View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Clemson University: TigerPrints

Clemson University
TigerPrints

All Theses

Theses

5-2014

MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH ALTERNARIA LEAF BLIGHT RESISTANCE AND ELEMENTAL SULFUR TOLERANCE IN MELON

James Daley
Clemson University, jdaley@g.clemson.edu

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

Part of the Genetics and Genomics Commons, and the Plant Sciences Commons

Recommended Citation
Daley, James, "MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH ALTERNARIA LEAF BLIGHT RESISTANCE AND ELEMENTAL SULFUR TOLERANCE IN MELON" (2014). *All Theses*. 1973.
https://tigerprints.clemson.edu/all_theses/1973

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.

MAPPING QUANTITATIVE TRAIT LOCI ASSOCIATED WITH ALTERNARIA LEAF BLIGHT RESISTANCE AND ELEMENTAL SULFUR TOLERANCE IN MELON

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Plant and Environmental Science

---

by
James Daniel Daley
May 2014

Accepted by:
Richard L Hassell, Committee Chair
W. Patrick Wechter, Project Chair
Jeff Adelberg

ABSTRACT

Melon (*Cucumis melo* L.) production is significantly affected by Alternaria leaf blight, caused by *Alternaria cucumerina*. Fungicide application is the primary control method; however, this could be mitigated through the increased use of resistant varieties. USDA-ARS breeding line MR-1 has been shown to have a high level of resistance to *Alternaria cucumerina*. However, molecular markers linked MR-1 *Alternaria* resistance have yet to be identified. In order in identify QTL associated with *Alternaria* resistance MR-1 x Ananas Yokneum (AY) derived recombinant inbred lines (RILs) were phenotyped using a modified wounded-leaf assay.

Elemental sulfur is an effective fungicide for several foliar pathogens in many crops and species, but severe phytotoxicity prohibits its use on many melon lines. Sulfur tolerance is a heritable trait and QTL have been identified. MR-1 is highly susceptible to sulfur and AY is completely resistant. In order to identify sulfur tolerance QTL, MR-1 x AY RILs were rated for tolerance using vaporized sulfur.

A genetic map of MR-1 x AY RILs was constructed using 198 dominant markers (1 SCAR, 24 HFO-TAG, 25 ISSR, and 152 RAPD). This linkage map contains 23 linkage groups and spans 400cM with a maximum marker interval of 10cM and an average marker interval of 2cM. Quantitative trait analysis of 56 RILs for *Alternaria* resistance and 57 RILs for sulfur tolerance detected three QTL: one *Alternaria* resistance QTL, *ac.1,* and two sulfur tolerance QTL, *st.1* and *st.2*. *Ac.1* represents the MR-1 resistance allele, is located on linkage group 11, and explains 25% of the variance. *St.1*

and *st.2* were located on linkage groups 1 and 12 and explain 30% and 18% of the variance, respectively. MR-1 allele, *st.1*, increased susceptibility while the MR-1 allele, *st.2*, increased tolerance. QTL for these important traits will be beneficial for MAS and genetic studies.

DEDICATION

This work would not have been completed without support and inspiration of my lovely wife. I would also like to dedicate this to my parents who raised me right and encouraged me to explore nature and to my sister who is an example of hard work, dedication, and sheer will.

ACKNOWLEDGMENTS

First and foremost, I would like to express my gratitude to my advisor, Dr. Patrick Wechter, for his guidance, sense of humor, and forthright advice. I aspire to his professionalism, scientific expertise, and sense ofpurpose. I would also like to thank other committee members: Dr. Richard Hassell, who gave a chance to a deployed soldier whom he had never met and made this whole experience possible, and Dr. Jeff Adelberg for this unique insight.

I have greatly benefitted from my association with the many Clemson and USDA ARS personnel who have graciously contributed their time and resources: Dr. William Bridges, Dr. Halina Knap, Dr. Amnon Levi, Dr. Chandrasekar S Kousik, Dr. Anthony Keinath, and Melanie McMillan. This work would not have been possible without their contributions.

I sincerely acknowledge The Agricultural Society of South Carolina and the Specialty Crops Research Initiative grant that provided the financial means to make this research possible.

I would like to thank my family for their constant support from afar. Most of all, I owe my deepest gratitude to my wife who has always been by my side during the ups and downs of this experience and will forever have a place in my heart.

TABLE OF CONTENTS

Page

TITLE PAGE i

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER

I. LITERATURE REVIEW 1
- *Cucumis melo* L. 1
- General Breeding for Resistance in *Cucumis melo* L. 2
- *Alternaria cucumerina* 4
- Management of *Alternaria cucumerina* 6
- Resistance Screening Methods: Spray, Wounded-leaf, and Field 8
- Inheritance of Alternaria Leaf Blight Resistance 11
- Elemental Sulfur as an Effective Fungicide 12
- Elemental Sulfur Application Methods 13
- Elemental Sulfur Phytotoxicity in Melon 15
- Elemental Sulfur Toxicity and Coping Mechanisms in Cucurbits 16
- Inheritance of Elemental Sulfur Tolerance in Melon 19
- Quantitative Trait Mapping in Melon 20
- Genetic Maps of Melon 33
- The Genome of Melon 35
- Literature Cited 37

II. MAPPING ALTERNARIA CUCUMERINA RESISTANCE IN CUCUMIS MELO 49
- Introduction 49
- Materials and Methods 52
- Results 63
- Discussion 67
- Literature Cited 70
- Tables and Figures 72

III. MAPPING ELEMENTAL SULFUR TOLERANCE IN CUCUMIS MELO 79
Introduction 79
Materials and Methods 81
Results 89
Discussion 93
Literature Cited 89
Tables and Figures 99

APPENDICES 105

A: Sulfur vaporization tolerance ratings of MR-1 x AY RILs 106
B: Alternaria resistance screening of MR-1 x AY RILs 108
C: Overview of MR-1 x AY RILs testing 111
D: Survey of Melon Genetic Maps 114

LIST OF TABLES

Table Page

1.1 Lesion areas of the five most susceptible and five most resistant RILs and parental genotypes ... 72

1.2 *Alternaria* spray inoculation and resistance screening of three MR-1 hybrids, parents, RIL61 and Jindaozi ... 73

1.3 Summary of QTL analysis of *Alternaria cucumerina* resistance ... 74

1.4 HFO-TAG and ISSR sequence, annealing temperatures, and number of markers mapped ... 75

2.1 Comparison of sulfur vaporization and sulfur spray tolerance ratings of a subset RILs with parental controls [MR-1, AY, and (AY x MR-1) F_1] ... 99

2.2 Sulfur vaporization tolerance ratings of three MR-1 hybrids [(AY x MR-1) F1, (MR-1 x HBJ) F_1, and (Perlita x MR-1) F_1], parents MR-1, AY, HBJ, and Perlita), Jindaozi, and RIL 61 ... 100

2.3 Summary of QTL analysis of elemental sulfur tolerance ... 101

2.4 HFO-TAG (HFOWEC; HFSW) and ISSR sequence, annealing temperatures, and number of markers mapped ... 102

LIST OF FIGURES

Figure Page

1.1 *Alternaria* lesion areas (mm^2) of wounded-leaf inoculated first, second, and third leaves of individual MR-1, AY, and (AY x MR-1) F_1 plants 76

1.2 *Alternaria* lesion areas (mm^2) of wounded-leaf inoculated MR-1 hybrids [(AY x MR-1) F_1, (MR-1 x HBJ) F_1, and (Perlita x MR-1) F_1], parents (MR-1, AY, HBJ, and Perlita), Jindaozi, and RIL61 77

1.3 Linkage map of 59 MR-1 x AY RIL and QTL locations (95% CI) for sulfur tolerance (red), *st.1* and *st.2*, and Alternaria resistance (blue), *ac.1* 78

2.1 Distribution of MR-1 x AY RILs and parental controls according to vaporized sulfur tolerance ratings rounded to the nearest whole number 103

2.2 Linkage map of 59 MR-1 x AY RIL and QTL locations (95% CI) for sulfur tolerance (red), *st.1* and *st.2*, and Alternaria resistance (blue), *ac.1* 104

CHAPTER ONE
LITERATURE REVIEW

Cucumis melo L.

Cucumis melo L., melon, is intensely grown in temperate regions around the world and is of the Cucurbitaceae family which includes other well-known crops such watermelon (*Citrullus lanatus* L.), cucumber (*Cucumis sativus* L.), squash (*Cucurbita maxima* D.), pumpkin (*Cucurbita pepo* L.) (Fernandez-Silva et al., 2008). Cucurbitaceae ranks second only to Solanaceae in economic importance among vegetable crops around the world (Garcia-Mas et al., 2012). *Cucumis melo* is further classified into seven groups of which Inodorus (Cassaba and Honeydew) and Cantalupensis (Cantaloupe or muskmelon) dominate the US and European markets (Staub et al., 2000). In 2007, 34,354 hectares of cantaloupe and 7,019 hectares of honeydew melon were harvested across the US, generating $312.7 million and $82.5 million, respectively (Economic Research Service 2011, USDA/NASS 2007). World production of melon reached 29 million tonnes in 2009 (http://faostat.fao.org). However, 2010 US production represented a mere 4% of worldwide production (Economic Research Service 2011). China produces 45% of all melons grown worldwide (Economic Research Service 2011). Melons are an important crop, not only because of their value as a sweet fruit or culinary vegetable, but also because they are an excellent source of vitamins A and C (Munger et al., 1995). In addition, melons have a seed oil content of 50%, which is comparable to other oilseed crops, and seeds stripped of oil are composed of about 60-70% protein and thus are an excellent source of protein (Munger et al., 1995).

General Breeding for Resistance in *Cucumis melo* L.

Despite primarily being a cross-pollinating crop, melons perform well in breeding programs designed for either cross- or self-pollination (Choudhary and Fageria, 2002). Melons, though phenotypically diverse, exhibit relatively low genetic variation (Wang et al., 1997) and do not suffer from inbreeding depression. Traits can be incorporated into a cultivar via several crossings, which can then be followed up with the backcross method to fix oligogenic traits, or the pedigree method for polygenic traits (Choudhary and Fageria, 2002). Hybrid varieties benefit from the combination of desirable traits, though heterosis is minimal (Sitterly, 1972).

The backcross method has successfully been used to incorporate disease resistance genes into melon, as seen in the following commercially-acceptable cultivars resistant to gummy stem blight (*Didymella bryoniae)* (Norton, 1971, 1972; Norton et al., 1985). 'Gulf Coast', 'Chilton', and 'Aurora' cultivars were bred specifically to provide disease-resistant varieties adapted to the southeastern climate of the US (Norton et al., 1985). 'Chilton' and 'Gulf Coast' were bred to meet the consumer demand of smaller melons (Norton, 1971; Norton, 1972) while 'Aurora' meets the qualification of a jumbo variety (Norton et al., 1985). All three varieties were derived via the backcross method, with the recurrent parent resistant to both Downy Mildew and Powdery Mildew and the donor parent [plant introduction (PI) 140471] (Norton, 1971, 1972; Norton et al., 1985) having a single dominant gene conferring a high level of resistance to Gummy Stem Blight (Mcgrath et al., 1993 citing Prasad and Norton, 1967). Incidentally, it was found

that under extreme disease conditions the resistance did not adequately protect the plants from succumbing to the disease (Mcgrath et al., 1993). Two other PI's have since been identified with resistance to Gummy Stem Blight, one offering a different kind of resistance (Mcgrath et al., 1993). Resistance could be incorporated into commercial cultivars via combination with a recurrent elite line (Mcgrath et al., 1993); this could be an opportunity to pyramid resistance genes, which may not provide absolute resistance, but could, in concert, provide a greater degree of resistance and durability. Resistance is considered more durable when multiple genes, controlling different mechanisms, are involved because the likelihood of the pathogen overcoming multiple mechanisms is less than if just one mechanism existed (Collard and Mackill, 2008). The general model for resistance to specific pathogens is the induction of a signal cascade and defense response based on the recognition of pathogen proteins by plant receptors (Joobeur et al., 2004). These recognition receptor proteins are coded by resistance (R) genes which are thought to be evolving new virulent protein specificities through "diversifying selection, interallelic recombination and gene conversion" (Joobeur et al., 2004).

One of the main difficulties with pyramiding R genes is that the required progeny test can be difficult because phenotypes can be challenging to discern (Collard and Mackill, 2008). However, this can be overcome through the use of tightly-linked molecular markers (Collard and Mackill, 2008). There are numerous examples of successful introgression in cereal crops via MAB given by Collard and Mackill (2008) which include QTLs on 3 chromosomes for maize corn borer resistance and QTLs on 5 different chromosomes for root traits and aroma in rice. Pyramiding was also highlighted

with examples of powdery mildew resistance in wheat from the cross of two parents, each possessing a different resistance gene (Collard and Mackill, 2008). In each case, markers enable breeders to identify parents and progeny possessing genes of interest.

Alternaria cucumerina

Alternaria Leaf Blight (ALB), caused by *Alternaria cucumerina* (E. & E.) Elliot is a major fungal pathogen that affects melon production throughout the world (Jackson and Weber, 1959). In the U.S., ALB is a significant problem in the melon-producing midwestern and eastern states (Evans et al., 1992). For example, ALB has been responsible for yield losses in Indiana approaching 50% (Evans et al., 1992). The *A. cucumerina* conidia, separated from the mycelia, are elliptical and are generally beaked. Both the conidia and mycelia are translucent brown and become darker as they age (Sitterly, 1972). *A. cucumerina* infects plant tissue via conidia on leaves which produce hyphae that directly penetrate the leaf epidermis (Jackson and Weber, 1959). The hyphae then divide within the plant cells and radiate away from the point of infection around and through the cells of the epidermis and parenchyma (Jackson and Weber, 1959). Rupturing of cell walls and membranes eventually leads to necrosis of the affected cells (Jackson and Weber, 1959). Symptoms of infection in melon leaves first appear as rings of yellow to light green chlorosis surrounding necrotic brown flecks which are about 0.5 mm in diameter on the adaxial surface of the leaf (Jackson and Weber, 1959). As the infection progresses, brown lesions develop (Carmody et al., 1985) which can grow to 5-20mm and coalesce, killing the leaf (Chandler and Thomas, 1991). These necrotic rings

progress concentrically, giving the appearance of a bull's eye, though this is not a definitive way of diagnosing ALB, because these rings are often absent if the infection occurs rapidly (Jackson and Weber, 1959). The merging of lesions and consequent necrosis ultimately lead to defoliation and full exposure of the fruit to the sun (Jackson and Weber, 1959).

Alternaria mycelia persist in infected field debris, but require development of conidia for infection (Jackson and Weber, 1959). Reportedly, the disease can be spread through infested seeds, which can contain conidia that infect cotyledons in the developing seedling (Jackson and Weber, 1959), though the actual mechanisms of seed transfer and seed-based disease development have not been well documented. Rapid production of conidia from overwintered mycelia in the spring was reported to occur when temperature ranged 20-32°C during periods of extended wetness by rain or dew (Jackson and Weber, 1959). The degree of *Alternaria* lesion formation on leaves is dependent on about 8-10 hours of foliage wetness and is further enhanced by any physical damage to the leaf such as puncturing by insects (Chandler and Thomas, 1991). Under controlled conditions, Evans et al. (1992) found that establishment and lesion formation on plants inoculated with conidia was dependent on both temperature and duration of wetness and that an initial 24 hrs (maximum wetness period tested) at 18°C, and then maintained at 18°C, resulted in the most intense lesion formation in plants under greenhouse conditions.

In order to meet USDA standards, cantaloupes must have a soluble solid content (SSC) of 9% (good internal quality) or greater (11% being considered very good internal quality) (USDA Agricultural Marketing Service, 2008). Latin et al. (1994) found a

significant decrease in SSC as *Alternaria* infection increased and that sub 9% SSC occurred in fields with high infection rates. Decreased fruit yield by *Alternaria* is primarily caused by the rapid defoliation of the plant, leaving the fruit vulnerable to sun scalding (Latin et al., 1994). The resulting rise in the unshaded fruit temperature causes an increase in metabolism, leading to a decrease in SSC (Chandler and Thomas, 1991 citing Bouwkamp et al., 1978). In addition, under severe disease pressure *Alternaria* also can infect the overripe, sun-scalded fruit (Jackson and Weber, 1959).

Management of *Alternaria cucumerina*

Fungicides

Current disease control strategies rely on removing melon debris from the field, rotating crops, treating with fungicides, and planting resistant varieties (Seebold et al., 2009). Crop rotation and plowing provide only limited control; so heavy reliance on repeated fungicide applications is required for ALB control in the absence of sufficiently resistant commercial melons (Latin et al., 1994; Suheri and Latin, 1991). Chlorothalonil and mancozeb are two common protective fungicides suitable for control of ALB with repeated applications (Suheri and Latin, 1991). Conventionally, fungicides are sprayed at 7 to14 day intervals, but it was shown that adequate control on ALB in a particular melon cultivar can be achieved by rotating Chlorothalonil with “reduced-risk” fungicides (short re-entry interval) or using Melcast (Latin and Egel, 2001) to strategically time

Chlorothalonil applications (Keinath et al., 2007). In order to reduce fungicide use while maintaining adequate protection, Latin and Egel (2001) introduced MelCast, which allows for dynamic fungicide use based on duration of temperature and wetness. For example, maximum ALB severity is observed after 24 hrs of wetness at 18^0C (Evans et al., 1992) MelCast has been used in Indiana since 1996 and has reduced fungicide use 10-20% (Latin and Egel, 2001).

Resistance to *Alternaria cucumerina*

Sitterly (1972) suggested that the absence of breeding for ALB resistance could be because earlier, more destructive pathogens attacked the plant so quickly that ALB wasn't viewed as important; as varieties resistant to those earlier pathogens have become more available, the effects of ALB have become more apparent. *Alternaria* resistant melon varieties have markedly decreased lesion growth (2-6% of susceptible lesion growth) and sporulation (9-47% of susceptible sporulation per lesion unit area) after 10 days of infection (Thomas, 1984). Lesions on resistant plants remained small, while the lesions eventually coalesce to overcome leaves of susceptible plants (Thomas, 1984). However, melon cultivars, lines, and PIs that exhibit degrees of resistance to ALB have been noted in the literature (Thomas et al., 1990; Thomas and Caniglia, 1997; Egel, 1999ab; Boyhan and Norton, 1992; Sitterly, 1972; Carmody et al., 1985). During preliminary inoculation tests, we found that melon cultivars 'MR-1', 'M024', and 'Jindaozi' exhibited moderate to significant levels of resistance, respectively, while

‘Hales Best Jumbo’, ‘Perlita’, and ‘Ananas Yokneum’ appeared to be susceptible. Notably, ‘Ananas Yokneum’ (AY) has been reported as resistant (Thomas and Caniglia, 1997), but preliminary inoculations of AY have suggested susceptibility relative to ‘MR-1’, ‘M024’, and ‘Jindaozi’ (data not published.)

Resistance Screening Methods: Spray, Wounded-leaf, and Field

Spray Method

The spray method evenly distributes conidia onto foliage of seedlings and has been the primary method of evaluating disease severity under greenhouse conditions. Greenhouse-based tests rely on humidity chambers to incite disease progression and simulate field humidity. Evans et al. (1992) conducted an exhaustive study to determine the duration of wetness and temperature at which maximum disease severity occurs using dew chambers and found that 18^0C for 24 hours produced maximum severity. According to Melcast (Latin, 2001), 18^0C for 24 hours also gives the maximum severity score.

Alternaria studies using the spray method vary in leaf position, rating scheme, and humidity chamber duration. Thomas et al. (1990) rotated inoculated plants between dew chambers and greenhouse benches for ten days and evaluated lesion diameters on second expanded leaves. Boyhan and Norton (1992) and Carmody et al. (1985) placed inoculated plants in a humidity chamber only once for an extended period of time and evaluated severity based based on percentage of infected leaf and lesion size and number

of lesions, respectively. Carmody et al. (1985) showed that disease severity of greenhouse spray tests correlated with field severity. The major advantages of this method are the high number of young plants that can be tested without the expense of growing in the field and the uniformity afforded by greenhouse conditions.

Wounded-leaf Method

This method exploits the rapid infection that occurs when conidia are applied to wounded leaf tissue. Thomas et al. (1990) observed that rapid lesion expansion occurred on inoculated plants that had mechanical damage and that the severity was increased in susceptible lines. Batta (2003) demonstrated that lesion diameters on inoculated wound sites on cucumber leaf disks distinguished resistant, intermediate resistant, and susceptible cucumber lines. Like the spray method, artificial humidity is required to promote lesion expansion. However, in contrast to the spray method, lesions originate from deliberately wounded sites of the leaf tissue, giving the researcher control over lesion number and placement. In early attempts to adapting this leaf disk method to melon, we achieved lesion expansion, but the results were confounded by early senescence of leaf tissue. However, when leaf tissue of intact plants were wound-inoculated and subjected to the same conditions as Thomas et al. (1990), we observed uniform lesion expansion that correlated with expected relative resistance levels (data not shown). In this study, the wounded leaf method appeared most effective on the expanded third leaves of individually potted plants. The number of plants that can be tested, similar

to the spray method, is limited only by the size of the humidity chamber and available greenhouse space.

Field Method

The most straight-forward method is to allow the natural disease progression under untreated field conditions. Plants can be naturally or artificially inoculated. This method, while highly subject to environmental effects, is a practical measure of disease severity. Because of environmental effects, this method requires a relatively large number of plants, investment in field space and resources, multiple testing locations, and significant time allotment. In order to increase the efficiency of field tests, the greenhouse-based test can serve as less resource intensive initial screen for interesting genotypes. The field method has been used in a several ALB studies: Egel (1999ab) in a two year resistance screen of around 20 melon varieties, in fungicide studies (Keinath et al., 2007; Egel and Harmon, 2001; Thomas, 1983), in comparisons of field results to greenhouse data (Carmody et al., 1985), and to examine models of ALB severity and yield loss (Latin, 1992). Disease severity scores have been based on visual estimates of the percentage of leaf area diseased at prescribed intervals, foliage loss per area, average diameter of lesions, and/or number of lesions per leaf (Keinath et al., 2007; Egel and Harmon 2001; Thomas, 1983; Egel 1999ab). Because melons are being grown under production conditions, the field method is the ultimate measure of resistant varieties, fungicide effectiveness, and the effectiveness of resistance markers.

Inheritance of Alternaria Resistance

Alternaria resistance has been reported as oligogenic (Boyhan and Norton, 1992), suggesting a model of resistance characterized by multiple genes and a major dominant gene, while a single dominant gene conferring resistance has been reported in the MR-1 breeding line (Thomas et al., 1990). Boyhan and Norton (1992) examined the progeny from crosses and backcrosses of highly susceptible PMR 6 and highly resistant AC-82-37-2 and estimated that the inheritance had significant additive effects, heterozygote x homozygote epistatic interactions, and no significant dominance effects. Within the limits of the plants tested, the broad- and narrow-sense heritabilities were reported as 0.57 and 0.45, respectively, meaning that resistance could be effectively improved in breeding programs based on the pedigree and backcross methods (Boyhan and Norton, 1992). ALB resistance screenings on 16 cultivars showed varying levels of resistance (Egel 1999ab), further suggesting an oligogenic inheritance. However, Thomas et al. (1990) examined a series of crosses between resistant MR-1 and a susceptible parent, 'Perlita', and reported a clear Mendelian ratio of 3:1, suggesting that inheritance of *Alternaria* resistance in MR-1 was conferred by a single dominant gene. This MR-1 resistance gene is designated *Ac* (Thomas et al., 1990). The MR-1 line was originally developed in 1984 from an inbred line of PI124111 for its exceptional level of resistance to powdery mildew and downy mildew (Thomas, 1986). Whether or not *Ac* is part of the multiple genes observed by Boyhan and Norton (1992) is not explicit in the literature. Though, the putative oligogenic resistance model had a low dominance effect, which

suggests that the dominant gene *Ac* observed may not be part of the multiple gene resistance. Further evidence would come from identifying markers associated with the resistance in MR-1 and validating these markers in lines used by Boyhan and Norton (1992).

Elemental Sulfur as an effective fungicide

Elemental sulfur has been recognized for thousands of years as a potent fungicide (Williams and Cooper, 2004). For cucurbits, sulfur is an inexpensive and effective organic method for controlling powdery mildew (*Podosphaera xanthii*) (Koller, 2010; Keinath and DuBose, 2012). Furthermore, sulfur effectiveness has been shown to be significantly enhanced when used on resistant plants (Koller, 2010). Sulfur can be used on many cucurbits, including melon, but phytotoxicity can be extreme in some lines (Perchepied et al., 2004; Gogoi et al., 2013; Johnson and Mayberry, 1980). Sulfur is considered a contact fungicide, and hence more effective against fungal pathogens on the surface of the leaf (Keinath and DuBose, 2012). Sulfur interacts with fungal pathogens by direct contact, diffusion through water, and by vapor action around sulfur particles (Bent, 1967). Despite thousands of years of experience with sulfur as a fungicide, the exact mechanism remains elusive, but it is thought that the sulfur interferes with mitochondrial respiration in the fungus (Cooper and Williams, 2004), resulting in inhibition of conidia germination (Gogoi et al., 2013). According to the Fungicide Resistance Action Committee (2013), sulfur's mode of action is defined as "multi-site contact activity" and considered "low risk" for pathogen resistance development. In

order to mitigate pathogen fungicide resistance, sulfur and similar low risk fungicides, should be used prior to adoption of fungicides with heightened risk for pathogen resistance (Keinath and DuBose, 2012).

"Sulfur induced resistance" (SIR) is the natural deployment of sulfur-containing compounds to protect against pests and disease (Bloem et al., 2004). Heightened research interest in sulfur stems from the discovery of sulfur produced in *Theobroma cacao* and localized in the xylem as a resistance reaction to the fungal pathogen *Verticillium dahlia* (Cooper et al., 1996). Sulfur has also been detected in several plant species, primarily in response to pathogens invading the plant vascular system (Cooper and Williams, 2004; Williams and Cooper, 2003; Williams et al., 2002). Innate sulfur production in cucurbits remains uninvestigated.

Elemental Sulfur Application methods

Sulfur Dust

Sulfur is applied as a micronized spray or dust, and the various formulations (sulfur $> 90\%$) differ primarily in the size of the sulfur particles (Emmett et al., 2003). Sulfur primarily forms an eight atom ring (S_8) at room temperature (Meyer, 1976). The majority of applied sulfur remains on the leaf surface or intergrates into the cuticle (McGrath and Johnston, 1986). The efficacy and adherence of sulfur increases as the particle size decreases and thus its surface area coverage increases (Wilcoxon and

McCallan, 1931; Gogoi et al., 2013; Motior et al., 2011). However, because the increased coverage accelerates vaporization and degradation, smaller particles have lowered persistence (Emmett et al., 2003). Through microorganism-mediated oxidation, sulfur is also used to amend soil pH, making available nutrients restricted in alkaline soils, and providing sulfate to the plants (Grayston and Germida 1991; Motior et al., 2011). Also, SIR is influenced by the availability of usable sulfur (Bloem et al., 2004). However, repeated applications as a fungicide can inadvertently wash into the soil and gradually decrease the pH, leading to diminished nutrient availability (Ngatunga et al., 2003; Owen et al., 1999).

Sulfur Vaporization

Sulfur vaporization by means of timer controlled vaporizers is a convenient method of powdery mildew control in greenhouses. Vaporization of sulfur forms a cloud of very fine sulfur particles that distribute over nearby plants (Barker and Wallace, 1922). Early vaporization of sulfur was done by applying sulfur to hot pipes, but commercial sulfur vaporizers can be now be purchased (Barker and Wallace, 1922). Solid sulfur melts at 119.6 ^{0}C and boils at 444.6 ^{0}C (Meyer, 1976), and sufficient vaporization occurs from 170^{0}C and 230^{0}C (Barker and Wallace, 1922). Vapor pressures at these temperatures are respectively ~75 Pa and ~874 Pa (West and Menzies, 1929). For example, the Wilmod Sulphur Evaporator WSE75 (www.wse75.com) heats sulfur (purity >99%) to 190 ^{0}C, at which point the vapor pressure is ~185 Pa (West and Menzies, 1929). This is sufficient for 100-700m^2 of greenhouse space, depending on the crop

(www.wse75.com). Vaporization provides an inconspicuous, pervasive fine layer of sulfur dust for powdery mildew control in a greenhouse.

Elemental Sulfur Phytotoxicity in Melon

Sulfur application is an effective method to control the casual agents of powdery mildew on cucurbits, but some melon cultivars are extremely susceptible to sulfur (Perchepied et al., 2004). The limited research on sulfur phytotoxicity in melon has focused on sulfur dust application for tolerance screening and QTL discovery (Johnson and Mayberry, 1980; Perchepied et al., 2004). Sulfur phytotoxicity is manifested as necrosis and pronounced "burning" on the leaf tissue starting 4 days after dusting fruiting melon plants in field conditions (Johnson and Mayberry, 1980). In greenhouse conditions, vaporized sulfur can cause symptoms within 24 hrs of application in susceptible melon lines at the second expanded leave stage. Symptoms of sulfur phytotoxicity appear preferentially on matures leaf tissue of susceptible lines, in a top-down, tolerance-to-susceptible pattern (Johnson and Mayberry, 1980). The observations by Johnson and Mayberry (1980) of sulfur phytotoxicity progression suggests that there is a developmental threshold that determines susceptibility of mature leaf tissue.

Research on the mechanism of sulfur tolerance and phytotoxicity in cucurbits is, as far as can be discerned, nonexistent. Nevertheless, extensive research (next section) on the susceptibility of cucurbits to oxidized and reduced forms of sulfur may provide indications of the underlying mechanism of sulfur tolerance in some melon lines.

Interestingly, it has been demonstrated that higher plants are able to directly metabolize foliar sulfur (Legris-Delaporte et al., 1987; Jolivet et al., 1995; Williams and Cooper, 2004; Vitti et al., 2007; McGrath and Johnston, 1986). If this also occurs in cucurbits, then it is plausible that mechanisms to prevent toxicity from excess sulfur intake from other sources could be the same for foliar sulfur intake.

Elemental Sulfur Toxicity and Coping Mechanism in Cucurbits

Plants take in sulfur through their roots as sulfate and through their leaves primarily as sulfur dioxide (SO_2) and hydrogen sulfide (H_2S), but excess sulfur accumulation can become toxic at levels that vary by species, varieties, soil-sulfur content, and environmental conditions (Rennenberg, 1984; Hawkesford and De Kok, 2006). The exact mechanism for phytotoxicity of sulfur in cucurbits is not explicit in the literature, but if direct oxidation of foliar sulfur is excessive, it could lead to the toxic accumulation of sulfur compounds. Because of the interest in the effects of excess sulfur from pollution on cucurbits; SO_2, H_2S, sulfate, and sulfur-containing products have been the focus of numerous research efforts. Nevertheless, the elucidated sulfur metabolic pathways and, importantly, coping mechanisms provide clues to how cucurbits may respond to excess sulfur from possible direct metabolism of foliar sulfur.

The mechanisms by which plants cope with excess sulfur are multifaceted (Rennenberg, 1984). Plant sulfur metabolism involves balancing sulfur needs with both sulfur uptake and source, and it is not completely understood (Hawkesford and De Kok,

2006). Sulfate is reduced through a series of enzymatic reactions to form primarily cysteine, methionine, and glutathione (Hawkesford and De Kok, 2006). Stomatal closure, storage, metabolism, emission, and translocation are observed mechanisms of sulfur management (Rennenberg, 1984). Plant SO_2 metabolism generally follows one of two pathways: oxidized to H_2SO_4 or reduced to H_2S and partially emitted (Heber and Hüve, 1997; Sekiya et al., 1982). Both pathways eventually integrate into sulfur metabolism; however, the former pathway increases hydrogen ions and sulfur compounds; and the latter pathway alleviates excess hydrogen ions and sulfur through partial emission (Heber and Hüve, 1997). Sub-injurious SO_2 concentrations, however, can be a valuable source of sulfur for otherwise deficient plants (Cowling et al., 1973); H_2S can be oxidized by the plant to sulfate, and reintroduced into the sulfur reduction pathway (Rennenberg and Filner, 1982). Emission of H_2S in response to excess sulfur is a feature of many plant species (Hällgren and Fredriksson, 1982; Rennenberg, 1984). Cucumber, pumpkin, and melon release H_2S when supplied with excess sulfate, possibly as a means to eliminate excess sulfur (Wilson et al., 1978). When pumpkin was fumigated with SO_2, it was shown that glutathione, an end product of sulfur metabolism, was inhibited, and H_2S was emitted as a mechanism to lessen precursor accumulation (Rennenberg and Filner, 1982). Concerns over sulfur pollution from fossil fuels has led to extensive desulphurization of natural gas (now the dominant source of sulfur production) (Meyer, 1976). Consequently, sweeping reductions of sulfur emissions across Europe have led to widespread sulfur deficiencies in some crops (Zhao et al., 1997). In fact, some plant species can rely solely on SO_2 or H_2S in the absence of sulfate

in the soil (Hawkesford and De Kok, 2006). However, excess sulfur can be toxic, and plants vary significantly in susceptibility (Mejstrik, 1980).

Acute foliar injury and lesion formation isn't necessarily a reliable method of evaluating the often subtle effects of SO_2 at low to moderate concentrations on plants (Eason et al., 1996; Mejstrik, 1980; Bressan et al., 1978). Cucumber (*C. sativus)*, fumigated at low concentrations of SO_2, caused significant reductions in root fresh weight (39%) and leaf area (46%) (Mejstrik, 1980). In another study it was shown that environmental concentrations of SO_2 decreased the dry mass of roots, without significantly affecting foliage of watermelon cultivars (Eason et al., 1996). Understanding the underlying mechanisms will guide breeding strategies for SO_2 tolerance (Bressan et al., 1978).

Among cucurbits, tolerance to SO_2 appears to be chiefly affected by absorption rates and, to a lesser extent, H_2S emission (Rennenberg, 1984; Wilson et al., 1978). However, the SO_2 tolerance of cucurbit young leaves was shown to be primarily determined by emission of H_2S in order to prevent toxic precursor accumulation (Rennenberg, 1984; Wilson et al., 1978). Cucurbit SO_2 tolerance and hydrogen sulfide production is lower in young plants than in old plants, but young leaf tissue, regardless of plant age or cultivar susceptibility, is more resistant than mature leaf tissue (Rennenberg, 1984; Bressan et al., 1978). Young cucurbit leaves were shown to actually absorb SO_2 faster than mature leaves (Bressan et al., 1979), but young leaf tissue converted 10% of absorbed SO_2 to H_2S (compared only 2% conversion in mature leaves) and emitted 10-100 times more H_2S than mature leaves (Sekiya et al., 1982). The tolerance of young

leaves in susceptible cucurbits leads to defoliation of lower leaves first, similar to the observations made by Johnson and Mayberry (1980) when sulfur was applied to susceptible melon cultivars. This young leaf SO_2 tolerance mechanism was found to be developmentally determined, metabolically controlled, and independent of absorptions rates (Bressan et al., 1978; Sekiya et al., 1982).

Inheritance of Elemental Sulfur Tolerance in Melon

Sulfur tolerance in melon is quantitatively inherited (Perchepied et al., 2004). Perchepied et al. (2004) successfully mapped one major and two minor QTL affecting sulfur tolerance in two recombinant inbred lines sharing a common resistant parent. The strong QTL exerted complete dominance in the cross ‘Vedrantais’ x PI124112 and incomplete dominance in ‘Vedrantais’ x PI161375 (Perchepied et al., 2004). The two minor QTL were only detected in the ‘Vedrantais’ x PI124112 population (Perchepied et al., 2004). Breeding for tolerance was first recorded in the 1930’s and eventually led to the 1942 introduction of the “V-1 Sulfur Resistant Cantaloupe” (Johnson and Mayberry, 1980). In 1979 a screen of 31 melon cultivars by Johnson and Mayberry (1980) showed that 23 were resistant and 8 were susceptible and that melons from the inodorous group were all tolerance. Two hundred thirty-six melon accessions from around the world were screened and 47% showed complete tolerance (Perchepied et al., 2004). ‘Top Mark’, a variety originally reported as resistant by Johnson and Mayberry (1980), was used as a source of sulfur tolerance in one melon breeding program (Zink and Gubler, 1990).

Preliminary Observations of Sulfur Tolerance Segregation

Varying degrees of sulfur tolerance were observed in the MR-1 x AY RILs. Introduction of sulfur vaporizers as a means of controlling greenhouse PM thwarted early attempts to increase and test MR-1 x AY RILs because of sulfur susceptibility in some of the RILs. A special sulfur-free greenhouse had to be utilized. However, in the presence of vaporized sulfur, MR-1, MR-1 x AY F_1, and some RILs suffered phytotoxicity to varying degrees, with the most susceptible exhibiting rapid defoliation of all but the youngest leaf tissue. AY and many RILs showed no symptoms. The symptoms of the parental lines and F_1 range from MR-1 (high damage) → MR-1 x AY F_1 (intermediate damage) → AY (no damage). The intermediate nature of the F_1 suggests an incompletely dominant gene action conferring tolerance, similar to the 'Vedrantais' x PI161375 cross reported by Perchepied et al. (2004), and the varying degrees of susceptibility suggest the effects of minor QTL. Initial observations of degrees of sulfur tolerance in MR-1 x AY RILs, MR-1 susceptibility, complete tolerance in AY, and intermediate susceptibility in MR-1 x AY F_1 suggest the existence of multiple QTL affecting tolerance to elemental sulfur.

Quantitative Trait Mapping in Melon

Historically, genetic knowledge has advanced through the study of 'macromutations' controlled by single genes; however, the majority of observed variation in plants is controlled by multiple loci, resulting in quantitatively inherited traits

(Tanksley, 1993). For example, Cuevas et al. (2008) identified seven quantitative trait loci (QTL) contributing to B-carotene accumulation in melon. Quantitatively inherited traits are often controlled by complex arrangements of multiple genes that display a continuous distribution of phenotypes (Kearsey, 1998). QTL contributing to a trait can operate in cis- or trans- to the actual gene, adding an additional level of complexity (Miles and Wayne, 2008). The interaction of allelic differences in structural and/or regulatory genes combined with additive contributions, gene-gene interactions (epistasis), and environmental effects contribute to a highly complex and difficult to describe inheritance (Kearsey, 1998). Even in cases where the number of contributing genes are few, the trait can be continuous because of subtle effects of the environment (Kearsey, 1998). There are generally three tiers of QTL: a few major genes contributing large effects, increasingly more QTL with medium effects, and numerous minor QTL (Semagn et al., 2010).

Because many important agronomic traits are controlled by polygenes, plant breeders have utilized statistical genetics to describe quantitative traits, make heritability estimates, and develop breeding schemes (Fehr, 1991). Heritability estimates predict the portion of the phenotypic variation (V_P) that is explained by genetic factors (V_G) that can be improved through breeding as opposed to environmental effects (V_E) which a breeder has no control over. This relationship is described by the simple equation $V_P = V_G + V_E$, and can be employed to make estimates of the combined effect of QTL, however the effects of the individual QTL cannot be described (Kearsey, 1998). Intricate genetic structures are frequently more accurately described by identifying marker linkage

patterns than by statistical analysis of inheritance studies (Perchepied et al., 2005) The majority of traits of agronomic interest tend to have heritabilities of less than 50% (Kearsey, 1998). Because traits with high heritabilities are easily distinguished using phenotypic selection, it is generally only cost effective to use MAS when heritibilities are low (Mauricio, 2001). Plant breeding schemes are adjusted according to heritability estimates and frequently take a conventional breeding approach, utilizing available molecular tools (Fehr, 1991).

QTL analysis is a statistical approach that attempts to explain complex traits by identifying contributing regions of the chromosomes (Miles and Wayne, 2008). The breakthrough for elucidating QTL was the introduction of molecular marker based mapping techniques that could be paired with phenotypic data from a segregating population (Kearsey, 1998). Regardless of the molecular marker type, differences within the genetic code can be mapped relative to each other; some of these differences can be associated with QTL which have measurable effects on the traits (Miles and Wayne, 2008). Subsequently, QTL analysis in plants has been extensive, and it is estimated that, as of 2008, 10,000 QTLs have been identified (Bernardo, 2008). The average observed plant trait variance, over a 20 year period up to 2008, attributed to identified QTL range from 40% to 60% (Bernardo, 2008). QTL analysis uses the combination of phenotype and genotype to explain gene action, epistatic interaction, level and direction of contribution, position, and the number of loci (Semagn et al., 2010).

Mapping Populations

There are various mapping population types: F_2, F_1 backcross (BC), $F_{2:3}$ (F_3 families), recombinant inbred lines (RILs), near isogenic lines (NILs), and double haploid lines (DHLs); but all share the common feature of being based on divergent parental phenotype for the trait of interest or are known to contain different alleles for the same trait (Miles and Wayne, 2008). In any case, segregation of a trait is measurable. Ideally, a small number of QTL provide strong cumulative contributions to the phenotype that are consistent across multiple environments (Zalapa et al., 2007). This was the case for six QTL identified in melon RILs in which sugar content increased according to the number of QTL, irrespective of which of the QTL were present (Harel-Beja et al., 2010). The incorporation of such cumulative QTL into a breeding program would be relatively straightforward; however, QTL are often strongly subject to environmental effects (have low heritability) and are inconsistent across environments (Harel-Beja et al., 2010; Paris et al., 2008; Monforte et al., 2004). To further complicate the inheritance, QTL can be both numerous and varied in their effect; QTL identified in a population under certain environmental conditions are sometimes not detected when a population is placed under other environmental conditions (Xu and Crouch, 2008). Studies commonly replicate experiments under varied conditions and have demonstrated that while some QTL are common to multiple environments, others are unique to particular areas (Xu and Crouch, 2008; Zalapa et al., 2007; Collard and Mackill, 2008; Monforte et al., 2004; Perchepied et

al., 2005). Common QTL may universally contribute to a particular phenotype and are of particular breeding value.

Experimental populations in melon are F_1-derived and are subject to the F_1 parent marker-QTL linkages (Semagn et al., 2010). This creates an ideal mapping situation because the uniform marker-QTL associations across the subsequent population change only by the frequency of recombination events (Semagn et al., 2010). Thus, heterozygous markers and alleles in an F_1 segregate and can be mapped given sufficient marker coverage, population size, and phenotype measurability (Kearsey, 1998). Marker density beyond one marker per every ten cM is subject to diminishing returns as increasing the population becomes more lucrative but is often constrained by resources (Kearsey, 1998).

F_2 Population

The F_2 population is the simplest breeding scheme that provides a temporary heterozygous population representing a theoretically even distribution of the parental alleles (Semagn et al., 2010). The high heterozygosity makes F_2 populations useful for studying both additive effects and dominance (Semagn et al., 2010). However, because of limited meiotic events, there are limited crossover opportunities and, thus, low resolution of QTL (Takagi et al., 2013). Decreased resolution also limits epistatic studies (Semagn et al., 2010).

Backcross Population

Similar to a F_2 population, the backcross population is a simple temporary population that has a short production time (Semagn et al., 2010). However, because the

F_1 is crossed with one of the parents, dominance can lead to skewing of the effects (Semagn et al., 2010). As with F_2 populations, the few meiotic events in BC creation decrease the number of crossover events so that even distant linkages can still exist (Semagn et al., 2010).

$F_{2:3}$ Family

The phenotypic mean of the F_3 family is used to estimate the phenotype of the F_2 parent (Fehr, 1991). This type of population is preferable when increased replicates are required for traits with low heritability (Fehr, 1991). In order to decrease linkage disequilibrium, the $F_{2:3}$ can be increased to any future generation $F_{X:Y}$ (Semagn et al., 2010). Limited testing over multiple locations is dependent on the amount of $F_{X:Y}$ seed available.

Recombinant Inbred Lines (RILs)

Highly inbred lines (F_6 or higher) are produced by single seed decent from an F_2 generation. The advanced generation of F_6 or higher increases the resolution of the genetic map because of the increased opportunities for crossover events between tightly linked markers and genes (Semagn et al., 2010). The high homozygosity allows potentially infinite replenishment of RIL seed; consequently, RILs can be replicated under different environments (Fukino et al., 2008; Semagn et al., 2010; Collard and Mackill, 2008). However, because of the costs associated with production, the population sizes are often smaller than other population types and have diminished QTL positioning accuracy (Semagn et al., 2010). QTL analysis of RILs can provide information about the additive effect of the QTL but not dominance of those QTL (Semagn et al., 2010).

Unintentional selection during the RIL development can increase heterozygosity and skew the distribution of alleles (Harel-Beja et al., 2010).

Near Isogenic Lines (NILs)

NILs are produced from repeated backcrossing to the recurrent parent that does not have the trait of interest in order to isolate and study the effects of targeted loci (Kooke et al., 2012). BC_6 or higher represents at least 99% genetic similarity to the recurrent parent and has undergone several recombination events that isolate target loci (Semagn et al., 2010). The genetic background becomes uniform except for the trait of interest, and other genetic effects that may have distorted phenotypic expression are diminished (Xu and Crouch, 2008). Being able to isolate QTL makes NILs ideal for studying phenotypic effects of individual QTL, high-resolution mapping, and genetic studies (Semagn et al., 2010). Development can be very expensive, but permanent population that can be transferred to other laboratories and repeatedly tested (Semagn et al., 2010). Like RILs, limited population sizes can affect the accuracy of QTL positioning and only the additive component can be quantified (Semagn et al., 2010).

Double Haploid Lines (DHLs)

Double haploidy allows the creation of fully homozygous lines in two generations (Semagn et al., 2010). DH production dramatically shortens the time to a RI-like population but with not as much recombination and thus lower resolution between even distantly linked genes/markers (Semagn et al., 2010). The sequencing of a DH melon and parents revealed that only 17 recombination events had occurred with an average of 1.4 per linkage group (Garcia-Mas et al., 2012). Consequently, DHL lines require large

numbers for increased resolution or bin mapping strategies (Deleu et al., 2009; Fernandez-Silva et al., 2008). As long as the lines can be perpetuated, DHLs are permanent and can be transferred to other laboratories for repeated testing (Semagn et al., 2010). Production costs can be relatively low, but double haploidy is only possible for species that have developed protocols (Semagn et al., 2010). DHL have been shown to suffer from high segregation distortion because of inadvertent selection of genes that promote DHL creation (Gonzalo et al., 2005).

Molecular Markers

Regardless of the marker type used, the corresponding genetic polymorphism represents a single point in the genome that can be positioned relative to other such points based on recombination frequencies. The utility of a genetic map, repeatability, codominance, and inter-map transferability of markers add value to the marker type chosen (Oliver et al., 2001).

Molecular marker types are judged useful based on five general criteria: reliability, DNA quantity and quality requirements, technical procedures, extent polymorphic, and costs (Semagn et al., 2006). The genetic maps of melon use a variety of markers types: random amplified polymorphic DNA (RAPD), sequence characterized amplified region marker (SCAR), restriction fragment length polymorphism (RFLP), cleaved amplified polymorphic sequences (CAPS), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP), inter simple

sequence repeat (ISSR), simple sequence repeats (SSR), sequence-tagged sites (STS), and single nucleotide polymorphism (SNP). The first molecular maps in melon were created using RAPD, RFLP, and AFLP (Baudracco-Arnas and Pitrat, 1996; Wang et al., 1997). These marker types can be used without prior knowledge of sequence data (Semagn et al., 2006), and are useful for initial genetic mapping. RAPD markers (and ISSR makers) are frequently criticized for reported non-reproducibility, potential comigration, and limited extrapolation beyond the studied population (Semagn et al., 2006; Wang et al., 1997). However, Park, Hwang, and Crosby (2009) found RAPD markers types to be very reliable and desirable because of their simplicity relative to other marker types. Dominant markers (RAPD, AFLP, SRAP, ISSR) provide similar information as codominate marker types (RFLP, CAPS, SSR, SNP) in populations devoid of heterozygotes (RILs, DHLs, NILs) (Semagn et al., 2006). Homozygous population types, particularly RILs, have been used frequently for genetic mapping in melon (Appendix D).

The general trend in molecular mapping in melon, evident in the latest consensus map (Díaz Bermúdez et al., 2011), has been toward EST-based RFLP, CAPS, SSR, and SNP. In particular, the latter two types dominate the latest melon consensus map (Díaz Bermúdez et al., 2011). EST-SNPs have successfully been gathered from EST databases (ICuGI.org), genome sequences, array hybridization, and amplicon sequencing (Deleu et al., 2009). EST-based markers are especially useful because they are found in gene sequences and hence highly conserved between species and valuable for QTL analysis (Fernandez-Silva et al., 2008; Collard and Mackill, 2008). EST-based markers are

commonly used as anchors to merge maps of various crosses (Fukino et al., 2008; Díaz Bermúdez et al., 2011). The proliferation of these markers types in melon is partly due to the growing EST database maintained by International Cucurbit Genomics Initiative (ICuGI, http://www.icugi.org/). The ICuGI, along with important genomic information for cucumber (*Cucumis sativus* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], and *Cucumis pepo* L., maintains a database of 129,067 melon ESTs from which over 3,200 candidate EST-SSRs and 1360 candidate EST-SNPs have been identified (May, 2010 EST collection). EST-based markers have been shown to be universally dispersed throughout the genome and very polymorphic, highly reproducible, frequently codominant, and amenable to gel based analysis (Fernandez-Silva et al., 2008).

QTL Mapping Methods

QTL are generally mapped for two reasons: (1) to understand the genetic mechanisms and inheritance of the trait and (2) for use in marker assisted selection (Semagn et al., 2010). Molecular mapping is made possible by recombination during meiosis and molecular marker segregation frequencies (Byrne, 2005). Prior to QTL analysis, polymorphic markers are first arranged into linkage groups using recombination frequencies at general minimum 3 LOD significance (Mauricio, 2001). The majority of melon maps (Appendix D) surveyed use JoinMap 4 (Kyazma B.V., Wageningen, Netherlands) or MapMaker (Lander et al., 1987) for linkage group assembly and map creation. While simple statistical models (Single-Factor ANOVA, regression analysis,

simple interval mapping, etc.) are sufficient for basic analysis, many statistical methods have been, and continue to be, developed to increase precision of extremely complex QTL analysis (Kearsey, 1998; Byrne, 2005). The methods most commonly used are variations of the concept on interval mapping (IM) which calculates the probability of a QTL being located in the interval between two markers (Kearsey, 1998). IM, when compared other simpler methods gives higher resolution with smaller population requirements (Kao et al., 1999). A popular method based on IM called composite interval mapping (CIM) uses probabilities that a marker or the interval between two markers is linked to a particular trait while taking into account the presence of other markers (Miles and Wayne, 2008; Zeng, 1994). CIM efficiently decreases the background "noise" that would otherwise make some QTL undetectable; and narrows the region likely to contain the QTL (Byrne, 2005; Zeng, 1994). CIM has been used in the majority of QTL analysis in melon (see Appendix D) and provides useful information about the QTL: likely position on the linkage group, statistical significance (LOD), explained variance (R^2), parental source of QTL, and dominance and additive effects (Byrne, 2005). However, CIM does not provide information about epistatic effects and can be affected by uneven marker dispersion (Semagn et al., 2010). In order to account for epistatic effects, decrease error rates, and improve QTL detection, additional statistical methods have been developed: multiple interval mapping (MIM) (Kao et al., 1999), muItiple-QTL mapping (MQM) (Arends et al., 2010), and inclusive composite interval mapping (ICIM) (Li et al. 2008).

There are several popular QTL analysis programs such as R/qtl (Broman 2013), MapQTL 4 (Van Ooijen, 2004), and QTL IciMapping 3.3 (The Quantitative Genetics Group, http://www.isbreeding.net). Although, the most popular program among the melon maps surveyed is the GUI-based software WinQTL cartographer 2.5 (Wang et al., 2012) provided by North Carolina State University. The free and easy-to-use interface make WinQTL Cartographer 2.5 an effective and user friendly option. WinQTL Cartographer 2.5 offers single maker analysis, IM, CIM, MIM and permutation tests to determine significance thresholds (Wang et al., 2012).

Challenges of QTL and their Validation

The identification of QTL relies on statistical procedures that inherently tolerate a level of error that can lead to both type 1 and type 2 errors (Semagn et al., 2010). To minimize error, it is generally accepted that an LOD score of at least three should be used to declare significance (1000 times more likely than not that a QTL exists in an interval) (Mauricio, 2001). For increased precision to a particular data set permutation tests set LOD thresholds at prescribed levels of significance (Mauricio, 2001). In addition, effects of population size, marker density and dispersion, and quality of phenotyping data can dramatically affect precision (Mauricio, 2001). Thus, validation of QTL should be done prior to employment in expensive breeding schemes (Collard and Mackill, 2008). QTL analysis inherently exaggerates the effects (Vg) of detected QTL because minor, undetected QTL are obscured. (Kearsey, 1998; Mauricio, 2001). QTL with opposite

effects (in dispersion) that have wide confidence intervals can overlap and diminish the perceived effects (Kearsey, 1998). When closely associated QTL both have a positive effect, then the combined effect can trigger a false positive between the two (Kearsey, 1998). QTLs with low heritabilities tend to have wide confidence intervals (+- 10-20cM), and even when the heritability is high, the interval is generally around 10cM; however, this level of accuracy can be suitable for MAS (Kearsey, 1998).

Validating previous QTL studies rely on confirming the effects of the QTL in various populations, often by targeting a particular QTL for analysis by means of NILs (Flint-Garcia et al., 2003). However, molecular markers associated with QTL developed in a particular cross may not be applicable to other populations even of the same species because the polymorphism that created the marker may not exist or may be alternately linked (Kearsey, 1998). Marker validation involves applying the marker to other populations and evaluating if the marker predicts the expected phenotype (Semagn et al., 2010). For example, markers for powdery mildew resistance QTL identified in a melon RI population were also shown to be effective in a F_2 population utilizing a related resistant parent but a different susceptible parent (Fukino et al., 2008). In the event that a QTL is identified in the region of a known gene, the gene itself can be implicated (Flint-Garcia et al., 2003). Testing whether MAS of a QTL is effective in increasing the trait is a practical way to validate a marker (Flint-Garcia et al., 2003). Despite the challenges and limitations, QTL analysis can identify contributing regions of the chromosomes that can be used for revealing the genetics of the trait or for use in MAS (Kearsey, 1998).

Genetic Maps of Melon

A complete genetic map of melon will have 12 linkage groups, corresponding to 12 chromosomes, and will have a length of 1500-2000 cM (Baudracco-Arnas and Pitrat, 1996). One consequence of the relatively high genetic uniformity in melon is the decreased efficiency of identifying polymorphic markers (Oliver et al., 2001). This narrow genetic base is in sharp contrast to the remarkable morphological variation that makes melon classification based solely on morphology very perplexing (Silberstein et al., 1999; Shattuck-Eidens et al., 1990). Hence, a strategy to increase marker polymorphism in melon has been to select genetically diverse parents for population development (Oliver et al., 2001).

Among the 27 leading melon QTL/mapping studies in Appendix D, population sizes range from 63 (Perin et al. 2002) to 218 (Baudracco-Arnas and Pitrat, 1996) with an average of 109 (bin mapping studies excluded); among immortal population types (RIL and DHL) the range is 63 (Perin et al. 2002) to 190 (Boissot et al., 2010) with an average size of 109 (bin mapping studies excluded). Immortal populations have been reused for additional QTL analysis, increasing marker saturation, and consensus mapping (Díaz Bermúdez et al., 2011; Zalapa et al., 2007; Cuevas et al., 2008; Deleu et al., 2009; Paris et al., 2008; Fernandez-Silva et al., 2008; Gonzalo et al., 2005; Boissot et al., 2010; Monforte et al., 2004; Perin et al. 2002). In one case, bin mapping strategy was used on DHLs to first identify SSRs and then to identify EST-SNPs (Deleu et al., 2009; Fernandez-Silva et al., 2008). RILs have been frequently have been used at the F_6-F_8

generation though Fukino et al. (2008) used a F_8-F_{12} population. RILs at the F_6 are theoretically $< 4\%$ heterozygous (Fehr, 1991) and have been used as the minimum level of acceptable inbreeding among RIL based melon maps. Appendix D includes the primary maps published to date but does not reflect private maps developed by seed companies such as a Syngenta Seed Co. map of 1092 SNPs in PI414723 and Dulce (Garcia-Mas et al., 2012). It should also be mentioned that, though not included in Appendix D, QTL discovery in melon also has been done using NILs (Moreno et al., 2008). One study used SRAP only markers for mapping and found them to provide excellent coverage, even distribution, and relatively low segregation distortion (Wang et al., 2007).

Increasingly, anchor markers, particularly SSRs and SNPs, based on the database available through the ICUGI website, have led to the formation of consensus maps including the most comprehensive and recent map by Díaz Bermúdez et al. (2011). Notably, the only marker type not included in the consensus map are SRAP, though they have been shown to be efficient and reliable (Wang et al., 2007). Consensus mapping rapidly creates high density genetic maps, increases marker accuracy, and can be used to predict phenotypes (Díaz Bermúdez et al., 2011). The consensus map merged by Díaz Bermúdez et al. (2011) includes 1592 markers at a density of .72 cM/marker and 370 QTL affecting 62 traits. A map that contains >1 marker/cM is considered high density (Howad, Yamamoto et al. 2005). The consensus map also makes it possible to identify collinear QTL (Díaz Bermúdez et al., 2011).

The Genome of Melon

The sequencing of the melon genome in 2012 by Garcia-Mas et al. represents an important step in melon genetic tool development. Using a DHL, ~375Mb of the estimated 450Mb of melon were sequenced via 454 pyrosequencing (Garcia-Mas et al., 2012). The melon genome sequence provides information about the organization of genes, SNP/INDEL locations, and a physical map on which to anchor genetic maps (Garcia-Mas et al., 2012). In addition, the sequence allows for comparative studies with other sequenced genomes from which gene functions can be inferred and phylogeny can be studied (Garcia-Mas et al., 2012). Of particular interest to this research is the identification and localization of 411 R-genes of various types, 79 of which are found in 16 clusters (Garcia-Mas et al., 2012). Also, 63 putative genes involved in sugar accumulation; and 26 genes affecting carotenoid accumulation were identified (Garcia-Mas et al., 2012). In total, 27,427 genes were predicted with EST/protein confirmation of 18,948 genes (Garcia-Mas et al., 2012). Important to genetic map saturation and genetic studies, 2.1 million SNPs and 413,000 INDELS, 4.0% and 3.1% in exons, were identified between Piel de Sapo and PI161375 at a density of one SNP per 176 bp and one INDEL per 907 bp (Garcia-Mas et al., 2012). This reference genome will provide a vast array of additional SNPs and SSRs that will complement the information found the ICuGI database.

The sequence of melon is not a panacea of gene identification and QTL analysis, but it does provide a vast array of potential markers and predicted genes by which QTL

analysis will be drastically enhanced. Importantly, various marker types can be purposefully created to target specific regions of the genome and used to map in other melon lines. For example, Weng et al. (2010) successfully used the cucumber genome to assemble polymorphic markers for mapping in another cucumber cultivar. Relevant to this research, it is reasonable to suppose that QTL for ALB resistance may also be associated with one of the identified R-gene clusters and that a strategic set of corresponding markers could accelerate QTL discovery. Garcia-Mas et al. (2012) were able to collocate resistance genes *Vat* and *Fom-1* with R-gene clusters. Similarly fruit quality genes could be associated with marker types for mapping analysis. Resequencing other melon lines would provide additional markers for analysis (Garcia-Mas et al., 2012). However, phenotyping a sufficiently large segregating population remains essential to any attempt at QTL analysis.

LITERATURE CITED

ARENDS, D., PRINS, P., BROMAN, K.W. and JANSEN, R.C., 2010. *Tutorial-Multiple-QTL Mapping (MQM) Analysis*.

BARKER, B. and WALLACE, T., 1922. A new method of sulphur fumigation. *Ann.Rept.Agric.and Hort.Res.Stat.* pp. 123-124.

BATTA, Y., 2003. Alternaria leaf spot disease on cucumber: susceptibility and control using leaf disk assay. *An-Najah Univ.J.Res.: Nat.Sci,* 17, pp. 269-279.

BAUDRACCO-ARNAS, S. and PITRAT, M., 1996. A genetic map of melon (Cucumis melo L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. *Theoretical and Applied Genetics,* 93(1-2), pp. 57-64.

BAUDRACCO-ARNAS, S. and PITRAT, M., 1996. A genetic map of melon (Cucumis melo L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. *Theoretical and Applied Genetics,* 93(1-2), pp. 57-64.

BENT, K., 1967. Vapour action of fungicides against powdery mildews. *Annals of Applied Biology,* 60(2), pp. 251-263.

BERNARDO, R., 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science,* 48(5), pp. 1649-1664.

BLOEM, E., RIEMENSCHNEIDER, A., VOLKER, J., PAPENBROCK, J., SCHMIDT, A., SALAC, I., HANEKLAUS, S. and SCHNUG, E., 2004. Sulphur supply and infection with Pyrenopeziza brassicae influence L-cysteine desulphydrase activity in Brassica napus L. *Journal of experimental botany,* 55(406), pp. 2305-2312.

BOISSOT, N., THOMAS, S., SAUVION, N., MARCHAL, C., PAVIS, C. and DOGIMONT, C., 2010. Mapping and validation of QTLs for resistance to aphids and whiteflies in melon. *Theoretical and Applied Genetics,* 121(1), pp. 9-20.

BOYHAN, G. and NORTON, J., 1992. Inheritance of Resistance to Alternaria Leaf-Blight in Muskmelons. *HortScience,* 27(10), pp. 1114-1115.

BRESSAN, R.A., WILSON, L.G. and FILNER, P., 1978. Mechanisms of resistance to sulfur dioxide in the Cucurbitaceae. *Plant Physiology,* 61(5), pp. 761-767.

BRESSAN, R.A., LECUREUX, L., WILSON, L.G. and FILNER, P., 1979. Emission of ethylene and ethane by leaf tissue exposed to injurious concentrations of sulfur dioxide or bisulfite ion. *Plant Physiology,* 63(5), pp. 924-930.

BROMAN, K.W., 2013. Thirteen years of R/qtl: Just barely sustainable. *arXiv preprint arXiv:*1309.1192.

BYRNE, P., 2005. Quantitative Trait Locus (QTL) Analysis 2. *Journal of Natural Resources and Life Sciences Education,* 34, pp. 123-124.

CARMODY, B.E., MILLER, M.E. and GRISHAM, M.P., 1985. A Technique to Screen Muskmelons for Resistance to Alternaria Leaf-Blight. *Plant Disease,* 69(5), pp. 426-428.

CHANDLER, L. and THOMAS, C., 1991. Effect of leaf miner feeding activity on the incidence of Alternaria leaf blight lesions on muskmelon leaves. *Plant Disease,* 75(9), pp. 938-940.

CHANDLER, L.D. and THOMAS, C.E., 1991. Effect of Leaf Miner Feeding-Activity on the Incidence of Alternaria Leaf-Blight Lesions on Muskmelon Leaves. *Plant Disease,* 75(9), pp. 938-940.

CHOUDHARY, B.R. and FAGERIA, M.S., 2002. Breeding for multiple disease resistance in cucurbits (water melon, musk melon, cucumber and squash) - a review. *Agric. Rev,* 23, pp. 300.

COBB, J.N., DECLERCK, G., GREENBERG, A., CLARK, R. and MCCOUCH, S., 2013. Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theoretical and Applied Genetics,* 126(4), pp. 867-887.

COLLARD, B.C.Y. and MACKILL, D.J., 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 363(1491), pp. 557-572.

COOPER, R.M., RESENDE, M.L., FLOOD, J., ROWAN, M.G., BEALE, M.H. and POTTER, U., 1996. Detection and cellular localization of elemental sulphur in disease-resistant genotypes of Theobroma cacao. *Nature,* 379(6561), pp. 159-162.

COOPER, R.M. and WILLIAMS, J.S., 2004. Elemental sulphur as an induced antifungal substance in plant defence. *Journal of experimental botany,* 55(404), pp. 1947-1953.

CUEVAS, H., STAUB, J., SIMON, P. and ZALAPA, J., 2009. A consensus linkage map identifies genomic regions controlling fruit maturity and beta-carotene-associated flesh color in melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 119(4), pp. 741-756.

CUEVAS, H., STAUB, J., SIMON, P., ZALAPA, J. and MCCREIGHT, J., 2008. Mapping of genetic loci that regulate quantity of beta-carotene in fruit of US Western

Shipping melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 117(8), pp. 1345-1359.

DANIN-POLEG, Y., TADMOR, Y., TZURI, G., REIS, N., HIRSCHBERG, J. and KATZIR, N., 2002. Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. *Euphytica,* 125(3), pp. 373-384.

DELEU, W., ESTERAS, C., ROIG, C., GONZÁLEZ-TO, M., FERNÁNDEZ-SILVA, I., GONZALEZ-IBEAS, D., BLANCA, J., ARANDA, M.A., ARÚS, P. and NUEZ, F., 2009. A set of EST-SNPs for map saturation and cultivar identification in melon. *BMC Plant Biology,* 9(1), pp. 90.

DÍAZ BERMÚDEZ, A., BLANCA POSTIGO, J.M., MONFORTE GILABERT, A.J., FERGANY, M., FORMISANO, G., ZIARSOLO, P., FEI, Z., STAUB, J.E., ZALAPA, J.E. and CUEVAS, H.E., 2011. A consensus linkage map for molecular markers and Quantitative Trait Loci associated with economically important traits in melon, *BMC PLANT BIOLOGY* 2011, BioMed Central.

EASON, G., REINERT, R.A. and SIMON, J.E., 1996. Sulfur dioxide-enhanced phytotoxicity of ozone to watermelon. *Journal of the American Society for Horticultural Science,* 121(4), pp. 716-721.

ECONOMIC RESEARCH SERVICE, U., 2011-last update, Vegetables and Melons Outlook. Available: http://www.ers.usda.gov/publications/vgs/tables/world.pdf.

EGEL, D.S., 1999a. Severity of Alternaria leaf blight on muskmelon varieties, 1996. *Biol. Cult. Tests,* 14, pp. 157.

EGEL, D.S., 1999b. Severity of Alternaria leaf blight on muskmelon varieties, 1997. *Biol. Cult. Tests,* 14, pp. 158.

EGEL, D.S. and HARMON, P., 2001. Effects of nozzle type and spray pressure on control of Alternaria leaf blight of muskmelon with chlorothalonil. *Plant Disease,* 85(10), pp. 1081-1084.

EMMETT, B., ROZARIO, S. and HAWTIN, J., 2003. Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines. *Final Report to GWRDC, Project NumberL DAV,* 98(1), pp. 220.

EVANS, K., NYQUIST, W. and LATIN, R., 1992. A Model Based on Temperature and Leaf Wetness Duration for Establishment of Alternaria Leaf-Blight of Muskmelon. *Phytopathology,* 82(8), pp. 890-895.

FEDERER, W.T. and RAGHAVARAO, D., 1975. On augmented designs. *Biometrics,* , pp. 29-35.

FEHR, W.R., 1991. *Principles of cultivar development. 1. Theory and technique.* Macmillan.

FERNANDEZ-SILVA, I., EDUARDO, I., BLANCA, J., ESTERAS, C., PICO, B., NUEZ, F., ARUS, P., GARCIA-MAS, J. and MONFORTE, A.J., 2008. Bin mapping of genomic and EST-derived SSRs in melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 118(1), pp. 139-150.

FLINT-GARCIA, S.A., DARRAH, L.L., MCMULLEN, M.D. and HIBBARD, B.E., 2003. Phenotypic versus marker-assisted selection for stalk strength and second-generation European corn borer resistance in maize. *Theoretical and Applied Genetics,* 107(7), pp. 1331-1336.

FUKINO, N., OHARA, T., MONFORTE, A.J., SUGIYAMA, M., SAKATA, Y., KUNIHISA, M. and MATSUMOTO, S., 2008. Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 118(1), pp. 165-175.

GARCIA-MAS, J., BENJAK, A., SANSEVERINO, W., BOURGEOIS, M., MIR, G., GONZÁLEZ, V.M., HÉNAFF, E., CÂMARA, F., COZZUTO, L. and LOWY, E., 2012. The genome of melon (Cucumis melo L.). *Proceedings of the National Academy of Sciences,* 109(29), pp. 11872-11877.

GOGOI, R., SINGH, P.K., KUMAR, R., NAIR, K.K., ALAM, I., SRIVASTAVA, C., YADAV, S., GOPAL, M., CHOUDHURY, S.R. and GOSWAMI, A., 2013. Suitability of Nano-sulphur for Biorational Management of Powdery mildew of Okra (Abelmoschus esculentus Moench) caused by Erysiphe cichoracearum. *Journal of Plant Pathology & Microbiology,* 2013.

GONZALO, M., OLIVER, M., GARCIA-MAS, J., MONFORT, A., DOLCET-SANJUAN, R., KATZIR, N., ARUS, P. and MONFORTE, A., 2005. Simple-sequence repeat markers used in merging linkage maps of melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 110(5), pp. 802-811.

GONZALO, M., OLIVER, M., GARCIA-MAS, J., MONFORT, A., DOLCET-SANJUAN, R., KATZIR, N., ARÚS, P. and MONFORTE, A., 2005. Development of a consensus map of melon (Cucumis melo L.) based on high quality markers (RFLPs and SSRs) using F2 and double-haploid line populations. *Theor Appl Genet,* 110, pp. 802-811.

GRAYSTON, S. and GERMIDA, J., 1991. SULFUR-OXIDIZING BACTERIA AS PLANT-GROWTH PROMOTING RHIZOBACTERIA FOR CANOLA. *Canadian journal of microbiology,* 37(7), pp. 521-529.

HÄLLGREN, J. and FREDRIKSSON, S., 1982. Emission of hydrogen sulfide from sulfur dioxide-fumigated pine trees. *Plant Physiology,* 70(2), pp. 456-459.

HAREL-BEJA, R., TZURI, G., PORTNOY, V., LOTAN-POMPAN, M., LEV, S., COHEN, S., DAI, N., YESELSON, L., MEIR, A. and LIBHABER, S., 2010. A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. *Theoretical and Applied Genetics,* 121(3), pp. 511-533.

HAWKESFORD, M.J. and DE KOK, L.J., 2006. Managing sulphur metabolism in plants. *Plant, Cell & Environment,* 29(3), pp. 382-395.

HEBER, U. and HÜVE, K., 1997. Action of SO2 on Plants and Metabolic Detoxification of SO2. *International review of cytology,* 177, pp. 255-286.

HOWAD, W., YAMAMOTO, T., DIRLEWANGER, E., TESTOLIN, R., COSSON, P., CIPRIANI, G., MONFORTE, A.J., GEORGI, L., ABBOTT, A.G. and ARÚS, P., 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the Prunus reference map. *Genetics,* 171(3), pp. 1305-1309.

JACKSON, C.R. and WEBER, G.F., 1959. Morphology and Taxonomy of Alternaria Cucumerina. *Mycologia,* 51(3), pp. pp. 401-408.

JOHNSON JR, H. and MAYBERRY, K., 1980. The effect of dusting sulfur on muskmelons. *HortScience,* 15(5), pp. 652-654.

JOLIVET, P., BERGERON, E., ZIMIERSKI, A. and MEUNIER, J., 1995. Metabolism of elemental sulphur and oxidation of sulphite by wheat and spinach chloroplasts. *Phytochemistry,* 38(1), pp. 9-14.

KAO, C., ZENG, Z. and TEASDALE, R.D., 1999. Multiple interval mapping for quantitative trait loci. *Genetics,* 152(3), pp. 1203-1216.

KEARSEY, M., 1998. The principles of QTL analysis (a minimal mathematics approach). *Journal of experimental botany,* 49(327), pp. 1619-1623.

KEINATH, A.P. and DUBOSE, V.B., 2012. Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides. *Crop Protection,* 42, pp. 338-344.

KEINATH, A., EVERTS, K., LANGSTON JR, D., EGEL, D. and HOLMES, G., 2007. Multi-state evaluation of reduced-risk fungicides and Melcast against Alternaria leaf blight and gummy stem blight on muskmelon. *Crop Protection,* 26(8), pp. 1251-1258.

KOLLER, M., 2010. Potassium Bicarbonate as a Potential Sulphur Substitute in Protected Organic Cropping, *I International Conference on Organic Greenhouse Horticulture 915* 2010, pp. 157-163.

KOOKE, R., WIJNKER, E. and KEURENTJES, J.J., 2012. Backcross populations and near isogenic lines. *Quantitative Trait Loci (QTL).* Springer, pp. 3-16.

KUZUYA, M., YASHIRO, K., TOMITA, K. and EZURA, H., 2006. Powdery mildew (Podosphaera xanthii) resistance in melon is categorized into two types based on inhibition of the infection processes. *Journal of experimental botany,* 57(9), pp. 2093-2100.

LANDER, E.S., GREEN, P., ABRAHAMSON, J., BARLOW, A., DALY, M.J., LINCOLN, S.E. and NEWBURG, L., 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics,* 1(2), pp. 174-181.

LATIN, R., 2001. *Melcast: Melon Disease Forecaster.* Purdue University, Cooperative Extension Service.

LATIN, R., 1992. Modeling the relationship between Alternaria leaf blight and yield loss in muskmelon. *Plant Disease,* 76(10), pp. 1013-1017.

LATIN, R., RANE, K. and EVANS, K., 1994. Effect of Alternaria Leaf-Blight on Soluble Solids Content of Muskmelon. *Plant Disease,* 78(10), pp. 979-982.

LATIN, R. and EGEL, D.S., 2001. *Melon Disease Forecaster.* Purdue Extension.

LEGRIS-DELAPORTE, S., FERRON, F., LANDRY, J. and COSTES, C., 1987. Metabolization of elemental sulfur in wheat leaves consecutive to its foliar application. *Plant Physiology,* 85(4), pp. 1026-1030.

LEVI, A., WECHTER, W.P., HARRIS, K.R., DAVIS, A.R. and FEI, Z., 2010. High-frequency Oligonucleotides in Watermelon Expressed Sequenced Tag-unigenes Are Useful in Producing Polymorphic Polymerase Chain Reaction Markers among Watermelon Genotypes. *Journal of the American Society for Horticultural Science,* 135(4), pp. 369-378.

LI, H., RIBAUT, J., LI, Z. and WANG, J., 2008. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. *Theoretical and Applied Genetics,* 116(2), pp. 243-260.

MAURICIO, R., 2001. Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nature Reviews Genetics,* 2(5), pp. 370-381.

MCGRATH, S. and JOHNSTON, A., 1986. Sulphur--crop nutrient and fungicide. *Span: progress in agriculture,* 1986.

MEJSTRIK, V., 1980. The influence of low SO2 concentrations on growth reduction of Nicotiana tabacum L. cv. samsun and Cucumis sativus L. cv. Unikat. *Environmental Pollution Series A, Ecological and Biological,* 21(1), pp. 73-76.

MEYER, B., 1976. Elemental sulfur. *Chemical reviews,* 76(3), pp. 367-388.

MILES, C. and WAYNE, M., 2008. Quantitative trait locus (QTL) analysis. *Nature Education,* 1(1),.

MONFORTE, A., OLIVER, M., GONZALO, M., ALVAREZ, J., DOLCET-SANJUAN, R. and ARUS, P., 2004. Identification of quantitative trait loci involved in fruit quality traits in melon (Cucumis melo L.). *Theoretical and Applied Genetics,* 108(4), pp. 750-758.

MORENO, E., OBANDO, J.M., DOS-SANTOS, N., FERNÁNDEZ-TRUJILLO, J.P., MONFORTE, A.J. and GARCIA-MAS, J., 2008. Candidate genes and QTLs for fruit ripening and softening in melon. *Theoretical and Applied Genetics,* 116(4), pp. 589-602.

MOTIOR, M., ABDOU, A., AL DARWISH, F., EL-TARABILY, K., AWAD, M., GOLAM, F. and SOFIAN-AZIRUN, M., 2011. Influence of elemental sulfur on nutrient uptake, yield and quality of cucumber grown in sandy calcareous soil. *Australian Journal of Crop Science,* 5(12), pp. 1610-1615.

MUNGER, H.M., STAUB, J.E., THOMAS, C.E. and MCCREIGHT, J.D., 1995-last update, 1995 Cucumis melo Crop Germplasm Committe Report. Available: http://www.ars-grin.gov/npgs/cgc_reports/melon.pdf2014.

NGATUNGA, E., DONDEYNE, S. and DECKERS, J.A., 2003. Is sulphur acidifying cashew soils of South Eastern Tanzania? *Agriculture, Ecosystems & Environment,* 95(1), pp. 179-184.

NORTON, J.D., 1972. Chilton, a high quality fruit for the commercial market. 84. Auburn University: Alabama Experiment Station.

NORTON, J.D., 1971. Gulfcoast- a sweet cantaloupe for the produce chain store market. 82. Auburn University: Alabama Experiment Station.

NORTON, J.D., COSPER, R.D., SMITH, D.A. and RYMAL, K.S., 1985. Aurora: a high quality "jumbo" cantaloupe. 278. Auburn University: Alabama Experiment Station.

OLIVER, M., GARCIA-MAS, J., CARDUS, M., PUEYO, N., LÓPEZ-SESÉ, A., ARROYO, M., GOMEZ-PANIAGUA, H., ARÚS, P. and VICENTE, M.D., 2001. Construction of a reference linkage map for melon. *Genome,* 44(5), pp. 836-845.

OWEN, K., MARRS, R., SNOW, C. and EVANS, C., 1999. Soil acidification—the use of sulphur and acidic plant materials to acidify arable soils for the recreation of heathland and acidic grassland at Minsmere, UK. *Biological Conservation,* 87(1), pp. 105-121.

PARIS, M.K., ZALAPA, J.E., MCCREIGHT, J.D. and STAUB, J.E., 2008. Genetic dissection of fruit quality components in melon (Cucumis melo L.) using a RIL population derived from exotic× elite US Western Shipping germplasm. *Molecular Breeding,* 22(3), pp. 405-419.

PARK, S.O., HWANG, H.Y. and CROSBY, K.M., 2009. A genetic linkage map including loci for male sterility, sugars, and ascorbic acid in melon. *Journal of the American Society for Horticultural Science,* 134(1), pp. 67-76.

PERCHEPIED, L., BARDIN, M., DOGIMONT, C. and PITRAT, M., 2005. Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. *Phytopathology,* 95(5), pp. 556-565.

PERCHEPIED, L., PÉRIN, C., GIOVINAZZO, N., BESOMBES, D., DOGIMONT, C., PITRAT, M., LEBEDA, A. and PARIS, H., 2004. Susceptibility to sulfur dusting and inheritance in melon. *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech Republic, 12-17 July, 2004.* 2004, Palacký University in Olomouc, pp. 353-357.

PERCHEPIED, L., DOGIMONT, C. and PITRAT, M., 2005. Strain-specific and recessive QTLs involved in the control of partial resistance to Fusarium oxysporum f. sp. melonis race 1.2 in a recombinant inbred line population of melon. *TAG.Theoretical and applied genetics.Theoretische und angewandte Genetik,* 111(1), pp. 65-74.

PERIN, C., HAGEN, L., DE CONTO, V., KATZIR, N., DANIN-POLEG, Y., PORTNOY, V., BAUDRACCO-ARNAS, S., CHADOEUF, J., DOGIMONT, C. and PITRAT, M., 2002. A reference map of Cucumis melo based on two recombinant inbred line populations. *Theoretical and Applied Genetics,* 104(6-7), pp. 1017-1034.

RENNENBERG, H., 1984. The fate of excess sulfur in higher plants. *Annual Review of Plant Physiology,* 35(1), pp. 121-153.

RENNENBERG, H. and FILNER, P., 1982. Stimulation of h2s emission from pumpkin leaves by inhibition of glutathione synthesis. *Plant Physiology,* 69(4), pp. 766-770.

SEEBOLD, K., COOLONG, T., BESSIN, R. and KAISER, C., 2009-last update, An IPM Scouting Guide for Common Problems of Cucurbit Crops in Kentucky. Available: http://www.ca.uky.edu/agc/pubs/id/id91/id91.pdf2012.

SEKIYA, J., WILSON, L.G. and FILNER, P., 1982. Resistance to injury by sulfur dioxide correlation with its reduction to, and emission of, hydrogen sulfide in Cucurbitaceae. *Plant Physiology,* 70(2), pp. 437-441.

SEMAGN, K., BJØRNSTAD, Å and NDJIONDJOP, M., 2006. Principles, requirements and prospects of genetic mapping in plants. *African Journal of Biotechnology,* 5(25).

SEMAGN, K., BJØRNSTAD, Å and XU, Y., 2010. The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology,* 13(5), pp. 16-17.

SHATTUCK-EIDENS, D.M., BELL, R.N., NEUHAUSEN, S.L. and HELENTJARIS, T., 1990. DNA sequence variation within maize and melon: observations from polymerase chain reaction amplification and direct sequencing. *Genetics,* 126(1), pp. 207-217.

SILBERSTEIN, L., KOVALSKI, I., BROTMAN, Y., PERIN, C., DOGIMONT, C., PITRAT, M., KLINGLER, J., THOMPSON, G., PORTNOY, V. and KATZIR, N., 2003. Linkage map of Cucumis melo including phenotypic traits and sequence-characterized genes. *Genome,* 46(5), pp. 761-773.

SILBERSTEIN, L., KOVALSKI, I., HUANG, R., ANAGNOSTOU, K., KYLE JAHN, M.M. and PERL-TREVES, R., 1999. Molecular variation in melon (Cucumis melo L.) as revealed by RFLP and RAPD markers. *Scientia horticulturae,* 79(1), pp. 101-111.

SITTERLY, W., 1972. Breeding for Disease Resistance in Cucurbits. *Annual Review of Phytopathology,* 10, pp. 471.

STAUB, J.E., DANIN-POLEG, Y., FAZIO, G., HOREJSI, T., REIS, N. and KATZIR, N., 2000. Comparative analysis of cultivated melon groups (Cucumis melo L.) using random amplified polymorphic DNA and simple sequence repeat markers. *Euphytica,* 115(3), pp. 225-241.

SUHERI, H. and LATIN, R., 1991. RETENTION OF FUNGICIDES FOR CONTROL OF ALTERNARIA LEAF-BLIGHT OF MUSKMELON UNDER GREENHOUSE CONDITIONS. *Plant Disease,* 75(10), pp. 1013-1015.

TAKAGI, H., ABE, A., YOSHIDA, K., KOSUGI, S., NATSUME, S., MITSUOKA, C., UEMURA, A., UTSUSHI, H., TAMIRU, M. and TAKUNO, S., 2013. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal,* 74(1), pp. 174-183.

TANKSLEY, S.D., 1993. Mapping polygenes. *Annual Review of Genetics,* 27(1), pp. 205-233.

THOMAS, C., 1986. Downy and Powdery Mildew Resistant Muskmelon Breeding Line Mr-1. *HortScience,* 21(2), pp. 329-329.

THOMAS, C., 1984. Resistant Reaction Type Against Alternaria Leaf-Blight in Cucumis-Melo. *Phytopathology,* 74(7), pp. 865-865.

THOMAS, C. and CANIGLIA, E., 1997. Evaluation of US honeydew-type melons for resistance against downy mildew and Alternaria leaf blight. *HortScience,* 32(6), pp. 1114-1115.

THOMAS, C., MCCREIGHT, J. and JOURDAIN, E., 1990. Inheritance of Resistance to Alternaria-Cucumerina in Cucumis-Melo Line Mr-1. *Plant Disease,* 74(11), pp. 868-870.

THOMAS, C.E., 1983. Fungicide Applications Based on Duration of Leaf Wetness Periods to Control Alternaria Leaf Blight of Cantaloup in South Texas. *Plant Disease,* 67(2), pp. 145.

USDA/NASS, 2007-last update, USDA National Agricultural Statistics Service, Quick Stats. Available: http://quickstats.nass.usda.gov/results/90E50984-9FBB-37D6-AB24-98BE3D09E8FA2012].

VITTI, G.C., FAVARIN, J.L., GALLO, L.A., PIEDADE, SÔNIA MARIA DE STEFANO, FARIA, MARIA REGINA MEIRELLES DE and CICARONE, F., 2007. Foliar elementary sulfur assimilation by soybean. *Pesquisa Agropecuária Brasileira,* 42(2), pp. 225-229.

WANG, J., YAO, J., LIU, L., WANG, Y. and LI, W., 2007. Construction of a molecular genetic map for melon (Cucumis melo L.) based on SRAP. *Acta Horticulturae Sinica,* 34(1), pp. 135.

WANG, X., GAO, X., LI, G., WANG, H., GENG, S., KANG, F. and NIE, X., 2011. Construction of a melon genetic map with fruit and seed QTLs. *Yi chuan= Hereditas/Zhongguo yi chuan xue hui bian ji,* 33(12), pp. 1398-1408.

WANG, Y., THOMAS, C. and DEAN, R., 1997. A genetic map of melon (Cucumis melo L.) based on amplified fragment length polymorphism (AFLP) markers. *Theoretical and Applied Genetics,* 95(5-6), pp. 791-798.

WANG, S., BASTEN, C.J. and ZENG, Z.-., 2012-last update, Windows QTL Cartographer 2.5. Available: http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

WANG, Y.H., THOMAS, C.E. and DEAN, R.A., 1997. A genetic map of melon (Cucumis melo L.) based on amplified fragment length polymorphism (AFLP) markers. *Theoretical and Applied Genetics,* 95(5-6), pp. 791-798.

WECHTER, W.P., DEAN, R.A. and THOMAS, C.E., 1998. Development of Sequence-specific Primers that Amplify a 1.5-kb DNA Marker for Race 1 Fusarium Wilt Resistance in Cucumis melo L. *HortScience,* 33(2), pp. 291-292.

WEST, W.A. and MENZIES, A., 1929. The Vapor Pressures of Sulphur Between 100 and 550 with Related Thermal Data. *The Journal of physical chemistry,* 33(12), pp. 1880-1892.

WILCOXON, F. and MCCALLAN, S., 1931. The fungicidal action of sulphur. III. *Physical factors affecting the efficiency of dusts.Contrib.Boyce Thompson Inst,* 3(509-528), pp. V0l.

WILLIAMS, J.S. and COOPER, R.M., 2003. Elemental sulphur is produced by diverse plant families as a component of defence against fungal and bacterial pathogens. *Physiological and Molecular Plant Pathology,* 63(1), pp. 3-16.

WILLIAMS, J. and COOPER, R., 2004. The oldest fungicide and newest phytoalexin–a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathology,* 53(3), pp. 263-279.

WILLIAMS, J.S., HALL, S.A., HAWKESFORD, M.J., BEALE, M.H. and COOPER, R.M., 2002. Elemental sulfur and thiol accumulation in tomato and defense against a fungal vascular pathogen. *Plant Physiology,* 128(1), pp. 150-159.

WILSON, L.G., BRESSAN, R.A. and FILNER, P., 1978. Light-dependent Emission of Hydrogen Sulfide from Plants. *Plant Physiology,* 61(2), pp. 184-189.

XU, Y. and CROUCH, J.H., 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Science,* 48(2), pp. 391-407.

YUSTE-LISBONA, F.J., CAPEL, C., SARRIA, E., TORREBLANCA, R., GÓMEZ-GUILLAMÓN, M.L., CAPEL, J., LOZANO, R. and LÓPEZ-SESÉ, A.I., 2011. Genetic linkage map of melon (Cucumis melo L.) and localization of a major QTL for powdery mildew resistance. *Molecular Breeding,* 27(2), pp. 181-192.

ZALAPA, J., STAUB, J., MCCREIGHT, J., CHUNG, S. and CUEVAS, H., 2007. Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. *Theoretical and Applied Genetics,* 114(7), pp. 1185-1201.

ZENG, Z., 1994. Precision mapping of quantitative trait loci. *Genetics,* 136(4), pp. 1457-1468.

ZHAO, F., WITHERS, P., EVANS, E., MONAGHAN, J., SALMON, S., SHEWRY, P. and MCGRATH, S., 1997. Sulphur nutrition: an important factor for the quality of wheat and rapeseed. *Plant nutrition for sustainable food production and environment.* Springer, pp. 917-922.

ZINK, F. and GUBLER, W., 1990. Resistant germplasm controls Fusarium wilt in cantaloupes. *California Agriculture,* 44(6), pp. 27-28.

CHAPTER TWO
MAPPING RESISTANCE TO *ALTERNARIA CUCUMERINA* IN *CUCUMIS MELO*

Introduction

Melon (*Cucumis melo* L.) is an economically important crop found around the world and is severely affected by fungal pathogens. *Alternaria cucumerina*, the causal agent of *Alternaria* leaf blight (ALB), is one such pathogen that has the potential to cause extensive damage (Latin, Egel 2001). In the United States, ALB is a significant problem in the melon-producing midwestern and eastern states (Evans et al., 1992). ALB has been responsible for yield losses in Indiana approaching 50% (Evans et al., 1992). *A. cucumerina* infects plant tissue via conidia which produce hyphae that penetrate the leaf epidermis, leading to lesion formation (Jackson and Weber, 1959). Under severe infection the merging of rapidly expanding lesions causes defoliation and sun-scalding of the fruit (Jackson and Weber, 1959). Decreased fruit yield by *Alternaria* is primarily caused by the rapid defoliation of the plant, leaving the fruit vulnerable to sun scalding (Latin et al., 1994). The severity of infection has been shown to vary according to the presence of overwintered mycelia in the field (Jackson and Weber, 1959), leaf wetness duration, temperature (Chandler and Thomas, 1991; Evans et al., 1992), damage to the foliage by insects (Chandler and Thomas, 1991), fungicide treatments (Thomas, 1984), and degree of cultivar resistance (Boyhan and Norton, 1992; Egel 1999ab; Thomas and Caniglia, 1997; Carmody et al., 1985). Fungicide application is the primary control method and has been enhanced by the introduction of MelCast by Latin and Egel (2001), a tool which allows for strategic fungicide use based on duration of temperature and wetness. However ALB severity could be further mitigated through the use of resistant

varieties (Thomas and Caniglia, 1997), which would have immediate financial benefits for growers who would potentially require no fungicide applications to control ALB.

Resistance to ALB has been identified in several muskmelons lines (Egel, 1999ab; Sitterly, 1972; Thomas et al., 1990; Thomas and Caniglia, 1997; Boyhan and Norton, 1992). *Alternaria* resistant melons, after 10 days of infection, were shown to have markedly decreased lesion growth (2-6% of susceptible plant lesion growth) and sporulation of lesion area (9-47% of susceptible plant sporulation) (Thomas, 1984). The genetic basis of resistance has been shown to be additive and multifactorial (Boyhan and Norton, 1992); in addition, Thomas, et al. (1990) described the inheritance of a single, dominant resistance gene, *Ac*, which confers a high degree of resistance in the USDA MR-1 breeding line. However, markers linked to ALB resistance in melon have yet to be identified.

There are two greenhouse inoculation methods used to evaluate resistance: spray and wounded-leaf. The spray method evenly distributes conidia onto foliage followed by exposure to high humidity in order to create favorable growth conditions for *Alternaria*. Following inoculation, lesion expansion occurs over a set amount of time. Spray-inoculated plants are then evaluated using rating schemes based on percent leaf damage, number of lesions present, or lesion size. This inoculation method has been the dominant method used in both greenhouse and field assays for resistance. However, spray inoculation tests can be partially confounded by enhanced infection caused by mechanical leaf injury, early leaf senescence, insect damage, or leaf necrosis (Thomas et al., 1990; Chandler and Thomas, 1991).

The second type of inoculation is called the wounded-leaf method and has been shown to be effective on cucumber leaf disks (Batta, 2003). This method involves placing droplets of inoculum on artificial foliar wounds and exposing plants to high humidity to encourage disease progression over a set period of time. This method allows for definitive evaluation of resistance by direct measurement of lesion area. The wounded-leaf method increases control of lesion placement to enhance uniformity of lesion expansion and thus increase overall differentiation between resistant and susceptible plants. The wounded-leaf method has been shown to be effective in cucumber (Batta, 2003), but has yet to be demonstrated in melon. Using a wounded-leaf inoculation assay on melon would exploit the rapid, differential lesion expansion observed by Thomas et al. (1990) on injured resistant and susceptible plants and allow for greater clarity in phenotyping.

In this study, a genetic map of MR-1 x AY recombinant inbred lines (RIL) was constructed through the use of dominant markers, and QTL analysis was conducted to detect QTL affecting *Alternaria* lesion area. In order to increase consistency of the phenotyping, RILs were evaluated using a modified wounded-leaf inoculation method. The effects of MR-1 alleles on *Alternaria* resistance was analyzed using both the wounded-leaf and spray inoculation methods. In addition, inoculation method correlation was tested. Lesion areas of first, second, and third leaves were measured to determine their relative efficiency in resolving resistant, intermediate resistant, and susceptible melon lines.

Materials and Methods

Plant Materials

MR-1 was selected and inbred from a plant introduction (PI) 124111, an Indian melon line, with high levels of disease resistance (Thomas, 1986). MR-1 has been shown to possess resistance to powdery mildew, downy mildew, *Fusarium* wilt *Fusarium oxysporum* f.sp *melonis,* and ALB, making it an excellent source for resistance breeding (Kuzuya et al., 2006; Thomas, 1986; Thomas et al., 1990). Ananas Yokneum (AY) is reported as susceptible to powdery mildew, downy mildew, and *Fusarium* wilt (Wang et al., 1997). Though AY is reported as resistant to *A. cucumerina* (Thomas and Caniglia, 1997), our preliminary results indicate susceptibility relative to MR-1. In order to exploit contrasting resistance levels to *Alternaria,* MR-1 x AY RILs (F_6-F_{10}) were used for genotyping and phenotyping.

Leaf position comparisons were conducted on the 1st, 2nd, and 3rd leaves of individual MR-1, AY, and (AY x MR-1) F_1 plants. In addition the effects of MR-1 alleles on resistance in susceptible lines were analyzed using Hales Best Jumbo (HBJ), Perlita, AY, MR-1 hybrids [(MR-1 x HBJ) F_1, (AY x MR-1) F_1, and (Perlita x MR-1) F_1], MR-1, Jindaozi, and RIL61. Also, using these same lines, the two inoculation methods (wounded-leaf and spray) were compared using Spearman's correlation.

Plants were grown using Metro-mix 360 (Sun Gro Horticulture, Agawam, MA) in 50 cell trays (PRO050R5; Myers Industries Lawn & Garden Group, Middlefield, OH) until the 1-2 fully expanded leaf at which time they were transferred to 4 inch plastic pots (SVT-400 4 x 5; T.O. Plastics, Inc., Clearwater, MN) and fertilized (Peters Professional

Water Soluble Fertilizer 20-20-20; Everris International B.V., Geldermalsen, Netherlands) at 10 g/L. Plants were grown in a temperature controlled greenhouse and year-round day-night temperatures averaged 22ºC and ranged 18-38 ºC. Pesticides were applied using a Mini AutoFog™ (Dramm Corporation, Manitowoc, WI) to control greenhouse pests. In preparation for inoculation, pesticides were no longer applied when plants reached the third-expanding leaf.

A. cucumerina Preparation

The *A. cucumerina* isolate, AC-4B, used in this study was collected in South Carolina in 2001 from melon (Anthony Keinath, 2014, personal communication). *A. cucumerina* cultures were grown for 30-40 days on quarter strength potato dextrose agar (Becton, Dickinson and Company; Franklin Lakes, NJ) on a 12hr light/dark cycle at room temperature under fluorescent lights. In order to avoid CO_2 inhibition of sporulation, the petri dishes were not sealed (Cotty, 1987). Conidia were harvested by flooding the 100 mm x 15 mm petri dish with sterile distilled H_2O to approximately half the volume and, using a 1ml pipette, repeatedly aspirating and pipetting directly into the dish across the range of culture for 30 sec in order to dislodge conidia. The dish was then poured into a 250 ml media bottle and vigorously shaken for 30 sec to separate clumped conidia. Conidia counts were performed using a hemacytometer. The concentration was adjusted with sterile dH_2O according to the test and immediately used for inoculation.

A. cucumerina Wounded-Leaf Inoculation and Lesion Measurements

The adaxial side of the third expanded leaf was lightly scratched using a 22 gauge needle to produce narrow perforating wounds that averaged 3.8mm in length. A series of these wounds were placed approximately equidistant between the midrib and margin. Additionally, a buffer zone of approximately 15mm between wound locations allowed for lesion expansion in even the most susceptible lines to occur unimpeded by the expansion of nearby lesions. The number of wounds per leaf ranged from 3-8, dependent on leaf area, which varied between recombinant inbred lines. A 10µl droplet of 2.5×10^4 conidia/ml inoculum (~250 condia) was pipetted directly on each wound and allowed to dry prior to be being placed in a humidity chamber. Lesion areas were recorded by measuring the length and width and using the equation for the area of an ellipse [(Diameter_A/2) * (Diameter_B/2) * π], because lesions were occasionally elliptical. All measurements were recorded in millimeters using digital calipers (Digimatic Caliper CD-6"B; Mitutoyo Corporation, Aurora, IL). Controls were wounded and inoculated with sterile dH_2O using both methods.

A. cucumerina Spray Inoculation and Evaluation

The adaxial side of third leaves were sprayed to runoff at 275 kPa with a suspension of 1.0×10^4 conidia/ml of *Alternaria cucumerina*, in accordance with Thomas and Caniglia (1997). Control plants were sprayed with sterile dH_2O. Spray-inoculated

plants were rated 1-5 based on the criteria used by Thomas and Caniglia (1997): 1= leaves necrotic with few to many large lesions, rapidly expanding lesions; 2 = leaves chlorotic with few to many large, rapidly expanding lesions; 3 = leaves green, but few to many large (6-8mm), expanding lesions; 4 = leaves green with few to many moderate-sized (5-6mm) expanding lesions; 5 = leaves green with few to many restricted, small (1-2mm) non-expanded lesions

Humidity Chamber Conditions

Inoculum on both wounded and spray inoculated plants was allowed to dry onto the leaf surface prior to being randomized and placed in a humidity chamber. The plastic covered humidity chamber (4.6m x 2.4m x 1.2m) had a capacity of ~500 plants and was arranged in two tiers with four expanded metal platforms. The humidifier (PA-600; Pharos Inc., Springdale, AR) was centrally located on one short end and was set to humidify for an initial 60 min and then evenly distributed 30 min intervals for a total of 3 hrs. The humidity chamber was located in a temperature controlled headhouse which nightly averaged 21ºC and ranged 16-27 ºC. Inoculated plants were placed on an alternating 10-day regimen used by Thomas et al. (1990): 16 hrs in the humidity chamber followed by 8 hrs of natural daylight in the greenhouse. On day ten, lesion areas of wounded plants were measured and 1-5 ratings (Thomas and Caniglia, 1997) of spray inoculated plants were recorded.

Experimental Design and Data Analysis: Effects Leaf Position on Lesion Area

A complete randomized design was used to analyze the effect of the 1st, 2nd, and 3rd leaf position of individual MR-1, AY, and (AY x MR-1) F_1 on lesion area. MR-1, AY, Hales Best Jumbo (HBJ), MR-1 hybrids [(Perlita x MR-1) F_1, (MR-1 x HBJ) F_1, and (AY x MR-1) F_1], Jindaozi, and RIL61 were replicated >7 times and the test was repeated once. Fischer's Protected LSD (P=0.05) was used to determine significance. All statistical analyses were conducted using JMP® Pro 10 (SAS Institute Inc., Cary, NC) software.

Experimental Design: Effects of MR-1 Alleles on *Alternaria* Resistance

A complete randomized design was used to analyze the effects of MR-1 alleles on *Alternaria* resistance using two *Alternaria* inoculation techniques: wounded-leaf and spray. In addition, Spearman's correlation was used to determine the strength of the relationship between the two inoculation techniques. MR-1, AY, Hales Best Jumbo (HBJ), MR-1 hybrids [(Perlita x MR-1) F_1, (MR-1 x HBJ) F_1, and (AY x MR-1) F_1], Jindaozi, and RIL61 were replicated >7 times and the test was repeated once. Fischer's Protected LSD (P=0.05) was used to determine significance.

Experimental Design: MR-1 x AY RILs Lesion Areas

Statistical analysis of RIL lesion area was conducted using augmented incomplete blocks. The RILs were divided into incomplete blocks (planting date) in which lines were completely randomized with at least 8 replications per line. Lines were repeated at least once in separate blocks; and MR-1, AY, and (AY x MR-1) F1 checks were included in every test. The use of checks in every block is a fundamental facet of augmented block design and provides an estimate of the blocking effect and experimental error variance, significantly reducing error (Federer and Raghavarao, 1975). Planting date and planting date x line effects were designated as a random and the means of individual plants from each planting date were compared using Fischer's Protected LSD (*P*=0.05). This statistical strategy was utilized because the recombinant populations at the beginning of testing included 59 individuals with the remaining 30 individuals being inbred to the F_7 generation, so the augmented design allowed genotypes to be tested as they became available.

DNA Isolation

Melon DNA was extracted using the method outlined by Dellaporta, etal. (1983) with some modifications. Leaf tissue (50mg) was placed in 1.5ml microcentrifuge tubes and either DNA-extracted immediately or stored at -80^{0}C. The extraction buffer contains 100mM Tris-EDTA ph 8, 50 mM EDTA ph 8, 500 mM sodium chloride, 1% SDS, 10 mM beta-mercaptoethanol. Tissue was ground by hand in 1.5ml microcentrifuge using a

polypropylene pestle after adding 566µl of extraction buffer and 10µl RNase. After 30sec of tissue grinding the tubes were vortexed for 30sec. All tubes were incubated at 65°C for 10mins. After incubation, 165µl 5 M potassium acetate was added, and the tubes were vortexed for 30 sec and centrifuged at 13,500 G for 15 min. The supernatant was transferred to new tubes. One half of the volume (~400µl) cold isopropanol (>99%) was added to the supernatant and mixed by inversion until DNA strands form, then centrifuged at 13,500 G for 5 mins. The supernatant was discarded, and the DNA pellet was dislodged from the bottom of the tube and washed twice 500µl ethanol (70%). The pellet was briefly spun down in order to aspirate off any remaining liquid and placed in the hood for 10 min. The DNA was resolublized with 100 µl of H_2O. For the quantification of DNA, 1ul of DNA was prepared using the Quant-iT™ dsDNA Assay Kit, broad range (Thermo Fisher Scientific Corporation, Waltham, MA) and quantified on a Qubit Fluorometer (Thermo Fisher Scientific Corporation, Waltham, MA). DNA concentration was adjusted to 10ng/µl for marker analysis.

Molecular Markers

The random amplified polymorphic DNA (RAPD) protocol optimized by Levi et al. (1993), with slight modifications, was used for PCR-based analysis of RAPD, sequence characterized amplified region marker (SCAR), cleaved amplified polymorphic sequences (CAPS), inter simple sequence repeat (ISSR), sequence-tagged sites (STS), high-frequency oligonucleotides-targeting active genes (HFO-TAG) (Levi et al., 2010)

markers. All RAPD markers were annealed at 48.5^{0}C, and HFO-TAG and ISSR markers were annealed at varying temperatures (Table 1.4). Ten µl reactions were composed of 5.3µl dH_2O, 1µl reaction buffer (200 µM NaCl, 500 mM Tris-HCl pH 9, 10% Triton-X-100, 0.1% Gelatin), 0.8µl $MgCL_2$ [25mM] (Promega Corporation, Madison, WI), 0.8µl dNTP Mix [2.5 mM] (Life Technologies, Carlsbad, CA), 0.1 Taq 5u/µl (GoTaq® DNA Polymerase; Promega Corporation, Madison, WI; FIREPol® DNA Polymerase; Solis Byodyne, Tartu, Estonia), 1µl DNA 10ng/µl and 1µl primer [10µM]. HFO-TAG primers and FOM1 (Wechter et al., 1998) (1µl per 24-mer) PCR samples used 2µl primer [10µM] and 4.3µl dH_2O. Samples were amplified via a MJ Research PTC 200 thermocycler (MJ Research, Waltham, MA) set to an initial denaturing at 94°C for 4mins followed by 45 cycles: 92°C for 60sec (denaturation), 48.5°C (varied for ISSR and HFO-TAG markers) for 70 sec (annealing), and 72°C for 120sec (elongation). FOM1, composed two 24-mer primers MUSKFOM I ($^{5'}$TCGACCAGACGAAGTTCTTCGAGC$^{3'}$) and MUSKFOM II ($^{5'}$GAACTAAGGTCACGTTTATCGATC$^{3'}$), was amplified using an initial 96°C for 5min followed by 37 cycles and a last 5min 72°C extension: 94°C for 60sec (denaturation), 68°C for 60 sec (annealing), and 72°C for 120sec (Wechter et al., 1998). Samples were then removed and prepared for agarose gel electrophoresis by adding 2 µl Blue/Orange Loading Dye, 6X (Promega Corporation, Madison, WI) to each 10µl sample. One Kb DNA Ladder (Life Technologies, Carlsbad, CA) was used to estimate band size. Electrophoresis was run at 180V for 90min at 4^{0}C on a HE99X Max Horizontal Unit (Hoefer Inc., Holliston, MA) using 1.5% agarose gel (Agarose BP160-500; Thermo Fisher Scientific Inc., Waltham, MA). Each marker was screened for

polymorphism between the MR-1 and AY parents, and markers that produced strong polymorphisms were selected for mapping and run against the full population and parents.

Marker Nomenclature

RAPD markers (Operon Technologies, Alameda, CA) were designated by the prefix 'OP' followed by the Operon kit letter(s), Operon primer number, and amplicon bp (e.g., OPW07_650). ISSR markers were designated by the prefix 'ISSR' followed by a number corresponding to Table 1.4 and amplicon bp (e.g., ISSR32_325). HFO-TAG markers are designated 'HFSW' or 'HFOWEC' followed by a number corresponding to Table 1.4 and amplicon bp (e.g., HFSW67_900 and HFOWEC33_725). The single SCAR is designated 'FOM1'.

Genotyping by Sequencing

DNA aliquots of 89 MR-1 x AY RILs, 3 MR-1 plants, and 3 AY plants were submitted for to Cornell University Institute for Genomic Diversity (IGD) for Genotyping by Sequencing (GBS). DNA was extracted using the ChargeSwitch gDNA Plant Kit (Life Technologies, Carlsbad, CA) from 100 mg of tissue from the same 59 MR-1 x AY RILs used for agarose-based genotyping (above), 30 addition MR-1 x AY RILs, and three individual MR-1 and AY plants. The tissues were first homogenized in

1.5ml microcentrifuge tubes in a solution containing 100 µl Reagent A [300 mM $CaCl_2$, 15% polyvinylpyrolidone (10,000 average molecular weight) and ChargeSwitch Lysis Buffer], 100 µl 10% SDS, 2 µl RNase A, and 900 µl ChargeSwitch Lysis Buffer using a FastPrep FP120 homogenizer (Thermo Fisher Scientific, Waltham, MA) with a ¼" Ceramic Sphere (MP Biomedicals, Santa Ana, CA) in each microcentrifuge tube. The manufacturer's recommended protocol was then followed to completion. For the quantification of DNA, 1 ul of DNA was prepared using the Quant-iT™ dsDNA Assay Kit, broad range (Thermo Fisher Scientific Corporation, Waltham, MA) and quantified on a Qubit Fluorometer (Thermo Fisher Scientific Corporation, Waltham, MA). Concentrations over 100 ng/µl were diluted to 50-100 ng/µl by adding more ChargeSwitch Elution Buffer.

DNA quality checks were conducted according to IGD instructions (http://sorghumdiversity.maize.cornell.edu/), and images submitted were for review. Once approved, 50 µl aliquots of the extracted DNA were pipetted into a 96 well plate (VWR 83007-374; VWR International, Radnor, PA) and sealed using PCR tube strip caps (VWR 20170-000; VWR International, Radnor, PA). The samples were immediately placed on dry-ice and shipped overnight to IGD for GBS.

GBS data is pending receipt and results will not be presented in this thesis.

Linkage Map Construction and QTL Analysis

The linkage map was constructed using JoinMap® 3.0 (Kyazma B.V., Wageningen, Netherlands), and linkage groups were assembled with a minimum LOD score of 4.0, Kosambi mapping function, with a recombination frequency of 0.4. The expected 1:1 segregation was tested using a chi-squared test to identify segregation distortion (p-value 0.01) (Zalapa et al., 2007), and markers exhibiting segregation distortion were excluded from linkage map construction.

QTL analysis was performed using the composite interval mapping function on WinQTL Cartographer 2.5 (North Carolina State University, Raleigh, NC). *Alternaria* mean lesion areas (Appendix A) were used as trait values for QTL analysis. Model 6 was selected using the forward regression procedure with a walking speed of 1cM and the default settings of a 10cM window and up to 5 background markers. QTL significance was declared based on a 1,000 permutations at a p-value = 0.05. Confidence intervals (95%) were calculated automatically with at least 10cM distance between QTL, and R^2 values were determined using the highest point within the confidence interval. QTL were be named after the putative resistance gene, *Ac* (Thomas et al., 1990).

Results

Linkage Map Assembly

A total of 198 dominant markers (1 SCAR, 24 HFO-TAG, 25 ISSR, and 152 RAPD) were mapped onto 23 linkage groups of 59 MR-1 x AY RILs using the Kosambi function and a recombination value of 0.4 (Figure 1.3). Out of 223 markers initially scored, 11 markers failed to map onto any linkage group and 14 showed linkage distortion using JoinMap® 3.0 (Kyazma B.V., Wageningen, Netherlands) χ^2 analysis (p-value 0.01) and were excluded. Linkage groups were assembled using LOD 3 (102 markers) and LOD 4 (96 markers), and total map coverage was 400cM with the largest marker interval of 10 cM and an average interval of 2 cM. Of the linkage-distorted markers, 8 were predominantly MR-1 and 6 were predominantly AY. The 198 markers mapped among the 59 individuals were equally distributed between the two parents: 49.9% MR-1; 50.1% AY.

Alternaria resistance screening of AY x MR-1 RILs

Lesion areas of among 88 MR-1 x AY RILs was continuous and ranged from $20mm^2$ to $111mm^2$; MR-1, (AY x MR-1) F_1, and AY had respective means of $25mm^2$, $37mm^2$, and $72mm^2$ (Table 1.1; Appendix A). Lesion formation at a wound site was visually detectable generally on day three post inoculation. Initially the wound site would show a slight chlorosis, progressing to minor lesion formation, symptomatically

similar to spray inoculation. Over the course of the infection, lesions expanded and dark brown rings, characteristic of *Alternaria cucumerina* infection, would often become evident within the lesion. In resistant lines, lesion formation was slower and often surrounded by a noticeable wider chlorotic ring. The perforations from the wounding expanded as lesions expanded, leading to ragged, gaping holes in highly susceptible plants. Spray inoculated plants usually did not have holes within their lesion sites after the ten day assay. Water-inoculated control plants showed no signs of infection (chlorotic ring and lesion formation).

Effect of Leaf Position of Wounded-leaf Lesion Area

Leaf position significantly affected lesion area means and statistical separation of resistant, intermediate resistant, and susceptible lines when inoculated using the wounded-leaf method (Figure 1.1). On planting date 1 MR-1 and (AY x MR-1) F_1 lesion means were significantly different from AY across all leaves, but MR-1 was significantly different from (AY x MR-1) F_1 only when second and third leaves were examined. Planting date 1 MR-1 lesion means significantly trended smaller from 1st leaves ($48mm^2$) to 3rd leaves($27mm^2$), while (AY x MR-1) F_1 mean lesions remained static (44-$45mm^2$). Planting date 1 AY lesion means were significantly higher in 3rd leaves ($89mm^2$) than 1st ($61mm^2$) and 2nd leaves ($64mm^2$). Planting date 2 results were similar to planting 1 except that MR-1 was only significantly different from (AY x MR-1) F_1 in the in third leaves and AY lesion area compared to planting date 1 was significantly increased in

first leaves (76mm^2) and significantly decreased in the 3rd leaves (64mm^2). MR-1 lesion area, again, trended significantly smaller from 1st leaves (46mm^2) to 3rd leaves(27mm^2), while (AY x MR-1) F_1 mean lesions remained static (44 to 45mm^2). In both planting dates 3rd leaves provided superior lesion mean separation.

Effect of MR-1 Alleles on *Alternaria* Resistance

Alternaria spray and wounded-leaf inoculations were conducted on two planting dates on three MR-1 hybrids [(AY x MR-1) F_1, (MR-1 x HBJ) F_1, and (Perlita x MR-1) F_1], parents (MR-1, AY, HBJ, and Perlita), Jindaozi, and RIL61 (Table 1.2 and Figure 1.2). Spray resistance ratings correlated with wounded-leaf mean lesion areas ($r = -.67$, $p < .0001$, Spearman correlation). Spray inoculation ratings did not differ significantly between planting dates, separating the lines into four groups: resistant [(Perlita x MR-1) F_1, 5.0; Jindaozi, 5.0; (MR-1 x HBJ) F1, 4.9; and MR-1, 4.8], intermediate resistant [(AY x MR-1) F_1, 4.5], intermediate susceptible (Perlita, 4.2 and HBJ, 4.0), and highly susceptible (AY, 3.1 and RIL61, 2.9). On both planting dates AY, Perlita, and HBJ all significantly increased in resistance when crossed with MR-1. Wounded-leaf inoculated plants differed significantly between planting dates as the second planting mean lesion area (43mm^2) was significantly lower than the first planting date mean lesion area (54mm^2), and there were Planting date x Line interactions, notably Perlita and Jindaozi which were both significantly larger in the first planting date when compared to the second planting date. Planting date 1 mean lesion area ranged from 111mm^2 (RIL61) to

$14mm^2$ [(Perlita x MR-1) F_1] and susceptible lines AY ($89mm^2$), Perlita ($66mm^2$),and HBJ ($54mm^2$) all significantly decreased in mean lesion area when crossed with MR-1: (AY x MR-1) F_1 ($44mm^2$), (MR-1 x HBJ) F_1 ($37mm^2$), and (Perlita x MR-1) F_1 ($14mm^2$). Planting date 2 mean lesion area ranged from $81mm^2$ (RIL61) to 23 (Jindaozi), and susceptible lines AY ($64mm^2$), Perlita ($41mm^2$), and HBJ ($57mm^2$) all significantly decreased in mean lesion area when crossed with MR-1: (AY x MR-1) F_1 ($39mm^2$), (MR-1 x HBJ) F_1 ($35mm^2$), and (Perlita x MR-1) F_1 ($25mm^2$).

QTL Analysis of RILs for *Alternaria* Resistance

Eighty-nine lines were tested for *Alternaria* resistance; the mean lesion area (Appendix A) was used for QTL analysis. Of the mapping population, 56 of the 59 individuals had mean lesion data useful for QTL analysis (Appendix C). Composite interval mapping, WinQTL Cartographer 2.5 (North Carolina State University, Raleigh, NC), detected one QTL, ac.*1,* affecting *Alternaria* resistance with opposite additive effects located on linkage group 11 (Table 1.3). QTL significance, LOD 2.9, was determined by using 1,000 permutations at p-value 0.05. The approximate position of *ac.1* on the linkage map is shown in Appendix D. The MR-1 allele at the *ac.1* has a R^2 value of 0.25 and an additive effect of -10.43, decreasing lesion area.

Discussion

Wounded-leaf inoculated resistant and susceptible lines differed significantly in lesion area, analogous to observations by Batta (2003) in cucumber leaf disk assays. Lesion size differences among the RILs became increasingly noticeable over the 10-day assay, thus clear differences between resistant, intermediate resistance, and susceptibility could be statistically determined. However, in leaf position and MR-1 hybrid tests, a significant environmental effect between the two planting dates was observed, although greenhouse temperatures throughout both dates ranged from 19^0C to 30^0C. In future testing, environmental conditions may have to be compensated for by dynamically adjusting the duration of the test to allow susceptible control lines (e.g., AY, Perlita, HBJ, or RIL61) to reach predetermined severity thresholds (lesion size) prior to evaluating the population. Also, although rare, some AY controls were excluded from analysis because of high levels of powdery mildew on third leaves, which severely restricted lesion area relative to the other AY controls.

Comparison of the first, second, and third leaves resulted in a significant effect of leaf position on differentiation between MR-1, AY, and (AY x MR-1) F_1 lesion sizes. In other greenhouse-based resistance assays in melon, leaf selection ranged from 4th leaf (Evans et al., 1992), to 2nd leaf (Thomas et al., 1990; Thomas and Caniglia, 1997; Chandler and Thomas, 1991) to 1st leaves and cotyledons (Carmody et al., 1985). In this study, 1st and 2nd leaves were often smaller and irregular, thus the number of possible wound sites was limited. In some cases, the size of the first leaves only permitted a single lesion, whereas third leaves were large enough to accommodate six or more lesions.

Lesion expansion on the first leaves was often accompanied by early senescence, particularly in MR-1 plants, which led to abnormal lesion expansion and inconsistent results, similar to observations by Thomas et al. (1990). Wounded-leaf inoculation of 3rd leaves provided superior resolution when compared to 1st and 2nd leaves in 4th expanding leaf stage plants.

Spray inoculated resistance ratings correlated to lesion size observed in the wounded-leaf tests, indicating that the wounded-leaf method is also a valid measure of resistance under greenhouse conditions. However, with the spray method only four resistance groups were identified, whereas the wounded-leaf method identified seven resistance levels in the first planting date and five in the second planting date. Compared to the spray method, the wounded-leaf method direct lesion measurement is likely more suited for QTL analysis where phenotyping precision enhances quality (Cobb et al., 2013). However, when large populations make direct lesion measurements impractical (e.g., a breeding program for *Alternaria* resistance or resistance screening), an arbitrary rating scheme or lesion size reference could be used.

Carmody et al. (1985) reported that lesion size and mean number of lesions on cotyledons were predictive of lesion size, number of lesions, and resistance rating in field conditions. In addition, Carmody et al. (1985) rated Perlita and HBJ as susceptible in both greenhouse and field conditions, comparable to our ratings for these two cultivars in third leaf spray and wounded-leaf assays. The similarities suggests that the wounded-leaf method may also be predictive of resistance and susceptibility under field conditions. Nevertheless, future field comparison studies will need to be conducted.

Contrary to expectations, MR-1 *Alternaria* resistance did not exert complete dominance in an MR-1 x AY RILs as was reported in a (Perlita x MR-1) F_2 inheritance study (Thomas et al., 1990) and supported by our observations of high resistance in (Perlita x MR-1) F_1 (Table 1.2; Figure 1.2). Because of the continuous distribution of lesion areas among the RILs and intermediate resistance of (AY x MR-1) F_1, we expected to identify several contributing QTL; however, only one major QTL was detected corresponding to an MR-1 resistance allele, *ac.1* (Table 1.3; Figure 1.3). It is very likely that with the increased population and marker density that will be provided by GBS other major/minor QTL affecting lesion area will be detected. However, a*c.1* poses interesting questions as to the inheritance of the *Alternaria* resistance. Additional molecular testing would be required, but the putative resistance gene, *Ac,* identified by Thomas (1990) could be located within the confidence intervals of *ac.1*. However, indicating that the dominance of *Ac* is dependent on the cross, this study found that both (AY x MR-1) F_1 and (MR-1 x HBJ) F_1 increased in resistance compared to AY and HBJ, respectively, but were both significantly less resistant than MR-1 while (Perlita x MR-1) F_1 was equal to or slightly more resistant than MR-1 (Figure 1.2). However, due to the observed overall additive nature of *Alternaria* resistance and the effects of *ac.1* in the MR-1 x AY RILs, this study is in agreement with Boyhan and Norton (1992) that a pedigree or backcross method would lead to improved resistance to *Alternaria cucumerina* in melon. MAS targeting *ac.1* would significantly accelerate the introgression of *Alternaria* resistance into commercial melon lines.

Literature Cited

BATTA, Y., 2003. Alternaria leaf spot disease on cucumber: susceptibility and control using leaf disk assay. *An-Najah Univ.J.Res.: Nat.Sci,* 17, pp. 269-279.

BOYHAN, G. and NORTON, J., 1992. Inheritance of Resistance to Alternaria Leaf-Blight in Muskmelons. *HortScience,* 27(10), pp. 1114-1115.

CARMODY, B.E., MILLER, M.E. and GRISHAM, M.P., 1985. A Technique to Screen Muskmelons for Resistance to Alternaria Leaf-Blight. *Plant Disease,* 69(5), pp. 426-428.

CHANDLER, L. and THOMAS, C., 1991. Effect of leaf miner feeding activity on the incidence of Alternaria leaf blight lesions on muskmelon leaves. *Plant Disease,* 75(9), pp. 938-940.

COBB, J.N., DECLERCK, G., GREENBERG, A., CLARK, R. and MCCOUCH, S., 2013. Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theoretical and Applied Genetics,* 126(4), pp. 867-887.

COTTY, P., 1987. Modulation of Sporulation of Alternaria-Tagetica by Carbon-Dioxide. *Mycologia,* 79(4), pp. 508-513.

DELLAPORTA, S.L., WOOD, J., and HICKS, J.B., 1983. A plant DNA minipreparation: Version II. *Mol. Biol. Reporter* 1(4), pp. 19-21.

EGEL, D.S., 1999a. Severity of Alternaria leaf blight on muskmelon varieties, 1996. *Biol. Cult. Tests,* 14, pp. 157.

EGEL, D.S., 1999b. Severity of Alternaria leaf blight on muskmelon varieties, 1997. *Biol. Cult. Tests,* 14, pp. 158.

EVANS, K., NYQUIST, W. and LATIN, R., 1992. A Model Based on Temperature and Leaf Wetness Duration for Establishment of Alternaria Leaf-Blight of Muskmelon. *Phytopathology,* 82(8), pp. 890-895.

FEDERER, W.T. and RAGHAVARAO, D., 1975. On augmented designs. *Biometrics*, pp. 29-35.

JACKSON, C.R. and WEBER, G.F., 1959. Morphology and Taxonomy of Alternaria Cucumerina. *Mycologia,* 51(3), pp. pp. 401-408.

KUZUYA, M., YASHIRO, K., TOMITA, K. and EZURA, H., 2006. Powdery mildew (Podosphaera xanthii) resistance in melon is categorized into two types based on inhibition of the infection processes. *Journal of experimental botany,* 57(9), pp. 2093-2100.

LATIN, R. and EGEL, D.S., 2001. Melon Disease Forecaster. Purdue Extension.

SITTERLY, W., 1972. Breeding for Disease Resistance in Cucurbits. *Annual Review of Phytopathology,* 10, pp. 471.

THOMAS, C., 1986. Downy and Powdery Mildew Resistant Muskmelon Breeding Line Mr-1. *HortScience,* 21(2), pp. 329-329.

THOMAS, C., 1984. Resistant Reaction Type Against Alternaria Leaf-Blight in Cucumis-Melo. *Phytopathology,* 74(7), pp. 865-865.

THOMAS, C. and CANIGLIA, E., 1997. Evaluation of US honeydew-type melons for resistance against downy mildew and Alternaria leaf blight. *HortScience,* 32(6), pp. 1114-1115.

THOMAS, C., MCCREIGHT, J. and JOURDAIN, E., 1990. Inheritance of Resistance to Alternaria-Cucumerina in Cucumis-Melo Line Mr-1. *Plant Disease,* 74(11), pp. 868-870.

WANG, Y.H., THOMAS, C.E. and DEAN, R.A., 1997. A genetic map of melon (Cucumis melo L.) based on amplified fragment length polymorphism (AFLP) markers. *Theoretical and Applied Genetics,* 95(5-6), pp. 791-798.

WECHTER, W.P., DEAN, R.A. and THOMAS, C.E., 1998. Development of Sequence-specific Primers that Amplify a 1.5-kb DNA Marker for Race 1 Fusarium Wilt Resistance in Cucumis melo L. *HortScience,* 33(2), pp. 291-292.

ZALAPA, J., STAUB, J., MCCREIGHT, J., CHUNG, S. and CUEVAS, H., 2007. Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. *Theoretical and Applied Genetics,* 114(7), pp. 1185-1201.

Tables and Figures

Table 1.1 Lesion areas of the five most susceptible and the five resistant RILs and parental checks [MR-1, AY, and (AY x MR-1) F_1].

Line	Planting Dates	Total plants	Mean Lesion Area (mm2)	
61	2	22	111	a
7	3	29	101	ab
17	2	17	100	a-c
9	2	41	90	a-e
45	2	20	89	a-e
AY	9	136	72	e-l
(AY x MR-1) F1	9	120	37	m-q
58	2	24	32	m-r
75	2	22	30	m-r
19	3	43	30	n-r
89	2	23	26	o-r
MR-1	9	138	25	p-r
93	2	19	20	q-r

Mean lesion areas were selected from the full population analysis across nine planting dates utilizing an augmented block design (Appendix A). Planting dates refers to the number of planting dates the line was tested and total plants is the number of plants tested across all planting dates. Mean lesion areas with different letters indicate a significant difference (Fischer's Protected LSD; P=0.05).

Table 1.2 *Alternaria* spray resistance ratings of three MR-1 hybrids, parents, RIL61 and Jindaozi

Line	Total Plants	Rating
(PERLITA x MR-1) F1	16	5.0a
JINDAOZI	22	5.0a
(MR-1 x HBJ) F_1	20	4.9ab
MR-1	20	4.8ab
(AY x MR-1) F_1	13	4.5b
PERLITA	24	4.2c
HBJ	19	4.0c
AY	22	3.1d
RIL61	21	2.9d

Lines from two planting dates (1 = 1/22/2014; 2 = 1/31/2014) were tested. The effects of plant date and line x plant date were insignificant, so data across the planting dates were combined. Ratings with different letters indicate a significant difference (Fischer's Protected LSD; P=0.05).

Table 1.3 Summary of QTL analysis of *Alternaria cucumerina* resistance (Ac).

Trait	QTL	Linkage Group	Position	LOD	R^2	a
Ac	*ac.1*	LG11	6.0cM	5.41	0.25	-10.43

Additive effects, a, for the MR-1 allele are given.

Table 1.4 HFO-TAG (HFOWEC; HFSW) and ISSR sequence, annealing temperatures, and number of markers mapped.

Marker	Sequence (5' -> 3')	Annealing Temp °C	Number of Markers
HFOWEC161	GACGGCCAGTCTCCGGCGA	50	3
HFOWEC170	GACGGCCAGTGCCGGCCA	50	1
HFOWEC33	GACGGCCAGTGCGGCGGAA	50	4
HFOWEC40	GACGGCCAGTTCGCCGTCG	50	1
HFOWEC46	GACGGCCAGTCCCTCCTCC	50	1
HFOWEC49	GACGGCCAGTGCCTCCTCC	50	1
HFOWEC65	GACGGCCAGTATCGCCGCCG	50	3
HFOWEC86	GACGGCCAGTTGCCGGCG	50	1
HFOWEC99	GACGGCCAGTGCGGCTGC	50	1
HFSW04	GGCGGCGG	48	1
HFSW113	GAGGCGGC	40	2
HFSW67	GCCGCTGC	45	5
HFWS112	GCCGCCTC	40	1
ISSR06	CTCTCTCTCTCTCTCTG	44	1
ISSR10	ACACACACACACACACT	50	3
ISSR11	ACACACACACACACACG	50	1
ISSR17	ACACACACACACACACGT	47	3
ISSR19	AGAGAGAGAGAGAGAGYC	52	1
ISSR21	GAGAGAGAGAGAGAGACTA	52	1
ISSR27	TGTGTGTGTGTGTGTGGA	50	2
ISSR31	ACACACACACACACACYA	50	1
ISSR32	TCCTCCTCCTCCTCCGT	50	3
ISSR33	ATGATGATGATGATGGA	50	1
ISSR35	GACAGACAGACAGACAGT	50	1
ISSR36	CTTCACTTCACTTCA	52	1
ISSR37	AGACAGACAGACAGACGC	50	1

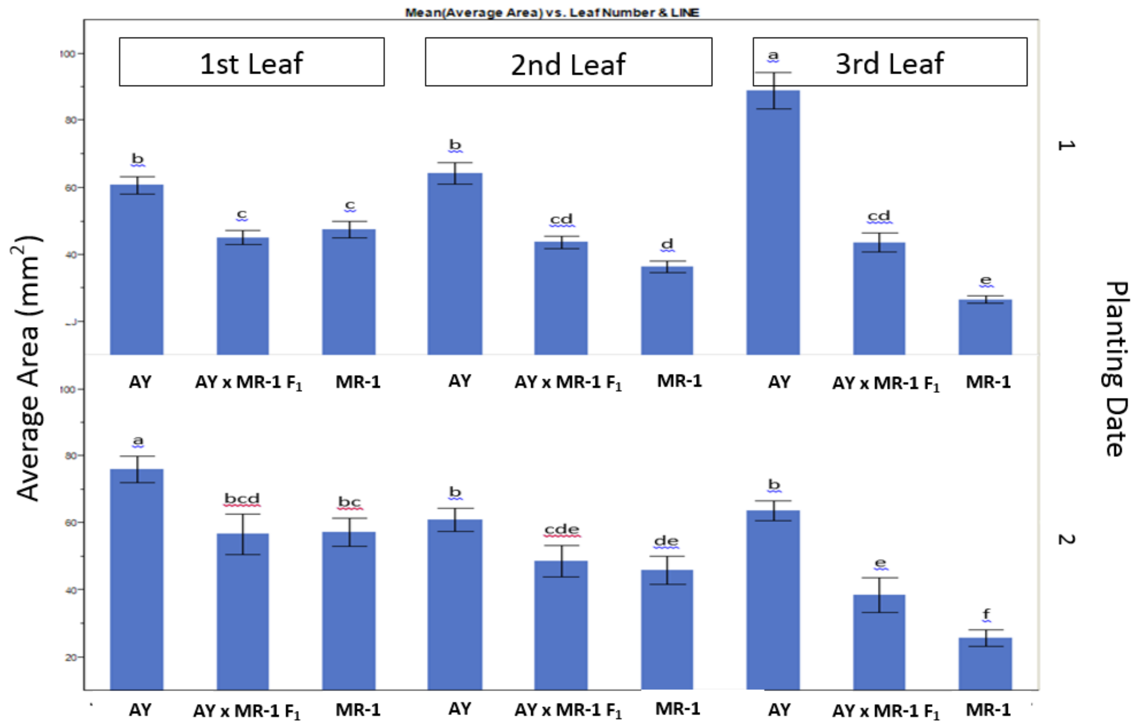

Figure 1.1 *Alternaria* lesion areas (mm^2) of wounded-leaf inoculated first, second, and third leaves of individual MR-1, AY, and (AY x MR-1) F_1 plants. The test was conducted on two groups from two different planting dates (1 = 1/22/2014; 2 = 1/31/2014). Plant date 1 included 11 AY, 12 (AY X MR-1) F_1, and 13 MR-1 plants; plant date 2 included 10 AY, 7 (AY X MR-1) F_1, 11 MR-1 plants. Different letters within a planting date indicate a significant difference (Fischer's Protected LSD; P=0.05). Errors bars indicate standard errors of the mean.

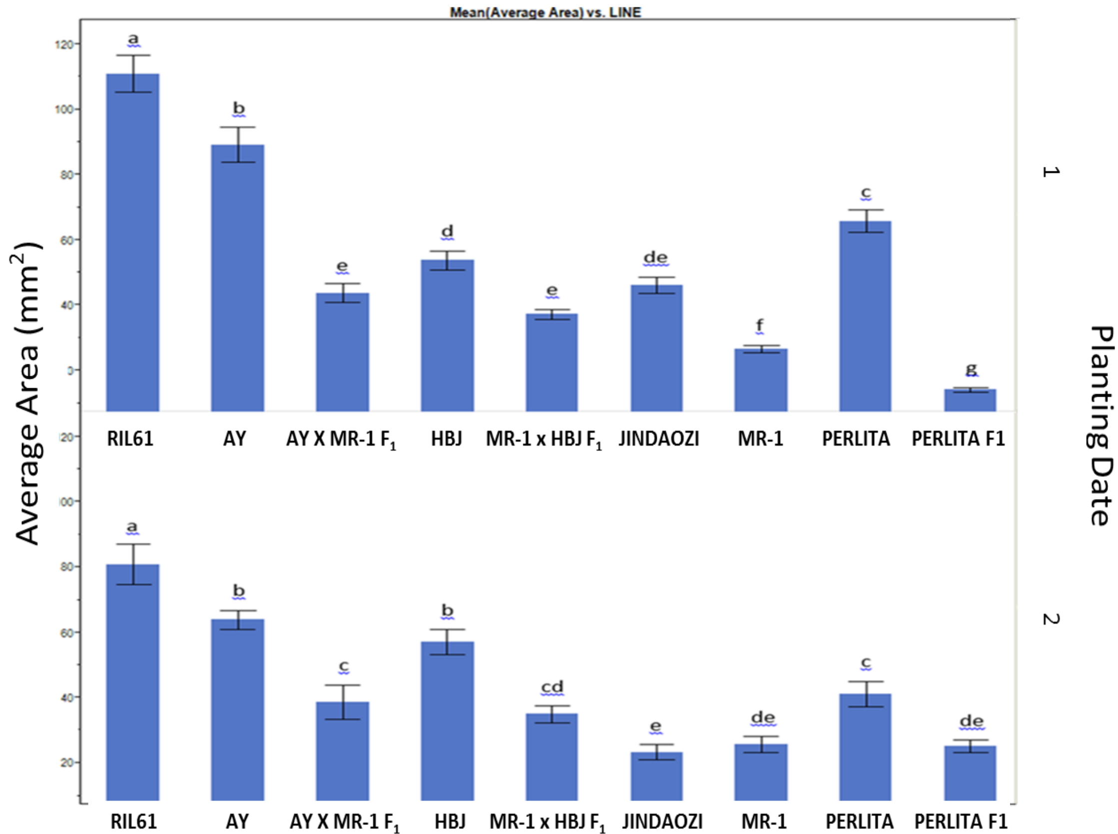

Figure 1.2 *Alternaria* lesion areas (mm^2) of wounded-leaf inoculated MR-1 hybrids [(AY x MR-1) F_1, (MR-1 x HBJ) F_1, and (Perlita x MR-1) F_1], parents (MR-1, AY, HBJ, and Perlita, Jindaozi, and RIL61. The test was conducted on two groups from two different planting dates (1 = 1/22/2014; 2 = 1/31/2014). Planting date 1 included 10 RIL61, 11 AY, 12 (AY x MR-1) F_1, 10 HBJ, 13 (MR-1 x HBJ) F_1, 10 Jindaozi, 13 MR-1, 13 Perlita, and 10 (Perlita x MR-1) F_1 plants; planting date 2 included 11 RIL61, 13 AY, 7 (AY x MR-1) F_1, 13 HBJ, 10 (MR-1 x HBJ) F_1, 12 Jindaozi, 11 MR-1, 11 Perlita, and 12 (Perlita x MR-1) F_1 plants. Different letters within a planting date indicate a significant difference (Fischer's Protected LSD; P=0.05). Errors bars indicate standard errors of the mean.

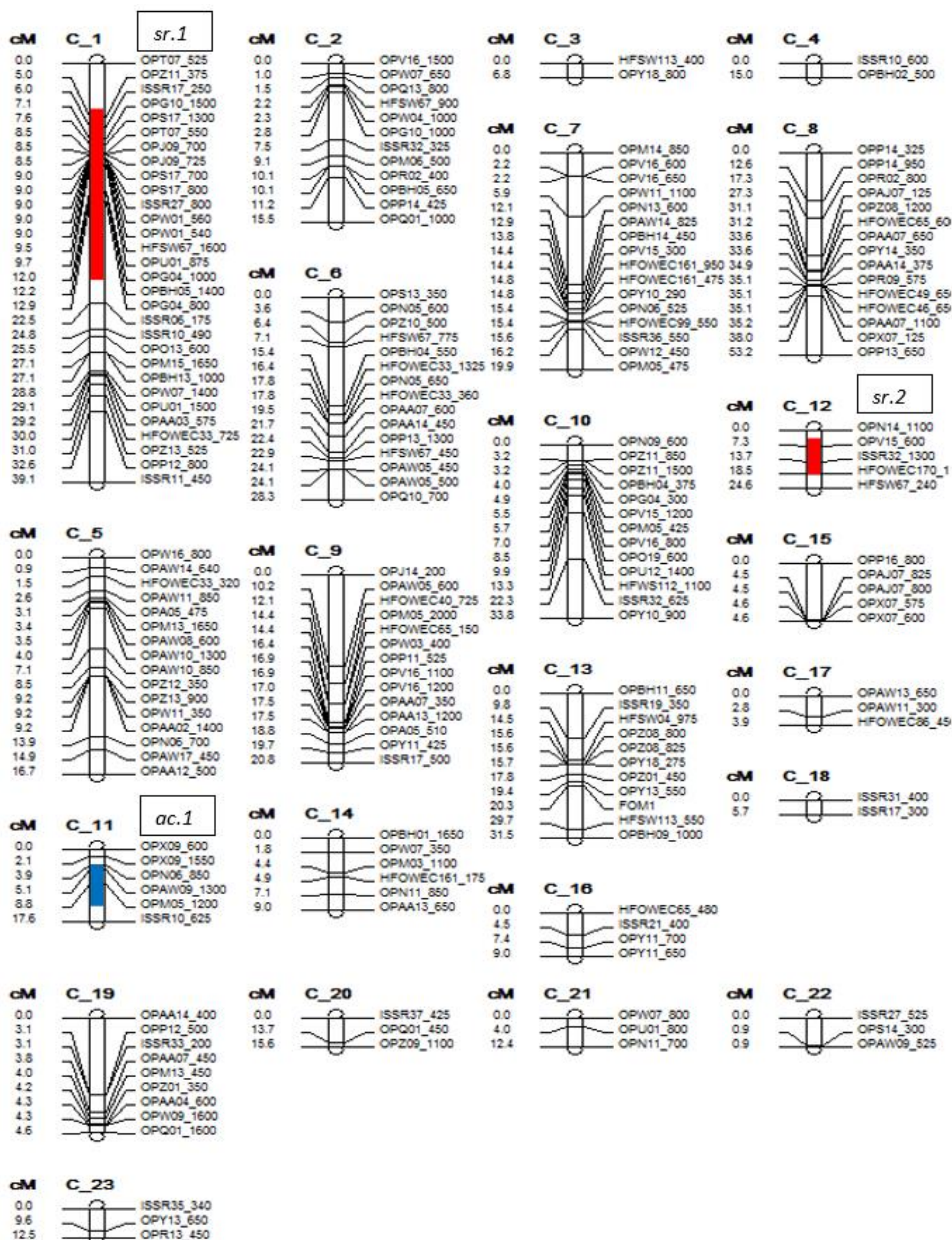

Figure 1.3 Linkage map of 59 (AY x MR-1) RIL and QTL locations (95% CI) for elemental sulfur tolerance (red), *st.1* and *st.2,* and *Alternaria* resistance (blue), *ac.1*.

CHAPTER THREE
MAPPING TOLERANCE TO ELEMENTAL SULFUR IN CUCUMIS MELO

Introduction

Elemental Sulfur is widely used as an organic fungicide in fruit and vegetable crops for control of powdery mildew and rusts (Williams and Cooper, 2004). For cucurbits, sulfur is an inexpensive and effective method for controlling powdery mildew (*Podosphaera xanthii*) (Koller, 2010; Keinath and DuBose, 2012). Sulfur is considered a contact fungicide, and hence more effective against fungal pathogens on the outside of the leaf (Keinath and DuBose, 2012). Sulfur makes contact with fungal pathogens by direct contact, diffusion through water, and by vapor action (Bent, 1967). Despite thousands of years of experience with sulfur as a fungicide, the exact mechanism remains elusive, but is thought that the sulfur permeates into the fungus and interferes with mitochondrial respiration (Cooper and Williams, 2004). The result is inhibition of conidia germination (Gogoi et al., 2013). According to the Fungicide Resistance Action Committee (2013), sulfur's mode of action is defined as "multi-site contact activity" and considered "low risk" for pathogen resistance development.

Sulfur can be used on many cucurbits, including melon, but phytotoxicity can be extreme in some lines while other lines are completely tolerant (Perchepied et al., 2004; Gogoi et al., 2013; Johnson and Mayberry, 1980). In a sulfur tolerance screen of 31 melon cultivars by Johnson and Mayberry (1980) 23 were shown to be tolerant and 8 were susceptible. In another study, melon 236 accessions from around the world were screened for sulfur tolerance, and 47% showed complete tolerance (Perchepied et al.,

2004). Sulfur phytotoxicity is manifested as necrosis and pronounced "burning" on the leaf tissue starting four days after dusting fruiting melon plants in field conditions (Johnson and Mayberry, 1980). In greenhouse conditions, vaporized sulfur causes symptoms in as little as 24hrs post-application in highly susceptible melon lines. Symptoms of sulfur phytotoxicity appear preferentially on matures leaf tissue of susceptible lines, in a top-down tolerance-to-susceptible pattern (Johnson and Mayberry, 1980). The observations by Johnson and Mayberry (1980) of phytotoxicity progression suggests that there is a developmental threshold that determines susceptibility of mature leaf tissue.

Sulfur tolerance in melon is quantitatively inherited (Perchepied et al., 2004). Perchepied et al. (2004) successfully mapped one major and two minor QTL affecting sulfur tolerance in two recombinant inbred lines (RILs) sharing a common resistant parent. The major QTL exerted complete dominance in the cross (Vedrantais x PI124112) F_1 and incomplete dominance in (Vedrantais x PI161375) F_1 (Perchepied et al., 2004). The two minor QTL were only detected in the Vedrantais x PI124112 population (Perchepied et al., 2004). Breeding for sulfur tolerance was first recorded in the 1930's and eventually led to the introduction of sulfur resistant commercial melon lines (Johnson and Mayberry, 1980).

Sulfur is generally applied as a micronized spray or dust, and the various formulations (sulfur >90%) differ primarily in the size of the sulfur particles (Emmett et al., 2003). Sulfur vaporization by means of timer controlled vaporizers is a convenient method of greenhouse powdery mildew control, as well as a means to screen for sulfur

tolerance. Vaporization of sulfur (>99%) forms very fine cloud of sulfur particles that distribute over nearby plants (Barker and Wallace, 1922). Early vaporization of sulfur was done by applying sulfur to hot pipes (Barker and Wallace, 1922), but commercial sulfur vaporizers can be now be purchased. The introduction of sulfur vaporizers as a means of controlling greenhouse powdery mildew led to discovery that USDA-ARS breeding line MR-1 is highly susceptible to sulfur, Ananas Yokneum (AY) is completely resistant, (AY x MR-1) F_1 was partially susceptible, and MR-1 x AY RILs were mixed resistant and susceptible.

In this study, MR-1 x AY RILs were rated for tolerance to vaporized sulfur and quantitative trait analysis was conducted to detect quantitative trait loci (QTL) affecting sulfur tolerance in melon. Additionally, the effects of sulfur vaporization and micronized sulfur spray were compared.

Materials and Methods

Plant Materials

MR-1 x AY derived RILs were used for linkage map construction, phenotypic analysis, and QTL analysis. Vaporized and micronized sulfur comparison tests used MR-1, AY, (AY x MR-1) F_1, and variously susceptible and tolerant RILs- 2, 18, 21, 23, 29, 30, 31, 44. The effects of MR-1 alleles on sulfur susceptibility were studied using MR-1, AY, Hales Best Jumbo (HBJ), MR-1 hybrids [(Perlita x MR-1) F_1, (MR-1 x HBJ) F_1, and

(AY x MR-1) F_1], Jindaozi, and RIL61. All control plants remained in a sulfur-free greenhouse.

Plants were grown using Metro-mix 360 (Sun Gro Horticulture, Agawam, MA) in 50 cell trays (PRO050R5; Myers Industries Lawn & Garden Group, Middlefield, OH) until the 1-2 fully expanded leaf at which time they were transferred to 4 inch plastic pots (SVT-400 4 x 5; T.O. Plastics, Inc., Clearwater, MN) and fertilized (Peters Professional Water Soluble Fertilizer 20-20-20; Everris International B.V., Geldermalsen, Netherlands) at 10 g/L. Plants were fertilized a second time at the same rate just prior to being moved for sulfur screening. Plants were grown in a temperature controlled greenhouse and year-round day-night temperatures averaged 22°C and ranged 18-38 ^{o}C. Pesticides were applied using a Mini AutoFog™ (Dramm Corporation, Manitowoc, WI) to control greenhouse pests. During the testing period no pesticides were used.

Sulfur Vaporization

At approximately the 7th leaf stage (RILs ranged from 6-9th leaf stage) plants were moved to a greenhouse utilized a vaporizer (Wilmod Sulfur Evaporator WSE75; Zoetermeer, Netherlands) to vaporize sulfur (>99% purity) nightly for 2.5 hrs. On the 5th day, vaporized-sulfur plants were visually rated 0 – 4 based on the percent of damaged foliage: 0 = No damage; 1 = 1-25%; 2 = 26-50%; 3 = 51-75% 4 = 76-100%.

Sulfur spray

Micronized sulfur dust (Bonide Products Inc., Oriskany, NY) was sprayed using a medium-high rate for listed fruits and vegetables (9 g/L). Sprayed plants were grown and rated under the same conditions as vapor-treated plants. Water spray controls remained in a sulfur-free greenhouse.

Experimental Design: Sulfur Tolerance Screening

Sulfur tolerance was evaluated using a complete randomized design. The RILs, MR-1, AY, and (AY x MR-1) F_1 were replicated four times, and the test was repeated once, and Fischer's Protected LSD (P=0.05) was used to declare significance. Control plants remained in a sulfur-free environment. All statistical analyses were conducted using JMP® Pro 10 (SAS Institute Inc., Cary, NC) software.

Experimental Design: Comparing the Effects of Sulfur Vaporization and Spray

A complete randomized design was used to compare two elemental sulfur application methods: vaporization and spray, and Spearman's correlation was used to determine the strength of the relationship between the two application methods. MR-1, AY, (AY x MR-1) F_1, and a subset of (AY x MR-1) RILs (Table 2.1) of known tolerance

level were replicated four times and the test was repeated once. Fischer's Protected LSD (*P*=0.05) was used to declare significance.

Effects of MR-1 Alleles on Sulfur Susceptibility

A complete randomized design was used to analyze the effects of MR-1 alleles on sulfur susceptibility. MR-1, AY, Hales Best Jumbo (HBJ), MR-1 hybrids [(Perlita x MR-1) F_1, (MR-1 x HBJ) F_1, and (AY x MR-1) F_1], Jindaozi, and RIL61 were replicated >9 times and the test was repeated once. Fischer's Protected LSD (*P*=0.05) was used to declare significance.

DNA Isolation

Melon DNA was extracted using the method outlined by Dellaporta, etal. (1983) with some modifications. Leaf tissue (50mg) was placed in 1.5ml microcentrifuge tubes and either DNA-extracted immediately or stored at -80^{0}C. The extraction buffer contains 100mM Tris-EDTA ph 8, 50 mM EDTA ph 8, 500 mM sodium chloride, 1% SDS, 10 mM beta-mercaptoethanol. Tissue was ground by hand in 1.5ml microcentrifuge using a polypropylene pestle after adding 566µl of extraction buffer and 10µl RNase. After 30sec of tissue grinding the tubes were vortexed for 30sec. All tubes were incubated at 65°C for 10mins. After incubation, 165µl 5 M potassium acetate was added, and the tubes were vortexed for 30 sec and centrifuged at 13,500 G for 15 min. The supernatant

was transferred to new tubes. One half of the volume (~400µl) cold isopropanol (>99%) was added to the supernatant and mixed by inversion until DNA strands form, then centrifuged at 13,500 G for 5 mins. The supernatant was discarded, and the DNA pellet was dislodged from the bottom of the tube and washed twice 500µl ethanol (70%). The pellet was briefly spun down in order to aspirate off any remaining liquid and placed in the hood for 10 min. The DNA was resolublized with 100 µl of H_2O. For the quantification of DNA, 1ul of DNA was prepared using the Quant-iT™ dsDNA Assay Kit, broad range (Thermo Fisher Scientific Corporation, Waltham, MA) and quantified on a Qubit Fluorometer (Thermo Fisher Scientific Corporation, Waltham, MA). DNA concentration was adjusted to 10ng/µl for marker analysis.

Molecular Markers

The random amplified polymorphic DNA (RAPD) protocol optimized by Levi et al. (1993), with slight modifications, was used for PCR-based analysis of RAPD, sequence characterized amplified region marker (SCAR), cleaved amplified polymorphic sequences (CAPS), inter simple sequence repeat (ISSR), sequence-tagged sites (STS), high-frequency oligonucleotides-targeting active genes (HFO-TAG) (Levi et al., 2010) markers. All RAPD markers were annealed at 48.5^0C, and HFO-TAG and ISSR markers were annealed at varying temperatures (Table 1.4). Ten µl reactions were composed of 5.3µl dH_2O, 1µl reaction buffer (200 µM NaCl, 500 mM Tris-HCl pH 9, 10% Triton-X-100, 0.1% Gelatin), 0.8µl $MgCL_2$ [25mM] (Promega Corporation, Madison, WI), 0.8µl

dNTP Mix [2.5 mM] (Life Technologies, Carlsbad, CA), 0.1 Taq 5u/µl (GoTaq® DNA Polymerase; Promega Corporation, Madison, WI; FIREPol® DNA Polymerase; Solis Byodyne, Tartu, Estonia), 1µl DNA 10ng/µl and 1µl primer [10µM]. HFO-TAG primers and FOM1 (Wechter et al., 1998) (1µl per 24-mer) PCR samples used 2µl primer [10µM] and 4.3µl dH_2O. Samples were amplified via a MJ Research PTC 200 thermocycler (MJ Research, Waltham, MA) set to an initial denaturing at 94ºC for 4mins followed by 45 cycles: 92°C for 60sec (denaturation), 48.5°C (varied for ISSR and HFO-TAG markers) for 70 sec (annealing), and 72°C for 120sec (elongation). FOM1, composed two 24-mer primers MUSKFOM I ($^{5'}$TCGACCAGACGAAGTTCTTCGAGC$^{3'}$) and MUSKFOM II ($^{5'}$GAACTAAGGTCACGTTTATCGATC$^{3'}$), was amplified using an initial 96ºC for 5min followed by 37 cycles and a last 5min 72ºC extension: 94°C for 60sec (denaturation), 68°C for 60 sec (annealing), and 72°C for 120sec (Wechter et al., 1998). Samples were then removed and prepared for agarose gel electrophoresis by adding 2 µl Blue/Orange Loading Dye, 6X (Promega Corporation, Madison, WI) to each 10µl sample. One Kb DNA Ladder (Life Technologies, Carlsbad, CA) was used to estimate band size. Electrophoresis was run at 180V for 90min at 4^0C on a HE99X Max Horizontal Unit (Hoefer Inc., Holliston, MA) using 1.5% agarose gel (Agarose BP160-500; Thermo Fisher Scientific Inc., Waltham, MA). Each marker was screened for polymorphism between the MR-1 and AY parents, and markers that produced strong polymorphisms were selected for mapping and run against the full population and parents.

Marker Nomenclature

RAPD markers (Operon Technologies, Alameda, CA) were designated by the prefix 'OP' followed by the Operon kit letter(s), Operon primer number, and amplicon bp (e.g., OPW07_650). ISSR markers were designated by the prefix 'ISSR' followed by a number corresponding to Table 1.4 and amplicon bp (e.g., ISSR32_325). HFO-TAG markers are designated 'HFSW' or 'HFOWEC' followed by a number corresponding to Table 1.4 and amplicon bp (e.g., HFSW67_900 and HFOWEC33_725). The single SCAR is designated 'FOM1'.

Genotyping by Sequencing

DNA aliquots of 89 MR-1 x AY RILs, 3 MR-1 plants, and 3 AY plants were submitted for to Cornell University Institute for Genomic Diversity (IGD) for Genotyping by Sequencing (GBS). DNA was extracted using the ChargeSwitch gDNA Plant Kit (Life Technologies, Carlsbad, CA) from 100 mg of tissue from the same 59 MR-1 x AY RILs used for agarose-based genotyping (above), 30 additional MR-1 x AY RILs, and three individual MR-1 and AY plants. The tissues were first homogenized in 1.5ml microcentrifuge tubes in a solution containing 100 µl Reagent A [300 mM $CaCl_2$, 15% polyvinylpyrolidone (10,000 average molecular weight) and ChargeSwitch Lysis Buffer], 100 µl 10% SDS, 2 µl RNase A, and 900 µl ChargeSwitch Lysis Buffer using a

FastPrep FP120 homogenizer (Thermo Fisher Scientific, Waltham, MA) with a ¼” Ceramic Sphere (MP Biomedicals, Santa Ana, CA) in each microcentrifuge tube. The manufacturer's recommended protocol was then followed to completion. For the quantification of DNA, 1ul of DNA was prepared using the Quant-iT™ dsDNA Assay Kit, broad range (Thermo Fisher Scientific Corporation, Waltham, MA) and quantified on a Qubit Fluorometer (Thermo Fisher Scientific Corporation, Waltham, MA). Concentrations over 100 ng/µl were diluted to 50-100 ng/µl by adding more ChargeSwitch Elution Buffer.

DNA quality checks were conducted according to IGD instructions (http://sorghumdiversity.maize.cornell.edu/), and images submitted were for review. Once approved, 50 µl aliquots of the extracted DNA were pipetted into a 96 well plate (VWR 83007-374; VWR International, Radnor, PA) and sealed using PCR tube strip caps (VWR 20170-000; VWR International, Radnor, PA). The samples were immediately placed on dry-ice and shipped overnight to Cornell University Institute for Genomic Diversity for GBS. GBS data is pending receipt and results will not be presented in this thesis.

Linkage Map Construction and QTL Analysis

The linkage map was constructed using JoinMap® 3.0 (Kyazma B.V., Wageningen, Netherlands), and linkage groups were assembled with a minimum LOD score of 4.0, Kosambi mapping function, with a recombination frequency of 0.4. The

expected 1:1 segregation was tested using a chi-squared test to identify segregation distortion (p-value 0.01) (Zalapa et al., 2007), and markers exhibiting segregation distortion were excluded from linkage map construction.

QTL analysis was performed using the composite interval mapping function on WinQTL Cartographer 2.5 (North Carolina State University, Raleigh, NC). Average sulfur ratings (0-4) (Appendix B) were considered trait values and directly used for QTL analysis. Model 6 was selected using the forward regression procedure with a walking speed of 1cM and the default settings of a 10cM window and up to 5 background markers. QTL significance was declared based on a 1,000 permutations at a p-value = 0.05. Confidence intervals (95%) were calculated automatically with at least 10cM distance between QTL, and R^2 values were determined using the highest point within the confidence interval.

Results

Linkage Map Assembly

A total of 198 dominant markers (1 SCAR, 24 HFO-TAG, 25 ISSR, and 152 RAPD) were assembled into 23 linkage groups of 59 MR-1 x AY RILs using the Kosambi function and a recombination value of 0.4 (Figure 2.2). Out of 223 markers initially scored, 11 markers failed to map onto any linkage group and 14 showed linkage distortion using JoinMap® 3.0 (Kyazma B.V., Wageningen, Netherlands) χ^2 analysis (p-value 0.01) and were excluded. Linkage groups were assembled using LOD 3 (102

markers) and LOD 4 (96 markers), and total map coverage was 400cM with the largest marker interval of 10 cM and an average interval of 2 cM. Of the linkage-distorted markers, 8 were predominantly MR-1 and 6 were predominantly AY. The 198 markers mapped among the 59 individuals were equally distributed between the two parents: 49.9% MR-1; 50.1% AY.

Recombinant inbred line elemental sulfur tolerance screening

Varying degrees of sulfur phytotoxicity were observed among the MR-1 x AY RILs in both spray and vaporized experiments. The phytotoxicity severity (0-4) of 107 RILs incited by sulfur vaporization was dominated by resistant lines, with 77 lines asymptomatic and 25 lines showing some degree of symptoms (Appendix B; Figure 2.1). Of the susceptible lines, 0 lines rated at 1 (0-25% damage); 3 lines rated at 2 (25-50% damage); 8 lines rated at 3 (50-75% damage); and 14 lines rated at 4 (75-100% damage). In less susceptible lines (rating 1 & 2), the damage was an slow leaf bronzing and marginal necrosis that progressed from the lower mature foliage upward over the five day period leading to dry, brittle lower leaves, while upper expanding leaf tissue remained asymptomatic. Of the highly susceptible lines (rating 3 & 4), MR-1 X AY F_1 and MR-1 showed symptoms of bronzing and even dry, brittle leaf tissue 24hrs after exposure and were rapidly damaged up to the youngest expanding and emerging leaf tissue by the end of the five day trial. Intermediate susceptible plants that remained in sulfur conditions beyond the five day trial eventually succumbed to the upward damage (data not shown).

Resistant lines, AY, and control plants (in a sulfur-free greenhouse) remained asymptomatic.

Comparing the Effects of Vaporization and Spray

Spray and vaporization application methods correlated ($r = .97$, $p < .0001$, Spearman correlation), though sulfur spray did incite significantly less severity than sulfur vaporization across both planting dates ($p = .0003$; $p < .0001$) (Table 2.1). This decreased severity of sprayed individuals compared to vaporized individuals is likely the result of dosage as arbitrary spray and vaporization rates were selected. Phytotoxicity symptoms for sprayed lines were the same as vaporization. Sulfur sprayed plants had visible foliar deposits of sulfur from the evaporation of sulfur-containing droplets which corresponded to pronounced areas of burning on some susceptible lines. Sulfur vaporized plants had no visible accumulation of sulfur and the only evidence that vaporization had occurred was a slight odor upon entry into the greenhouse. Control plants remained asymptomatic.

Effects of MR-1 Alleles on sulfur Susceptibility

Sulfur resistant (Perlita, AY) and moderately susceptible (HBJ) lines significantly increased in susceptibility when crossed with MR-1 (Table 2.2). (AY x MR-1) F_1 and (MR-1 x HBJ) F_1 plants increased from 1.0 to 2.9 and 1.3 to 3.3, respectively. Perlita had

the largest increase (0 to 4) in sulfur susceptibility when crossed with MR-1, and the (Perlita x MR-1) F_1 response to sulfur was similar to the MR-1 sulfur response. Sulfur tolerance observed in Perlita corresponds to observations by Johnson and Mayberry (1980). Jindaozi and RIL61 were included for comparison and were rated as 1.1 and 0 respectively. Control plants remained asymptomatic.

QTL Analysis of RILS for Vaporized Sulfur

Of the 109 lines rated for sulfur tolerance one was excluded for segregation and seven only appeared one of the two planting dates because of low germination (Appendix B). Phenotypic data, the average rating across the planting dates, was used for QTL analysis. Of the mapping population of 59 individuals, 58 had sulfur data from at least one planting date, which was used for QTL analysis (Appendix C). One line, 37, showed segregation across the two planting dates and was excluded from the analysis (data not shown). Composite interval mapping, WinQTL Cartographer 2.5 (North Carolina State University, Raleigh, NC), detected two QTL affecting sulfur tolerance, *st.1* and *st.2*, with opposite additive effects located on linkage groups 1 and 12, respectively (Table 2.3). QTL significance, LOD 2.8, was determined by using 1,000 permutations at p-value 0.05. The approximate positions of *st.1* and *st.2* on the linkage map are shown in Figure 2.2. Interestingly, the direction of allelic effects at two QTL was opposite. The MR-1 allele at the stronger QTL, *st.1*, has a R^2 value of 0.45 and an additive effect of 1.1, increasing susceptibility. In contrast the MR-1 allele at *st.2* has an R^2 value of 0.18 and an additive effect of -0.8, decreasing susceptibility.

Discussion

Varying degrees of sulfur susceptibility were observed with the most susceptible suffering rapid defoliation of all but the youngest leaf tissue and the most resistant showing slight bronzing and necrosis on mature leaves. Other studies also report varying degrees of susceptibility and the distinct pattern of defoliation of leaf tissue starting on mature leaves and progressing upward toward young leaf tissue (Perchepied et al., 2004, Johnson and Mayberry, 1980). The observations of this study and those reported by Johnson and Mayberry (1980) of phytotoxicity progression suggests that there is a developmental threshold that determines susceptibility of mature leaf tissue, though the exact mechanism of elemental sulfur tolerance in melon, as far as can be discerned, has yet to be elucidated. However, it has been shown that the air pollutant sulfur dioxide (SO_2) at high enough concentrations can lead to acute injury in cucurbits, affecting the mature leaves first (Sekiya et al., 1982), resembling symptoms of sulfur phytotoxicity. The young cucurbit leaf tissue SO_2 tolerance mechanism has been explained as a heightened capability to reduce SO_2 to, and emit, hydrogen sulfide (H_2S), ridding excess sulfur. In another study, cucurbits emitted H_2S when exposed to excess sulfate through their roots and that when detached petioles were fed a sulfate solution, H_2S emission increased substantially (Wilson et al., 1978). In addition, it has been demonstrated that higher plants are able to directly metabolize foliar sulfur (Legris-Delaporte et al., 1987; Jolivet, et al., 1995; Williams and Cooper, 2004; Vitti et al., 2007; McGrath, 1986). However, the exact mechanism for phytotoxicity of sulfur in cucurbits is not explicit in the literature, but if direct oxidation of foliar sulfur is excessive, it could lead to the toxic

accumulation of sulfur compounds. If foliar sulfur occurs at significant levels in cucurbits, then it is conceivable that the biomechanical mechanism of H_2S emission to expel excess sulfur could partly explain the observed sulfur tolerance of certain melon lines. Hence, it is possible that sulfur tolerance loci, *st.1* and *st.2,* may affect melon H_2S emission.

MR-1 is known to be highly resistant to powdery mildew, downy mildew, Fusarium wilt *Fusarium oxysporum f.sp melonis,* and *Alternaria* leaf blight, making it an excellent source for resistance breeding (Kuzuya et al., 2006; Thomas, 1986; Thomas et al., 1990); however, breeders will need to take measures to avoid inadvertent introgression of sulfur susceptibility. The degree of sulfur susceptibility increase when MR-1 was crossed with relatively resistant varieties depended on the cross. Unexpectedly, when crossed with MR-1 the already susceptible HBJ only moderately increased in susceptibility, whereas completely resistant Perlita became as susceptible as MR-1 (Table 2.1).

St.1 and *st.2,* appear to segregate independent of each other, as well as *Alternaria* resistance QTL (*ac.1*) and the *Fusarium* race 1 resistance marker, FOM1 (Wechter et al., 1998) (Figure 2.2), indicating favorable conditions for selecting against the MR-1 allele at *st.1*. Indeed, the breeding value of MR-1 itself would be enhanced by the introgression of sulfur tolerance. This could be accomplished through marker-assisted-backcrossing with MR-1 as the recurrent parent, or from the identification of elite lines among the MR-1 x AY RILs. In this regard, MR-1 x AY RIL6 has already been found to be sulfur resistant (Appendix B), resistant to *Alternaria* leaf blight (Appendix A), resistant to

powdery mildew race 1 (unpublished data), and has the SCAR marker for *Fusarium* wilt race 1 resistance. Further testing will be required, but RIL6 could already contain all of the known fungal resistance of MR-1.

Sulfur tolerance is an important trait for melon production that can be selected for by means of phenotypic assays using micronized sulfur application or vaporization. Additionally, *st.1* and *st.2* provide interesting insight into the underlying genetic structure of sulfur tolerance and the potential to accelerate sulfur tolerance breeding through MAS.

LITERATURE CITED

BARKER, B. and WALLACE, T., 1922. A new method of sulphur fumigation. *Ann.Rept.Agric.and Hort.Res.Stat.* pp. 123-124.

BENT, K., 1967. Vapour action of fungicides against powdery mildews. *Annals of Applied Biology,* 60(2), pp. 251-263.

COOPER, R.M. and WILLIAMS, J.S., 2004. Elemental sulphur as an induced antifungal substance in plant defence. *Journal of experimental botany,* 55(404), pp. 1947-1953.

DELLAPORTA, S.L., WOOD, J., and HICKS, J.B., 1983. A plant DNA minipreparation: Version II. *Mol. Biol. Reporter* 1(4), pp. 19-21.

EMMETT, B., ROZARIO, S. and HAWTIN, J., 2003. Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines. *Final Report to GWRDC, Project NumberL DAV,* 98(1), pp. 220.

GOGOI, R., SINGH, P.K., KUMAR, R., NAIR, K.K., ALAM, I., SRIVASTAVA, C., YADAV, S., GOPAL, M., CHOUDHURY, S.R. and GOSWAMI, A., 2013. Suitability of Nano-sulphur for Biorational Management of Powdery mildew of Okra (Abelmoschus esculentus Moench) caused by Erysiphe cichoracearum. *Journal of Plant Pathology & Microbiology*, 2013.

JOHNSON JR, H. and MAYBERRY, K., 1980. The effect of dusting sulfur on muskmelons. *HortScience,* 15(5), pp. 652-654.

JOLIVET, P., BERGERON, E., ZIMIERSKI, A. and MEUNIER, J., 1995. Metabolism of elemental sulphur and oxidation of sulphite by wheat and spinach chloroplasts. *Phytochemistry,* 38(1), pp. 9-14.

KEINATH, A.P. and DUBOSE, V.B., 2012. Controlling powdery mildew on cucurbit rootstock seedlings in the greenhouse with fungicides and biofungicides. *Crop Protection,* 42, pp. 338-344.

KOLLER, M., 2010. Potassium Bicarbonate as a Potential Sulphur Substitute in Protected Organic Cropping, *I International Conference on Organic Greenhouse Horticulture 915* 2010, pp. 157-163.

KUZUYA, M., YASHIRO, K., TOMITA, K. and EZURA, H., 2006. Powdery mildew (Podosphaera xanthii) resistance in melon is categorized into two types based on inhibition of the infection processes. *Journal of experimental botany,* 57(9), pp. 2093-2100.

LEGRIS-DELAPORTE, S., FERRON, F., LANDRY, J. and COSTES, C., 1987. Metabolization of elemental sulfur in wheat leaves consecutive to its foliar application. *Plant Physiology,* 85(4), pp. 1026-1030.

LEVI, A., WECHTER, W.P., HARRIS, K.R., DAVIS, A.R. and FEI, Z., 2010. High-frequency Oligonucleotides in Watermelon Expressed Sequenced Tag-unigenes Are Useful in Producing Polymorphic Polymerase Chain Reaction Markers among Watermelon Genotypes. *Journal of the American Society for Horticultural Science,* 135(4), pp. 369-378.

MCGRATH, S. and JOHNSTON, A., 1986. Sulphur--crop nutrient and fungicide. *Span: progress in agriculture,* 1986.

PERCHEPIED, L., PÉRIN, C., GIOVINAZZO, N., BESOMBES, D., DOGIMONT, C., PITRAT, M., LEBEDA, A. and PARIS, H., 2004. Susceptibility to sulfur dusting and inheritance in melon. *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czech Republic, 12-17 July, 2004.* 2004, Palacký University in Olomouc, pp. 353-357.

SEKIYA, J., WILSON, L.G. and FILNER, P., 1982. Resistance to injury by sulfur dioxide correlation with its reduction to, and emission of, hydrogen sulfide in Cucurbitaceae. *Plant Physiology,* 70(2), pp. 437-441.

THOMAS, C., 1986. Downy and Powdery Mildew Resistant Muskmelon Breeding Line Mr-1. *HortScience,* 21(2), pp. 329-329.

THOMAS, C., MCCREIGHT, J. and JOURDAIN, E., 1990. Inheritance of Resistance to Alternaria-Cucumerina in Cucumis-Melo Line Mr-1. *Plant Disease,* 74(11), pp. 868-870.

VITTI, G.C., FAVARIN, J.L., GALLO, L.A., PIEDADE, SÔNIA MARIA DE STEFANO, FARIA, MARIA REGINA MEIRELLES DE and CICARONE, F., 2007. Foliar elementary sulfur assimilation by soybean. *Pesquisa Agropecuária Brasileira,* 42(2), pp. 225-229.

WECHTER, W.P., DEAN, R.A. and THOMAS, C.E., 1998. Development of Sequence-specific Primers that Amplify a 1.5-kb DNA Marker for Race 1 Fusarium Wilt Resistance in Cucumis melo L. *HortScience,* 33(2), pp. 291-292.

WILLIAMS, J. and COOPER, R., 2004. The oldest fungicide and newest phytoalexin–a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathology,* 53(3), pp. 263-279.

WILSON, L.G., BRESSAN, R.A. and FILNER, P., 1978. Light-dependent Emission of Hydrogen Sulfide from Plants. *Plant Physiology,* 61(2), pp. 184-189.

ZALAPA, J., STAUB, J., MCCREIGHT, J., CHUNG, S. and CUEVAS, H., 2007. Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. *Theoretical and Applied Genetics,* 114(7), pp. 1185-1201.

Tables and Figures

Table 2.1 Comparison of sulfur vaporization and sulfur spray tolerance ratings of a subset RILs with parental controls [MR-1, AY, and (AY x MR-1) F_1].

	11/20/2013 Planting Date		1/31/2014 Planting Date	
Line	Spray	Vaporization	Spray	Vaporization
2	3.0	3.8	1.2	3.0
18	0.0	0.0	0.0	0.0
21	4.0	4.0	2.5	3.8
23	0.0	0.0	0.0	0.0
29	3.3	4.0	1.0	3.3
30	0.0	0.0	0.0	0.0
31	0.0	0.0	0.0	0.0
44	4.0	4.0	1.0	3.0
AY	0.0	0.0	-	0.0
(AY x MR-1) F_1	2.5	3.1	-	3.3
MR-1	3.8	4.0	-	4.0

Lines from two planting dates (1 = 11/20/2014; 2 = 1/31/2014) were tested. Each line included four test plant and one control plant. Tolerance ratings (0-4) values, representative of the percent foliar damage after five days in a greenhouse with nightly sulfur vaporization or one spray application in a sulfur-free greenhouse.

Table 2.2 Sulfur vaporization tolerance ratings of three MR-1 hybrids [(AY x MR-1) F_1, (MR-1 x HBJ) F_1, and (Perlita x MR-1) F_1], parents (MR-1, AY, HBJ, and Perlita), Jindaozi, and RIL 61.

Line	Total Plants	Rating
(Perlita x MR-1) F_1	23	4.0a
MR-1	20	4.0a
(MR-1 x HBJ) F_1	20	3.3b
(AY x MR-1) F_1	19	3.0c
HBJ	21	1.5d
Jindaozi	24	1.1e
Perlita	20	0.0f
AY	24	0.0f
RIL 61	22	0.0f

Lines from two planting dates (1/22/2014; 1/31/2014) were tested. The effects of plant date and line x plant date were insignificant, so data across the planting dates were combined. Tolerance ratings (0-4) values, representative of the percent foliar damage after five days in a greenhouse with nightly sulfur vaporization. Ratings with different letters indicate a significant difference (Fischer's Protected LSD; P=0.05).

Table 2.3 QTL analysis of sulfur tolerance (St).

Trait	QTL	Linkage Group	Position	LOD	R^2	a
St	*st.1*	LG1	6.0cM	5.56	0.30	0.95
	st.2	LG12	14.7cM	3.30	0.18	-0.81

Additive effects, a, for the MR-1 allele are given.

Table 2.4 HFO-TAG (HFOWEC; HFSW) and ISSR sequence, annealing temperatures, and number of markers mapped.

Marker	Sequence (5' -> 3')	Annealing Temp °C	Number of Markers
HFOWEC161	GACGGCCAGTCTCCGGCGA	50	3
HFOWEC170	GACGGCCAGTGCCGGCCA	50	1
HFOWEC33	GACGGCCAGTGCGGCGGAA	50	4
HFOWEC40	GACGGCCAGTTCGCCGTCG	50	1
HFOWEC46	GACGGCCAGTCCCTCCTCC	50	1
HFOWEC49	GACGGCCAGTGCCTCCTCC	50	1
HFOWEC65	GACGGCCAGTATCGCCGCCG	50	3
HFOWEC86	GACGGCCAGTTGCCGGCG	50	1
HFOWEC99	GACGGCCAGTGCGGCTGC	50	1
HFSW04	GGCGGCGG	48	1
HFSW113	GAGGCGGC	40	2
HFSW67	GCCGCTGC	45	5
HFWS112	GCCGCCTC	40	1
ISSR06	CTCTCTCTCTCTCTCTG	44	1
ISSR10	ACACACACACACACACT	50	3
ISSR11	ACACACACACACACACG	50	1
ISSR17	ACACACACACACACACGT	47	3
ISSR19	AGAGAGAGAGAGAGAGYC	52	1
ISSR21	GAGAGAGAGAGAGAGACTA	52	1
ISSR27	TGTGTGTGTGTGTGTGGA	50	2
ISSR31	ACACACACACACACACYA	50	1
ISSR32	TCCTCCTCCTCCTCCGT	50	3
ISSR33	ATGATGATGATGATGGA	50	1
ISSR35	GACAGACAGACAGACAGT	50	1
ISSR36	CTTCACTTCACTTCA	52	1
ISSR37	AGACAGACAGACAGACGC	50	1

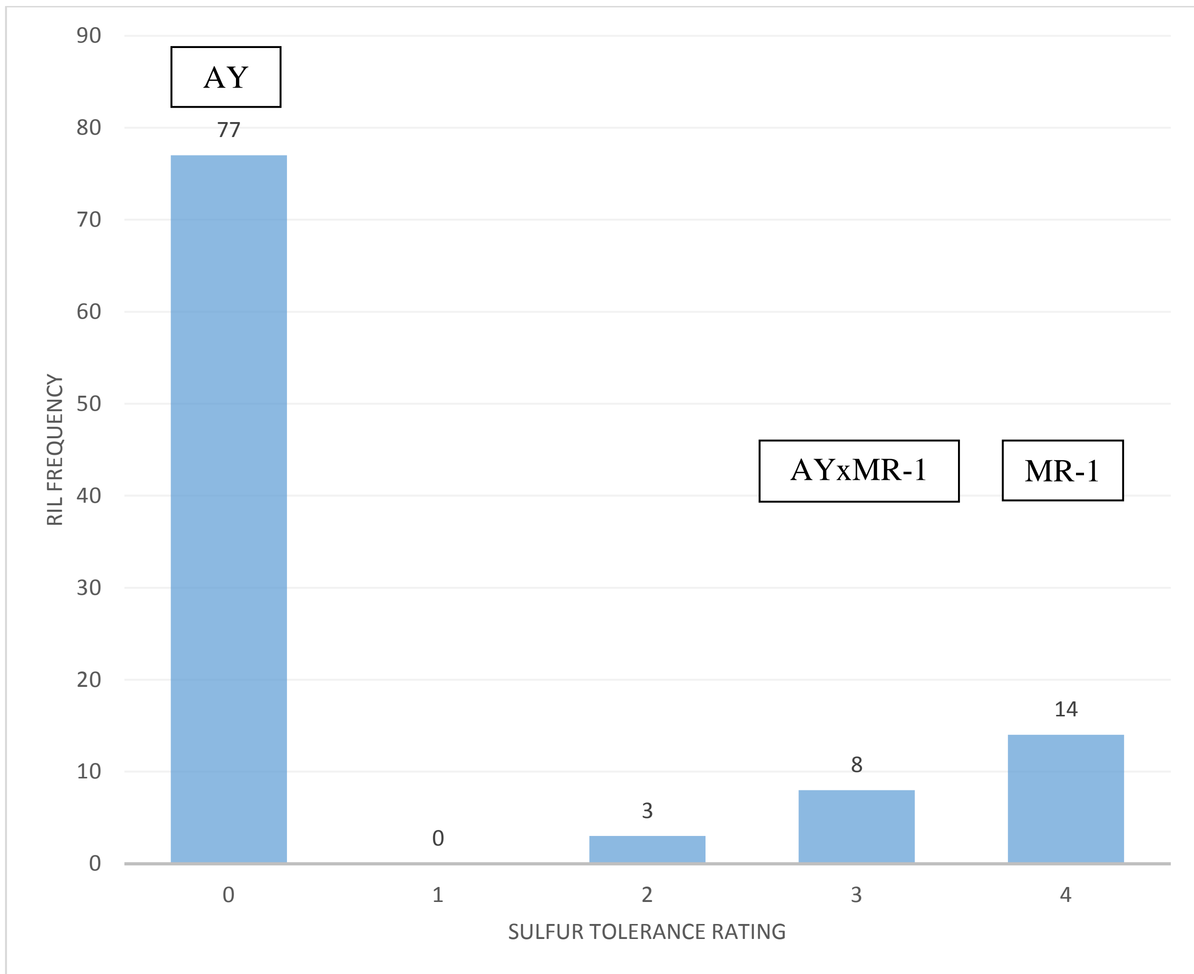

Figure 2.1 Distribution of MR-1 x AY RILs and parental controls (above bars) according to vaporized sulfur tolerance ratings rounded to the nearest whole number.

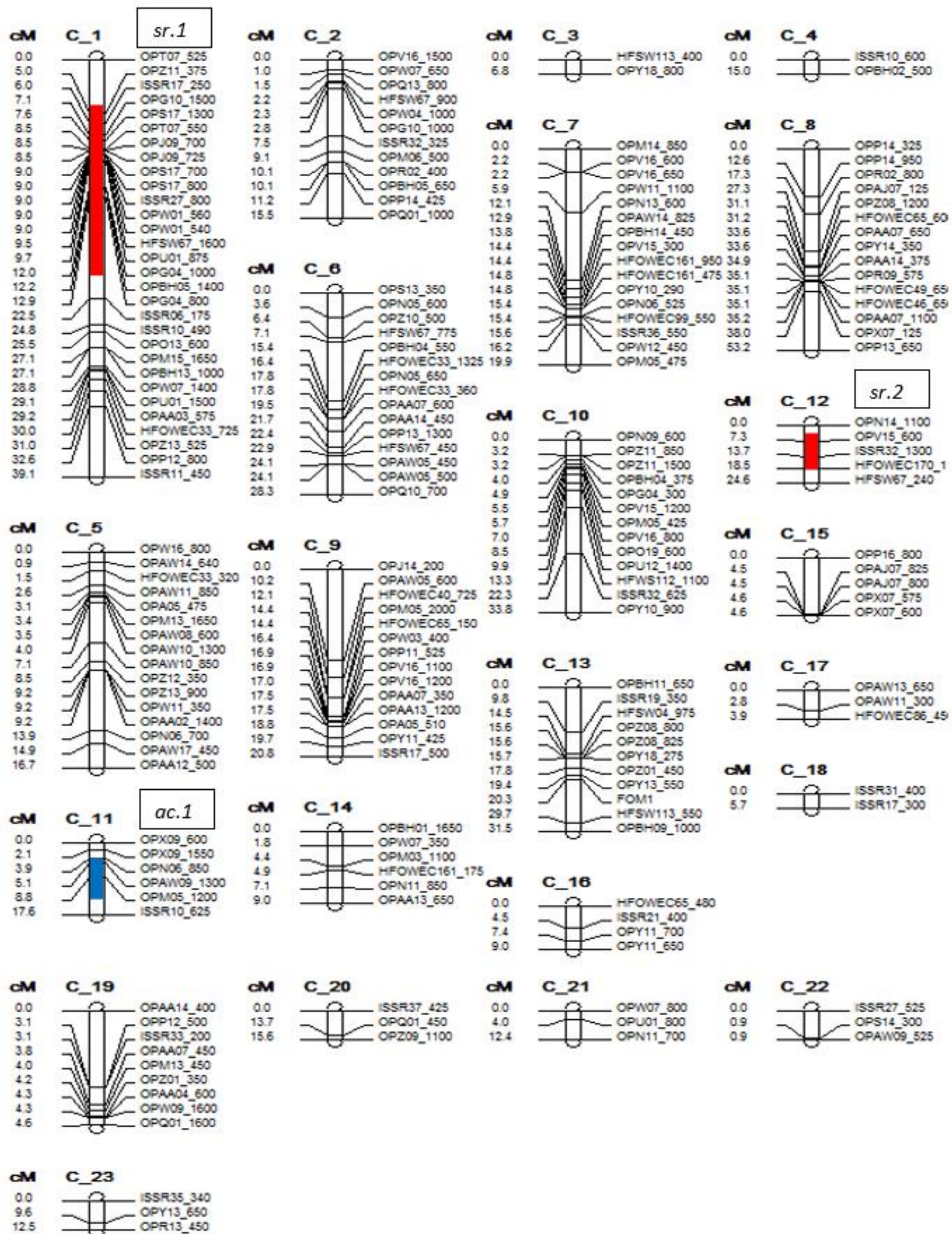

Figure 2.2 Linkage map of 59 (AY x MR-1) RIL and QTL locations (95% CI) for elemental sulfur tolerance (red), *st.1* and *st.2,* and *Alternaria* resistance (blue), *ac.1*

APPENDICES

Appendix A

Alternaria Resistance Screening of MR-1 x AY RILs

Alternaria resistance screening of the RILs with parental checks [MR-1, AY, and (AY x MR-1) F_1] on nine planting dates using an augmented block design. Planting dates is the number of planting dates the line was tested and total plants is the number of plants tested across all planting dates. Different letters indicate a significant difference (Fischer's Protected LSD; *P*=0.05).

Line	Planting Dates	Total plants	Mean Lesion Area (mm2)	
61	2	22	111	a
7	3	29	101	ab
17	2	17	100	a-c
9	2	41	90	a-e
45	2	20	89	a-e
96	2	23	88	a-f
59	4	40	87	b-d
29	2	21	86	b-h
44	2	20	85	b-g
82	2	21	85	b-h
5	3	45	85	b-f
50	3	35	83	b-h
28	3	35	82	b-i
18	3	44	80	c-j
55	3	55	78	c-k
39	2	21	77	c-m
13	2	27	75	d-o
80	3	34	73	d-n
99	3	30	73	d-o
AY	9	136	72	e-l
41	2	20	72	d-p
36	2	11	72	d-q
11	4	22	72	d-o
51	2	19	71	d-q
37	2	18	70	d-r
16	2	23	67	d-s
15	3	35	64	g-t
12	2	32	64	f-u
88	5	56	63	j-s
68	2	23	62	g-w
115	2	21	61	h-x
85	3	40	61	j-v
14	3	31	61	j-v
78	2	23	60	i-x
46	2	41	60	k-x
64	2	26	59	i-y
67	2	23	59	j-z
10	2	31	58	j-aa
87	2	23	58	j-ab

Appendix A continued

Line	Planting Dales	Total Plants	Mean Lesion Area (mm^2)	
72	3	27	58	l-y
23	2	32	57	k-ac
52	2	24	57	k-ae
57	2	19	57	k-ae
98	2	24	53	m-af
25	2	28	53	o-af
35	2	16	52	n-af
76	2	22	52	m-ag
40	3	25	51	p-af
49	3	38	50	p-ae
22	2	16	49	p-ah
71	1	14	49	l-ai
8	8	23	49	p-ah
62	2	19	47	q-ah
34	2	22	47	q-ah
60	2	21	46	r-ah
70	2	18	45	r-ah
43	2	15	45	r-ah
102	3	34	44	t-ah
32	2	8	44	s-ak
4	2	35	43	t-ak
79	2	20	43	s-ak
77	2	24	42	t-ak
1	3	17	42	u-aj
26	3	31	42	v-aj
31	2	16	42	t-ak
2	2	41	41	u-ak
92	2	23	41	u-ak
83	2	21	39	u-ak
24	2	22	39	w-ak
27	2	19	38	w-ak
54	2	18	38	w-ak
AYxMR-1 F_1	9	120	37	af-aj
21	2	19	37	x-ak
56	2	20	36	y-ak
100	3	27	36	aa-ak
119	2	21	35	y-ak
33	2	33	35	aa-ak
86	2	23	34	z-ak
81	2	20	34	aa-ak
6	3	24	34	ad-ak
3	2	31	34	af-ak
30	2	21	33	ab-ak
65	3	32	33	af-ak
20	2	21	33	ad-ak
74	2	23	33	ac-ak
66	3	30	32	af-ak
58	2	24	32	af-ak
75	2	22	30	af-ak
19	3	43	30	af-ak
89	2	23	26	ah-ak
MR-1	9	138	25	ai-ak
93	2	19	20	aj-ak

Appendix B

Sulfur vaporization resistance ratings of MR-1 x AY RILs

Resistance ratings (0-4) values, representative of the percent foliar damage, were averaged across the testing dates and used for QTL analysis.

Line	11/20/2014	1/8/2014	AVG
1	-	4.0[a]	4.0
2	3.0	3.8	3.4
3	4.0	4.0	4.0
4	0.0	0.0	0.0
5	0.0	0.0	0.0
6	0.0	0.0	0.0
7	-	4.0	4.0
8	4.0	4.0	4.0
9	2.5	3.4	3.0
10	1.6	1.8	1.7
11	4.0	4.0	4.0
13	0.0	0.0	0.0
14	0.0	0.0	0.0
15	0.0	0.0	0.0
16	0.0	0.0	0.0
17	0.0	0.0	0.0
18	0.0	0.0	0.0
19	0.0	0.0	0.0
20	0.0	0.0	0.0
21	4.0	4.0	4.0
22	0.0	0.0	0.0
23	0.0	0.0	0.0
24	0.0	0.0	0.0
25	4.0	4.0	4.0
26	0.0	0.0	0.0
27	0.0	0.0	0.0
28	0.0	0.0	0.0
29	4.0	4.0	4.0
30	0.0	0.0	0.0
31	0.0	0.0	0.0
32	0.0	0.0	0.0
33	0.0	0.0	0.0
34	3.2	4.0	3.6
35	0.0	0.0	0.0
36	0.0	0.0	0.0
38	0.0	0.0	0.0
39	0.0	0.0	0.0
40	3.7	2.8	3.2
41	0.0	0.0	0.0
43	0.0	0.0	0.0
44	2.8	4.0	3.4
45	3.8	4.0	3.9
46	0.0	0.0	0.0
47	0.0	0.0	0.0
49	0.0	0.0	0.0
50	2.5	2.8	2.6
51	0.0	0.0	0.0

Appendix B continued

Line	3/6/2014	1/8/2014	AVG
52	-	3.0	3.0
53	0.0	0.0	0.0
54	0.2	0.0	0.1
55	0.0	0.0	0.0
56	0.0	0.0	0.0
57	0.0	0.0	0.0
58	0.0	0.0	0.0
59	0.0	0.0	0.0
60	0.0	0.0	0.0
61	0.0	0.0	0.0
62	0.0	0.0	0.0
64	0.0	0.0	0.0
65	0.0	0.0	0.0
66	0.0	0.0	0.0
67	0.0	0.0	0.0
68	2.0	2.8	2.4
69	0.0	-	0.0
70	0.0	0.0	0.0
71	0.0	0.0	0.0
72	0.0	0.0	0.0
74	-	0.0	0.0
76	-	0.0	0.0
77	0.0	0.0	0.0
78	0.0	0.0	0.0
79	0.0	0.0	0.0
80	0.0	0.0	0.0
81	0.0	0.0	0.0
82	0.0	0.0	0.0
83	0.0	0.0	0.0
85	0.0	0.0	0.0
86	0.0	0.0	0.0
87	0.0	0.0	0.0
88	0.0	0.0	0.0
89	0.0	0.0	0.0
92	0.0	0.0	0.0
93	0.0	0.0	0.0
95	2.8	3.0	2.9
96	3.0	4.0	3.5
98	0.0	0.0	0.0
99	0.0	0.0	0.0
100	0.0	0.0	0.0
102	0.0	0.0	0.0
104	3.8	4.0	3.9
109	2.5	2.5	2.5
111	0.0	0.0	0.0
112	0.0	-	0.0
114	0.0	0.0	0.0
115	0.0	0.0	0.0
117	0.0	0.0	0.0
119	0.0	0.0	0.0
121	0.0	0.0	0.0
123	0.0	0.0	0.0
131	3.8	4.0	3.9
134	3.8	3.8	3.8
135	3.5	3.2	3.4
136	0.0	0.0	0.0
137	0.0	0.0	0.0
141	-	0.0	0.0
146	1.5	2.5	2.0
147	0.0	0.0	0.0

Appendix B continued

Line	3/6/2014	1/8/2014	AVG
148	3.8	4.0	3.9
150	0.0	0.0	0.0
160	3.8	3.8	3.8
162	-	2.8	2.8
AY	0.0	0.0	0.0
AYF1[b]	2.6	3.1	2.9
MR-1	3.9	4.0	3.9

[a] Due to low germination, rating based on one plant.

[b] (AY x MR-1) F_1

Appendix C

Overview of MR-1xAY RILs: Included in Genetic Map, Alternaria Resistance Screened, and Sulfur Resistance Screened

RIL	Gen	Fruit	Map	*Alternaria*	Sulfur
1	9		X	X	X
2	9		X	X	X
3	8	B	X	X	X
4	10		X	X	X
5	9		X	X	X
6	10		X	X	X
7	7		X	X	X
8	8	1	X	X	X
9	9		X	X	X
10	9		X	X	X
11	8	1	X	X	X
12	10		X	X	
13	9		X	X	X
14	9		X	X	X
15	9		X	X	X
16	7			X	X
17	10		X	X	X
18	9		X	X	X
19	10		X	X	X
20	10		X	X	X
21	7			X	X
22	10		X	X	X
23	10		X	X	X
24	9		X	X	X
25	9		X	X	X
26	10		X	X	X
27	10		X	X	X
28	9		X	X	X
29	7	1	X	X	X
30	8			X	X
31	8		X	X	X
32	8		X	X	X
33	8			X	X
34	8			X	X
35	8		X	X	X
36	9		X	X	X
37	10	A	X	X	
39	9		X	X	X
40	8	1	X	X	X
41	8	1	X	X	X
43	9	B	X	X	X
44	9			X	X
45	8		X	X	X
46	10		X	X	X
47	6				X
49	9		X	X	X
50	8	1	X	X	X
51	10		X	X	X

Appendix C continued

RIL	Gen	Fruit	Map	*Alternaria*	Sulfur
52	8		X	X	X
53	9		X		X
54	8		X	X	X
55	10		X	X	X
56	8		X	X	X
57	10		X	X	X
58	9		X	X	X
59	10		X	X	X
60	8	2	X	X	X
61	7		X	X	X
62	8	1		X	X
64	8			X	X
65	7	1	X	X	X
66	8			X	X
67	7			X	X
68	7			X	X
69	6				X
70	8	1		X	X
71	7	1	X	X	X
72	7			X	X
74	7			X	X
75	7			X	
76	7			X	X
77	7	A		X	X
78	8	1	X	X	X
79	8		X	X	X
80	8		X	X	X
81	7			X	X
82	7			X	X
83	7	2		X	X
85	8			X	X
86	8	2	X	X	X
87	8			X	X
88	7		X	X	X
89	8			X	X
92	7	1		X	X
93	8			X	X
95	6				X
96	6			X	X
98	8			X	X
99	7			X	X
100	7			X	X
102	7				X
104	6			X	X
109	6				X
111	6				X
112	6				X
114	6				X
115	6			X	X
117	6				X
119	6			X	X
121	6				X
123	6				X

Appendix C continued

RIL	Gen	Fruit	Map	*Alternaria*	Sulfur
131	6				X
134	6				X
135	6				X
136	6				X
137	6				X
141	6				X
146	6				X
147	6				X
148	6				X
150	6				X
160	6				X
162	6				X
148	6				X
150	6				X
160	6				X
162	6				X

Appendix D

Survey of Melon Genetic Maps

Parents	Population Type	Number of Ind.	Marker Types and Number	Map Length (cM)	Marker Density	Linkage Groups	Trait and QTLs Identified	QTL R^2 Range & Mapping Method	Reference
TGR-1661 (agrestis) & Bola de O	F2	195	AFLP- 188 RAPD- 39 SSR- 111 SCAR, CAPS- 14 Trait- 2	1285	3.6	14	Podosphaera xanthii race 1,2,5 resistance- 1	8.2-65.9% ICIM MQM	(Yuste-Lisbona, Capel et al. 2011)
Vedrantais & Songwhan Charmi	F2	218	RFLP- 34 RAPD- 64 Isozyme- 1 Trait- 5	1390	13.3	14	N/A	N/A	(Baudracco-Arnas and Pitrat, 1996)
AR 5 & Harukei 3	RILs (F8-F12)	93	SSR-157 SCAR/CAPS-7 Trait-3	877	5.3	20	Podosphaera xanthii resistance – 2	12-46% CIM	(Fukino et al., 2008)
Chinese Q 3-2-2 &Top Mark (cantalupensis)	F2:3	116	SSR- 154 CAPS- 8 SNP- 7	1095	6.5	12	Early Fruit Maturity (FM) - 3 B-carotene (Flesh Color) - 3	4-50% CIM	(Cuevas, Staub et al. 2009)
PI414723 (subsp. agrestis var. momordica) & Dulce (subsp. reticulatus)	RIL (F6-F8)	99	SSR-386 SNP- 76 INDEL- 6 AFLP- 200	1,222	2.7	12	Fruit Quality traits- 44 Fruit Sugar- 6 Carotenoid Content- 3	.01-61.7% IM MQM	(Harel-Beja et al., 2010)
PI414723-S5 (var. acidulous) & Dulce (var. reticulatus)	F2	112	SSR- 22 RAPD- 46 ISSR- 2 Trait- 4			14	N/A	N/A	(Danin-Poleg, Tadmor et al. 2002)
Piel de Sapo & PI161375	F2	93	RFLP- 234 AFLP- 94 RAPD- 47 SSR- 29 ISSR- 5 Isozyme- 2 Trait- 1	1197	3	12	N/A	N/A	(Oliver et al., 2001)
PI414723 (momordica) & Top Mark (cantalupensis)	F2	113	RAPD-74 AFLP-42 ISSR-3 SSR-16	1421	7.9	24	N/A	N/A	(Silberstein, Kovalski et al. 2003)

			RFLP-41 Trait –3						
Ano2 & K413	F2	143	AFLP- 121 SSR- 16 STS- 3 Trait- 2	1014.2	7.1	12	Fruit length- 1 Fruit width- 1 Fruit shape- 4 Center sugar- 1 Edge sugar- 3 Seed length- 2 Seed width- 2 Seed shape- 4 Seed weight- 4	6-56% CIM	(Wang, Gao et al. 2011)
MR-1 & Ananas Yokneum	BC	66	AFLP- 197 RAPD- 6 SSR- 1	1942	10	20	N/A	N/A	(Wang et al., 1997)
USDA 846-1 & Top Mark	RIL (F6)	81	RAPD – 114 SSR – 43 AFLP – 32 Trait – 1	1116	5.9	15	Primary Branching - 6 Fruit number - 9 Fruit weight per plant- 12 Average fruit weight per plot -5 Mature fruit per plot - 5	4-43% CIM	(Zalapa et al., 2007)
Vedrantais & PI124111	RIL (F6-F8)	120	AFLP- 28 SSR- 45 IMA- 12 Trait- 2	1150	4.2	35	Psuedoperonospora cubensis resistance - 9 Podosphaera xanthii race 1 resistance- 2 Podosphaera xanthii race 2 resistance- 2 Podosphaera xanthii race 3 resistance- 1 Podosphaera xanthii race 5 resistance- 1 Golovinomyces cichoracearum race 1 resistance-1	14-93% IM CIM	(Perchepied, Bardin et al. 2005)
USDA 846 & Top Mark	RIL (F7)	81	RAPD- 104 AFLP- 29 SSR-111 CAPS- 7 SNP- 4 Trait- 1	1180	4.6	12	B-Carotene- 7	8-31% CIM	(Cuevas et al., 2008)
Piel de Sapo & PI161375	DHL, F2	77, 93	RFLP- 226 SSR- 97 SNP- 3 Trait- 1	1021	3.1	12	N/A	N/A	(Gonzalo et al., 2005)
Piel de Sapo & PI161375	DHL, F2	77, 93	107	992	9.3	12	Earliness- 9 Fruit shape- 8 Fruit weight- 6	7-34% CIM	(Monforte et al., 2004)

							Sugar Content- 5 External color- 1 Flesh color- 2		
Deltex (reticulatus) & TGR1551 (PI482420)	F2	108	RAPD- Deltex- 171 TGR1551- 138 Combined-76 Trait-1	1182 1163 1394	6.9 8.4 18.3	12 12 12	Sucrose- 6 TSS- 3 RSTS- 4 Ascorbic Acid- 1	N/A	(Park, Hwang et al. 2009)
Vedrantais & PI161375 Vedrantais & PI414723 Composite Map	RIL (F6-F7) RIL (F6-F7)	163 63 226	AFLP- 346 IMA- 113 AFLP-233 IMA- 65 SSR- 5 RFLP- 2 Trait- 13 668	1411 1180 1654	3.2 3.7 2.5	12 19 12	N/A	N/A	(Perin et al. 2002)
Piel de Sapo & PI161375	DHL	14	SNP- 200 RFLP- 80 SSR- 212 SNP- 3 Trait- 35	1240	2.35	145 bins	N/A	N/A	(Deleu et al., 2009)
Piel de Sapo & PI161375	DHL	14	RFLP- 80 SSR- 212 SNP- 3 Trait- 1	1244	4.2	122 bins	N/A	N/A	(Fernandez-Silva et al., 2008)
Vedrantais & PI161375	RIL	190	SSR- 88 AFLP- 98 ISSR- 17 RFLP- 5 RAPD- 3 Trait- 5	1312	6.1	12	Aphis gossypii resistance- 6 Bemisia tabaci resistance- 2	2.9-71% CIM	(Boissot et al., 2010)
Vedrantais & Isabelle	RIL (F6-F8)	120	AFLP- 39 SSR- 45 IMA- 46 SCAR- 2	641	4.9	16	Fusarium oxysporum f. sp. melonis race 1.2- 9	44-66% (total) CIM	(Perchepied et al., 2005)
USDA-846-1 & Top Mark	RIL (F7)	81	RAPD – 114 SSR – 43 AFLP – 32 Trait – 1	1116	5.9	15	Soluble solid content- 10 Mesocarp pressure- 8 Fruit diameter- 6 Seed cavity diameter- 9 Cavity/fruit diameter- 9 Fruit shape- 10 Netting- 6	4-29% CIM	(Paris et al., 2008)

4G21 (chinensis) & 3A832 (saccherinus)	F2	114	SRAP- 152	2077	13.7	12	N/A	N/A	(Wang et al., 2007)
Consensus Map	N/A	N/A	SSR- 640 SNP- 330 AFLP- 252 RFLP- 239 RAPD- 89 ISSR- 15 INDEL- 16 Trait- 11	1150	.73	12	370	N/A	(Díaz Bermúdez et al., 2011)