colletotrichum truncatum* to four fungicides and characterization of thiabendazole-resistant isolates. Plant Disease, 99, 1590-1595.

Tucker, M., Lopez-Ruiz, F., Jayasena, K., & Oliver, R. 2015. Origin of Fungicide-Resistant Barley Powdery Mildew in Western Australia: Lessons to Be Learned. Fungicide Resistance in Plant Pathogens. 329-340. Springer Japan.

Velho, A., Alaniz, S., Casanova, L., Mondino, P., and Stadnik, M. 2015. New insights into the characterization of *Colletotrichum* species associated with apple diseases in southern Brazil and Uruguay. Fungal Biology, 119, 229-244.

Waller, J. M. 1992. *Colletotrichum* diseases of perennial and other cash crops. *Colletotrichum*: Biology, Pathology and Control. J. A. Bailey and M. J. Jeger, eds. CABI, Wallingford, UK. 131-142.

Weber, N., Veberic, R., Mikulic-Petkovsek, M., Stampar, F., Koron, D., Munda, A., and Jakopic, J. 2015. Metabolite accumulation in strawberry (Fragaria× ananassa Duch.) fruits and runners in response to *Colletotrichum nymphaeae* infection. Physiological and Molecular Plant Pathology, 92, 119-129.

Weir, B., Johnston, P., and Damm, U. 2012. The *Colletotrichum gloeosporioides* species complex. Studies in Mycology, 73, 115-180.

Wong, F., and Midland, S. 2007. Sensitivity distributions of California populations of *Colletotrichum cereale* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. Plant Disease, 91, 1547-1555.

Wyand R., Brown J. 2005. Sequence variation in the CYP51 gene of *Blumeria graminis* associated with resistance to sterol demethylase inhibiting fungicides. Fungal Genetics and Biology 42, 726–735.

Yamagishi, N., Fujinaga, M., Ishiyama, Y., Ogiso, H., Sato, T., and Tosa, Y. 2015. Life cycle and control of *Colletotrichum nymphaeae*, the causal agent of celery stunt anthracnose. Journal of General Plant Pathology, 81, 279-286.

Evaluation of the RIMpro predictive model for control of apple scab in New England Apple Orchards

Matt Wallhead[1] and Kirk Broders[1]
[1]Department of Biological Sciences, University of New Hampshire, Durham, NH 03824

Abstract

Apple scab, caused by the ascomycete *Venturia inaequalis* (conidial stage: *Spilocaea pomi*), is globally the most important disease in the production of apples. Numerous simulation models are currently available for assessing *V. inaequalis* primary infection risks, with RIMpro being the scab model most commonly used in Europe at present. RIMpro is capable of reducing fungicide treatments against apple scab by 79% if properly implemented. Use of RIMpro has the capacity to result in optimal apple scab control and reduced expenses for labor, fungicides, and fuel. The objective of this project was to evaluate the ability of RIMpro to control apple scab in New England apple orchards in addition to evaluating the performance of potassium bicarbonate + sulfur as a low-cost alternative spray material for the control of apple scab suitable for organic apple production. Use of RIMpro allowed for the reduction in the total number of spray applications made during the primary scab season by two sprays in 2013 and one spray in 2014 (28% and 25% reductions, respectively). The results from this investigation clearly emphasizes the importance of having an effective spray program in place and the usefulness of DSSs such as RIMpro. Finally, the potassium bicarbonate + sulfur treatment was shown to provide the same level of control as Captan used alone or when Captan was tank-mixed with Fontelis.

Introduction

Apple scab, caused by the ascomycete *Venturia inaequalis* (conidial stage*: Spilocaea pomi*), is worldwide the most important disease in the production of apples as measured by the potential economic loss, the cost and amount of fungicides necessary to control the disease each year, the constant consideration of the growers, and the environmental impact of the control measures (MacHardy 1996). Apple scab occurs wherever apples are grown. It is not possible to produce apples commercially without an effective fungicide program to control apple scab.

The story of apple domestication is well documented. Historical information and genetic evidence suggests that the center of origin of the cultivated apple (*M.* × *domestica*) was Central Asia (Harris et al. 2002, Juniper et al. 2006, Velasco et al. 2010). From Central Asia, the domesticated apple was introduced westward to Europe and eastward to China following the Silk Road trade route. Population genetics studies on *V. inaequalis* showed that the pathogen shared a common origin and spread with its host starting in Central Asia (Gladieux et al. 2008).

Venturia inaequalis reproduces asexually during spring and summer and sexually during winter-spring. Sexual reproduction occurs between strains of opposite mating types that have infected the same leaf. The fungus overwinters in the leaves remaining on the ground on the orchard floor. The ascospores, which are the primary inoculum, mature during spring and are discharged during rain events (MacHardy 1996). Timing of fungicide applications during the primary season is critical to prevent apple scab with calendar-based spray programs oftentimes beginning at silver-tip, the growth stage immediately following dormant period. In many cases, failures in scab control during primary scab season in the spring cannot be ameliorated by fungicide applications later in the season (Kohl et al. 2015). Sanitation can be used to significantly decrease primary inoculum in spring (MacHardy 1996, Didelot et al. 2016). The destruction of leaf litter though shredding, urea application, or leaf litter removal from the alleys combined with leaf ploughing within rows can reduce ascospore release by 50–95% (Gomez et al. 2007, Sutton et al. 2000, Vincent et al. 2004). The epidemiology of *V. inaequalis* during primary infections (until the end of ascospore release) and during secondary infections (in summer) is well known (Carisse et al. 2009, Holb et al. 2003, MacHardy 1996) and warning systems based on meteorological data can be used to apply fungicides for the prevention of apple scab at the correct time (MacHardy 1996).

While understanding the weather variables that are important for inoculum production growers in the Northeast are also contending with the consequences of climate change and variability in climate patterns. Heat waves, intense rainfall events and sea level rise pose growing challenges to many aspects of life in the Northeast (IPCC 2014). Recently trends in climate change in the northeastern U.S. have revealed an increase of high intensity rainfall events experienced each year. Climate change related risks from extreme events, such as heat waves, heavy precipitation and coastal flooding, are already moderate (high confidence), and with 1°C additional warming, risks will become high (medium confidence) (IPCC 2014). Once this happens, apple production will continue to rely on the prophylactic use of fungicides for the control of apple scab. One practice that conventional and organic growers alike can readily adapt into their IPM plan is the use of Decision Support Systems (DSSs).

Numerous simulation models are currently available for assessing *V. inaequalis* primary infection risks (Trapman et al. 1997, Rossi et al. 2007) with RIMpro being the scab model most commonly used in Europe at present. It is used by some 200 to 250 growers and experimental stations and 25 public and private disease warning services. The model is the basis for agricultural warnings in Quebec, Australia, New Zealand, Belgium, Denmark, France, the Netherlands, Sweden and some parts of Italy. RIMpro provides hour-by-hour data and is considered by most users to be a reliable model (Giraud and Trapman, 2006). Additional DSS's that growers could choose to use include NEWA, SkyBit and AgRadar. NEWA (Network for Environment and Weather Applications) Apple Disease Models is a service provided by Cornell Cooperative Extension (Carroll 2011). SkyBit is a proprietary DSS operated out of Bellefonte, Pennsylvania (Gleason 1997), and finally there is AgRadar, which is provided by Glen Kohler at the University of Maine Cooperative Extension (Orono, Maine) (Kohler 2015). Despite the availability of various simulation models to aid the management of scab, the practice of using DSS's has not been widely adopted to date. Instead, many growers rely on a calendar-based spray program. Calendar-based spraying starts at silver-tip and continues at intervals based on the label of the products being applied or by the growth stage of the apple tree until primary scab season is completed, typically around petal-fall. In low scab risk orchards some growers delay sprays as late as pink Following petal-fall, growers move to cover sprays for the rest of the season keeping the trees protected until harvest. Historically growers have relied on the Mills system in conjunction with calendar-based spray programs. The Mills system is a model usually presented as a table or figure for predicting three levels of infection by ascospores on the basis of temperature and the duration of leaf wetness and the criterion that the time required for infection by conidia is about one-third shorter than that indicated in the table for infection by ascospores (MacHardy 1989). Unfortunately, the prophylactic use of fungicide for the control of apple scab has led to the development of *V. inaequalis* populations that are resistance to an increasing number of fungicides in major apple-growing areas (Beresford et al 2013, Fiaccadori et al 2011), thereby implicating the unsustainability of calendar-based spray programs.

The RIMpro Scab model was developed by Marc Trapman, a consultant to a network of orchardists in the Netherlands, within the framework of a team of researchers, experimenters and technicians. The group's initial aim was to produce an ascospore infection simulation tool that would provide a better epidemiological approach than that offered by Mills system alone, that could run with any weather station and that would be easy for growers and orchard consultants to use (Trapman and Polfliet 1997).

In addition to the Apple Scab module, RIMpro contains models for sooty bloch, codling moth, fire blight, and a first version of a model for downy mildew. In all cases, the models were developed in close collaboration with a project or expert group on the pest or disease in question, and with the potential end-users of the decision support system (DSS). RIMpro is updated annually following progress in knowledge and experience, as well as technical developments and possibilities.

To run the RIMpro program a Biofix (starting point) and local weather data are needed to initiate the calculations. Biofix is the date where the first ascospores in nature are observed to be mature and ready for discharge at the next rain event. This moment can be established accurately by making regular observations of the development of pseudothecia under a microscope, or by wetting leaves and monitoring the ability of the pseudothecia to eject spores. The Biofix date can also be defined from the first ascospore projection as monitored in the field. In this case Biofix should be set a few days before the date where the first spores were observed. If none of this information is available, Biofix is set to the date of green tip of the main apple variety (usually MacIntosh in the Northeast), presuming a similarity between development of the fungus and the host due to coevolution. In most apple production regions, the Biofix date is found to be a date within a few days of green tip of apple. Local weather data requirements include measures of temperature, relative humidity, rainfall and leaf wetness.

RIMpro is capable of reducing fungicide treatments against apple scab by 79% if properly implemented. Use of RIMpro will result in optimal apple scab control and reduced expenses for labor, fungicides, and fuel. The number of fungicide treatments per year is timed by RIMpro; this timing depends on the climate in an individual year. In some years no fungicide treatments can be saved, but the optimal timing of the fungicides application and excellent control of apple scab is provided.

Objective

The objectives of this study were to: (1) evaluate the potential of RIMpro to provide satisfactory control of apple scab in Northeastern apple orchards; and (2) evaluate the effectiveness of potassium bicarbonate + sulfur tank-mix to provide similar levels of control against apple scab as compared to Captan when applied to RIMpro's predictions.

Materials and Methods

The research was conducted at the University of New Hampshire's Kingman Farm located in Madbury, NH in a stand of mature McIntosh trees over the course of the 2013 and 2014 growing seasons. The stand had been unmanaged (feral) for the past dozen years and due to high levels of *V. inaeuqalis*, was utilized as an inoculum source for other experiments and represents an ideal high inoculum sites to evaluate management strategies. Pruning the orchard back into a managed state occurred over the winter of 2012-2013. Due to being unmanaged for an extended period of time, apple scab inoculum levels were considered extremely high at the study site. The experimental design included four treatments: 1 = untreated; 2 = a calendar-based Captan (48.0% Captan) spray program (5kg/ha every 5-7 days starting at green-tip ending at petal-fall); 3 = 5kg/ha Captan 50 WG (wettable granules) (Micro Flo Co., Sparks, GA) applied according to RIMpro recommendations; and 4 = 5kg/ha potassium bicarbonate USP (KBC) (Armand Products Co., Princeton, NJ) + 5kg/ha Kumulus DF (dry-flowable) (80% sulfur) (BASF Corp., Florham Park, NJ) applied according to RIMpro recommendations. In 2014 Fontelis (20.4% penthiopyrad) (DuPont, Research Triangle Park, NC) was added as a tank-mix to the last two of the four sprays made during the primary scab-season for the Captan calendar-based treatment (TRT2) (Table 1). Calendar-based sprays were initiated at green-tip and continued at five to seven day intervals until petal-fall. Sprays were made according to RIMpro predictions whenever an infection period was predicted or when the predicted RIM value exceeded 300. For the KBC+S treatment, depending on the severity of the infection period (RIM value <900) multiple applications of KBC+S were made in what is known as the sandwich method. This is where applications are made before a predicted infection period, during the infection period and immediately following the infection period (Trapman et al 2012). The experimental design was a randomized complete block design (RCBD) with two trees per plot for each treatment and three replications (Figure 2). A buffer row separated individual treatments with at least one tree between treatments in the same row. No insecticide or fertilizer applications were performed for either year of the study. Fungicide applications were made using a Solo 451 Motorized Mist Blower (Solo. Newport News, VA).

Disease assessment. Foliar scab severity, quantified as number of lesions per leaf, was assessed on August 14 in 2013 and September 29 in 2014, with 50 and 30 leaves per tree being assessed, respectively. In addition to visual assessment, measures of reflectance were recorded. Reflectance measurements were obtained by creating a stack of seven leaves placed on top of one another with three scans per stack of leaves taken at 0, 90 and 270° using the VIRIS (Spectra

Vista Corp. Poughkeepsie, NY) in 2013 and an ASD FieldSpec4 in 2014 (ASD Inc. Boulder, CO). Six stacks of leaves were measured for each plot in the experiment. Reflectance measurements were collected with the aid of a hemispherical halogen light source mounted at a 45̊ angle with the distance of the light source from the sample being equidistant to the distance of the sensor to the sample. The spectral reflectance data compliments the visual scab ratings and provides finer scale quantitative data than visual assessments alone. The ANOVA and mean separation procedure were performed using the statistical software JMP Pro 12 (SAS Institute, Cary, NC).

Results

RIMpro effectiveness at reducing apple scab infection levels – Overviews of 2013 and 2014 primary scab season are shown in Figures 5 and 6. The intensity of apple scab severity was similar among years with the overall level of disease on unsprayed apples being 5.4 and 4.8 lesions per leaf for 2013 and 2014 respectively. Averaged across years, the mean number of lesions for unsprayed MacIntosh apple leaves was 4.9 lesions per leaf, and 0.71 lesions per leaf for fungicide treated trees. Among the individual fungicide treatments, the number of lesions per leaf was nearly the same with 0.58 lesions per leaf for Captan applied with a Calendar-spray program, 0.66 lesions per leaf for Captan applied to RIMpro's predictions, and 0.90 lesions per leaf for Potassium Bicarbonate + Sulfur (Kumulus) applied to RIMpro's predictions. In both years, all treated plots had lower mean levels of apple scab estimated as number of lesions per leaf with either Captan applied with a calendar spray program or Captan applied according to RIMpro generally having the lowest levels of disease severity (Figure 3 and 9). All three fungicide treatments were significantly different than the untreated control ($P \leq 0.05$) (Figure 3). There was no significant difference between any of the three fungicide treatments ($P \geq 0.05$). This can also be observed in Figure 4 where reflectance curves comparing fungicide treated apple leaves (Series 2-4) and unsprayed apple-scab infected leaves (Series 1) are shown. The blue line (Series 1) represents the untreated control and differences in reflectance values can be observed within the 568-686nm (red chlorophyll well) and 735-1370nm (near infrared or NIR) regions of the spectrum. Both the increased reflectance in the red chlorophyll well and the narrowing of that well are indicative of reduced amounts of chlorophyll in the untreated control, while the lower reflectance values in the near infrared region indicate that the untreated leaves are less healthy than fungicide treated leaves (Rock et al.1986; 1988). These differences are especially pronounced in the 2014 reflectance curves (Figure 4). Since NIR wavelengths of solar radiation are not absorbed by any pigments within the plant, they travel through most of the leaf and interact with both the palisade and spongy mesophyll cells (Gates 1965, Rock et al. 1986). This interaction (transmission and refraction) causes about half of the energy to be reflected and the other half to be transmitted through the leaf (Gates 1965, Stephens and Rasmussen 2010). In plants with turgid and healthy mesophyll cell walls and in dense canopies, more NIR energy will be reflected and less transmitted (Gates 1965). This cell wall/air space interaction within these cells causes healthy vegetation to look very bright in the NIR. In fact, much more NIR is reflected than in the visible, due to the strong absorption in the visible by chlorophylls and other plant pigments. By monitoring the amount of NIR and visible energy reflected from the plant, it is possible to determine the health of the plant (Stephens and Rasmussen 2010). Vegetation indices (NDVI, REIP and MSI) produced from data generated with the ASD FieldSpec4 show significant differences ($P \leq 0.05$) in reflectance properties between fungicide treated apple leaves and untreated apple leaves (Table 2).

For both 2013 and 2014, fungicide treatment had a significant effect on the number of apple scab lesions per leaf (Tables 3 and 4). The treatment x block effect was not significant in either year. Based on mean separation tests and pairwise comparisons between treatments, there was generally no statistical difference ($P>0.05$) between the three spray programs (TRT2-4) used in either year of the study. In 2014, although treatments where Captan was used generally had fewer average lesions per leaf (Table 4), they were not significantly different from the number of lesions per leaf when KBC+S was applied according to RIMpro.

Number of applications through primary scab season. Throughout the 2013 primary scab season the Captan Calendar treatment received 7 applications, Captan applied according to RIMpro received 5 applications and the KBC+S applied according to RIMpro received 8 applications (Figures 7 and 8, Table 1). While KBC+S applied according to RIMpro received the greatest number of fungicide applications throughout the growing season it should be noted that the spray materials used are relatively low-cost as compared to conventional synthetic spray materials ($23/acre for KBC+S vs $38/acre for Captan).

In 2014 Fontelis was added as a tank-mix to the latter two of the four sprays made during the primary scab-season for the Captan Calendar treatment (TRT2), Captan applied according to RIMpro received three applications and the KBC+S applied according to RIMpro received three applications (Table 1). Overall, the total number of sprays using the RIMpro model was reduced by two applications in 2013 and one application in 2014 as compared to the calendar-based spray program (28% and 25 % reduction in sprays for 2013 and 2014, respectively).

Discussion

Following completion of this research we have confirmed that a practice that conventional and organic grower alike could readily adapt into their IPM plan is the use of RIMpro. Decision support systems such as RIMpro results have been shown to provide effective apple scab control. The number of fungicide treatments per year is timed by RIMpro; this timing depends on the actual climate in an individual year. In the first year of this study two fungicide treatments were saved, in the second one fungicide treatment was saved. While RIMpro has been shown to be effective at reducing the total number of sprays in a season it is not the only DSS that growers located in the northeastern U.S. could choose to implement. Other suitable DSS's that growers could choose to use include NEWA, SkyBit and AgRadar.

In a 2014 study conducted by Jon Clements, at Cold Spring Orchard located in Belchertown, MA, roughly 2 hours from Durham NH, NEWA's apple scab model predicted 9 primary infection periods, Skybit predicted 10 primary infection periods, AgRadar and RIMpro both predicted 6 primary infections periods. Results of the Cold Spring study align well with the results of the 2013 and 2014 Kingman Farm study. With Cold Spring having an average disease severity of 7.15 for unsprayed apple trees, estimated as the number of infected leaves per terminal in contrast to 0.66 and 0.65 disease severity for the Calendar and RIMpro-based treatments respectively (unpublished results).

Results of the Kingman Farm and Cold Spring Orchard study indicate that excellent control of apple scab is possible as long as an effective spray program is in place. AgRadar and RIMpro predicted the fewest number of primary infections for Cold Springs in 2014 although the timing of predicted infection events varied between the 2 models with AgRadar predicting the start of primary scab season between Apr 29 –May 2 and ending June 4-5 and RIMpro predicting the start of primary scab season as being between Apr 22-24 and ending May 22-24.

Results from the Kingman Farm trial indicate that RIMpro is a suitable DSS to be used to support the IPM decision making process. RIMpro was able to reduce the total number of spray applications made during the primary scab season by two sprays in 2013 and one spray in 2014 although if RIMpro had not been used to decide when to stop spraying in 2014 the total number of sprays saved would have increased from one spray to four, which would have been a 57% reduction in fungicide applications instead of the 28% and 25% reductions in fungicide applications observed in 2013 and 2014, respectively. An accurate Biofix date continues to be of utmost importance when deciding when to start the model each season, the Biofix date between the two years of the study was similar occurring on April 22 in 2013 and April 25 in 2014.

The use of spectrometers to assess the reflectance properties of apple leaves was shown to be useful in this study. Vegetation indices (NDVI, REIP and MSI) generated from data collected using the ASD FieldSpec 4 show that apple leaves that were treated with a fungicide during primary scab season were healthier than leaves left. These vegetation indices compliment data collected in this study showing that untreated apple leaves had a greater number of lesions per leaf when compared to fungicide treated leaves.

The impact on high intensity rainfall events on the epidemiology of the apple scab pathogen warrants further investigation. It would be expected that high intensity rainfall events early in the primary scab season would shorten the duration of primary scab season in orchards due to early depletion of ascospores. This effect was observed during the 2014 growing season at Kingman Farm where after the third infection event RIMpro predicted that the asci would have released all their ascospores. This is despite the fact three additional infections periods were predicted following the rain event where RIMpro predicted that all ascospores had been released. For both 2013 and 2014 RIMpro predicted seven infection events during primary scab season (Figure 5, Figure 6). The model seems to have been accurate as fungicide application ceased following the third infection event in 2014 and average disease levels were similar to those observed in 2013 when sprays continued for the entire primary scab season.

One of the difficulties growers face when choosing to implement a given DSS is the challenge of spraying a large number of acres within a small time-frame. Meteorological conditions can shift unexpectedly leaving a grower with a small window in which to spray an entire orchard. This challenge can be addressed through the use of fixed-spray systems (Agnello 2005, 2007 and 2015, Sharda 2015, Tennes 1976). Fixed sprays systems are an excellent candidate for variable-rate application of spray materials based on DSS predictions. A fixed spray system has potential as an alternative method of precision and timely delivery of pesticides while providing equivalent spray coverage compared to orchard airblast sprayers in tree fruit orchards. A fixed spray application system consists of hoses and emitters through which a spray solution is delivered (Sharda 2015). Spraying an entire orchard using a fixed system could have several advantages that would justify the initial establishment costs and reduce pesticide-associated risks. Spray drift would be minimized without sacrificing adequate crop protection. Pesticide application could be a more efficient process, achievable in a fraction of the time of tractor spraying, during shorter windows of acceptable spraying conditions, and at times of the year (i.e., early season) when ground conditions might make it impractical to drive through the orchard (Agnello 2005). Because multiple sprays and re-sprays would be much easier, this enhanced efficiency would make it more practical to use lower rates of pesticides and more "least-toxic" alternative or organically approved materials that have relatively short residual effectiveness, such as botanicals, microbials, oils, soaps, or insect growth regulators. To the extent that alternative pest management programs would be more realistic options in such plantings, such a system could favor growing fruit profitably for organic or niche specialty markets in selected blocks (Agnello 2007).

The results from this investigation clearly emphasizes the importance of having an effective spray program in place and the usefulness of DSSs such as RIMpro. Also the potassium bicarbonate + sulfur treatment (KBC+S) was shown to provide the same level of control as Captan used alone or when tank-mixed with Fontelis. This finding indicates that KBC+S is a suitable candidate for use in organic orchard operations or for those interested in using low-cost, low-hazard alternative spray materials as part of their IPM programs.

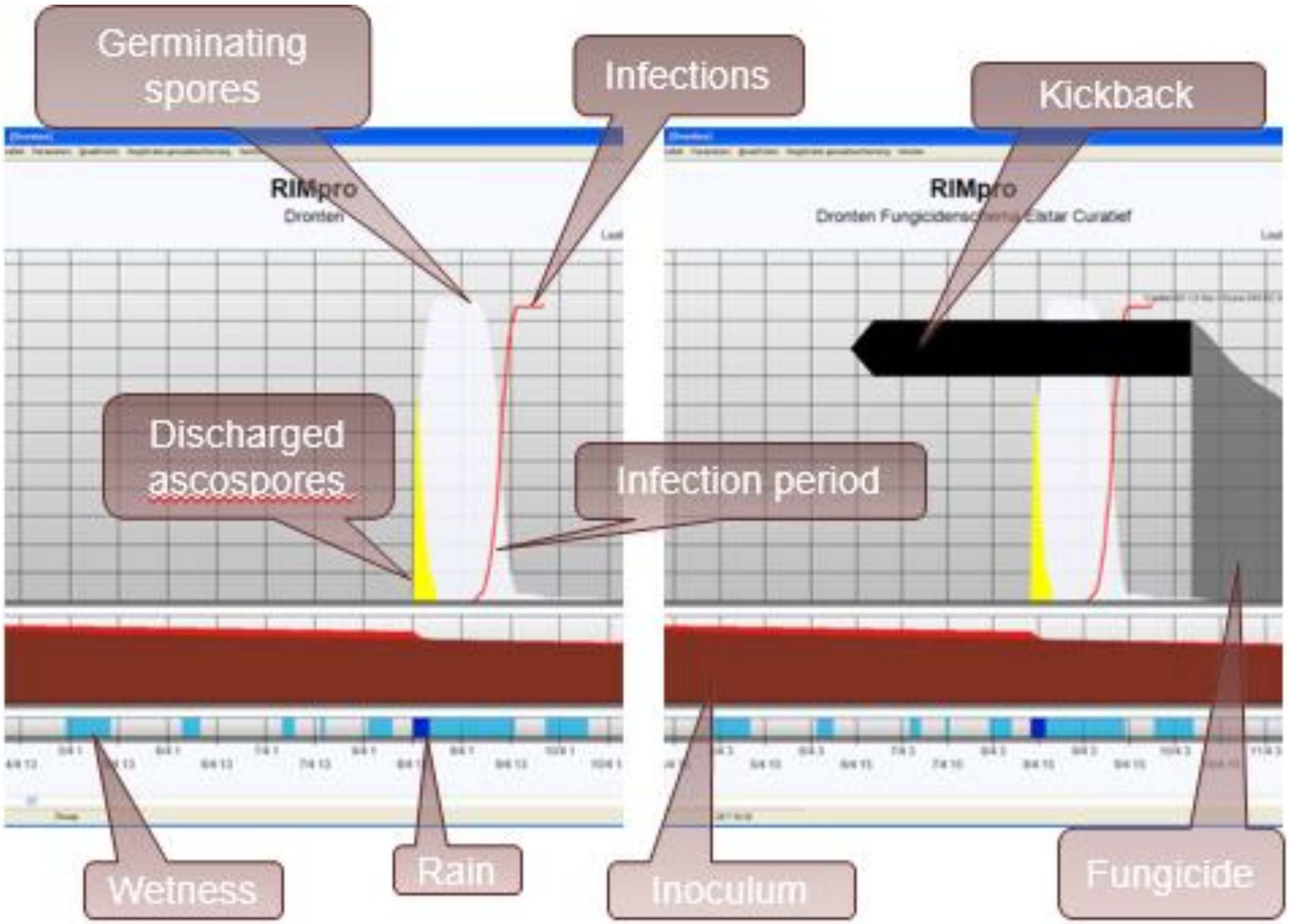

Figure 1. Overview of the RIMpro Disease Forecasting Model GUI (graphical user interface) for apple scab. Graph showing applied fungicide cover (grey) on a given plot superimposed on ascospore discharge graphs (yellow) and RIM infections (red line). Degradation is simulated according to active substance degradation, growth and leaching parameters (dark blue). The back arrow symbolizes protection by the curative effect (black). The potential ascospore dose is represented in the middle box either in red (ripe) or in brown (unripe). Periods of leaf wetting are shown in light blue.

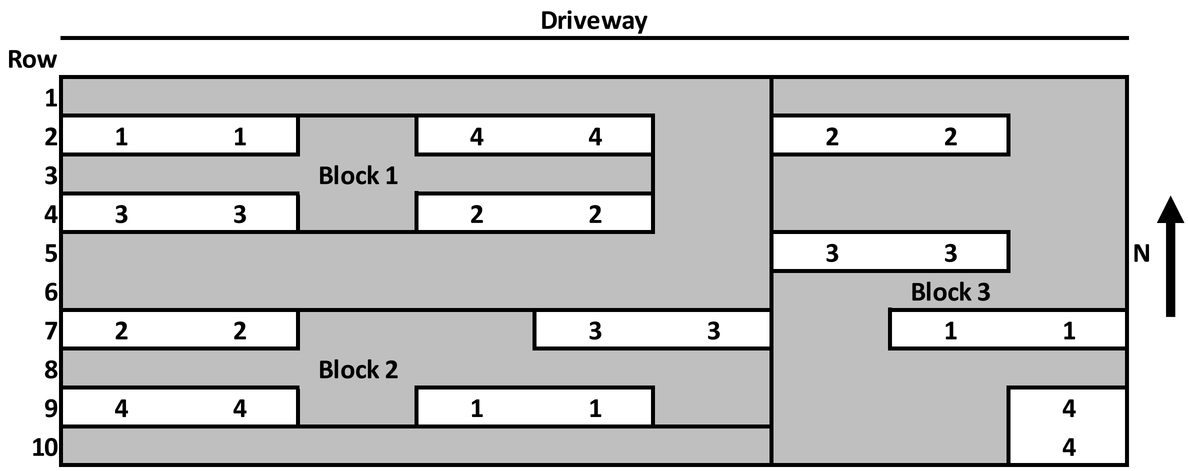

Figure 2. Plot map showing layout of experiment for 2013 and 2014 field trials located at Kingman Farm in Madbury, NH. Numbers 1-4 represent individual trees receiving different fungicide treatments: untreated control, UTC = 1; Captan Calendar-based = 2; Captan RIMpro = 3; and KBC+S Rimpro = 4.

Table 1. Table showing spray dates and rates for products used during the 2013 and 2014 growing season at Kingman Farm located in Madbury, NH.

2013

TRT#	TRT*	19-Apr	27-Apr	6-May	8-May	10-May	11-May	12-May
1	UTC	...	...	...	...	...	...	...
2	Captan (Calendar)	4.5lb/A	4.5lb/A	4.5lb/A	...	...	4.5lb/A	...
3	Captan (RIMpro)	...	...	...	4.5lb/A	...	4.5lb/A	...
4	KBC + S (RIMpro)	...	...	...	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A		4.5lb/A +4.5lb/A

2013 cont,

TRT#	TRT*	21-May	23-May	24-May	29-May	3-Jun	11-Jun
1	UTC	...	...	...	...	...	...
2	Captan (Calendar)	4.5lb/A	...	...	4.5lb/A	4.5lb/A	...
3	Captan (RIMpro)	...	...	...	4.5lb/A	4.5lb/A	4.5lb/A
4	KBC + S (RIMpro)	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A

2014

TRT#	TRT*	Rate 4/21	Rate 4/28	Rate 5/10	Rate 5/17
1	UTC	...	...	...	...
2	Captan (Calendar)	1.5lb/A	1.5lb/A	4.5lb/A	4.5lb/A
2	Fontelis	...	...	20 oz/A	10 oz/A
3	Captan (RIMpro)	...	1.5lb/A	4.5lb/A	4.5lb/A
4	KBC + S (RIMpro)	...	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A	4.5lb/A +4.5lb/A

*Treatments were applied using a Solo 451 Motorized Mist Blower (Solo. Newport News, VA) calibrated to deliver 35 gallon of water per acre.

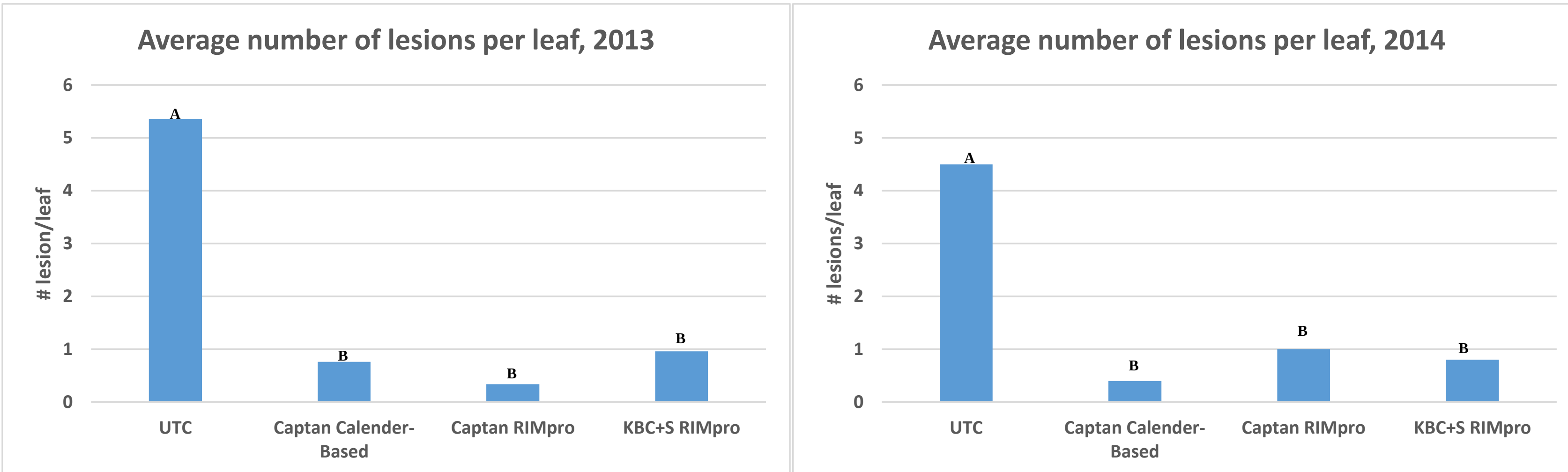

Figure 3. Average number of apple scab lesions per leaf for the 2013 (left) and 2014 (right) growing seasons. Means were compared using Student's t test with the statistical software JMP Pro 12 (SAS Institute, Cary, NC).

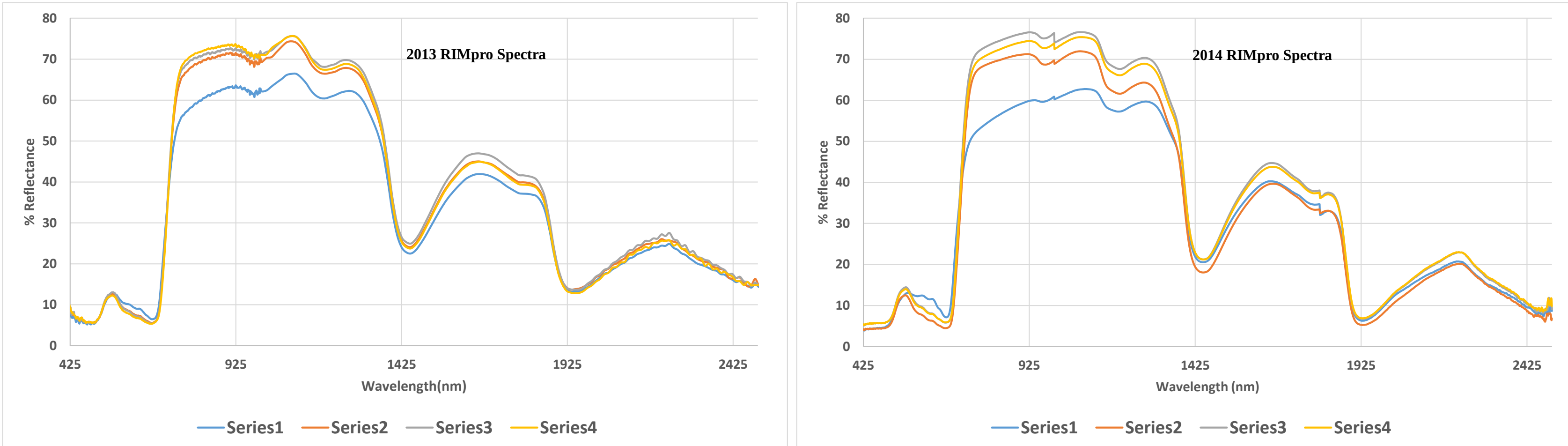

Figure 4. Reflectance curves comparing fungicide treated apple leaves (Series 2-4) and unsprayed apple-scab infected leaves (Series 1). The blue line (Series 1) represents the untreated control and obvious differences in reflectance values can be observed within the 568-686nm (red chlorophyll well) and 735-1370 (near infrared or NIR) regions of the spectrum. Results were obtained by scanning six stacks of seven leaves with three scans per leaf taken at 0, 90 and 270° using the VIRIS (GER. Poughkeepsie, NY) in 2013 (left) and an ASD FieldSpec4 in 2014 (right) (ASD. Boulder, CO). UTC = Series 1; Captan Calender-based = Series 2; Captan RIMpro = Series 3; and KBC + S Rimpro = Series 4.

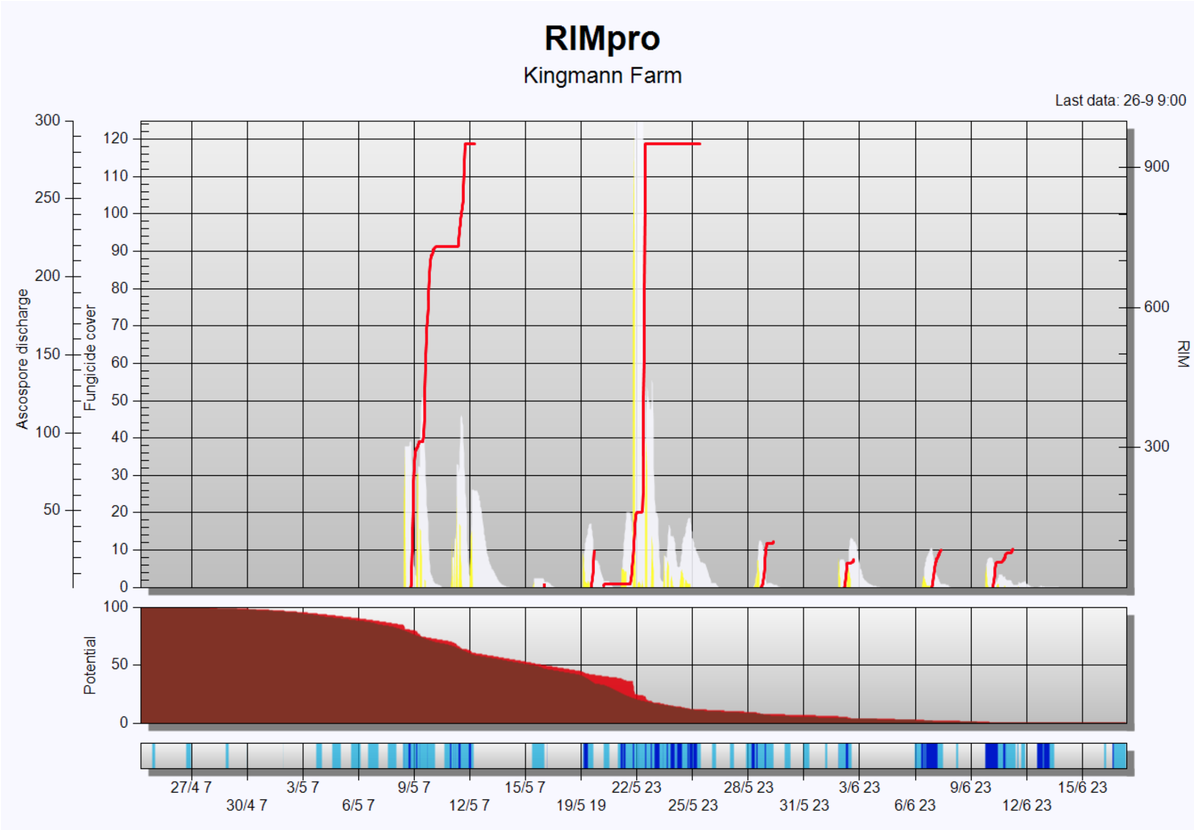

Figure 5. Overview of 2013 primary scab season at Kingman Farm in Madbury, NH. Graph showing ascospore discharge graphs (yellow) and RIM infections (red line). Periods of leaf wetting are shown in light blue and periods of rainfall in dark blue. The potential ascospore dose is represented in the middle box either in red (ripe) or in brown (unripe). An infection period is considered severe if the RIM value exceeds 900.

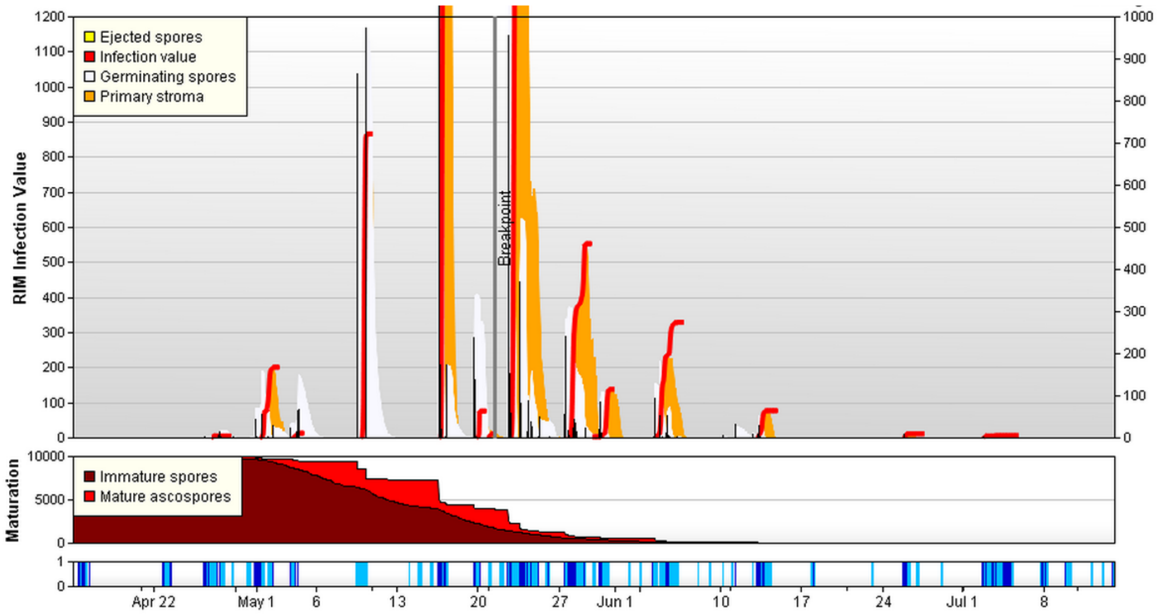

Figure 6. Overview of 2014 primary scab season at Kingman Farm in Madbury, NH. Graph showing ascospore discharge graphs (yellow) and RIM infections (red line). Periods of leaf wetting are shown in light blue and periods of rainfall in dark blue. The potential ascospore dose is represented in the middle box either in red (ripe) or in brown (unripe). An infection period is considered severe if the RIM value exceeds 900.

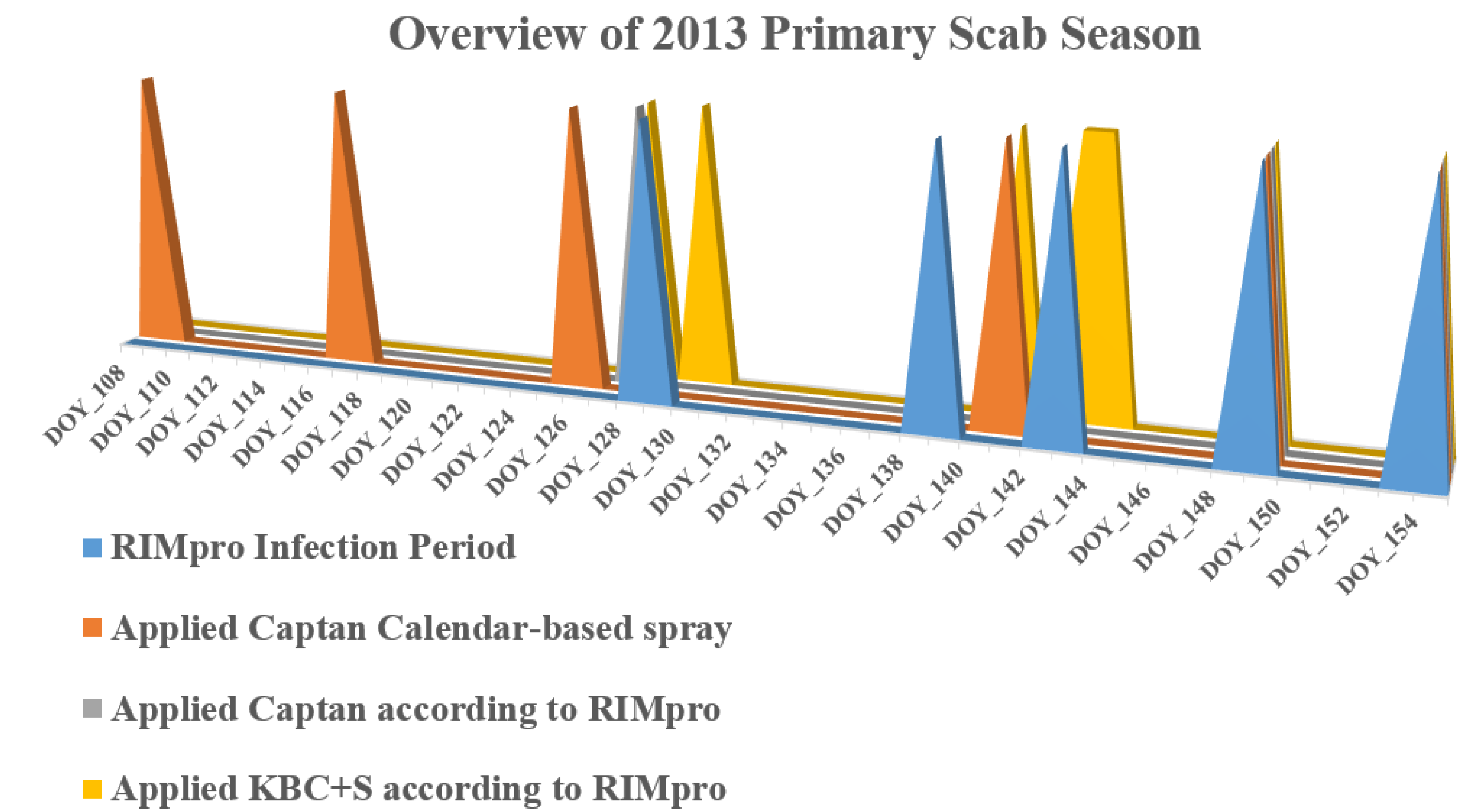

Figure 7. Overview of 2013 primary scab season showing infection periods predicted by RIMpro (blue), day of year when Captan Calendar-based treatments were made (red), day of year when Captan RIMpro-based treatments were made (grey), day of year when KBC+S RIMpro-based treatments were made (orange). Notice that 3 Captan Calendar-based applications were applied prior to RIMpro predicting the first infection event.

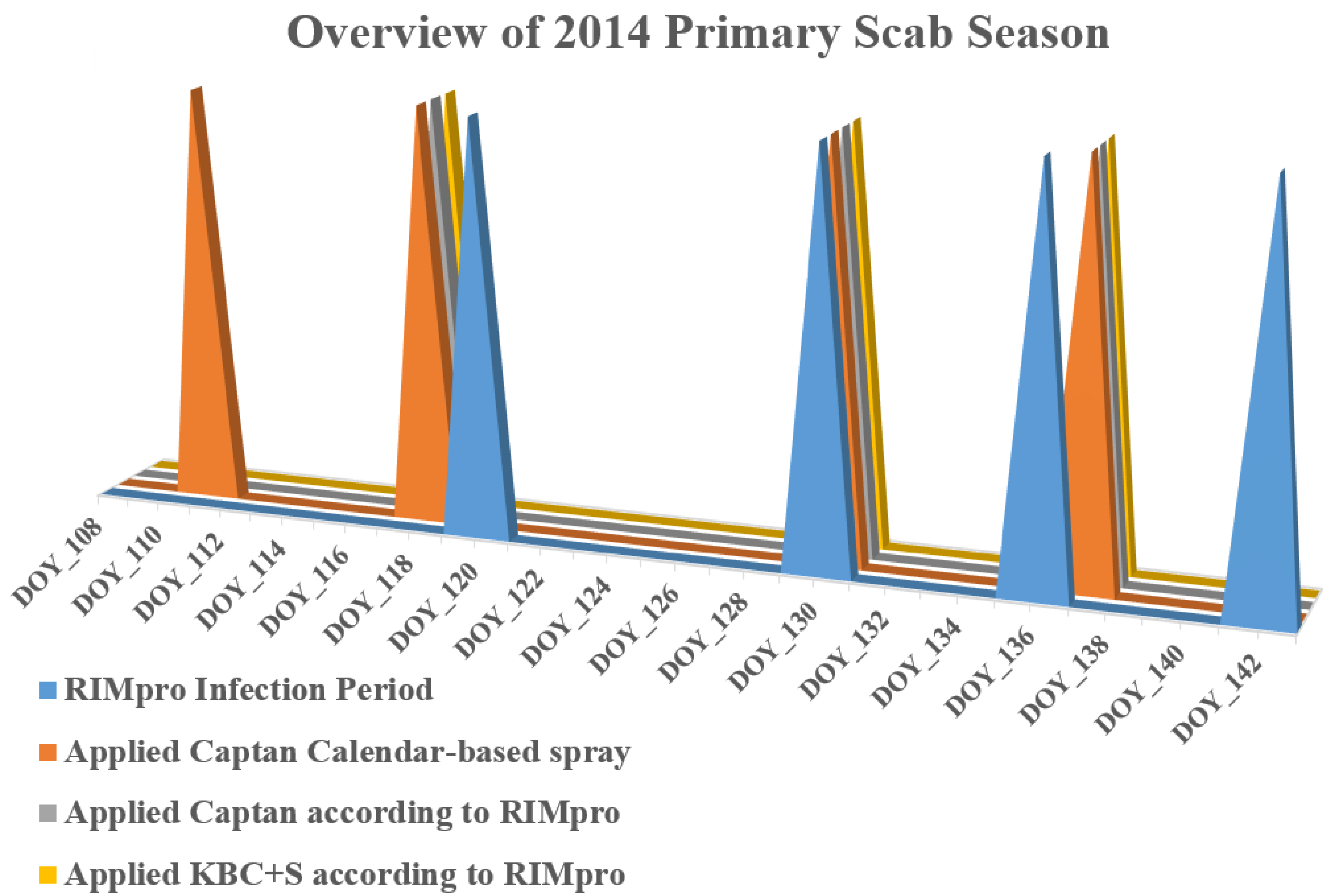

Figure 8. Overview of 2013 primary scab season showing infection periods predicted by RIMpro (blue), day of year when Captan Calendar-based treatments were made (red), day of year when Captan RIMpro-based treatments were made (grey), day of year when KBC+S RIMpro-based treatments were made (orange). Notice that 2 Captan Calendar-based applications were applied prior to RIMpro predicting the first infection event.

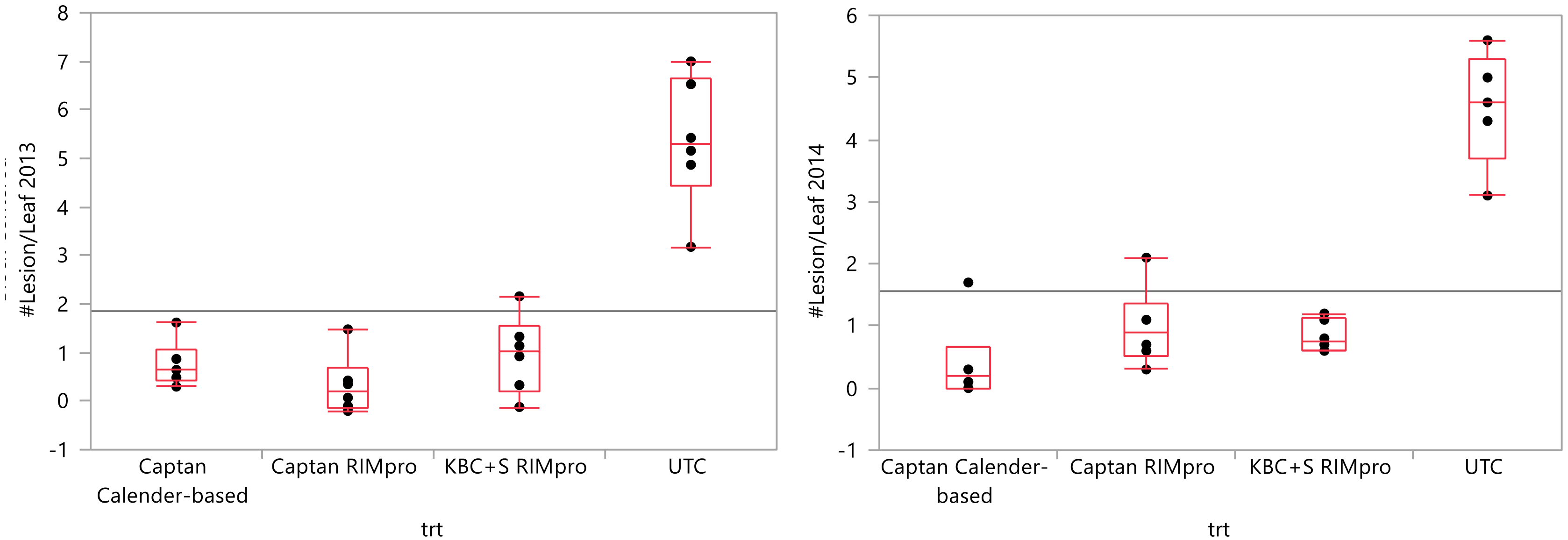

Figure 9. Boxplots for the 2013 (left) and 2014 (right) RIMpro trials. The ANOVA was completed with the statistical software JMP Pro 12 (P≤0.05) (SAS Institute, Cary, NC).

Table 2. Vegetation indices produced from reflectance measurements comparing untreated apple leaves to several fungicide programs.

Index	**Reference**	**Related to**	**Computation**	**Unsprayed**		**Captan Calendar-based**		**Captan RIMpro**		**KBC+S RIMpro**		***LSD***
NDVI	Rouse et al. (1974)	Chlorophyll, stress	((μR760-900)-(μR630-690))/((μR760-900)+(μR630-690))	0.72	b[x]	0.86	a	0.83	a	0.83	a	*0.06*
REIP	Rock et al. (1988)	Chlorophyll, stress	R740*((((R680+780)/2)-700)/(R740-700))	713	b	721	a	719	a	720	a	*3.22*
MSI	Hunt and Rock (1989)	Water content	(R1550–1750)/(R760–800)	0.68	a	0.53	c	0.56	b	0.57	b	*0.02*

[x] Index means within a row followed by the same letter are not significantly different at $P \leq 0.05$.

References

Agnello, A., Landers, A., and Loeb, G. 2015. A fixed-spray system for Spotted Wing Drosophila management in high tunnel bramble crops. Journal of Berry Research, 5, 1-8.

Agnello, A., Landers, A., 2007. Current progress in developing a fixed-spray application system for high density plantings. Published online. http://web.entomology.cornell.edu/agnello/assets/Agnello_Fixed_Spray_05.pdf

Agnello, A., Landers, A., 2005. Optimization of a fixed spraying system for commercial high-density apple plantings. Final Report. Published online. http://web.entomology.cornell.edu/agnello/assets/Agnello_Fixed_Spray_07.pdf

Beresford, R., Wright, P., Wood, P., Park, N., Larsen, N., and Fisher, B. 2013. Resistance of *Venturia inaequalis* to demethylation inhibitor and dodine fungicides in four New Zealand apple-growing regions. N. Z. Plant Prot., 66, 274-283.

Carroll, J., Weigle, T. and Petzoldt, C. 2011. The network for environment and weather applications (NEWA). NY Fruit Q, 19, 5-9.

Carisse, O., Philion, V., Rolland, D., and Bernier, J. 2000. Effect of fall application of fungal antagonists on spring ascospore production of the apple scab pathogen, *Venturia inaequalis*. Phytopathology, 90, 31-37.

Didelot, F., Caffier, V., Orain, G., Lemarquand, A., and Parisi, L. 2016. Sustainable management of scab control through the integration of apple resistant cultivars in a low-fungicide input system. Agriculture, Ecosystems & Environment, 217, 41-48.

Fiaccadori, R., Cicognani, E., Alberoni, G., Collina, M., and Brunelli, A. 2011. Sensitivity to strobilurin fungicides of Italian *Venturia inaequalis* populations with different origin and scab control. Pest Manag. Sci., 67, 535-540.

Gates, D.M., Keegan, H.J., Schleter, J.C., and Weidner, V.R. 1965. Spectral properties of plants. Applied Optics, 4.

Giraud, M. and Trapman, M. 2006. The Rimpro model: Interest in the management of apple scab. L'arboriculture fruitière, 603, 29-32.

Gladieux, P., Zhang, X., Afoufa-Bastien, D., Sanhueza, R., Sbaghi, M., and Le Cam, B. 2008. On the origin and spread of the scab disease of apple out of Central Asia. PLoS One3e1455.

Gleason, M., Parker, S., Pitblado, R., Latin, R., Speranzini, D., Hazzard, R. and Biederstedt, D. 1997. Validation of a commercial system for remote estimation of wetness duration. Plant Disease, 81, 825-829.

Gomez, C., Brun, L., Chauffour, D., and De Le Vallée, D. 2007. Effect of leaf litter management on scab development in an organic apple orchard. Agriculture, Ecosystems & Environment, 118, 249-255.

Harris, S., Robinson, J., and Juniper, B. 2002. Genetic clues to the origin of the apple. Trends in Genetics, 18, 426–430.

Hickory K. 1991. Fungicide benefits assessment fruit and nut crops - East. USDA National Agricultural Pesticide Impact Assessment Program.

Holb, I., Heijne, B., Withagen, J., Gáll, J., and Jeger, M. 2005. Analysis of summer epidemic progress of apple scab at different apple production systems in the Netherlands and Hungary. Phytopathology, 95, 1001-1020.

Hunt, E. and Rock, B. 1989. Detection of changes in leaf water content using near-and middle-infrared reflectances. Remote Sensing of Environment, 30, 43-54.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151.

Juniper, B., and D. Mabberley. 2006. The Story of the Apple. Timber Press, Portland, OR.

Koehler, G. 2001. Apple spray materials cost per dose. http://umaine.edu/ipm/files/2010/10/AppleSprayDoseCost.pdf

Koehler, G. 2015. Ag-Radar – Apple. http://extension.umaine.edu/ipm/ag-radar-background/ag-radar-apple-sites/

Köhl, J., Scheer, C., Holb, I., Masny, S., and Molhoek, W. 2015. Toward an Integrated Use of Biological Control by *Cladosporium cladosporioides* H39 in Apple Scab (*Venturia inaequalis*) Management. Plant Disease, 99, 535-543.

MacHardy, W. E., & Gadoury, D. M. 1989. A revision of Mills criteria for predicting apple scab infection periods. Phytopathology, 79, 304-310.

MacHardy, W. 1996. Apple Scab: Biology, Epidemiology, and Management. APS Press, St. Paul, MN.

MacHardy W. 1998. Action thresholds increase fungicide and sanitation efficiency in integrated management of apple scab. Proceedings of the 7th International Congress of Plant Pathology.

MacHardy W., Gadoury D., and Rosenberger D. 1993. Delaying the onset of fungicide programs for control of apple scab in orchards with low potential ascospore dose of *Venturia inaequalis*. Plant Disease, 77, 372-375.

Rock, B., Hoshizaki, T., and Miller, J. 1988. Comparison of in situ and airborne spectral measurements of the blue shift associated with forest decline. Remote Sensing of Environment, 24, 109-127.

Rock, B., Vogelmann, J., Williams, D., Vogelmann, A., and Hoshizaki, T. 1986. Remote detection of forest damage. BioScience, 36, 439-445.

Rouse Jr, J., Haas, R., Schell, J., and Deering, D. 1974. Monitoring vegetation systems in the Great Plains with ERTS. NASA special publication, 351, 309.

Rossi, V. and Bugiani, R. 2007. A-scab (Apple-scab), a simulation model for estimating risk of Venturia inaequalis primary infections. EPPO Bulletin, 37, 300–308.

Sharda, A., Karkee, M., Zhang, Q., Ewlanow, I., Adameit, U., and Brunner, J. 2015. Effect of emitter type and mounting configuration on spray coverage for solid set canopy delivery system. Computers and Electronics in Agriculture, 112, 184-192.

Stephens, C., and Rasmussen, P. 2010. On target near infrared tutorial. Utah State University Extension. Logan, UT.

Sutton, D., MacHardy, W., & Lord, W. 2000. Effects of shredding or treating apple leaf litter with urea on ascospore dose of *Venturia inaequalis* and disease buildup. Plant Disease, 84, 1319-1326.

Tennes, B., Burton, C., and Reichard, D. 1976. Concepts for metering sprays and spraying in high density fruit culture. Paper No. 76-1505. ASAE. St Joseph, MI: American Soc. of Agric. Engineers.

Trapman, M., Polfliet M. 1997. Management of primary infections of Apple-scab with the simulation program RIMpro: review of four years of field trials. In Proc. of the 4th workshop on integrated control of pome fruit diseases. Bull. OILB-SROP, 20, 241–250.

Trapman, M., Triloff, P., Philion, V. and Scholffer, K. 2012. The European Apple Scab Sandwich. The European Fruit Magazine, 2012-4.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P., 2010. The genome of the domesticated apple (Malus × domestica Borkh.). Nature Genetics, 42, 833–839.

Vincent, C., Rancourt, B., & Carisse, O. 2004. Apple leaf shredding as a non-chemical tool to manage apple scab and spotted tentiform leaf miner. Agriculture, ecosystems & environment, 104, 595-604.

UAVs for orchard management: Prospects and procedures for aerial monitoring of plant health status in the Northeastern U.S.

Matt Wallhead and Kirk Broders[1]
[1]Department of Biological Sciences, University of New Hampshire, Durham, NH 03824

Abstract

Disease outbreaks, insect infestation, nutrient deficiencies, and weather variation constantly threaten to diminish annual yields and profits in orchard crop production systems. Automated crop inspection with an unmanned aerial vehicle (UAV) would allow growers to regularly survey crops and detect areas affected by disease or stress and lead to more efficient targeted applications of pesticides, water and fertilizer. The overall goal of this project was to develop a low cost (~$2000USD) aerial imaging platform coupling low cost sensors with low cost unmanned aerial vehicles (UAVs) to be used for monitoring crop health. Following completion of this research, we have identified a useful tool for agricultural and ecological applications. For commercial agriculture, farmers using UAV-based remote sensing will be able to detect disease outbreaks, determine fertility requirements and assess overall crop health and yield potential. This project emphasizes the interrelationships between robotics, imaging systems, geospatial technologies, and agricultural production systems to develop the next generation of tools for monitoring crop health.

Introduction

Orchard crop production is vulnerable to a number of potential risks such as apple scab, fire blight, frost damage and insect damage. A particular disease of importance is Apple scab, caused by the ascomycete pathogen *Venturia inaequalis* (conidial stage: *Spilocaea pomi*). *Venturia inaequalis* is worldwide the most important disease in the production of apples as measured by the potential economic loss, the cost and the amount of fungicides necessary to control the disease each year, the constant consideration of the growers, and the environmental impact of the control measures (MacHardy, 1996). Apple scab occurs wherever apples are grown. It is not possible to produce apples commercially without an effective fungicide program to control apple scab. In fact, the USDA National Agricultural Pesticide Impact Assessment Program estimated that 100% of Eastern apple orchards are affected by apple scab, and without fungicide treatments, yield losses would be as high as 90% (Hickory 1991).

Disease outbreaks, insect infestation, nutrient deficiencies, and weather variation constantly threaten to greatly diminish annual yields and profits. Ground and aerial based remote sensing research has proven that many types of diseases, including pre-visual disease symptoms for pathogens, invasive species and crop health markers can be detected through airborne hyperspectral imaging (Rock et al. 1988), but this type of workflow does not appreciably increase efficiency of plant inspection. While experienced growers can identify many diseases and pests, they may not have the time to thoroughly inspect entire orchards. What is needed is a cost-effective way for growers to identify crop problems in their fields without manually scouting the entire farm. Automated crop inspection with an unmanned aerial vehicle (UAV) would allow growers to easily and regularly survey large areas. However, the issue with automated crop inspection using digital imagery alone is that identifying crop problems can be challenging and labor intensive, as automated techniques are not robust (Slaughter 2008). Hyper or multispectral imagery allows for a great deal more information to be captured about different light bands, allowing for much easier identification of crop problems (Garcia-Ruiz 2012).

The solar-reflected optical spectrum spans a wavelength range of 400 nm to 3000nm (Asner 1998). Of this range, the 400 nm to 2500 nm region is routinely measured using a variety of optical sensors ranging from multispectral (for example, Landsat TM and OLI) to hyperspectral (for example, AVIRIS) (Asner 1998). The relationship between plant growth and spectral response in the visible and infra-red wavelengths has been well established using the ratio of red and infra-red reflectance, or other indices based on this ratio (Jackson 1984, Bauer 1985). Early research with color and color infra- red air photos demonstrated that a number of crop infestations could be detected on the photographs including apple scab, wheat stem rust, corn leaf blight, and root rot in field beans (Hatfield and Pinter 1993). Stress caused by the disease infestation results in reflectance changes in the vegetation which can then be detected with the remote sensing data (Garcia-Ruiz 2012). Many of the soil and crop parameters of interest to the grower are very dynamic with time and thus repetitive coverage made possible with remote sensing platforms, especially UAV platforms, are an attractive source of monitoring information. This information can then be used in conjunction with management units, in order to quickly evaluate potential problems and to provide an effective management solution.

Analyzing vegetation using remote sensing data requires knowledge of the structure and function of vegetation and its reflectance properties (Goetz et al. 1983, Rock et al. 1986). This knowledge enables one to link vegetative structures and their condition to their reflectance behavior in an ecological system of interest. However, an effective multispectral UAV platform must flag problem areas, identify the source of the problem, and delineate its location and spatial extent. Progress has been made in this regards, especially in the field of weed management (Barragan 2012, Pena 2013, Torres-Sanchez 2013) but also for Huanglongbing-infected citrus trees (Garcia-Ruiz 2013), Verticillium wilt of olive (Calderon 2013), and downy mildew of poppy (Calderon 2014).

Vegetation reflectance properties are used to derive vegetation indices (VIs). VIs are constructed from reflectance measurements in two or more wavelengths across the optical spectrum to analyze specific characteristics of vegetation, such as total leaf area, chlorophyll content and water content (Rock et al 1986, Hunt and Rock 1987). For different properties and field conditions, some indices within a category provide results with higher validity than others. By comparing the results of different VIs in a category, and correlating these to field conditions measured on site, you can assess which indices in a particular category perform the best at modelling the variability in your scene (Asner 1998). The VIs provided by many software packages are not designed to quantify the exact concentration or abundance of any given vegetation component. Instead, they are intended for use in geographically mapping relative amounts of vegetation components, which can then be interpreted in terms of ecosystem conditions (Asner 1998). This technology could potentially improve grower profits by reducing pesticide use, improving yields, and reducing labor and operating costs.

Based on this information the overall goals of this project were to develop an aerial imaging platform coupling low cost sensors with low cost unmanned vehicles, and then evaluate the effectiveness of this platform at detecting disease and abiotic stress of orchard crops. This will lead to the development of tools that allow growers to identify crop problems through periodic surveillance of their crops. This product would contribute a cost-effective and valuable tool for farm-scale pest management and research.

Objectives:

1) Evaluate unmanned aerial systems (UAV + sensor) to maximize stability and minimize cost;

2) Determine whether orthomosaics with sub-centimeter ground resolution could be produced using a commercial-off-the-shelf (COTS) imaging system mounted onboard an UAV.

3) Determine the optimal spectral bands within the 350 to 2500nm range for detection of apple scab.

Materials and Methods

Evaluation of UAV platforms to maximize stability and minimize cost.

Sensor selection. The Canon Powershot S100 and S110 (Canon U.S.A Inc. Melville, NY) were selected as a good base for this application due to its global shutter, 12MP resolution, relatively low weight and compactness. The 12MP resolution is more than adequate for this application. The global shutter is highly desirable for snapshot imaging applications like this one. Most image sensors used for video use a rolling shutter, where the pixels are exposed at different times and overlap with the readout. This adds significant temporal distortion when vibration or motion are present which is difficult to remove. With a global shutter all pixels are exposed simultaneously, eliminating this problem. CHDK (Canon Hack Developers Kit), a program loaded on the memory card of the cameras add additional functionality, most importantly the ability to trigger camera to capture images based on a user-defined interval. To install CHDK onto the camera's memory card the program STICK (Dave Mitchell) (zenoshrdlu.com) was used. Camera settings change depending on environmental conditions but it is advised to use as low an ISO as possible, maximizing both shutter speed and aperture and setting the focus to infinity. For example, a common setting used to capture aerial photos would be ISO100, shutter speed

1/1600, F2.5, and infinity focus. CHDK is used to trigger the camera every 4 seconds and Mission Planner software (Michael Osborne) (ardupilot.com/downloads) was used to geotag the acquired imagery.

Step 2. Air Vehicle Integration: For the proposed research, a six-rotor multicopter platform was chosen to be implemented (Figure 1 A. The frame is a 550mm hexacopter frame (H550-V1 Hobbyking Hong Kong, China) modeled after DJI's Flamewheel platform (DJI Shenzhen, Gaundong, China). The platform was assembled and prepared for flight by Rotary Robotics (Rotary Robotics Boston, MA) The 550 hexacopter frame type allows for stable hovering in moderate winds with a payload of up to 1 lb. Flight duration was approximately 5-7 minutes, depending on weather conditions. Hovering platforms (VTOL, vertical take-off and landing) allow for easy variation of altitude and viewing angle. Many applications for UAVs may find that fixed wing platforms are more suitable for the area to be imaged but for this research (small-scale orchards surrounded by forest) the multirotor platform is better suited.

The research vehicle was initially equipped with an APM2.5 (ArduPilotMega autopilot system) (copter.ardupilot.com), this hardware is specifically designed to allow for flexible vehicle control in a package that weighs less than 15 grams. The board is equipped with all necessary sensors to control the vehicle as well as a GPS receiver. In May 2014 the APM2.5 flight controller was replaced with a NAZA-M V2 (DJI Shenzhen, Gaundong, China). Additionally, to add autonomous capabilities to the Naza-M V2 a 2.4GHz Bluetooth datalink was required (DJI Shenzhen, Gaundong, China).

During the mid-summer 2014, a second UAV, the 3D Robotics X8 platform (3DR Berkeley, CA, Figure 1 B), was also evaluated. The X8 is a coaxial octocopter with flight times of 15 minutes and a payload capacity of 2 pounds. The X8 has an APM2.6 flight controller with

3DR u-blox GPS with compass (3DR Berkeley, CA). The X8 was fitted with a 3-axis DYS gimbal (Huizhou, Guangdong, China) and 8-bit AlexMoS brushless gimbal stabilization board and inertial measurement (IMU) unit (Krasnodar, Krasnodar Krai, Russia) in order to stabilize the camera to enhance image quality.

Evaluation of ability to produce orthomosaics with sub-centimeter ground resolution produced using commercial-off-the-shelf (COTS) imaging system mounted onboard an UAV.

In order to determine the capacity of a UAV mounted imaging system to accurately monitor and detect biotic and abiotic stresses in orchard production systems, multiple flights were made over the Woodman Farm Orchard located in Durham, NH. Only flights with the X8 are reported in this study due to the limited mission planning capabilities provided by the Naza-M V2 in the 550 hexacopter and the lack of ability to geo-tag images. Flights were made on August 30, September 26, October 15, Oct 18, November 13 and November 27 in 2014 and April 30 and May 9 in 2015. Flight planning was completed using the software Mission Planner V1.3.25 for Windows, Droidplanner 2 application (Arthur Benemann) (play.google.com) for Android or the Tower application (3DR Berkeley, CA) (play.google.com) for Android. Flights were flown at altitudes between 75 and 100 meters with the April 30 2015 flight being the one exception and was flown at an altitude of 30 meters. Photoscan Agisoft V1.1.6 was used create orthomosaics. Agisoft setting used were Align Photos; Accuracy=high, Pair Selection=reference, Key point limit=40000, Tie Point limit=1000. Build Dense Cloud; Quality=High, Depth Filtering=Mild. Build Mesh; Surface Type=height field, Source data=dense cloud, Face Count=high, Interpolation=enabled, Point Classes=All. Build Texture; Mapping Mode=Adaptive orthomosaic, Blending mode=mosaic, Texture size/count=4096. After exporting the resulting orthomosaic, the resulting image was imported into QGIS 2.8.1 where it was georeferenced to produce the final rendered orthomosaic. At this point the images are ready for visual image analysis.

Evaluation of 2500 spectral bands for detection of apple scab.

In order to determine the proper optical bandpass filters for the detection of apple scab two years of field trials were conducted. This was complete by evaluating the effectiveness of the RIMpro model with low-cost alternative fungicide compounds at the University of New Hampshire's Kingman Farm located in Madbury, NH in a stand of mature McIntosh trees over the course of the 2013 and 2014 growing seasons. The experimental design included four treatments (untreated, 4.5lb/A KBC+ 4.5lb/A Kumulus applied according to RIMpro recommendations, 4.5 lb/A Captan applied according to RIMpro recommendations, and a calendar-based Captan spray program (4.5lb/A)) (See Chapter 2 for additional details). The experimental design was a randomized complete block design (RCBD) with two trees per treatment and three replications. A buffer row separated individual treatments with at least one tree between treatments in the same row. Foliar scab severity represented as number of lesions per leaf was assessed on August 14 in 2013 and September 29 in 2014. No insecticide or fertilizer applications were performed for either year of the study. Fungicide applications were made using a Solo 451 Motorized Mist Blower (Solo. Newport News, VA). Reflectance measurements were obtained by scanning six stacks of seven leaves with three scans per leaf taken at 0, 90 and 270° using the VIRIS (GER. Poughkeepsie, NY) in 2013 and an ASD FieldSpec4 in 2014 (ASD. Boulder, CO) with the aid of a hemispherical halogen light source mounted at a 45° angle with the distance of the light source from the sample being equidistant to the distance of the sensor to the sample. Spectral data were analyzed using the ANOVA procedure in R (R Foundation for Statistical Computing. Vienna, Austria) (Table 3, Figure 10). Vegetation indices evaluated include NDVI (Rouse et al. 1974), REIP (Rock et al 1988), NIR3/1 (Vogelmann and Rock 1989), MSI (Hunt and Rock 1989), Scab Index 1 (Delalieux et al. 2009), Scab Index 2 (Delalieux et al. 2009), Green Peak (Thenkabail et al. 2000), Red Edge (Merton et al. 1999), and ZTM (Zarco-Tejada et al. 2001). Vegetation indices were analyzed using Proc GLM in SAS 9.4 (SAS Institute. Cary, NC) (Table 2, Figure 11). Descriptions, computation and references for each index are provided in Table 2.

Results

Evaluate UAV platforms to maximize stability and minimize cost.

Following testing and evaluation of two multirotor UAV platforms, 3DR's X8 proved to be a low-cost platform suitable for monitoring agricultural environments (Figure 1-B). Flight testing of the 550 hexacopter resulting in the conclusion that the platform is suitable for some uses (single-frame imagery) but overall inadequate for precision agriculture applications (mapping). The main failings of the 550 hexacopter were due to limitations of the flight controller (autopilot), although, the frame style and flight times were acceptable. The original flight controller tested was an APM2.5. This flight controller failed to meet our requirements for several reasons. The main reason for moving on from the APM 2.5 to Naza-M V2 was power issues experienced when triggering a dual-camera setup. The dual-USB camera trigger was shown to cause "brown-outs" (power problems) which resulted in several "fly-aways". The Naza-M V2 was selected at this time for evaluation based on it being the main competitor for the APM 2.5 and for its reliability and stability in flight. The first limitation of the Naza-M V2 is the number of waypoints that can be added to autonomous missions or flights. The Naza-M V2 is capable of flying missions with up to 14 waypoints. This is in contrast to 3DR's APM and PX4 (Pixhawk) flight controllers which are able to fly 127 and 200+ waypoints respectively. The second limitation of the Naza-M V2 was it lacked the ability to geo-tag images, APM and PX4 flight controllers both possess this feature, which is critical for precision agriculture purposes.

To acquire images with the 550 hexacopter a Canon S100 was hard-mounted vertically to the bottom of the hexacopter. This method proved inadequate as image quality was low (below 0.50). To estimate quality, images were loaded into Agisoft Photoscan software, images were selected, and then using the Estimate Image Quality procedure image quality was determined. Image quality is scored on a scale of 0-1 with images being considered useful if the image quality score is greater than 0.50. Images with quality scores below 0.50 were discarded. After transitioning to the X8 platform, which included the addition of a gimbal, image quality was greatly improved resulting in a greater number of useful images (quality >0.50) from each flight or mission and reduced post-processing time as it is not necessary to select and discard poor quality images. At an estimated cost of $1900 for the platform, gimbal and camera and flight times ranging from 8-12 minutes 3DRs X8 proved to be a stable, efficient and low-cost platform useful for precision agriculture purposes.

Evaluation of ability to produce orthomosaics with sub-centimeter ground resolution produced using commercial-off-the-shelf (COTS) imaging system mounted onboard an UAV.

Georeferenced orthomosaics were produced for August 30, September 26, October 15, October 18, and November 13, November 27 in 2014 and May 9 in 2015 (Figures 2-8). Spatial resolution for orthomosaics resulting from images captured at a flying height of 100m on August

30, September 26, October 15, October 18, and November 13 was 3.02, 6.82, 3.40, 5.32, 4.13 cm/pix, respectively (Table 1). Flying heights between 15 and 33 meters are able to provide observable features at the leaf level, although orthomosaic generation at low-altitudes is difficult due to the software having difficulty finding common points in images during the alignment process. At this point in time single-frame imagery is required to observe stressors at the leaf level. We wanted to determine whether orthomosaics with sub-centimeter ground resolution could be produced using a commercial-off-the-shelf (COTS) imaging system mounted onboard an UAV, however this was not accomplished as the finest-scale spatial resolution achieved in this study was 1.13 cm/pix (Table 1) achieved by flying at 33m above ground level (AGL). Overall spatial resolution was inadequate to detect apple scab lesion at the leaf-level. Visual image analysis is limited to whole tree effects or phenomenon. The orthomosaics produced throughout 2014 using the X8 octocopter are useful for visually identifying areas of interest (AOIs) for further investigation or scouting. Also, tree phenology and stand counts are easily determined in the orthomosaics. Calculation of vegetation indices and change detection for temporal analysis is not valid unless image pixel values are converted to reflectance values. This requires the use of a calibrated reflectance panel, which was not used in this study, therefore this approach is not suitable for the orthomosaics produced in this study.

Evaluation of 2500 spectral bands for detection of apple scab.

Results of the ANOVA performed on the non-imaging spectrometer data identified 873 bands suitable for the detection of visible apple scab lesions (P-value ≤ 0.01) (Figure 10 and 11). Of the 873 bands, 205 were in the visible spectrum (400-790nm), 595 bands in the near-infrared (791-1400nm) and 73 bands in the short-wave infrared region of the electromagnetic spectrum (1401-3000nm) (Table 11). Vegetation indices evaluated include NDVI (Rouse et al. 1974), REIP (Rock et al 1988), NIR3/1 (Vogelmann and Rock 1989), MSI (Hunt and Rock 1989), Scab Index 1 (Delalieux et al. 2009), Scab Index 2 (Delalieux et al. 2009), Green Peak (Thenkabail et al. 2000), Red Edge (Merton et al. 1999), and ZTM (Zarco-Tejada et al. 2001) and are included in Table 2 and Figure 11. The NDVI value for the unsprayed treatment was 0.72 as compared to the three fungicide programs where the values were 0.86, 0.83 and 0.83 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The REIP value, which is a measure of chlorophyll content or stress, for the unsprayed treatment was 713nm as compared to the three fungicide programs where the values were 721, 719 and 720nm for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The NIR3/1 value, which is a measure of moisture or stress, for the unsprayed treatment was 1.04 as compared to the three fungicide programs where the values were 0.90, 0.93 and 0.93 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The MSI value for the unsprayed treatment was 0.68 as compared to the three fungicide programs where the values were 0.53, 0.56 and 0.57 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The Scab Index-1 value for the unsprayed treatment 0.38 as compared to the three fungicide programs where the values were 0.73, 0.73 and 0.74 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The Scab Index-2 value for the unsprayed treatment 0.31 as compared to the three fungicide programs where the values were 0.12, 0.15 and 0.15 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The Green Peak value for the unsprayed treatment 0.13 as compared to the three fungicide programs where the values were 0.12, 0.14 and 0.14 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. The Red Edge value for the unsprayed treatment 0.17 as compared to the three fungicide programs where the values were

0.35, 0.35 and 0.34 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively. Finally, the ZTM value for the unsprayed treatment 1.61 as compared to the three fungicide programs where the values were 2.66, 2.37 and 2.44 for the Captan-calendar based, Captan RIMpro and KBC+S RIMpro treatments, respectively (Table 2, Figure 11).

Discussion

Following completion of this research, we have identified a useful tool for agricultural and ecological applications. For commercial agriculture, farmers using UAV-based remote sensing will be able to detect disease outbreaks, determine fertility requirements and assess overall crop health and yield potential. This will allow them to increase yields while reducing labor costs and eliminating unnecessary fungicide and fertilizer applications. Although this research is focusing on orchard crops, the same techniques can be translated to other fruit and vegetable crops as well as conventional row crops, including corn, soybeans, rice, and wheat. There are several remote sensing applications in precision agriculture using small UAVs. Sankaran et al. (2015) summarized many of these uses in a recent review, including the use of UAVs for: weed detection (Herwitz et al., 2004, Göktoğan et al., 2010, Torres-Sánchez et al., 2013 and Rasmussen et al., 2013), aerobiological sampling (Techy et al., 2008, Schmale et al., 2008, Gonzalez et al., 2011 and Aylor et al., 2011), leaf area index estimation (Hunt et al., 2008), soil characterization (Sugiura et al., 2007 and d'Oleire-Oltmanns et al., 2012), water status (Sullivan et al., 2007, Berni et al., 2009, Suárez et al., 2010, Zarco-Tejada et al., 2012 and Gago et al., 2015), diseases (Garcia-Ruiz et al., 2013, Sankaran et al., 2013, Calderón et al., 2013 and Calderón et al., 2014), pest management (Huang et al., 2009) and yield estimation (Irmak et al., 2000 and Swain et al., 2010) among others.

The Naza-M V2 in the 550 hexacopter proved inadequate for precision agriculture purposes due to the limited number of waypoints possible in a single flight (16) and the lack of the ability to geotag images. Hard-mounting the camera to the frame produced useable images although image quality was low and post-processing time was increased. Flight times and platform stability was acceptable.

The X8 platform with an APM 2.6 proved to be the most low-cost and reliable platform evaluated in this study (<$2000). The inclusion of a gimbal on the X8 greatly enhanced image quality and reduced post-processing time. It should be noted that while an APM 2.6 flight controller was used in this study, as of 05/26/15, APM 2.5 and 2.6 flight controllers are no longer supported by the development community (MacKay 2015). Future platforms should utilize PX4 (Pixhawk 1 and 2) flight controllers for scientific research. At an altitude of 100m image resolution was adequate enough for whole-tree observations, but not of high enough resolution to identify pests at the leaf-level. Ground resolutions ranging from 1-8 cm/pixel were reported for flights made between altitude of 33 and 100 meters (Table 1). Flying heights between 15 and 33 meters have been shown to provide observable features at the leaf level, although orthomosaic generation at low-altitudes is difficult due to the software having difficulty finding common points in images during the alignment process. At this point in time single-frame imagery is required to observe stressors at the leaf level.

This study has identified 873 spectral bands useful for distinguishing apple scab infected leaves from non-infected leaves. Narrow-band optical filters in the visible, near- and short-wave infrared region of the electromagnetic spectrum suitable for detecting healthy from scab-infected leaves included bands in the visible spectrum ranging from 400-790, 791-1385nm in the near-infrared and 1801-1873nm in the short-wave infrared region. Our study confirms the results of

Delalieux et al. (2009) where the group identified 2 vegetation indices that were superior for distinction of scab infected leaves and non-infected leaves (Table 2, Figure 11). These two indices were R440/R690 and R695/R760 and have been confirmed to excel at distinguishing healthy from scab-infected apple leaves (Table 2, Figure 11). Additional indices that were found to be useful for distinguishing apple scab infected leaves from non-infected leaves include; NDVI, REIP, NIR3/1, MSI, Red Edge and ZTM (Table 2, Figure 11). The only value or biophysical indicator evaluated that was found to be unable to distinguish healthy from infected apple leaves was the Green Peak (555nm) which is the reflectance value at for a single band at 555nm (Table 2, Figure 11). While indices derived from single bands has been shown to be useful, narrow-band filters commonly used in commercially available multispectral cameras are typically at least 5-10nm wide.

A potential limitation of this study is the flight time of the UAV, which depends on the payload. The UAV used in this study has a flight time of 8-12 min depending on the payload (up to 2lb). Fixed wing aerial platforms could be flown for 2.5 hours with a 2.2lb payload (Garcia-Ruiz et al. 2013, Zarco-Tejada et al. 2011), although these platforms exceed our targeted final cost for the platform (~$2000) at the initiation of this research. Presently costs have come down for certain platforms making fixed-wing UAVs a more attractive choice. Unmanned aerial hybrids such as tilt-rotor and tail-sitter UAV platforms have been released which combine the benefits of multirotor UAVs in that it vertical take-off and landing is possible combined with the forward flight capabilities of a fixed-wing UAV (Saeed 2015). Another potential limitation would be the lack of available low-cost commercial-off the-shelf (COTS) sensors capable of sensing wavelengths exceeding 1000 nm. Previous research has shown that narrow-waveband ratios consisting of wavelengths of approximately 1500 nm and 2250 nm to be the most appropriate for detecting apple scab at early developmental stages serving as a promising tool to identify scab stress before symptoms become visible to the naked eye (Delalieux et al. 2009).

Additional limitations would include public perception and federal regulations regarding the use of UAVs for commercial purposes. The federal government took an important step in 2012 when Congress passed, and the president signed into law, the Federal Aviation Administration (FAA) Modernization and Reform Act. The FAA has been tasked with integrating UAV into federal airspace and have already begun allowing wider use of unmanned aircraft in the U.S. airspace in the first half of 2015 (GAO report Sept 2012). COA (Certificate of Authorization) and Section 333 exemptions have been the most recent introduction to UAV regulations. A COA is an authorization issued by the Air Traffic Organization to a public operator for a specific UA activity. At the time of publication 74 COA's have been publically issued, but are less common and more difficult to obtain than a Section 333 exemption. Examples of entities that have received COAs are Department of Defense, Defense Advanced Research Projects Agency, Department of Energy, Department of Interior, Federal Bureau of Investigation, Army, Air Force, NOAA, Cornell University, Virginia Tech and Colorado State University to name a few. Again, these permits are for a specific use and may be the best option for commercial UAV use.

As of March 23, 2015, the FAA will automatically grant a "blanket" COA for flights at or below 400 feet to any UAS operator with a Section 333 exemption, provided the aircraft weighs less than 55 pounds, operations are conducted during daytime Visual Flight Rules (VFR) conditions and within visual line of sight (VLOS) of the pilots, and stay certain distances away (3-5 miles) from airports or heliports. Since the permiting programs inception on March 23, 2015 the FAA has granted 2331 permits (as of Nov 20, 2015). Section 333 permitting is a much more attractive option for agricultural use or for commercial UAV use in general.

Future research should focus on the use of multispectral camera arrays such as MicaSense's RedEdge (MicaSense Inc. Seattle, WA) precision agriculture sensor. The RedEdge is multispectral camera capable of producing 5-banded images with bands in the blue, green, red, red edge and near-infrared bands. This sensor utilizes a calibrated reflectance panel on the ground and downwelling light sensor so images can be adjusted to obtain reflectance values. This will allow for the calculation of vegetation indices and change-detection analysis for rendered orthomosaics.

As a result of this research we have identified multirotor UAVs as a useful tool for agricultural and ecological applications. With the aid of commercial-off-the-shelf cameras we were able to produce orthomosaics with a resolution of 1.13 cm/pixel. Finally, we confirmed the results of Delalieux et al. (2009) where the group identified vegetation indices useful for distinguishing healthy from scab infected apple foliage, additionally we identified several additional useful indices for distinguishing healthy from scab-infected foliage.

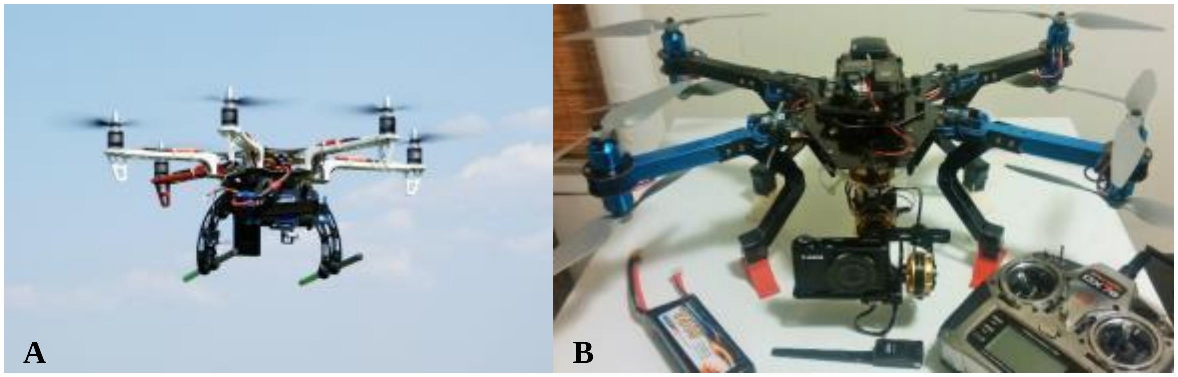

Figure 1. 550 hexacopter w/ Naza-M V2 (A) and 3DR X8 octocopter w/ APM2.6 (B)

Figure 2. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on August 30, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 3. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on September 26, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 4. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on October 15, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 5. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on October 18, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 6. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on November 13, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 7. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on November 27, 2014 overlaid on publically available aerial imagery (Google 2015).

Figure 8. Rendered orthomosaic (center) of Woodman Horticultural Research farm located in Durham, NH on May 9, 2015 overlaid on publically available aerial imagery (Google 2015).

Table 1. Orthomosaic parameter summary for 7 flights made over Woodman Farm in 2014.

Date	Altitude (m)	Number of images	Ground Resolution (cm/pix)	Tie-points	Projections	Error (pix)
8/30/2014	100	55	3.02	121126	311660	0.839
9/26/2014	100	61	6.82	232625	696554	0.877
10/15/2014	100	32	3.40	17008	48438	1.535
10/15/2014	33	38	1.13	7389	16912	1.700
10/18/2014	100	69	5.32	34755	118074	2.040
11/13/2014	100	96	4.13	200980	620932	0.748

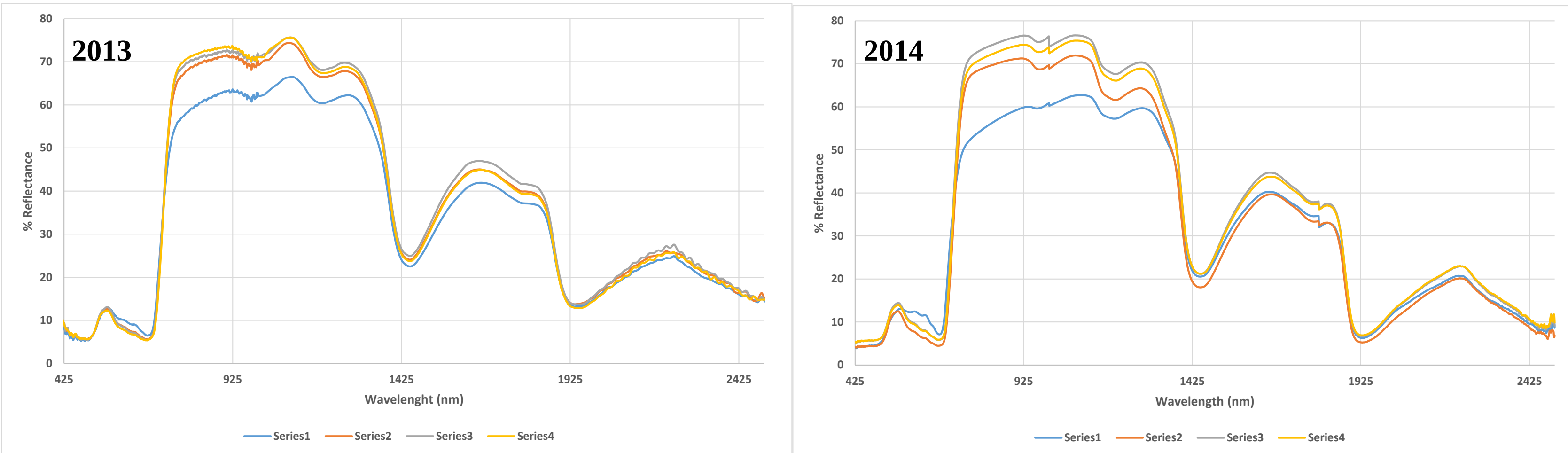

Figure 9. Reflectance curves comparing fungicide treated apple leaves (Series 2-4) and unsprayed apple-scab infected leaves (Series 1) for the 2013 and 2014 growing seasons. The blue line (Series 1) represents the untreated control and obvious differences in reflectance values can be observed within the 568-686nm (red) and 735-1370 (infrared) regions of the spectrum. Results were obtained by scanning six stacks of seven leaves with three scans per leaf taken at 0, 90 and 270° using the VIRIS (GER. Poughkeepsie, NY) in 2013 (left) and an ASD FieldSpec4 in 2014 (right) (ASD. Boulder, CO). UTC = Series 1; Captan Calendar-based = Series 2; Captan RIMpro = Series 3; and KBC + S Rimpro = Series 4.

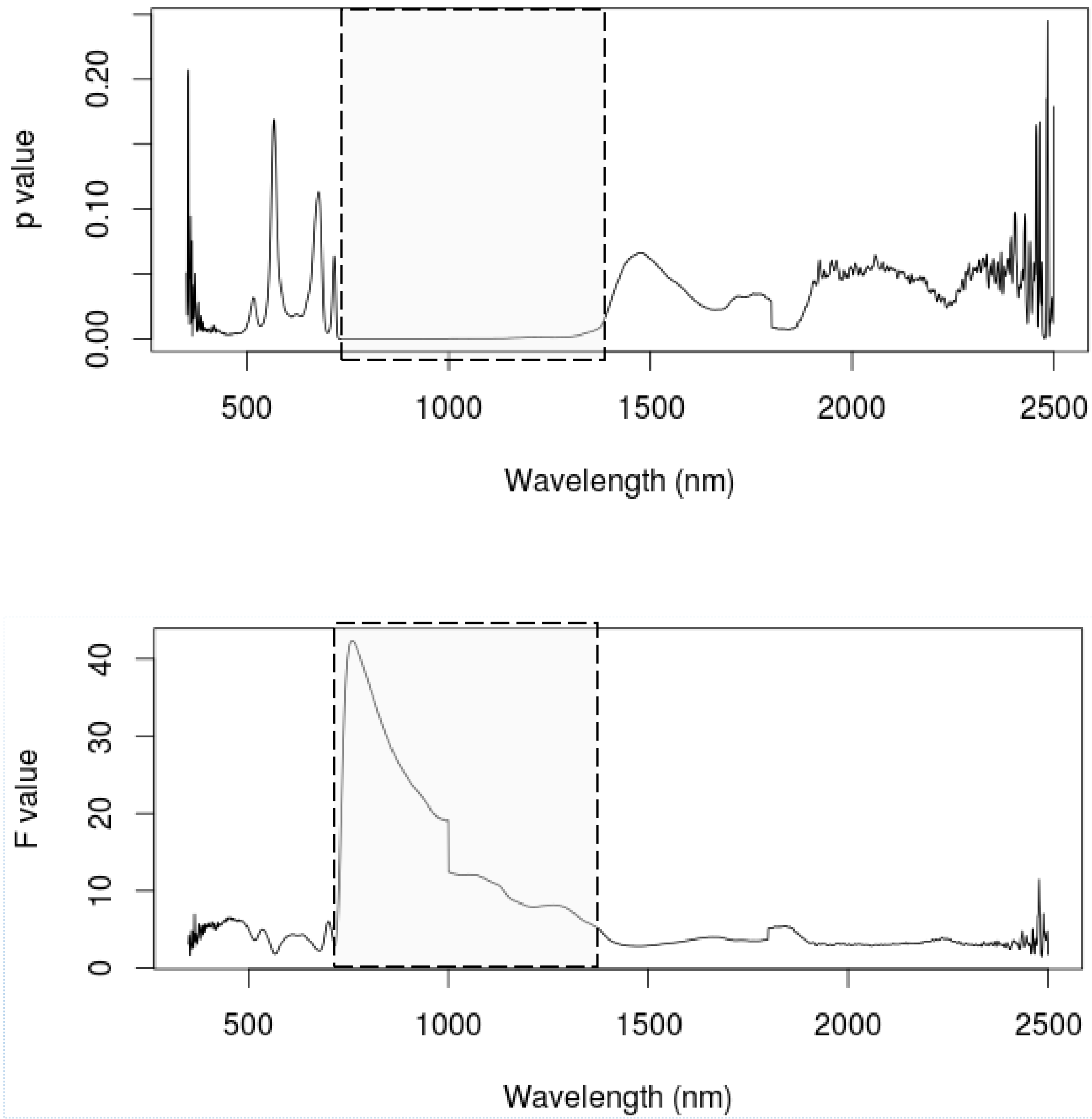

Figure 10. Plot of P-values (top) and F-values (bottom) resulting from performing an ANOVA for all 2500 bands of spectrometer data collected with the ASD FieldSpec 4. The shaded box (near-infrared region) emphasizes the portion of the spectrum where maximum difference are observed between the 4 fungicide programs used in the study.

Table 2. Vegetation indices produced from reflectance measurements comparing untreated apple leaves to several fungicide programs.

Index	Reference	Related to	Computation	Unsprayed	Captan Calendar-based	Captan RIMpro	KBC+S RIMpro	*LSD*
NDVI	Rouse et al. (1974)	Chlorophyll, stress	((µR760-900)-(µR630-690))/((µR760-900)+(µR630-690))	0.72 B[x]	0.86 A	0.83 A	0.83 A	*0.06*
REIP	Rock et al. (1988)	Chlorophyll, stress	R740*((((R680+780)/2)-700)/(R740-700))	713 B	721 A	719 A	720 A	*3.22*
NIR3/1	Vogelman and Rock (1986)	Water content	(µR1250-1325)/(µR794-900)	1.04 A	0.90 C	0.93 BC	0.93 B	*0.03*
MSI	Hunt and Rock (1989)	Water content	(R1550–1750)/(R760–800)	0.68 A	0.53 C	0.56 B	0.57 B	*0.02*
Scab Index 1	Delalieux et al. (2009)	Apple Scab, Chlorophyll	R440/690	0.38 B	0.73 A	0.73 A	0.74 A	*0.08*
Scab Index 2	Delalieux et al. (2009)	Apple Scab, Stress	R695/760	0.31 A	0.12 B	0.15 B	0.15 B	*0.07*
Green Peak	Thenkabail et al. (2000)	Chlorophyll, Stress	R555	0.13 A	0.12 A	0.14 A	0.14 A	*ns*
Red Edge	Merton et al. (1999)	Chlorophyll, Stress	R740-710	0.17 B	0.35 A	0.35 A	0.34 A	*0.04*
ZTM	Zarco Tejada et al. (2001)	Chlorophyll, Stress	R750/R710	1.61 C	2.66 A	2.37 B	2.44 AB	*0.21*

[x] Index means within a row followed by different letters differ significantly ($P < 0.05$).

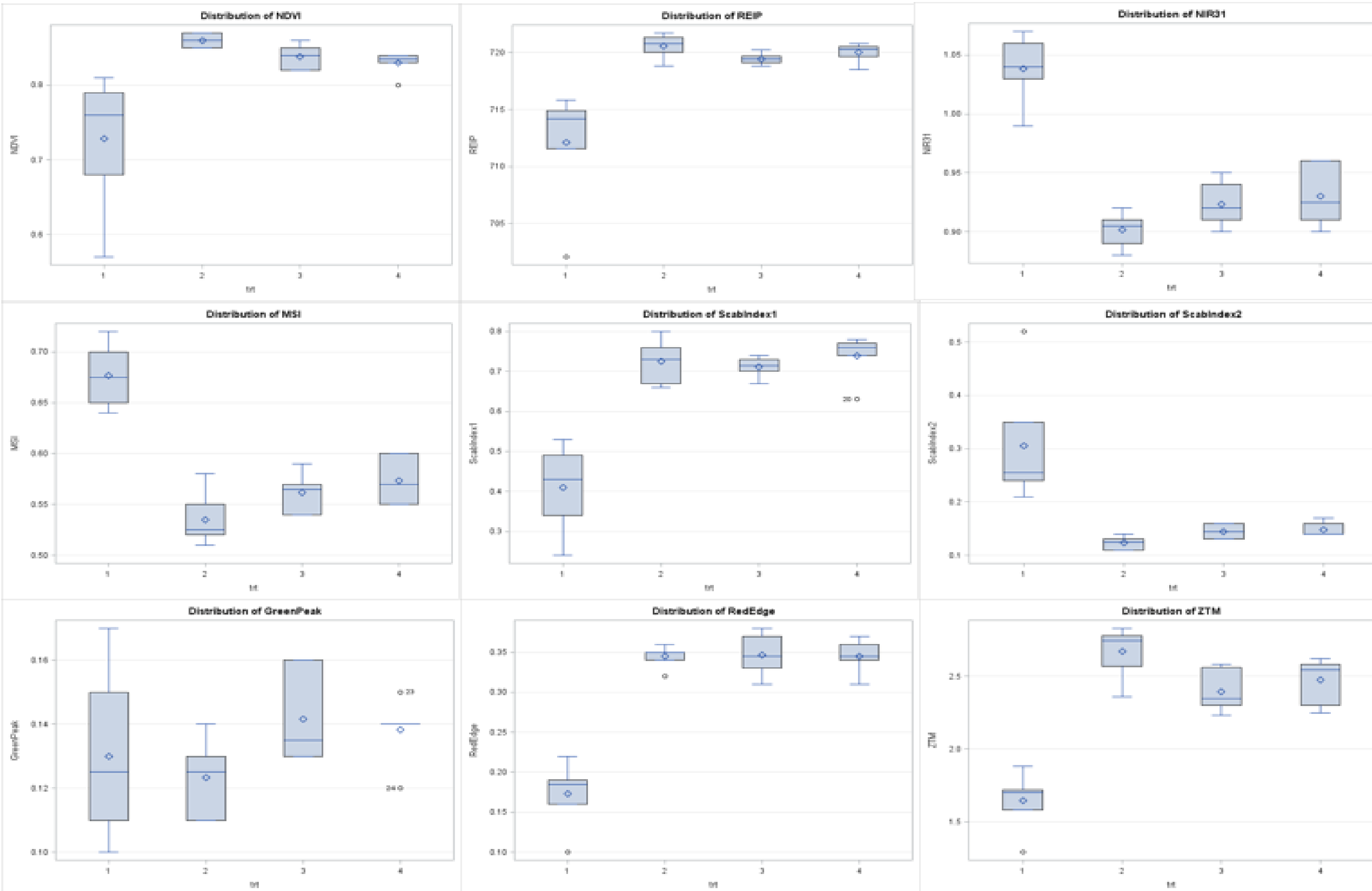

Figure 11. Box-plots for 9 vegetation indices evaluated in this study. From top left, NDVI, REIP, NIR3/1, MSI, Scab Index 1, Scab Index 2, Green Peak, Red Edge and ZTM. Significant differences (Tukey's HSD, *P≤0.05*) were observed between the unsprayed and fungicide treated apple foliage for all indices evaluated except for Green Peak (555nm) (bottom left).

Table 3. Wavelengths with corresponding F and P-values for 873 spectral bands.

Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value
400	5.64	0.006	450	6.51	0.003	500	4.90	0.011	736	27.16	<0.001	786	39.20	<0.001	836	31.33	<0.001
401	5.80	0.005	451	6.52	0.003	501	4.83	0.012	737	28.84	<0.001	787	39.02	<0.001	837	31.18	<0.001
402	5.52	0.007	452	6.52	0.003	502	4.75	0.012	738	30.41	<0.001	788	38.87	<0.001	838	31.04	<0.001
403	5.33	0.008	453	6.51	0.003	503	4.65	0.013	739	31.86	<0.001	789	38.73	<0.001	839	30.90	<0.001
404	5.32	0.008	454	6.48	0.003	528	4.66	0.013	740	33.25	<0.001	790	38.57	<0.001	840	30.75	<0.001
405	5.37	0.008	455	6.43	0.003	529	4.78	0.012	741	34.53	<0.001	791	38.40	<0.001	841	30.63	<0.001
406	5.38	0.008	456	6.36	0.004	530	4.87	0.011	742	35.66	<0.001	792	38.27	<0.001	842	30.48	<0.001
407	5.74	0.006	457	6.40	0.004	531	4.92	0.011	743	36.65	<0.001	793	38.12	<0.001	843	30.33	<0.001
408	5.97	0.005	458	6.55	0.003	532	4.94	0.011	744	37.56	<0.001	794	37.96	<0.001	844	30.20	<0.001
409	5.97	0.005	459	6.21	0.004	533	4.97	0.010	745	38.37	<0.001	795	37.81	<0.001	845	30.05	<0.001
410	5.81	0.005	460	6.19	0.004	534	5.00	0.010	746	39.08	<0.001	796	37.63	<0.001	846	29.94	<0.001
411	5.54	0.007	461	6.45	0.003	535	4.99	0.010	747	39.69	<0.001	797	37.46	<0.001	847	29.83	<0.001
412	5.13	0.009	462	6.46	0.003	536	4.97	0.010	748	40.21	<0.001	798	37.31	<0.001	848	29.68	<0.001
413	5.04	0.010	463	6.39	0.004	537	4.93	0.011	749	40.64	<0.001	799	37.11	<0.001	849	29.55	<0.001
414	5.73	0.006	464	6.31	0.004	538	4.87	0.011	750	41.01	<0.001	800	36.95	<0.001	850	29.42	<0.001
415	5.76	0.006	465	6.27	0.004	539	4.85	0.011	751	41.37	<0.001	801	36.81	<0.001	851	29.30	<0.001
416	5.55	0.007	466	6.33	0.004	540	4.83	0.012	752	41.64	<0.001	802	36.66	<0.001	852	29.19	<0.001
417	5.45	0.007	467	6.28	0.004	541	4.76	0.012	753	41.84	<0.001	803	36.47	<0.001	853	29.04	<0.001
418	5.10	0.009	468	6.18	0.004	542	4.65	0.013	754	41.99	<0.001	804	36.29	<0.001	854	28.92	<0.001
419	4.96	0.010	469	6.14	0.004	543	4.57	0.014	755	42.14	<0.001	805	36.14	<0.001	855	28.81	<0.001
420	4.97	0.010	470	6.21	0.004	693	4.70	0.013	756	42.24	<0.001	806	36.00	<0.001	856	28.69	<0.001
421	5.09	0.009	471	6.21	0.004	694	5.00	0.010	757	42.28	<0.001	807	35.85	<0.001	857	28.56	<0.001
422	5.57	0.006	472	6.11	0.004	695	5.29	0.008	758	42.32	<0.001	808	35.69	<0.001	858	28.44	<0.001
423	5.92	0.005	473	6.02	0.005	696	5.54	0.007	759	42.34	<0.001	809	35.53	<0.001	859	28.33	<0.001
424	5.83	0.005	474	6.09	0.004	697	5.74	0.006	760	42.34	<0.001	810	35.38	<0.001	860	28.20	<0.001
425	5.36	0.008	475	6.20	0.004	698	5.90	0.005	761	42.31	<0.001	811	35.23	<0.001	861	28.09	<0.001
426	5.53	0.007	476	6.25	0.004	699	6.00	0.005	762	42.26	<0.001	812	35.07	<0.001	862	27.98	<0.001
427	5.64	0.006	477	6.11	0.004	700	6.04	0.005	763	42.21	<0.001	813	34.90	<0.001	863	27.88	<0.001
428	5.55	0.007	478	6.09	0.004	701	6.03	0.005	764	42.15	<0.001	814	34.73	<0.001	864	27.75	<0.001
429	5.69	0.006	479	6.13	0.004	702	5.96	0.005	765	42.06	<0.001	815	34.55	<0.001	865	27.66	<0.001
430	5.61	0.006	480	6.12	0.004	703	5.84	0.005	766	41.99	<0.001	816	34.38	<0.001	866	27.56	<0.001
431	5.53	0.007	481	6.14	0.004	704	5.67	0.006	767	41.89	<0.001	817	34.24	<0.001	867	27.45	<0.001
432	5.59	0.006	482	6.09	0.004	705	5.47	0.007	768	41.77	<0.001	818	34.12	<0.001	868	27.33	<0.001
433	5.62	0.006	483	6.03	0.005	706	5.23	0.008	769	41.64	<0.001	819	33.93	<0.001	869	27.24	<0.001
434	5.74	0.006	484	6.08	0.004	707	4.97	0.010	770	41.54	<0.001	820	33.76	<0.001	870	27.14	<0.001
435	5.91	0.005	485	6.10	0.004	708	4.70	0.013	771	41.44	<0.001	821	33.63	<0.001	871	27.03	<0.001
436	6.03	0.005	486	6.05	0.005	722	4.82	0.012	772	41.31	<0.001	822	33.44	<0.001	872	26.91	<0.001
437	5.92	0.005	487	5.99	0.005	723	5.67	0.006	773	41.19	<0.001	823	33.32	<0.001	873	26.83	<0.001
438	5.98	0.005	488	5.95	0.005	724	6.70	0.003	774	41.04	<0.001	824	33.18	<0.001	874	26.73	<0.001
439	6.16	0.004	489	5.88	0.005	725	7.89	0.001	775	40.87	<0.001	825	32.97	<0.001	875	26.59	<0.001
440	6.10	0.004	490	5.79	0.005	726	9.25	0.001	776	40.71	<0.001	826	32.81	<0.001	876	26.51	<0.001
441	6.23	0.004	491	5.71	0.006	727	10.75	<0.001	777	40.59	<0.001	827	32.68	<0.001	877	26.43	<0.001
442	6.31	0.004	492	5.64	0.006	728	12.39	<0.001	778	40.45	<0.001	828	32.53	<0.001	878	26.35	<0.001
443	6.18	0.004	493	5.60	0.006	729	14.12	<0.001	779	40.29	<0.001	829	32.37	<0.001	879	26.26	<0.001
444	5.99	0.005	494	5.53	0.007	730	15.94	<0.001	780	40.13	<0.001	830	32.23	<0.001	880	26.17	<0.001
445	6.21	0.004	495	5.40	0.007	731	17.81	<0.001	781	39.98	<0.001	831	32.07	<0.001	881	26.07	<0.001
446	6.52	0.003	496	5.39	0.007	732	19.74	<0.001	782	39.82	<0.001	832	31.90	<0.001	882	25.98	<0.001
447	6.46	0.003	497	5.36	0.008	733	21.66	<0.001	783	39.65	<0.001	833	31.74	<0.001	883	25.88	<0.001
448	6.25	0.004	498	5.22	0.008	734	23.55	<0.001	784	39.53	<0.001	834	31.61	<0.001	884	25.78	<0.001
449	6.29	0.004	499	5.03	0.010	735	25.37	<0.001	785	39.38	<0.001	835	31.48	<0.001	885	25.68	<0.001

Table 3 continued. Wavelengths with corresponding F and P-values for 873 spectral bands.

Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value
886	25.60	<0.001	936	22.03	<0.001	986	19.29	<0.001	1036	12.06	<0.001	1086	11.76	<0.001	1136	10.19	<0.001
887	25.52	<0.001	937	21.97	<0.001	987	19.29	<0.001	1037	12.06	<0.001	1087	11.73	<0.001	1137	10.13	<0.001
888	25.42	<0.001	938	21.91	<0.001	988	19.25	<0.001	1038	12.06	<0.001	1088	11.71	<0.001	1138	10.07	<0.001
889	25.33	<0.001	939	21.87	<0.001	989	19.22	<0.001	1039	12.03	<0.001	1089	11.68	<0.001	1139	10.00	<0.001
890	25.25	<0.001	940	21.77	<0.001	990	19.18	<0.001	1040	12.03	<0.001	1090	11.64	<0.001	1140	9.94	<0.001
891	25.17	<0.001	941	21.72	<0.001	991	19.14	<0.001	1041	12.04	<0.001	1091	11.61	<0.001	1141	9.87	<0.001
892	25.08	<0.001	942	21.68	<0.001	992	19.11	<0.001	1042	12.04	<0.001	1092	11.58	<0.001	1142	9.80	<0.001
893	25.01	<0.001	943	21.58	<0.001	993	19.11	<0.001	1043	12.04	<0.001	1093	11.56	<0.001	1143	9.73	<0.001
894	24.93	<0.001	944	21.49	<0.001	994	19.05	<0.001	1044	12.05	<0.001	1094	11.53	<0.001	1144	9.66	<0.001
895	24.84	<0.001	945	21.43	<0.001	995	19.09	<0.001	1045	12.05	<0.001	1095	11.50	<0.001	1145	9.59	<0.001
896	24.76	<0.001	946	21.38	<0.001	996	19.17	<0.001	1046	12.04	<0.001	1096	11.47	<0.001	1146	9.52	<0.001
897	24.67	<0.001	947	21.30	<0.001	997	19.17	<0.001	1047	12.06	<0.001	1097	11.45	<0.001	1147	9.45	<0.001
898	24.58	<0.001	948	21.24	<0.001	998	19.08	<0.001	1048	12.09	<0.001	1098	11.43	<0.001	1148	9.38	<0.001
899	24.51	<0.001	949	21.15	<0.001	999	19.12	<0.001	1049	12.10	<0.001	1099	11.41	<0.001	1149	9.32	<0.001
900	24.42	<0.001	950	21.04	<0.001	1000	19.16	<0.001	1050	12.11	<0.001	1100	11.39	<0.001	1150	9.27	<0.001
901	24.34	<0.001	951	20.94	<0.001	1001	12.43	<0.001	1051	12.11	<0.001	1101	11.37	<0.001	1151	9.22	<0.001
902	24.28	<0.001	952	20.89	<0.001	1002	12.43	<0.001	1052	12.10	<0.001	1102	11.34	<0.001	1152	9.16	<0.001
903	24.19	<0.001	953	20.81	<0.001	1003	12.42	<0.001	1053	12.10	<0.001	1103	11.31	<0.001	1153	9.11	<0.001
904	24.13	<0.001	954	20.72	<0.001	1004	12.41	<0.001	1054	12.10	<0.001	1104	11.27	<0.001	1154	9.06	<0.001
905	24.05	<0.001	955	20.62	<0.001	1005	12.40	<0.001	1055	12.09	<0.001	1105	11.24	<0.001	1155	9.02	<0.001
906	23.97	<0.001	956	20.44	<0.001	1006	12.38	<0.001	1056	12.08	<0.001	1106	11.21	<0.001	1156	8.98	<0.001
907	23.94	<0.001	957	20.37	<0.001	1007	12.36	<0.001	1057	12.08	<0.001	1107	11.19	<0.001	1157	8.95	<0.001
908	23.87	<0.001	958	20.34	<0.001	1008	12.34	<0.001	1058	12.08	<0.001	1108	11.16	<0.001	1158	8.92	<0.001
909	23.77	<0.001	959	20.30	<0.001	1009	12.31	<0.001	1059	12.08	<0.001	1109	11.14	<0.001	1159	8.89	<0.001
910	23.69	<0.001	960	20.21	<0.001	1010	12.28	<0.001	1060	12.09	<0.001	1110	11.13	<0.001	1160	8.86	<0.001
911	23.65	<0.001	961	20.12	<0.001	1011	12.25	<0.001	1061	12.10	<0.001	1111	11.11	<0.001	1161	8.83	<0.001
912	23.59	<0.001	962	20.03	<0.001	1012	12.24	<0.001	1062	12.09	<0.001	1112	11.08	<0.001	1162	8.80	<0.001
913	23.52	<0.001	963	19.97	<0.001	1013	12.23	<0.001	1063	12.08	<0.001	1113	11.05	<0.001	1163	8.76	<0.001
914	23.47	<0.001	964	19.95	<0.001	1014	12.21	<0.001	1064	12.09	<0.001	1114	11.03	<0.001	1164	8.73	<0.001
915	23.44	<0.001	965	19.92	<0.001	1015	12.20	<0.001	1065	12.08	<0.001	1115	11.00	<0.001	1165	8.71	<0.001
916	23.34	<0.001	966	19.89	<0.001	1016	12.21	<0.001	1066	12.07	<0.001	1116	10.98	<0.001	1166	8.69	<0.001
917	23.25	<0.001	967	19.85	<0.001	1017	12.18	<0.001	1067	12.06	<0.001	1117	10.96	<0.001	1167	8.67	<0.001
918	23.19	<0.001	968	19.76	<0.001	1018	12.15	<0.001	1068	12.05	<0.001	1118	10.94	<0.001	1168	8.66	<0.001
919	23.14	<0.001	969	19.66	<0.001	1019	12.14	<0.001	1069	12.04	<0.001	1119	10.91	<0.001	1169	8.64	<0.001
920	23.09	<0.001	970	19.60	<0.001	1020	12.13	<0.001	1070	12.03	<0.001	1120	10.88	<0.001	1170	8.63	<0.001
921	23.00	<0.001	971	19.58	<0.001	1021	12.13	<0.001	1071	12.04	<0.001	1121	10.85	<0.001	1171	8.62	<0.001
922	22.92	<0.001	972	19.60	<0.001	1022	12.14	<0.001	1072	12.03	<0.001	1122	10.83	<0.001	1172	8.61	<0.001
923	22.91	<0.001	973	19.53	<0.001	1023	12.13	<0.001	1073	12.02	<0.001	1123	10.80	<0.001	1173	8.58	<0.001
924	22.83	<0.001	974	19.56	<0.001	1024	12.11	<0.001	1074	12.00	<0.001	1124	10.76	<0.001	1174	8.55	<0.001
925	22.76	<0.001	975	19.55	<0.001	1025	12.09	<0.001	1075	11.98	<0.001	1125	10.73	<0.001	1175	8.53	<0.001
926	22.70	<0.001	976	19.38	<0.001	1026	12.09	<0.001	1076	11.95	<0.001	1126	10.70	<0.001	1176	8.50	<0.001
927	22.62	<0.001	977	19.46	<0.001	1027	12.07	<0.001	1077	11.92	<0.001	1127	10.66	<0.001	1177	8.48	<0.001
928	22.55	<0.001	978	19.41	<0.001	1028	12.06	<0.001	1078	11.90	<0.001	1128	10.63	<0.001	1178	8.46	<0.001
929	22.50	<0.001	979	19.34	<0.001	1029	12.05	<0.001	1079	11.88	<0.001	1129	10.59	<0.001	1179	8.45	<0.001
930	22.45	<0.001	980	19.37	<0.001	1030	12.06	<0.001	1080	11.86	<0.001	1130	10.55	<0.001	1180	8.42	<0.001
931	22.34	<0.001	981	19.33	<0.001	1031	12.05	<0.001	1081	11.85	<0.001	1131	10.50	<0.001	1181	8.40	<0.001
932	22.27	<0.001	982	19.31	<0.001	1032	12.06	<0.001	1082	11.83	<0.001	1132	10.45	<0.001	1182	8.37	<0.001
933	22.25	<0.001	983	19.30	<0.001	1033	12.07	<0.001	1083	11.81	<0.001	1133	10.39	<0.001	1183	8.34	<0.001
934	22.21	<0.001	984	19.27	<0.001	1034	12.07	<0.001	1084	11.79	<0.001	1134	10.33	<0.001	1184	8.32	<0.001
935	22.09	<0.001	985	19.27	<0.001	1035	12.05	<0.001	1085	11.77	<0.001	1135	10.26	<0.001	1185	8.30	<0.001

Table 3 continued. Wavelengths with corresponding F and P-values for 873 spectral bands.

Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value	Wavelength (nm)	F-value	P-Value
1186	8.28	0.001	**1236**	8.04	0.001	**1286**	7.97	0.001	**1336**	6.23	0.004	**1801**	5.13	0.009	**1851**	5.27	0.008
1187	8.25	0.001	**1237**	8.05	0.001	**1287**	7.95	0.001	**1337**	6.19	0.004	**1802**	5.15	0.009	**1852**	5.31	0.008
1188	8.23	0.001	**1238**	8.05	0.001	**1288**	7.93	0.001	**1338**	6.15	0.004	**1803**	5.15	0.009	**1853**	5.31	0.008
1189	8.20	0.001	**1239**	8.06	0.001	**1289**	7.91	0.001	**1339**	6.12	0.004	**1804**	5.15	0.009	**1854**	5.34	0.008
1190	8.18	0.001	**1240**	8.06	0.001	**1290**	7.89	0.001	**1340**	6.09	0.004	**1805**	5.14	0.009	**1855**	5.32	0.008
1191	8.15	0.001	**1241**	8.07	0.001	**1291**	7.88	0.001	**1341**	6.06	0.004	**1806**	5.14	0.009	**1856**	5.25	0.008
1192	8.13	0.001	**1242**	8.07	0.001	**1292**	7.85	0.001	**1342**	6.04	0.005	**1807**	5.16	0.009	**1857**	5.21	0.009
1193	8.11	0.001	**1243**	8.07	0.001	**1293**	7.83	0.001	**1343**	6.01	0.005	**1808**	5.17	0.009	**1858**	5.16	0.009
1194	8.08	0.001	**1244**	8.08	0.001	**1294**	7.80	0.001	**1344**	5.98	0.005	**1809**	5.20	0.009	**1859**	5.13	0.009
1195	8.06	0.001	**1245**	8.08	0.001	**1295**	7.76	0.001	**1345**	5.96	0.005	**1810**	5.18	0.009	**1860**	5.13	0.009
1196	8.04	0.001	**1246**	8.09	0.001	**1296**	7.74	0.001	**1346**	5.94	0.005	**1811**	5.19	0.009	**1861**	5.15	0.009
1197	8.02	0.001	**1247**	8.10	0.001	**1297**	7.71	0.001	**1347**	5.91	0.005	**1812**	5.19	0.009	**1862**	5.11	0.009
1198	8.01	0.001	**1248**	8.11	0.001	**1298**	7.67	0.001	**1348**	5.89	0.005	**1813**	5.22	0.009	**1863**	5.08	0.009
1199	7.99	0.001	**1249**	8.12	0.001	**1299**	7.65	0.002	**1349**	5.86	0.005	**1814**	5.28	0.008	**1864**	5.01	0.010
1200	7.98	0.001	**1250**	8.12	0.001	**1300**	7.62	0.002	**1350**	5.83	0.005	**1815**	5.32	0.008	**1865**	4.89	0.011
1201	7.97	0.001	**1251**	8.12	0.001	**1301**	7.59	0.002	**1351**	5.81	0.005	**1816**	5.35	0.008	**1866**	4.80	0.012
1202	7.96	0.001	**1252**	8.12	0.001	**1302**	7.56	0.002	**1352**	5.79	0.005	**1817**	5.36	0.008	**1867**	4.69	0.013
1203	7.95	0.001	**1253**	8.13	0.001	**1303**	7.53	0.002	**1353**	5.77	0.006	**1818**	5.33	0.008	**1868**	4.62	0.014
1204	7.94	0.001	**1254**	8.14	0.001	**1304**	7.50	0.002	**1354**	5.75	0.006	**1819**	5.31	0.008	**1869**	4.59	0.014
1205	7.93	0.001	**1255**	8.15	0.001	**1305**	7.47	0.002	**1355**	5.73	0.006	**1820**	5.31	0.008	**1870**	4.56	0.014
1206	7.92	0.001	**1256**	8.15	0.001	**1306**	7.43	0.002	**1356**	5.70	0.006	**1821**	5.29	0.008	**1871**	4.56	0.014
1207	7.91	0.001	**1257**	8.16	0.001	**1307**	7.39	0.002	**1357**	5.67	0.006	**1822**	5.30	0.008	**1872**	4.59	0.014
1208	7.90	0.001	**1258**	8.16	0.001	**1308**	7.36	0.002	**1358**	5.64	0.006	**1823**	5.34	0.008	**1873**	4.57	0.014
1209	7.90	0.001	**1259**	8.16	0.001	**1309**	7.32	0.002	**1359**	5.61	0.006	**1824**	5.35	0.008			
1210	7.90	0.001	**1260**	8.17	0.001	**1310**	7.29	0.002	**1360**	5.59	0.006	**1825**	5.39	0.007			
1211	7.90	0.001	**1261**	8.17	0.001	**1311**	7.25	0.002	**1361**	5.56	0.007	**1826**	5.38	0.007			
1212	7.90	0.001	**1262**	8.18	0.001	**1312**	7.21	0.002	**1362**	5.53	0.007	**1827**	5.29	0.008			
1213	7.90	0.001	**1263**	8.17	0.001	**1313**	7.17	0.002	**1363**	5.50	0.007	**1828**	5.28	0.008			
1214	7.90	0.001	**1264**	8.17	0.001	**1314**	7.13	0.002	**1364**	5.48	0.007	**1829**	5.29	0.008			
1215	7.90	0.001	**1265**	8.16	0.001	**1315**	7.09	0.002	**1365**	5.45	0.007	**1830**	5.29	0.008			
1216	7.90	0.001	**1266**	8.15	0.001	**1316**	7.05	0.002	**1366**	5.42	0.007	**1831**	5.30	0.008			
1217	7.90	0.001	**1267**	8.15	0.001	**1317**	7.01	0.002	**1367**	5.39	0.007	**1832**	5.37	0.008			
1218	7.91	0.001	**1268**	8.15	0.001	**1318**	6.97	0.002	**1368**	5.36	0.008	**1833**	5.38	0.007			
1219	7.91	0.001	**1269**	8.15	0.001	**1319**	6.93	0.002	**1369**	5.33	0.008	**1834**	5.35	0.008			
1220	7.92	0.001	**1270**	8.15	0.001	**1320**	6.88	0.003	**1370**	5.30	0.008	**1835**	5.35	0.008			
1221	7.92	0.001	**1271**	8.14	0.001	**1321**	6.84	0.003	**1371**	5.27	0.008	**1836**	5.35	0.008			
1222	7.93	0.001	**1272**	8.14	0.001	**1322**	6.80	0.003	**1372**	5.23	0.008	**1837**	5.36	0.008			
1223	7.93	0.001	**1273**	8.13	0.001	**1323**	6.76	0.003	**1373**	5.20	0.009	**1838**	5.37	0.008			
1224	7.94	0.001	**1274**	8.13	0.001	**1324**	6.71	0.003	**1374**	5.17	0.009	**1839**	5.39	0.007			
1225	7.95	0.001	**1275**	8.12	0.001	**1325**	6.67	0.003	**1375**	5.13	0.009	**1840**	5.40	0.007			
1226	7.96	0.001	**1276**	8.11	0.001	**1326**	6.63	0.003	**1376**	5.09	0.009	**1841**	5.43	0.007			
1227	7.97	0.001	**1277**	8.10	0.001	**1327**	6.59	0.003	**1377**	5.05	0.010	**1842**	5.44	0.007			
1228	7.98	0.001	**1278**	8.09	0.001	**1328**	6.55	0.003	**1378**	5.00	0.010	**1843**	5.45	0.007			
1229	7.99	0.001	**1279**	8.08	0.001	**1329**	6.51	0.003	**1379**	4.95	0.010	**1844**	5.44	0.007			
1230	8.00	0.001	**1280**	8.07	0.001	**1330**	6.47	0.003	**1380**	4.89	0.011	**1845**	5.41	0.007			
1231	8.00	0.001	**1281**	8.05	0.001	**1331**	6.42	0.003	**1381**	4.83	0.012	**1846**	5.38	0.007			
1232	8.01	0.001	**1282**	8.03	0.001	**1332**	6.38	0.004	**1382**	4.77	0.012	**1847**	5.33	0.008			
1233	8.02	0.001	**1283**	8.02	0.001	**1333**	6.33	0.004	**1383**	4.70	0.013	**1848**	5.31	0.008			
1234	8.03	0.001	**1284**	8.01	0.001	**1334**	6.30	0.004	**1384**	4.64	0.013	**1849**	5.27	0.008			
1235	8.04	0.001	**1285**	7.99	0.001	**1335**	6.26	0.004	**1385**	4.58	0.014	**1850**	5.26	0.008			

References

Asner, G. 1998. Biophysical and Biochemical Sources of Variability in Canopy Reflectance. Remote Sensing of Environment 64, 234-253.

Aylor, D., Schmale, D., Shields, E., Newcomb, M., and Nappo, C. 2011. Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agricultural and Forest Meteorology, 151, 251-260.

Bauer, M. 1985. Spectral inputs to crop identification and condition assessment. Proceedings of the IEEE 73, 1071–1085.

Barragán, J., Kelly, M., Castro, A., & López-Granados, F. 2012. Object-based approach for crop row characterization in UAV images for site-specific weed management. Proceedings of the 4th GEOBIA: 426-430.

Berni, J., Zarco-Tejada, P., Suárez, L., and Fereres, E. 2009. Thermal and narrowband multispectral remote sensing for vegetation monitoring from an unmanned aerial vehicle. Geoscience and Remote Sensing, IEEE Transactions on 47.3, 722-738.

Calderón, R., Navas-Cortés, J., Lucena, C., and Zarco-Tejada, P. 2013. High-resolution airborne hyperspectral and thermal imagery for early detection of Verticillium wilt of olive using fluorescence, temperature and narrow-band spectral indices. Remote Sensing of Environment, 139, 231-245.

Calderón, R., Montes-Borrego, M., Landa, B., Navas-Cortés, J., & Zarco-Tejada, P. 2014. Detection of downy mildew of opium poppy using high-resolution multi-spectral and thermal imagery acquired with an unmanned aerial vehicle. Precision Agriculture, 15, 639-661.

Delalieux, S., van Aardt, J., Keulemans, W., Schrevens, E., Coppin, P. 2007. Detection of biotic stress (*Venturia inaequalis*) in apple trees using hyperspectral data: Non-parametric statistical approaches and physiological implications European Journal of Agronomy, 27, 130–143.

Delalieux, S., Somers, B., Verstraeten, W., van Aardt, J., Keulemans, W., Coppin, P. 2009. Hyperspectral indices to diagnose leaf biotic stress of apple plants, considering leaf phenology. International Journal of Remote Sensing, 8, 1887-1912.

Delalieux, S., Somers, B., Verstraeten, W., van Aardt, J., Keulemans, W., Coppin, P. 2009. Hyperspectral Reflectance and Fluorescence Imaging to Detect Scab Induced Stress in Apple Leaves. Remote Sensing. 1, 58-874

d'Oleire-Oltmanns, S., Marzolff, I., Peter, K., and Ries, J. 2012. Unmanned Aerial Vehicle (UAV) for monitoring soil erosion in Morocco. Remote Sensing, 4, 3390-3416.

Gago, J., Douthe, C., Coopman, R., Gallego, P., Ribas-Carbo, M., Flexas, J., and Medrano, H. 2015. UAVs challenge to assess water stress for sustainable agriculture. Agricultural Water Management, 153, 9-19.

Garcia-Ruiz, F., Sankaran, S., Mari Maja, J., Lee, W., Rasmussen, J., Ehsani, R. 2013. Comparison of two aerial imaging platforms for identification of Huanglongbing-infected citrus trees. Computers and Electronics in Agriculture, 91, 106-115.

GAO. Measuring Progress and Addressing Potential Privacy Concerns Would Facilitate Integration into the National Airspace System. Reissued on September 18, 2012. GAO-12-981.

Goetz, A., Rock, B., and Rowan, L. 1983. Remote sensing for exploration: an overview. Economic Geology, 78, 573-590.

Göktoğan, A., Sukkarieh, S., Bryson, M., Randle, J., Lupton, T., and Hung, C. 2010. A rotary-wing unmanned air vehicle for aquatic weed surveillance and management. In Selected papers from the 2nd International Symposium on UAVs, Reno, Nevada, USA June 8–10, 2009 (pp. 467-484). Springer Netherlands.

Gonzalez, F., Castro, M., Narayan, P., Walker, R., and Zeller, L. 2011. Development of an autonomous unmanned aerial system to collect time-stamped samples from the atmosphere and localize potential pathogen sources. Journal of Field Robotics, 28, 961-976.

Herwitz, S., Johnson, L., Dunagan, S., Higgins, R., Sullivan, D., Zheng, J., and Brass, J. 2004. Imaging from an unmanned aerial vehicle: agricultural surveillance and decision support. Computers and Electronics in Agriculture, 44, 49-61.

Hatfield, P., Pinter, P. 1993. Remote sensing for crop protection. Crop Protection, 12, 403-413.
Hickory K. 1991. Fungicide benefits assessment fruit and nut crops - East. USDA National Agricultural Pesticide Impact Assessment Program.

Huang, Y., Hoffmann, W., Lan, Y., Wu, W., and Fritz, B. 2009. Development of a spray system for an unmanned aerial vehicle platform. Applied Engineering in Agriculture, 25, 803-809.

Hunt, E. and Rock, B. 1989. Detection of changes in leaf water content using near-and middle-infrared reflectances. Remote Sensing of Environment, 30, 43-54.

Hunt, E., Hively, W., Daughtry, C., McCarty, G., Fujikawa, S., Ng, T., and Yoel, D. 2008. Remote sensing of crop leaf area index using unmanned airborne vehicles. In Proceedings of the Pecora 17 Symposium, Denver, CO.

Irmak, S., Haman, D., and Bastug, R. 2000. Determination of crop water stress index for irrigation timing and yield estimation of corn. Agronomy Journal, 92, 1221-1227.

Jackson, R.D. 1984. Remote sensing of vegetation characteristics for farm management. SPIE Proceedings, 475, 81-96.

MacHardy, W. 1996. Apple Scab: Biology, Epidemiology, and Management. APS Press, St. Paul, MN.

MacKay, R. 2015. APM: Copter Release Notes. github.com/diydrones/ardupilot.

Merton, R 1999. Multi-temporal analysis of community scale vegetation stress with imaging spectroscopy. Ph.D. thesis, Department of Geography, University of Auckland, Auckland, New Zealand.

Peña, J., Torres-Sánchez, J., de Castro, A., Kelly, M., and López-Granados, F. 2013. Weed mapping in early-season maize fields using object-based analysis of unmanned aerial vehicle (UAV) images. PLoS One, 8.10.

Rasmussen, J., Nielsen, J., Garcia-Ruiz, F., Christensen, S., and Streibig, J. 2013. Potential uses of small unmanned aircraft systems (UAS) in weed research. Weed Research, 53, 242-248.

Ray, S., Singh, P., Panigrahy, S. 2010. Use of Hyperspectral Remote Sensing for Crop Stress Detection: Ground-Based Studies. International Archives of the Photogrammetry: Remote Sensing and Spatial Information Science. Volume XXXVIII, Part 8, Kyoto, Japan.

Rock, B., Hoshizaki, T., and Miller, J. 1988. Comparison of in situ and airborne spectral measurements of the blue shift associated with forest decline. Remote Sensing of Environment, 24, 109-127.

Rock, B., Vogelmann, J., Williams, D., Vogelmann, A., and Hoshizaki, T. 1986. Remote detection of forest damage. BioScience, 86, 439-445.

Rouse Jr, J., Haas, R., Schell, J., and Deering, D. 1974. Monitoring vegetation systems in the Great Plains with ERTS. NASA special publication, 351, 309.

Saeed, A., Younes, A., Islam, S., Dias, J., Seneviratne, L., and Cai, G. 2015. A review on the platform design, dynamic modeling and control of hybrid UAVs. Unmanned Aircraft Systems (ICUAS), 2015 International Conference, 806-815.

Sankaran, S., Khot, L., Maja, J., and Ehsani, R. 2013. Comparison of two multiband cameras for use on small UAVs in Agriculture. In 5th IEEE Workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing (WHISPERS), 25-28.

Sankaran, S., Khot, L., Espinoza, C., Jarolmasjed, S., Sathuvalli, V., Vandemark, G., and Pavek, M. 2015. Low-altitude, high-resolution aerial imaging systems for row and field crop phenotyping: A review. European Journal of Agronomy, 70, 112-123.

Schmale, D., Dingus, B., and Reinholtz, C. 2008. Development and application of an autonomous unmanned aerial vehicle for precise aerobiological sampling above agricultural fields. Journal of Field Robotics, 25, 133.

Slaughter, D., Giles, D., and Downey, D. 2008. Autonomous robotic weed control systems: A review. Computers and electronics in agriculture, 61, 63-78.

Suárez, L., Zarco-Tejada, P., González-Dugo, V., Berni, J., Sagardoy, R., Morales, F., and Fereres, E. 2010. Detecting water stress effects on fruit quality in orchards with time-series PRI airborne imagery. Remote Sensing of Environment, 114, 286-298.

Sugiura, R., Noguchi, N., & Ishii, K. 2007. Correction of low-altitude thermal images applied to estimating soil water status. Biosystems engineering, 96, 301-313.

Sullivan, D., Fulton, J., Shaw, J., & Bland, G. 2007. Evaluating the sensitivity of an unmanned thermal infrared aerial system to detect water stress in a cotton canopy. Transactions of the ASABE, 50, 1955-1962.

Swain, K., Thomson, S., and Jayasuriya, H. 2010. Adoption of an unmanned helicopter for low-altitude remote sensing to estimate yield and total biomass of a rice crop. Transactions of the ASAE (American Society of Agricultural Engineers), 53, 21.

Techy, L., Woolsey, C., and Schmale, D. 2008. Path planning for efficient UAV coordination in aerobiological sampling missions. In Decision and Control, 2008. CDC 2008. 47th IEEE Conference, 2814-2819.

Thenkabail, P., Smith, R., and De Pauw, E. 2000. Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. Remote sensing of Environment, 71, 158-182.

Torres-Sánchez, J., López-Granados, F., De Castro, A., & Peña-Barragán, J. 2013. Configuration and specifications of an unmanned aerial vehicle (UAV) for early site specific weed management. PLoS ONE, 8.3.

Vogelmann, J., and Rock, B. 1986. Assessing forest decline in coniferous forests of Vermont using NS-001 Thematic Mapper Simulator data. International Journal of Remote Sensing, 7, 1303-1321.

Zarco-Tejada, P., 2011. High resolution hyperspectral and thermal remote sensing from UAV platforms for stress detection and vegetation monitoring. In: Remote Sensing Photogrammetry Society (RSPSoc) and National Center for Earth Observation (NERC) Technology Cluster UAV Workshop, Durham.

Zarco-Tejada, P., González-Dugo, V., and Berni, J. 2012. Fluorescence, temperature and narrow-band indices acquired from a UAV platform for water stress detection using a micro-hyperspectral imager and a thermal camera. Remote Sensing of Environment, 117, 322-337.

Zarco-Tejada, P., Miller, J., Noland, T., Mohammed, G., and Sampson, P. (2001). Scaling-up and model inversion methods with narrowband optical indices for chlorophyll content estimation in closed forest canopies with hyperspectral data. Geoscience and Remote Sensing, IEEE Transactions on 39.7, 1491-1507.