PRACTICAL TESTING OF ONION HYBRIDS FEATURING NOVEL BOTRYTIS LEAF BLIGHT RESISTANCE OR DOUBLED HAPLOID PARENTS.

A Thesis

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ABSTRACT

Onion (*Allium cepa*) is an important vegetable crop in the United States and worldwide. In 2009, *ca.* 103,960 acres of onions were harvested in the United States at an estimated value of nearly $473 million including the 10,000 acres harvested in New York, valued at $46 million (U.S. Department of Agriculture, 2009).

Onion breeding has, for the last 60 years, focused largely on production of single cross hybrid varieties, created by crossing male sterile female lines with male fertile inbred lines. The shift from open pollinated varieties to hybrid varieties occurred in the 1950's after reports by Jones and Clark (1943) and Jones and Davis (1944) outlined how the cytoplasmic male sterility system allowed large scale production of hybrid seed, and demonstrated the significant increase in productivity of the resulting hybrids over standard open pollinated onion varieties. These hybrid varieties were adapted rapidly by onion growers, due to the increased yield (i.e. hybrid vigor) and uniformity.

The creation of new male lines with novel beneficial traits for use in hybrid combination is a major strategy of onion breeding programs. When a desired trait, such as disease resistance, cannot be found in a crop species, it must be transferred from a related wild species through inter-specific crosses. Resistance to Botrytis leaf blight (BLB) was transferred to onion *A. Cepa* through inter-specific crossing with *Allium roylei* and backcross breeding. Another procedure used to develop homozygous inbred lines in crops such as maize, rice and Brassicas, is the creation of doubled haploid inbred lines. Trial

results of two types of inbred lines, created using both inter-specific backcross breeding and doubled haploids, are presented.

Two onion lines 07-801 (BC2F3) and 07-808 (BC1F3), resistant to *Botrytis squamosa* (Walker 1925) causal agent of Botrytis leaf blight (BLB), were produced through inter-specific backcross breeding to introgress the resistance gene Bs1 from *A. roylei* to *A. cepa*. BLB resistance levels of these lines were compared in inoculated trials with the susceptible commercial variety Festival. Four evaluations, the two in the greenhouse and two in the field, found these lines to be significantly more resistant the susceptible control Festival. In non-inoculated trials in commercial fields Bs1 heterozygous hybrids, created by crossing the resistant inbred line 07-801 with a susceptible female, were intermediate in BLB resistance. They showed disease levels that differed significantly in resistance from both the resistant male line and susceptible female. In an inoculated greenhouse trial with multiple *B. squamosa* isolates, the homozygous Bs1 male, heterozygous Bs1 resistant hybrid, and susceptible female had a similar pattern of resistance as that seen in the field. The inbred lines 07-801 and 07-808 could be used as males in hybrid combination to reduce yield loss due to BLB. The homozygous lines showed stronger resistance indicating that Bs1 is partially dominant and that using male and female parents, both homozygous for Bs1resistance, would create a more resistant hybrid.

Twenty doubled haploid (DH) onion lines, produced from two different sources from the Cornell onion breeding program (Alan et al., 2003, 2004), were comparable in bulb weight with their source lines and Festival. Twenty-five DH hybrids, created using DH lines as males, were equivalent or significantly greater in bulb size and yield when compared with current commercial onion hybrids that share the same female parents. The ability of DH lines to be exceptionally productive, both as inbred lines and in hybrid combinations, may be attributed to the removal of deleterious sub-lethal genes that are fully expressed in the homozygous state during gynogenesis. These results suggest that doubled haploids could be used to both speed up that process of inbred line development and create higher yielding varieties.

BIOGRAPHICAL SKETCH

Peter Hyde began gardening at a young age. His father, Dr. Greg Hyde a biology professor and single parent, diligently used gardening and natural ecosystems as a teaching medium for biologic fundamentals. After receiving a B.S. in Wildlife Biology in 2002 from the University of Vermont he lived in western Kenya, volunteering for the Kakamega environmental education program (KEEP), a community based education center. While in Kenya he developed the desire to become active in development programs aimed at poverty reduction and natural resource conservation. Upon returning to the US in 2003 he moved to Ithaca, NY and took a field technician position with Martha Mutschler in the Department of Plant Breeding with the goal of learning “a little” about agricultural systems and how they relate to resource conservation and development work. After two years of work, he enrolled in the employee degree program aspiring to earn a Masters degree. Upon completion of his degree Peter will move to Ghana to volunteer for the Alliance for a Green Revolution in Africa with his partner Liz.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

EVALUATION OF ONION LINES AND HYBRIDS HOMOZYGOUS AND HETEROZYGOUS FOR RESISTANCE TO *Botrytis squamosa.*

1.1 Abstract

Onion (*Allium cepa*) lines resistant to the fungal pathogen *Botrytis squamosa*, the causal agent of Botrytis leaf blight (BLB), were produced by backcrossing the BLB resistance of the wild onion relative *Allium roylei* (the Bs1 resistance gene) into cultivated onion, *A. cepa*. The goal of this work was to evaluate resistant lines to determine whether they were homozygous for this resistance, and to test the relative degree of BLB control when this resistance is homozygous vs. heterozygous in resistant lines and hybrids. Lines were evaluated for their BLB resistance, using a visual assessment of number and size of lesions along with a numeric count of lesions on leaf segments of a known area. Two lines 07-808 (a BC1F3) and 07-801(a BC2F3) were identified as being fixed for resistance because all plants in these populations were uniformly resistance in two greenhouse and two field inoculation trials when compared to the susceptible variety Festival. Regional trials in productions fields comparing 07-801, a F1 hybrid created using 07-801 as a male, the susceptible female and susceptible controls showed that the resistant line had significantly lower symptoms than the heterozygous resistant hybrid and susceptible controls. The heterozygous resistant hybrid also had moderate resistance showing significantly lower symptoms than the susceptible female and controls. Greenhouse chamber inoculated trials evaluated resistant line 07-801, the F1 hybrid created using 07-801 as a male,

the female used to create the hybrid and a susceptible control for resistance against varied strains of *B. squamosa* collected from different onion growing regions in New York. Across all isolated the resistant lines showed significantly lower BLB symptoms than susceptible varieties, and the heterozygous resistant lines were intermediate between homozygous resistant and susceptible controls. These results show that resistance to BLB derived from *A. roylei* is heritable and can be transferred into the backcross three level, and that this resistance controls various strains of BLB. Resistance is shown to be partial dominance because the hybrids heterozygous for the Bs1 resistant gene have an intermediate level of resistance between the homozygous resistant line and susceptible female.

1.2 Introduction

Onion (*Allium cepa*) is an important vegetable crop in the United States and worldwide. In 2009, *ca.* 103,960 acres of onions were harvested in the United States at an estimated value of nearly $473 million including the 10,000 acres harvested in New York, valued at $45.5 million (U.S. Department of Agriculture, 2009). Botrytis leaf blight (BLB) caused by *Botrytis squamosa* (Walker 1925) is an important foliar disease of onions in temperate regions around the world (Lacy and Pontius, 1983; Lorbeer, 1992; Lorbeer et. al, 2007; Shoemaker and Lorbeer, 1977; Sutton et. al, 1986). BLB is endemic to eastern Canada, New York and Michigan (Tremblay et. al, 2003); BLB epidemics can occur when moist conditions persist (Lorbeer and Andaloro, 1983; Lorbeer, 1992).

Primary infection of *B. squamosa* on onion leaves occurs from airborne conidia (asexual reproductive structures) produced by conidiophores growing on sprouting onions and leaf debris infected in the previous growing season as well as on sclerotia (compact masses of fungi) that over-winter in the soil (Ellerbrock and Lorbeer, 1977a; Ellerbrock and Lorbeer, 1977b; Lorbeer, 1983, 1992). The perfect apothecial stage of *B. squamosa,* by which sexual reproduction occurs and creates new strains of the fungus, also has been identified in New York (Bergquist and Lorbeer 1972; Ellerbrock and Lorbeer, 1977a). Multiple cycles of secondary inoculum, also in the form of airborne conidia, are produced throughout the growing season by conidiophores on blighted leaves (Lorbeer 1983, 1992, 2007) when leaf wetness is sustained for 12 hours and temperatures are between 8 and 22°C (Sutton et. al, 1983, 1984). The initial symptoms of BLB infection are lesions *ca.* 2 mm in diameter surrounded by a light green halo (Lorbeer et. al, 2007). In 3 to 5 days, these lesions become sunken and straw colored, and if moist conditions persist, blighting and significant leaf die-back occur in 5 to 12 days (Lorbeer et. al, 2007). Unchecked, BLB epidemics can drastically reduce marketable yields by stunting plant growth and bulb development (Alderman et. al, 1987; Lorbeer et. al, 2007).

Growers rely heavily on fungicide to control BLB. In the northeastern United States and southeastern Canada spraying occurs 6 to 14 times per season (Carisse and Willocquet, 2008; Lacy and Pontius, 1983; Tremblay et. al, 2003). In New York, onions rank the highest in pesticide use among vegetable crops (Anon, 1999). In New York and eastern Canada onions are mostly grown on muck soil of which limited acreage is available. Therefore, crop rotation, a significant tool for reducing disease pressure, is not usually practiced in large-scale onion production (Tremblay et. al, 2003). The development of fungicide resistant strains of *B. squamosa* is becoming a major concern due to this lack of crop rotation coupled with the high level of fungicide use (Tremblay et. al, 2003). The three classes of fungicides currently in use are benzonitriles, dicarboximids, and ethylene bis dithiocarbamates (EBDC) (Lorbeer and Vincelli, 1989; Carisse and Tremblay, 2007; Carisse and Willocquet, 2008; Tremblay et. al, 2003). Incidents of strains resistant to two forms of EBDC, iprodione, and vinclozin have been detected in the laboratory (Tremblay et. al, 2003) and strains resistant to dicarboximides have been isolated in fields in Quebec, Canada (Carisse and Tremblay, 2007). It can be expected that overuse of fungicides will cause more resistant strains to develop (Tremblay et. al, 2003), especially in New York where the perfect stage has been identified (Ellerbrock and Lorbeer, 1977a). To slow the development of resistant strains, it is recommended that anti-resistant integrated pest management (IPM) strategies be employed (Tremblay et. al, 2003).

Current IPM strategies focus on reducing sources of primary inoculum and forecasting epidemics to maximize efficacy of the fungicide sprays (Lorbeer, 1997; Lorbeer et. al, 2002). Primary inoculum is reduced by destroying cull piles, removing volunteer onions from fields, separating seed production fields from bulb production areas, and removing leaf debris after harvesting (Lorbeer and Andaloro, 1983; Lorbeer et. al, 2007). Several models have been developed to predict BLB epidemics leading to efficient fungicide applications (Lorbeer et. al, 2002). Blight-Alert in New York is based on a spray regime after a lesion threshold has been reached and lengthy periods of leaf moisture are expected (Vincelli and Lorbeer, 1987, 1988a, 1988b, 1989). Bot-Cast in eastern Canada is based on duration of leaf wetness (Sutton et. al, 1986). Predictive models in Michigan are based on expected conidia production (Lacy and Pontius, 1983). Further refinement of predictive models has recently focused on using qPCR for real-time quantification of airborne spore counts and its ability to predict outbreaks (Carisse et. al, 2009; Carisse and Willocquet, 2008).

Use of resistant cultivars is a major strategy of IPM that is easily integrated into horticultural practices. However, it is not currently being used in large-scale onion production because all onion cultivars are susceptible to BLB (Maude, 1990; Tremblay et. al, 2003). Previous research has identified strong resistance to BLB in *Allium roylei* (De Vries et. al, 1992b; Kik, 2002; Walters et. al, 1996). Interspecific hybridization and subsequent backcross breeding has been utilized in numerous crops including tomato, wheat, rice, cotton, and maize to expand the genome and introduce novel traits, including resistance genes from wild relatives (Kik, 2002). These techniques, however, have been underutilized in onion breeding (Chuda and Adamus, 2009; Kik, 2002). *Allium altaicum, Allium fistulosum, Allium galanthum, Allium oschaninii, A. roylei,* and *Allium vavilovii* are wild relatives that were identified as having potential for interspecific crossing with *A. cepa* because they share a common center of origin with *A. cepa* (Chuda and Adamus, 2009; Rabinowitch, 1997). Crosses between *A. cepa* and *A. roylei* have been reported by McCollum (1982) and Van der Meer and De Vries (1990) and crosses with *A. fistulosum* and *A. galanthum* have also been reported (Kik, 2002). Through the screening of backcross progenies, the resistance was determined to have high partial dominance controlled by a single gene (Bs1) (De Vries et. al, 1992b). Male and female sterility problems have been noted in interspecific crosses with *A. roylei* and other wild relatives, however, they can be overcome by subsequent backcrossing (Van der Meer and De Vries, 1990).

Onion (*Allium cepa*) BC1F3 and BC2F3 lines resistant BLB were produced by backcrossing the BLB resistance of the wild onion relative *Allium roylei* (the Bs1 resistance gene) into cultivated onion, *A. cepa* (Mutschler et al, in preparation). The goal of this work was to evaluate the lines to determine that these lines were homozygous for this BLB resistance, and to test the relative degrees of BLB control when this resistance is homozygous vs. heterozygous in resistant lines and hybrids which closely resemble cultivated onion under both inoculated field trials and field trials in New York commercial fields.

1.3 Materials and methods

Plant material.

The genotypes used for these trials were 07-808, 07-801, GAL-cms X 07-801, GAL-cms, Candy and Festival (Table 1.1). The lines 07-808 and 07-801 were F3 breeding lines expected to be homozygous for the Bs1, a resistance gene for the onion pathogen *B. squamosa.* The female line (GAL-cms) has male cytoplasm sterility derived from *Allium gallanthum* cytoplasm, the nuclear

Table 1.1. Onion lines created by backcrossing *A. cepa* onto interspecific F1 hybrid (*A. cepa* x *A. roylei*).

Entry[a]	Type	R/S[b]
07-808	BC1F3	Homozygous Bs1 resistant
07-801	BC2F3	Homozygous Bs1 resistant
GAL-cms X 07-801	BC3F1	Heterozygous Bs1 resistant
GAL-cms	*Onion line with A. galanthum* sterile cytoplasm	Susceptible
Candy	Seminis commercial F1 Hybrid	Highly susceptible
Festival	Bejo commercial F1 Hybrid	Susceptible

[a] Different experimental lines tested in field and chamber trials.
[b] Resistance or susceptibility of line.

genome of GAL-cam is that of the maintainer line B2215C (Havey, 1999). The entry GAL-cms X 07-801 is an F1 hybrid between using the female GAL-cms. Festival (BEJO ZADEN B.V, Warmenhuizen, The Netherlands) is a commercial yellow storage hybrid variety commonly grown in New York. Candy (Seminis Vegetable Seeds, Inc. Saint Louis, MO) is a mild hybrid that was used as a highly susceptible control.

Onions seedlings used for summer trials were sown in March in Metro mix 200 (The Scotts Company LLC, Marysville, OH) in 288 cell trays, 3.8 cm deep (model 720532, T.O. Plastics, Clearwater, MN) and transplanted into the field *ca.* eight weeks later in May. For the first week while the seeds were germinating the greenhouse temperature was set at 22C. Thereafter, it was set at 18C and 13C during the day and night respectively. The seedlings were fertilized with 67% m/v (67g per 100L water) 15-5-15 water soluble fertilizer. Seedlings were hardened off in an outside cold frame, without fertilizer application ca. 12 days prior to transplanting. If nighttime temperatures dropped below 0C a clear plastic cover was placed over the seedlings and removed in the morning.

Botrytis squamosa strains used for inoculations.

Five strains of *B. squamosa* collected from commercial field in different counties in New York were used for inoculations (Table 1.2). MD16 was collected in 1990 and identified as an exceptionally virulent strain (Walters et al. 1996), therefore, MD16 was used for all inoculated field and greenhouse trials. Isolates ELMT2, OSMT4, WCSS5 and OCMT1 isolated in 2009 were used in chamber evaluations in 2010.

Table 1.2. *Botrytis squamosa* isolates collected in New York and used for inoculations.

Isolate[a]	Location Collected	Reference	Year Collected
MD16	Orange County, NY	Walters et al. 1996	1990
ELMT2	Genesee County, NY	Current publication	2009
OSMT4	Oswego County, NY	Current publication	2009
WCSS5	Wayne County, NY	Current publication	2009
OCMT1	Orange County, NY	Current publication	2009

[a] Collection culture code *B. squamosa* strains.

Culture media.

Botrytis squamosa cultures were maintained on potato dextrose agar (PDA) as described by Walters et al. (1996). Acidified PDA (APDA) contained 0.1% lactic acid. Inoculum was produced in potato dextrose broth (PDB) as described by Walters et al. (1996).

B. squamosa *isolation from mycelia growth in onion leaf tissue.*

Onion leaves with BLB lesions from natural infection in commercial fields cut from the plants ca. 15 cm sections and placed into sterile sealable plastic vials for transport and storage. Under sterile conditions 5 x 5 mm sections of leaf containing a lesion were cut from the leaf. Sections were surface disinfected in a 0.3 w/v sodium hypochlorite solution for three minutes and then sections rinsed in dH2O laid on a sterile surface and allowed to dry for ten minutes. The sections later were plated on acidified PDA and incubated at 20C with 14:10 day:night lighting. Hyphal tips were subcultured 1-2 days after incubation and transferred to new APDA. Subcultures were incubated for 2 weeks under the conditions previously stated and then used to prepare inoculum for plant inoculations. Isolates were identified visually as *B.*

squamosa using the identifying characteristics outlined in Chilvers and du Toit (2006).

Inoculum preparation.

Under sterile conditions a 5 x 5mm plug was cut from the PDA and transferred to PDB and incubated at 20C for 14 days under 14:10 day:night. The mycelial mat that formed after incubation was used for inoculum preparation, however, due to weather constraints for inoculation of onion plants, such as rain and temperature, mats were used between 12 and 16 days.

Mycelial mates were separated from PDB and rinsed two times in dH2O for 5 seconds each. Inoculum was prepared by disposing of the liquid media in each flask and rinsing the mycelial mats in the flasks two times with dH2O. Two mats were placed in a blender (Waring products corporation, model DS-7, Winsted, CT) with 200 ml dH2O and blended for one minute on high. The solution was filtered through two layers of cheese cloth. The solution was blended and filtered a second time as stated previously and distilled H2O was added to adjust the optical density to OD_{450}=0.70 and measured using a spectrophotometer. One drop of Tween 20 was added for every 200 ml of inoculum prepared to assists in spreading the mycelial fragments evenly on the leaves. Plants were inoculated by spraying to evenly coat the leaves with inoculum as described by Walters et al. (1996).

Inoculated field trials.

Field trials were laid out in a complete randomized block design. There were four replications with each entry planted with 15 or 18 plants per row. These rows were randomized within each replicated block. Field inoculations in 2007, 2008, and 2009 were conducted with *B. squamosa* isolate MD16 (Table 1.2).

The field used for all BLB inoculations was prepared with 565 kg per hectare (500 lb per acre) 20-10-10 fertilizer and irrigated as needed. Radiant™ SC (spinetoram) (Dow AgroSciences LLC, Indianapolis, IN) was used to control onion thrips (*Thrips tabaci*) as needed. Weeds were controlled by hand cultivation. No fungicides were applied.

Seedlings were transplanted in rows with 7.6 cm spacing between seedling and 0.6 m spacing between rows. To maintain leaf moisture during inoculations misting lines were placed down every other rows. Misting lines had nozzles (Baumac, model FN-1018-12, Hummert International, East City, Mo.) every 1.2 m at a height of 0.6 m, thereby, creating a grid with misting nozzles evenly spaced 1.2 meters in each direction, ensuring uniform leaf wetness.

Field inoculations were conducted in mid July 2007, 2008, and 2009, *ca.* 10 weeks after the seedlings were transplanted. The day and time of inoculation was chosen based on weather conditions in order to maximize disease because the optimal growth conditions for *B. squamosa* are below 20C. Therefore, inoculations were attempted when overnight temperatures were predicted to be between 16-20C on the day of inoculation.

The misting system was run from 7-12 pm for 4 nights after inoculating to increase leaf moisture and promote fungal growth and infection. A timer was used to run the misters for 2 minutes on and 3 minutes off to keep the leaves lightly coated with water, but extreme caution was taken to not apply excess water that could wash off the inoculum. Slight adjustments were made to compensate for weather conditions. Visual ratings were conducted one week after inoculation. If the disease development was poor one week after inoculation the plants were re-inoculate before rating.

Chamber inoculations.

Onion plants in the chamber trials during 2008 and 2009 were inoculated with *B. squamosa* isolate MD16 (Table 2). Plants were placed in a randomized order within the chamber, however, these populations were not large enough to permit a randomized block design.

The chamber trial in 2010 contained onion lines 07-801, GAL-cms X 07-801, GAL-cms, and Candy and were inoculated with *B. squamosa* strains MD16, ELMT2, OSMT4, WCSS5 and OCMT1. The four genotypes and five isolates were evaluated in a complete randomized design with 3 replications.

Chamber inoculations were preformed on plants grown from bulbs harvested from the summer field trials. The bulbs were stored at 4C and re-grown in 1 gallon pots filled with Cornell mix (Boodley and Sheldrake 1972) in the greenhouse for 6-8 weeks starting in December and January for inoculation in February and March, respectively.

The mist chamber was constructed of clear plastic with 4 humidifiers (model 707SM; Herrmidifier Co., Lancaster, Pa.) placed inside to create a uniform distribution of moisture. Humidifiers were placed on a timer which was adjusted to create an atmosphere of 100% humidity while not allowing excess moisture to condense on leaves and create run-off. Timing of the humidifiers varied greatly due to chamber volume, relative humidity, and air temperature. Prior to inoculation the leaves were wiped with non-absorbent cotton (Absorbent Cotton Co., Valley Park, MI) to remove leaf wax and the soil was watered heavily so that the plants would not need additional watering during the trial. The plants were placed in the chamber with the humidifiers already running to ensure that the leaves did not dry out after the plants were inoculated. Plants were removed from the chamber and leaves were air dried 7 days after inoculation and the visual evaluation and the number of lesion per cm^2 were evaluated as described in the lesion evaluation section.

Mini-plot trials.

Mini-plot trials in 2009 evaluated onion lines 07-801, 07-808, GAL-cms X 07-801, GAL-cms, Festival and Candy in a complete randomized block design with 3 replications. Rows were at 30 cm spacing and plants were transplanted at 7.6 cm spacing. A total of 15 plants were evaluated per row. The mini-plots in Sodus and Oswego on muck soil, were grown on conventionally managed onion farms, without the use of fungicides. Onions in the mini-plot in Elba on upland soil, were grown on a certified USDA organic farm, among seven acres of production onions and managed by the grower in the same manner as the rest of the onion field. Onions in the inoculated mini-plot in Cornell's East Ithaca research farm on sandy loam, were inoculated with MD16, and managed as previously described in *Inoculated field trials*.

Lesion evaluations.

A lesion size rating (0-5) and lesion count rating (0-5) on the entire plant was conducted (Table 1.3) (Figure 1.1). These ratings were added together to produce one 0-10 visual rating of plant resistance. The oldest leaves, that were not dead or dying, were sampled by cutting three 15 cm long sections of leaf. All the lesions were counted and lesions per cm^2 were calculated.

Table 1.3. Visual ratings scale of lesion count and size, for visual evaluation of BLB symptoms on onion leaves.

Rating	Count [a]	Size	
	Ave. lesion per leaf	Ave. Size [b]	Description [c]
0	0-10	ca. 0.1mm	hypersensitive type
1	11-20	ca. 0.5 mm	not sunken
2	21-40	ca. 1.0 mm	sunken straw colored center
3	41-80	ca. 2.0 mm	cracked, white halo present
4	81-160	ca.10 mm	grayish blighted areas
5	160-320	>12 mm	large blighted sections

[a] Estimated number of lesions per leaf averaged across entire plant.
[b] Estimated average diameter of lesions.
[c] Visual description of lesions.

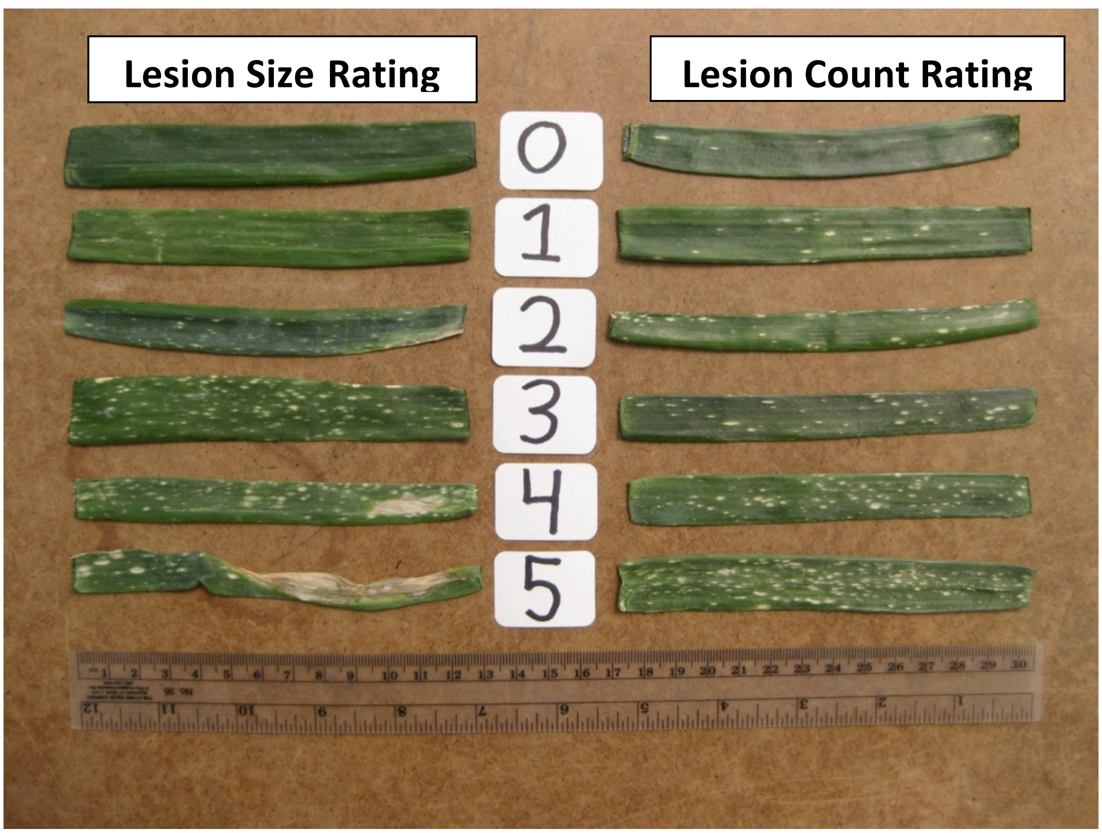

Figure 1.1. Samples of lesion size rating and lesion count rating.

Data analysis.

Means were compared with Tukey–Kramer HSD test using the generalized linear modeling procedures of JMP® (SAS Institute Inc., Cary, NC) to account for replication and location variation.

1.4 Results

Inoculated field and chamber trial.

Inoculated trials in the field and chamber were used to identify lines homozygous and uniform for resistance to BLB. Two lines which consistently showed resistance to BLB were used for hybrid production and trials. The lines 07-808 and 07-801 were evaluated two times each in the field and chamber in which they had significantly lower visual ratings and lesions per cm^2 than that of the susceptible control Festival (Table 1.4). Disease pressure is typically higher in the chamber than field. The visual rating for Festival in the chamber averaged 6.9 while in the field it averaged 2.5. The visual rating for 07-808 averaged 1.15 in the field and 0.70 in the chamber, while the visual rating for 07-801 averaged 0.59 in the field and 0.55 in the chamber.

Table 1.4. Field and chamber mean visual rating and mean lesion per cm^2 of lines 07-808 and 07-801 fixed for BLB resistance and susceptible variety Festival.

	Field 2007		Chamber 2008		
Pedigree	No.	Visual Rating	No.	Lesion per cm^2	Visual Rating
Festival	70	2.39 a[a]	15	0.69 a	7.07 a
07-808	63	0.87 b	11	0.05 b	1.59 b
07-801	68	0.79 b	14	0.05 b	0.61 b
	Field 2008		Chamber 2009		
Pedigree	No.	Visual Rating	No.	Lesion per cm^2	Visual Rating
Festival	55	2.58 a	7	0.42 a	6.57 a
07-808	30	0.37 b	5	0.11 b	0.20 b
07-801	55	0.35 b	7	0.05 b	0.43 b

[a] Means followed by the same letter are not different by Tukey HSD ($P \leq 0.05$)

Lesion per cm^2 of Festival in 2008 and 2009 was 0.69 and 0.42, respectively. Lesion per cm^2 of the BLB resistant BC1F3 line 07-808 was 0.05 and 0.11 in 2008 and 2009, respectively, which were significantly lower than Festival in both years. Lesion per cm^2 of the BLB resistant BC2F3 line 07-801 was 0.05 in both 2008 and 2009, which were significantly lower than the lesions per cm^2 Festival in both years, and not significantly different than the lesions per cm^2 the line 07-808 in 2008 and 2009. The two BLB resistant lines 07-801 and 07-808 had similar levels of resistance.

Mini-plots 2009.

Weather during 2009 was exceptionally conducive to BLB infection. The levels of disease pressure varied between locations. Sodus and Oswego, the two large scale conventional farms, had similar and the highest levels of infection and blighting. The inoculated field in East Ithaca had a moderate level and the organic field in Elba had the lowest level of infection and blighting.

When assessing all locations combined for mean visual rating, the ratings of the two susceptible cultivars Candy (at 5.6) and Festival (at 5.5), were significantly higher than that of the other genotypes (Table 1.5). GAL-cms had a mean visual rating of 4.3, GAL-cms x 07-801 had a mean visual rating of 3.0 and 07-801 had a mean visual rating of 1.5; all of these means were significantly different.

Genotypes in individual mini-plots had a similar pattern of resistance among the genotypes for combined evaluations, however, much of the significance between genotypes was lost, probably due in some extent to lowered numbers of plants evaluated in the mini-plots.

Table 1.5. Visual ratings of homozygous resistant line 07-801, heterozygous resistant F1 GAL-cms x 07-801, susceptible female GAL-cms, and susceptible control cultivars Candy and Festival at four separate locations and combined.

Location	Pedigree	No.	Visual Rating
All Mini-plots	Candy	174	5.6 a[a]
	Festival	194	5.3 a
	GAL-cms	173	4.3 b
	GAL-cms x 07-801	186	3.0 c
	07-801	183	1.5 d
Sodus	Candy	33	6.5 a
	Festival	46	6.7 a
	GAL-cms x 07-801	41	4.3 b
	GAL-cms	38	4.1 b
	07-801	37	2.7 c
Oswego	Candy	41	6.6 a
	Festival	45	6.8 a
	GAL-cms	38	6.2 ab
	GAL-cms x 07-801	40	5.5 b
	07-801	44	2.2 c
East Ithaca	Candy	55	5.2 a
	Festival	57	4.0 a
	GAL-cms	51	3.6 a
	GAL-cms x 07-801	58	2.0 b
	07-801	58	0.7 c
Elba	Candy	45	4.4 a
	Festival	46	4.3 a
	GAL-cms	46	3.6 a
	GAL-cms x 07-801	47	1.0 b
	07-801	44	1.0 b

[a]Means followed by the same letter are not different by Tukey HSD ($P \leq 0.05$)

Chamber isolate trial.

The average lesions per cm^2 among plant entries, averaged across all isolates, varied from 24.3 to 7.3 (Table 1.6). Across isolates the average lesions per cm^2 of the heterozygous and homozygous resistant lines were significantly different from both controls (GAL-cms and Candy), which were also significantly different from each other. However, the average lesions per cm^2 of the heterozygous and homozygous resistant lines were not significantly different from each other.

The visual ratings among plant entries averaged across all isolates varied from 8.02 to 4.28 (Table 1.6). There were significant differences for visual rating among all four plant entries. The visual rating of the heterozygous lines were both intermediate between that of the two parent lines GAL-cms and 07-801, and the visual rating of the heterozygous F1 hybrid and homozygous resistant line 07-801 were different, significantly.

When compared across all plant entries, there was little indication of significance among isolates (analysis not shown). The greatest disease was always seen in Candy, with lesions per cm^2 of 18.7 to 27.6 and visual ratings of 7.2 to 8.4. The least disease was always seen in the homozygous resistant line 07-801, with lesions per cm^2 of 9.7 to 4.5 and visual ratings of 5.32 to 3.00.

Table 1.6. Chamber analysis of homozygous resistant, heterozygous resistant, and susceptible lines against different Isolates 2010.

Isolate	Pedigree	No.	Lesion per cm^2	Visual Rating
All Isolates	Candy	60	24.3 a[a]	8.02 a
	GAL-cms	60	17.2 b	6.98 b
	GAL-cms x 07-801	60	9.9 c	5.50 c
	07-801	60	7.3 c	4.28 d
ELMT2	Candy	12	25.4 a	7.92 a
	GAL-cms	12	17.7 ab	7.58 a
	GAL-cms x 07-801	12	10.1 b	6.08 a
	07-801	12	9.7 b	5.33 b
MD16	Candy	12	27.6 a	8.42 a
	GAL-cms	12	18.3 ab	7.33 ab
	GAL-cms x 07-801	12	11.9 bc	4.92 bc
	07-801	12	7.9 c	3.92 c
OSMT4	Candy	12	23.3 a	8.17 a
	GAL-cms	12	16.6 ab	6.67 ab
	GAL-cms x 07-801	12	11.0 b	5.67 b
	07-801	12	8.9 b	5.75 b
WCSS5	Candy	12	26.3 a	8.33 a
	GAL-cms	12	16.6 b	6.92 ab
	GAL-cms x 07-801	12	10.5 b	6.33 b
	07-801	12	4.5 c	3.42 c
OCMT1	Candy	12	18.7 a	7.25 a
	GAL-cms	12	17.1 a	6.42 a
	GAL-cms x 07-801	12	6.2 b	4.50 b
	07-801	12	5.2 b	3.00 c

[a]Means followed by the same letter are not different by Tukey HSD ($P \leq 0.05$)

1.5 Discussion

Natural infection of *B. squamosa* occurs via conidia, however, sporulation of wild strains under laboratory conditions is too unpredictable for proper timing of inoculations (Lorbeer, 1997). Mycelial fragment inoculation shows disease development and symptoms identical to those seen in commercial fields (Lorbeer, 1992) and allows for evaluation with a broader diversity of *B. squamosa* than only using strains selected for sporulation under laboratory condition (Lorbeer, 1997). Therefore, mycelia fragments were used for inoculations.

In all four inoculated trials lines 07-808 and 07-801 had significantly lower visual rating and lesions per cm^2 than the susceptible control. This indicates that these lines are fixed for BLB resistance and all individuals in the population are homozygous for the Bs1gene conferring BLB resistance. The fixed status of these lines was further confirmed in the mini-plot evacuation in 2009 and the chamber trial in 2010. Line 07-801 consistently had a significantly lower visual rating than the both GAL-cms and GAL-cms x 07-801. The chamber isolate trial confirmed that resistance holds up to different strains of *Botrytis squamosa*, collected from four different growing regions in New York.

The resistance gene Bs1 was identified as partially dominant because GAL-cms x 07-801 was intermediate between GAL-cms and 07-801 in BLB symptoms. This indicates that breeding efforts should be directed to create homozygous male and female lines to produce hybrids varieties with the maximal level of resistance. The organic field in Elba was one location where there was not a significant difference between GAL-cms x 07-801 and 07-801. This field had lower disease pressure than the other mini-plots indicating that, under low disease pressure, heterozygous resistance might provide sufficient control to minimize yield reductions.

Resistance to downy mildew caused by *Peronospora destructor* was identified in *A. roylei* as two weakly linked genes, Pd1 and Pd2, and has been transferred into cultivated onion (De Vries et. al, 1992a; Kofoet et. al, 1990; Scholten et. al, 2007). This transfer is seen as a large step in reducing fungicide use and subsequent environmental impacts (Scholten et. al, 2007). The utilization of onions resistant to BLB, similar to downy mildew resistance, in conjunction with current IPM strategies and predictive models, could aid in reducing the use of fungicide sprays. This would reduce the risk of developing *Botrytis squamosa* strains resistant to fungicides. These factors indicate the need for further development of commercial varieties expressing resistance toward BLB. Furthermore, onion varieties resistant to BLB could be grown using fungicides with a lower environmental impact quotients (EIQ) (a measure of pesticide risk to farmworkers, consumers, ground water, and non-human biota (Kovach et. al, 1992)), and have the same level of disease suppression. This production practice would be applicable for use in both conventional and organic production. Further evaluations should focus on evaluating how resistant varieties could change the current spray thresholds and current spray regimes.

Partial or complete resistance can lead to a reduction in pesticide use (Chuda and Adamus, 2009) which is desirable because of the increased socio-environmental pressure against pesticides and the need to decrease the chance of strains of *B. squamosa* resistant to fungicides from developing (Tremblay et. al, 2003). There are no previous reports of BLB resistant onions in higher level backcross lines. The results presented indicate that the incorporation of BLB resistance via the Bs1 gene into commercial varieties would reduce the use of fungicide and decrease yield loss due to BLB.

REFERENCE

Alderman, S. C., J. A. Biernbaum, M. Hoffhines, D. M. Timberlake, D. P. Welch, M. L. Lacy and R. O. Barr. 1987. A simulation model of the spread of *Botrytis* leaf blight of onion and its effect on onion yields. Agriculture, Ecosystems & Environment 19:55-70.

Anonymous. 1999. Agricultural chemical usage: 1998 Vegetable summary. New York state agricultural statistics service. Albany, NY. 48 pp

Bergquist, R. R. and J. W. Lorbeer (1972) Apothecial production, compatibility and sex in *Botryotinia squamosa*. Mycologia 64:1270-1281.

Boodley, J. W., and Sheldrake, R. 1972. Cornell peat-lite mixes for commercial plant growing. Cornell University Cooperative Extension Division Informational Bulletin 43.3.

Carisse, O., D. M. Tremblay, C. A. Lévesque, K. Gindro, P. Ward and A. Houde. 2009. Development of a TaqMan Real-Time PCR Assay for Quantification of Airborne Conidia of Botrytis squamosa and Management of Botrytis Leaf Blight of Onion. Phytopathology 99:1273-1280.

Carisse, O., and D.M. Tremblay. 2007. Incidence and significance of iprodione-insensitive isolates of *Botrytis squamosa*. Plant Disease 91:41-46.

Carisse, O., S. Savary, and L. Willocquet. 2008. Spatiotemporal relationships between disease development and airborne inoculum in unmanaged and managed botrytis leaf blight epidemics. Phytopathology 98:38-44.

Chilvers, M.I., and L.J. du Toit. 2006. Detection and identification of *Botrytis* species associated with neck rot, scape blight, and umbel blight of onion. Online. Plant Health Progress doi:10.1094/PHP-2006-1127-01-DG.

Chuda, A. and A. Adamus. 2009. Aspects of interspecific hybridization within edible Alliaceae. Acta Plant Physiology 31:223–227.

De Vries, J.N., W.A. Wietsma and M.C. Jongerius 1992a. Linkage of downy mildew resistance genes Pd1 and Pd2 from *Allium roylei* Stearn in progeny of its interspecific hybrid with onion (*A.cepa*). Euphytica 64:131–137.

De Vries, J.N., W.A. Wietsma and M.C. Jongerius. 1992b. Introgression of leaf blight resistance from *A. roylei* Stearn into onion (*A.cepa* L.). Euphytica 62:127–13.

Ellerbrock, L.A. and J.W. Lorbeer. 1977a. Sources of primary inoculum of *Botrytis squamosa*. Phytopathology 67:363-372.

Ellerbrock, L.A. and J.W. Lorbeer. 1977b. Survival of sclerotia and conidia of *Botrytis squamosa*. Phytopathology 67: 219-225.

Havey, M. J. 1999. Seed yield, floral morphology, and lack of male-fertility restoration of male-sterile onion (*Allium cepa*) populations possessing the cytoplasm of *Allium galanthum.* Journal of American Society of Horticultural Science. 124:626–629.

Kik, C. 2002. Exploitation of wild relatives for the breeding of cultivated Allium species, p 145-158. In: H.D. Rabinowitch, L. Currah (eds.), Allium Crop Science: Recent Advances. CABI Publishing, Wallingford, UK.

Kofoet, A., C. Kik, W.A. Wietsma and J.N. de Vries. 1990. Inheritance of resistance to downy mildew from *Allium roylei* Stearn in the backcross *Allium cepa* L. x (*A. roylei* x *A. cepa*). Plant Breeding 105:144–149.

Kovach, J., C. Petzoldt, C. Degni, and J. Tette. 1992. A method to measure the environmental impact of pesticides. New York Agricultural Experiment Station, Geneva, New York's Food and Life Science Bulletin 139. Cornell University, Ithaca, NY, 8 pp.

Lacy, M. L. and G.A. Pontius. 1983. Prediction of weather-mediated release of conidia of *Botrytis squamosa* from onion leaves in the fields. Phytopathology 73:670-676.

Lorbeer , J.W. and J. Andaloro. 1983. Diseases of onion. Botrytis leaf blight, Vegetable Crops. New York State Agricultural Experimental Station, NY. 737 pp. http://www.nysipm.cornell.edu/factsheets/vegetables/ onion/blb.pdf

Lorbeer, J.W. and P.C. Vincelli. 1990. Efficacy of dicarboximide fungicides and fungicide combinations for control of Botrytis leaf blight of onion in New York. Plant Disease 74:235-237.

Lorbeer, J.W. 1992. Botrytis leaf blight of onion. pp. 186-211. In: Chaube H., J. Kumar, A. Mukhopadhyay and U. Singh(eds.), Plant Diseases of International Importance. Volume II. Diseases of Vegetables and Oil Seed Crops. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

Lorbeer , J.W. 1997. Management of diseases in Alliums. Acta Horticulturae 433: 585-591.

Lorbeer , J.W., T. Kuhar, and M. Hoffman 2002. Monitoring and forecasting for disease and insect attack in onions and *Allium* crops within IPM strategies, p. 293-309. In: H.D. Rabinowitch, L. Currah (eds.), Allium Crop Science: Recent Advances. CABI Publishing, Wallingford, UK.

Lorbeer , J.W., A.M. Seyb, M. de Boer and J. E. van den Ende. 2007. p. 273-294. In: Elad Y., B. Williamson, P. Tudzynski and N. Delen (eds.), Botrytis: Biology, Pathology and Control In: Botrytis Species on Bulb Crops. Springer Netherlands.

McCollum, G.D. 1982. Experimental hybrids between *Allium fistulosum* and *A. roylei.* Botanical Gazette 143:238–242.

Maude, R.B. 1990. Leaf diseases of onions. p. 173-189. In: Rabinowitch H.D. and J .L. Brewster (eds.), Onions and allied crops, Vol. II. CRC Press, Inc., Boca Raton, Florida.

Rabinowitch, H.D. 1997. Breeding alliaceous crops for pest resistance. Acta Horticulturae 433:223–246.

Scholten, O.E., A.W. van Heusden, L.I. Khrustaleva, K. Burger-Meijer, R.A. Mank, R.G.C. Antonise, J.L. Harrewijn, W. van Haecke, E.H. Oost, R.J. Peters, and C. Kik. 2007. The long and winding road leading to the successful introgression of downy mildew resistance into onion. Euphytica 156:345–353.

Shoemaker , P.B., and J.W. Lorbeer. 1977. Timing initial fungicide application to control *Botrytis leaf* blight epidemics on onions. Phytopathology 67:409-414.

Sutton, J.C., T.D.W. James and P.M. Rowell. 1983. Relation of weather and host factors to an epidemic of Botrytis leaf blight in onions. Canadian Journal of Plant Pathology 5:256-265.

Sutton, J.C., T.D.W. James and P.M. Rowell. 1986. Botcast: a forecasting system to time the initial fungicide spray for managing Botrytis leaf blight of onions. Agriculture, Ecosystems and Environment 18:123-144.

Sutton, J.C., P.M. Rowell and T.D.W. James. 1984. Effects of leaf wax, wetness duration and temperature on infection of onion leaves by *Botrytis squamosa*. Phytoprotection 65:65-68.

Tremblay, D. M., B.G. Talbot, and O. Carisse. 2003. Sensitivity of *Botrytis squamosa* to different classes of fungicides. Plant Disease 87:573-578.

U.S. Department of Agriculture. 2009. National agricultural statistics service. Washington, D.C. 1 May 2010. <http://www.nass.usda.gov/>

Van der Meer, Q.P., and J.N. De Vries. 1990. An interspecific cross between *Allium roylei* Stearn and *Allium cepa* L., and its backcross to *A. cepa*. Euphytica 47:29–31.

Vincelli, P. C. and Lorbeer, J. W. 1987. Sequential sampling plan for timing initial fungicide application to control Botrytis leaf blight of onion. Phytopathology 77:1301-1303.

Vincelli, P. C. and Lorbeer, J. W. 1988a. Forecasting spore episodes of *Botrytis squamosa* in commercial onion field in New York. Phytopathology 78:1078-1982.

Vincelli, P. C. and Lorbeer, J. W. 1988b. Relationship of precipitation probability to infection potential of *Botrytis squamosa* on onion. Phytopathology 78:1078-1982.

Vincelli, P. C., and J.W. Lorbeer. 1989. BLIGHT-ALERT: A weather-based predictive system for timing fungicide applications on onion before infection periods of *Botrytis squamosa*. Phytopathology 79:493-498.

Walker, J. C. 1925. Two undescribed species of *Botrytis* associated with the neck rot diseases of onion bulbs. Phytopathology 15:708-713.

Walters, T.W., L.A. Ellerbrock, J.J. van der Heide, J.W. Lorbeer and D.P. LoParco. 1996. Field and greenhouse procedures to evaluate onions for botrytis leaf blight resistance. HortScience 31:436-438.

CHAPTER 2

EVALUATION OF DOUBLED HAPLOID ONION LINES, AND THEIR USE IN HYBRID COMBINATION

Abstract

Doubled haploid (DH) onions lines were produced from diverse material in development within the Cornell onion breeding program (Alan et al., 2003, 2004). Twenty of these DH lines were evaluated to compare their vegetative productivity with their sources, and with a commercial hybrid. Sixteen of these DH lines were used to test the effect of using DH onion lines vs. conventional male inbred lines as male parents in hybrid production. To do this experimental DH hybrids were created using DH lines as males, and were compared with current commercial onion hybrids that share the same female parent. The vegetative vigor, of the DH lines, measured by row weight and average bulb weight, was comparable to that of the source varieties, showing minimal if any inbreeding depression. Hybrids using DH lines as males were either not significantly different or significantly greater in vegetative vigor compared to traditionally hybrids with the same female parent. The vegetative vigor of DH lines and in hybrid combinations is attributed to selection of cells without deleterious sub-lethal genes during gynogenesis. Evaluation of bulb quality traits showed that DH lines are comparable to commercially hybrids. These results suggest that doubled haploid lines could both speed up the process of inbred line development and could also create higher yielding varieties of acceptable horticultural quality.

Introduction

Breeding of dry storage onion (*Allium cepa*) for the last 60 years has largely focused on production of single cross hybrid varieties. Single cross hybrids are created by crossing two inbred lines, with the female line being a cytoplasmic male sterile line to insure that the seed produced is hybrid rather than a mixture of hybrid and selfed seed of the female parent. The major shift from open pollinated varieties to hybrid varieties occurred in the 1950s after reports by Jones and Clark (1943) and Jones and Davis (1944) outlined how the cytoplasmic male sterility system allowed large scale production of hybrid seed, and the significant increase in vegetative vigor of the resulting hybrids over standard open pollinated onion varieties. The increased hybrid vigor of hybrids leads to greater productivity, which in turn lead to greater marketable yields. Rapid adaptation of hybrids by onion growers ensued due to this increased yield and superior uniformity of horticultural traits in the hybrids. Numerous reports have addressed the benefits of hybrid onions (Dowker and Gordon, 1983; Evoor, 2007; Hosfield et al., 1977; Joshi and Tandon, 1976).

Commercially acceptable hybrid varieties must be uniform for critical traits such as days to maturity, bulb shape, and pungency. This uniformity is achieved by increasing the homozygosity of the inbred parent lines. However, as with most cross pollinated crops, onions suffer from severe inbreeding depression when self pollinated for several generations (Bohanec, 2002). This limits the cycles of self pollination that are possible while also maintaining lines that are vigorous enough to produce hybrid seed on the large scale required for commercial sales (Bohanec, 2002). The inability to repetitively self-pollinate a line greatly reduces the ability to create uniform inbred lines (Alan et al., 2004; Bohanec, 2002). Any heterozygosity and heterogeneity of the inbred parent lines leads to some degree of non-uniformity of the hybrids (Alan et al., 2004) and can also impede hybrids from reaching their maximal heterotic potential (Bohanec, 2002).

Biological constraints of the onion create an exceptionally lengthy development period for inbred lines. The biennial nature of the onion and requirements of bulb vernalization prior to flowering push the seed to seed generation time of onion to 2 years; therefore, typical selection practices to develop inbred lines using the single-seed-descent method take 10 to 12 years (Bohanec, 2002). The time to develop double haploid (DH) lines, which are completely homozygous, can be much quicker and cost effective than conventional breeding procedures (Alan et al., 2003, 2004; Bohanec, 2002; Röber et al., 2005).

DH onion lines were produced from diverse material in development within the Cornell onion breeding program, through a cooperative project with Dr. Lisa Earle (Alan et al., 2003, 2004). The objectives of the current study were to evaluate 20 of these DH onion lines for their ability to produce high quality hybrids to be used in commercial onion production. The initial set of DH lines was evaluated in 2006 and 2008 to compare their vegetative productivity with their sources, two different Cornell YIX lines, and with a commercial hybrid. A subset of these DH lines was used to test the effect of using DH onion lines vs. conventional male inbred lines as male parents in hybrid production. Experimental DH hybrids were evaluated by comparing them to current commercial onion hybrids that share the same female parent as the 20 DH derived hybrids. These trials were intended to determine the impact of using DH lines as males on the performance of onion hybrids both in terms of vegetative vigor measured by row weight, and average bulb weight.

Material and Methods

Plant material

Four DH lines from the Cornell open pollinated developing line YIX C, and 16 from the Cornell open pollinated developing line YIX E were generated through gynogenesis (Alan et al., 2003, 2004). Nineteen of these DH onion lines were tested in the 2006 trial and 14 were tested in the 2008 trial, eliminating 5 weaker lines (Table 2.1). The controls for the DH line trials were Festival, a commercially hybrid commonly grown in New York, as well as YIX E and YIX C, the two the open pollinated lines used in the development of the DH onion lines.

Table 2.1. Doubled haploid lines and hybrids in respective trials.

Doubled Haploid Line	From	Line Trial		Hybrid Trial			
		2006 Line	2008 Line	2008 Am	2008 AmSp	2009 Am	2009 AmSp
DH CU 066600	YIX E	X	X	X	X	X	X
DH CU 066601	YIX E	X	X	X	X	X	X
DH CU 066604	YIX E	X	X	X	X	X	X
DH CU 066607	YIX E	X					
DH CU 066608	YIX E	X					
DH CU 066613	YIX E	X		X	X	X	X
DH CU 066614	YIX E		X	X	X		
DH CU 066615	YIX E	X	X	X	X	X	X
DH CU 066616	YIX E	X	X	X	X		
DH CU 066619	YIX E	X	X	X	X	X	X
DH CU 066627	YIX E	X	X	X		X	
DH CU 066628	YIX E	X	X	X			
DH CU 066630	YIX E	X	X	X		X	
DH CU 066634	YIX E	X	X	X		X	
DH CU 066635	YIX E	X	X	X		X	
DH CU 066637	YIX E	X	X	X		X	
DH CU 066612	YIX C	X					
DH CU 066621	YIX C	X	X	X	X		
DH CU 066631	YIX C	X	X	X		X	
DH CU 066633	YIX C	X					

The DH hybrids evaluated in 2008 and 2009 were created using the DH lines as males and one or both of two sterile lines as the female parent. One sterile female was a American long storage pungent onion female (Am type) more typical Northeast pungent long day onion. Twelve of the experimental DH hybrids were produced using this Am type female, which is also the female parent of the Am type pungent storage commercial onion hybrids Safrane and Crockett (Bejo Zaden B.V, Warmenhuizen, The Netherlands). The other female was an American Spanish style onion line (AmSp type). Six of the experimental DH hybrids were produced using this AmSp female, which is also the female parent of the American Spanish-type commercial onion control hybrids Medeo and Calibra (Bejo Zaden B.V, Warmenhuizen, The Netherlands).

Seeds for direct sown trials in 2008 and 2009 were treated prior to sowing to control onion maggot and onion smut. The seeds were coated with Raxcil 2.6 F (tebuconazole) 250 mg /100g seed, Thiram 42 S (thiram) 188 mg/100g seed, Mundial (fipronil) 2.5 g/100g seed and the binding agent DISCO at a 1:1 ratio with control agents. This mixture was applied at 150 micro-liters per 250 seeds

Doubled haploid trial 2006.

The 2006 trial evaluated DH onion lines and the cultivars used to produce them. Seeds were sown on March 28, 2006, grown under greenhouse conditions in 288 cell plug trays filled with Cornell mix (Boodley and Sheldrake 1972) until *ca.* 7 weeks of age. Plants then were hardened off for several days in a cold frame and transplanted on May 15, 2006 to the mineral soil field plots at the Cornell East Ithaca farm, Ithaca, NY using a complete randomized block design. Seed limitations that year restricted plantings of each genotype to four 18 plant replications in a complete randomized block design.

Bulbs were lifted on September 15, 2006. The bulbs were dried for 2 weeks, weighed after room temperature curing, and put into cold storage. Grading was performed as described below, with the first grading for yield on October 10, 2006, and the second grading for storability on January 9, 2007.

Doubled haploid and DH hybrid trials in 2008.

The 2008 trial of DH lines was grown at Triple G farms in Elba, NY, in a plot embedded within this commercial onion field. The trial consisted of 15 DH lines and the control line Festival in a complete randomized block with three replications. Each entry was represented by one 20 foot row directly sown with 200 seeds in each replicate. In addition to this replicated trial of DH lines, an additional planting with DH hybrids also was grown in a neighboring plot for observation. This trial was planted on April 22, 2008. Throughout the season, the plot was treated by the grower in the same manner as the commercial planting in which it was embedded, providing commercial conditions for this trial. Stand counts were conducted on June 20, and maturity ratings were made on August 15. The onions were lifted on September 4, and harvested on September 10, 2008. Gradings were performed as described below, with the first grading for yield on October, 8 and the second grading for storability on February 6, 2009.

The 2008 trial of DH hybrids was grown at Star Growers in Elba, New York, in a plot embedded within this commercial onion field. The trial consisted of 25 double haploid hybrids and 4 controls in a complete randomized block with four replications. Each genotype was represented by two 25 foot rows that were planted with 250 seeds evenly spaced in each replicate. Each row was spaced one foot apart with the seeds sown at 1/2 inch depth. This trial was planted on April 16, 2008. Throughout the season, the plot was treated by the grower in the same manner as the commercial planting in which it was embedded, providing commercial conditions for this trial. Stand counts were conducted on June 20, 2008. Maturity ratings were conducted on August 15, 2009. These onions were lifted on September 3, 2008. The onions were harvested September 10, 2008. Gradings were performed as described below, with the first grading for yield on October 8, 2008 and the second grading for storability on February 6, 2009.

DH Hybrid trials 2009.

The DH hybrid trial grown at Triple G Farms, Elba, NY, was sown on May 21, 2009. This trial included 4 replications of the Am type DH hybrids and 4 replications of the AmSp type DH hybrids listed in Table 2.1. Each entry was represented by two 20 foot rows with 200 seeds in each rep. Seed were planted at a 1/2 inch depth and one foot spacing between rows. Stand counts were conducted on June 24, 2009. Maturity ratings were conducted on August 26, 2009. Onion were lifted on September 3, 2009 and harvested on September 15, 2008. Gradings were performed as described below, with the first grading for yield on October 20-21 2009, and the second grading for storability on January 12, 2010.

The DH hybrid trial grown at Gianetto Farms, in Oswego, NY, was sown on May 25, 2009. This trial included 4 replications of the Am type DH hybrids and 3 replications of the AmSp type DH hybrids as listed in Table 2.1. Each entry was represented by two 20 foot rows with 200 seeds in each rep. Seed were planted at a 1/2 inch depth and one foot spacing between rows. Stand counts were conducted on June 24, 2009 and maturity ratings were conducted on August 26, 2009. The onions were lifted on September 1, 2009 and harvested on September 22, 2009. Gradings were performed as described below, with the first grading (for yield) on October 15, 2009 and the second grading (for storability) on January 19, 2010.

Field evaluations and harvest.

Stand counts of direct sown trials were conducted by measuring off 10 feet in the middle of the 20 foot row and counting all the seedlings that had emerged. Maturity ratings, a visual assessment of percent of plants in each row in which the leaves had fallen over and begun to dry down, were conducted in August.

The onions were manually pulled from the ground when approximately 75 percent of the leaves in the trial had fallen over. The onions were laid in the field with their leaves covering the bulbs to dry for approximately 2 weeks. After the leaves had dried down, the onions were harvested by cutting off the dried leaves and placing all the onions from each entry into a 50 pound plastic mesh onion bag. The bags then were placed in wooden onion crates 4 feet square by 3 feet tall. These were covered to protect them from rain and left outside to cure for approximately 1 month.

Grading.

Grading was done after onions had finished curing. During the first grading for yield, each entry was run across the grader and rotten or damaged bulbs were removed. The marketable onions were tallied and weighed. Culls were tallied into one of five categories, maggot damage, bacterial decay, Fusarium basal rot and thick necks and then weighed as a group. All the marketable onions were re-bagged and put into on-farm commercial onion cold storage in large wooden crates until the second grading, 8 to 12 weeks later, depending on the year.

In the second grading for storability, the onions were rated for quality traits from 0-10, with 10 being the highest quality. Traits evaluated were uniformity, skin darkness, roots sprouting, tightness of necks, cleaning ability, firmness, brown stain damage, *B. cineria damage*, and overall appearance. Rotten or sprouting bulbs were discarded. All other bulbs were weighed as a group to calculate weight loss from the first to second grading.

Data analysis.

Grading data were used to generate values of row weight and average bulb weight for analysis. Row weight was calculated by totaling the weight of all size categories excluding the culls. Average bulb weight was calculated by dividing the row weight by the number of bulbs per row. Line means for row weight and average bulb weight were compared, with a student's t-test against the control lines using a Bonferroni correction to account for multiple comparisons, using the generalized linear modeling procedures of JMP® (SAS Institute Inc., Cary, NC) to account for replication and location variation. The assumptions of the data that need to be satisfied are independence of the entries, normality of the data, and homogeneity of the variances. Independence is satisfied by using a randomized block design to layout the planting plan. Normality of the data was proven using a Shapiro-Wilk test of the distribution of the residuals. A P-value over 0.05 indicated normality. Homogeneity of the variances is assessed by plotting the residuals by the predicted values. Even distribution of the data points indicates homogeneity of the variances. All data satisfied these assumptions if used.

Due to the subjective nature of the rating scales and non-parametic nature of the data, the quality trait evaluation data was not subject to statistical analysis.

Results

Doubled haploid line average bulb weight 2006.

An evaluation of average bulb weight was used to determine the yield potential in this trial, since the onions were transplanted at an even spacing providing a uniform stand count. Eleven of fifteen double haploid lines derived from YIX E were not significantly different in average bulb weight from the source line YIX E (Table 2.2). Eight of fifteen DH lines derived from YIX E were not significantly different than the commercial control, Festival (Table 2.2).

Table 2.2. Bulb weight (g) of DH lines compared with source line YIX E and hybrid control Festival in 2006 trial.

Pedigree	Bulb Weight (g)	p-value different than source	p-value different than Festival
Festival	276	0.1120	
YIX E	236		0.1120
DH CU 066608	233	0.9213	0.0923
DH CU 066628	230	0.8229	0.0358
DH CU 066615	228	0.7638	0.0608
DH CU 066604	217	0.4720	0.0235
DH CU 066627	211	0.3379	0.0129
DH CU 066635	210	0.3090	0.0111
DH CU 066630	208	0.2709	0.0089
DH CU 066616	200	0.1635	0.0040
DH CU 066634	196	0.1205	0.0025*
DH CU 066619	185	0.0449	0.0005*
DH CU 066601	168	0.0101	0.0001*
DH CU 066613	145	0.0005*	0.0001*
DH CU 066600	143	0.0007*	0.0001*
DH CU 066637	135	0.0002*	0.0001*
DH CU 066607	125	0.0001*	0.0001*

Bonferroni corrected alpha for multiple comparisons 0.0033.
* indicates 95 percent confidences of significantly different from the source line or Festival.

Two out of four double haploid lines derived from YIX C were not significantly different in average bulb weight from the source line YIX C. All of the DH lines derived from YIX C were significantly different than Festival (Table 2.3).

Table 2.3. Bulb weight of DH lines compared with source line YIX C and hybrid control Festival in 2006.

Pedigree	Bulb Weight (g)	p-value different than source	p-value different than Festival
Festival	276	0.5692	
YIX C	266		0.5692
DH CU 066631	223	0.0267	0.0083*
DH CU 066612	222	0.0258	0.0080*
DH CU 066633	136	0.0001*	0.0001*
DH CU 066621	107	0.0001*	0.0001*

Bonferroni corrected alpha for multiple comparisons 0.0125.
* indicates 95 percent confidences of significantly different from the source line or Festival.

Doubled haploid lines and hybrids in 2008 trials.

Seven of fifteen DH lines were not significantly different in row weight than Festival and the line DH CU 0666631 was greater (Table 2.4).

Table 2.4. DH row weight in 2008 compared to control line Festival.

Pedigree	Row weight (kg)	p-value different than Festival
DH CU 0666631	14.41	0.3781
Festival	12.92	
DH CU 0666619	12.31	0.7172
DH CU 0666630	12.47	0.7890
DH CU 0666627	11.08	0.2810
DH CU 0666634	9.66	0.0611
DH CU 0666635	9.99	0.0908
DH CU 0666604	9.23	0.0354
DH CU 0666615	8.72	0.0177*
DH CU 0666621	7.12	0.0016*
DH CU 0666600	6.71	0.0008*
DH CU 0666614	6.70	0.0004*
DH CU 0666616	4.87	0.0001*
DH CU 0666628	3.98	0.0001*
DH CU 0666601	4.52	0.0001*
DH CU 0666637	3.50	0.0001*

Bonferroni corrected alpha for multiple comparisons 0.0033.
* indicates 95 percent confidences of significantly different from Festival.

Seven out of fifteen DH lines were not significantly different in mean bulb weight than Festival and the lines DH CU 0666631 and DH CU 0666628 were greater (Table 2.5). The mean for row weight varied from 14.41 kg to 3.50 kg and the mean average bulb weight varied from 102.4 g to 45.4 g. The nine highest performing lines for both yield and average bulb weight included the hybrid control Festival and were not significantly different from each other. Three DH lines (DH CU 0666631, DH CU 0666619 and DH CU 0666630) had higher yields than the hybrid Festival and the two lines DH CU 0666631 and DH CU 0666628 had higher average bulb weight than Festival.

Table 2.5. DH mean bulb weight 2008 compared to control line Festival.

Pedigree	Bulb Weight (g)	p-value different than Festival
DH CU 0666631	102	0.8038
DH CU 0666628	100	0.6146
Festival	99	
DH CU 0666630	99	0.9862
DH CU 0666619	97	0.7180
DH CU 0666615	82	0.0471
DH CU 0666604	81	0.0105
DH CU 0666627	80	0.0277
DH CU 0666635	74	0.0031*
DH CU 0666634	73	0.0024*
DH CU 0666616	65	0.0001*
DH CU 0666600	61	0.0001*
DH CU 0666621	52	0.0001*
DH CU 0666614	51	0.0001*
DH CU 0666601	45	0.0001*
DH CU 0666637	45	0.0001*

Bonferroni corrected alpha for multiple comparisons 0.0033.
* indicates 95 percent confidences of significantly different from Festival.

All of the DH hybrids have higher row weight than their corresponding DH male parent (Table 2.6). The difference in row weight, between the double haploid male and the corresponding hybrid, generally increased as the row weight of the double haploid line decreased. The double haploid males with the highest row weight did not produce hybrids with the largest weight.

Table 2.6. DH line row weight (kg) difference compared to F1 hybrids produced with either Am or AmSp female in 2008.

Pedigree	Row weight (kg)			Diff. in Row weight of line vs. F1 (kg)	
	DH line	F1 Am A x DH line	F1 AmSp A x DH line	Am female	AmSp female
DH CU 0666631	14.41	17.65		3.23	
DH CU 0666630	12.47	18.47		6.01	
DH CU 0666619	12.31	15.47	26.27	3.16	13.97
DH CU 0666627	11.08	20.31		9.23	
DH CU 0666635	9.99	14.06		4.07	
DH CU 0666604	9.23	16.33	26.49	7.10	17.26
DH CU 0666621	7.12	18.33	17.03	11.21	9.91
DH CU 0666600	6.71	13.96	18.03	7.25	11.32
DH CU 0666614	6.70	13.58	14.10	6.88	7.40
DH CU 0666616	4.87	17.72	21.66	12.85	16.79
DH CU 0666601	4.52	17.13	22.02	12.61	17.50
DH CU 0666628	3.98	19.82		15.84	
DH CU 0666637	3.50	15.55		12.05	

All of the DH hybrids had a higher average bulb weight than their corresponding DH male parent (Table 2.7). The difference in average bulb weight, between the double haploid male and the corresponding hybrid, generally increased as the average bulb weight of the double haploid line decreased and the double haploid males with the highest average bulb weight did not produce the hybrids with the largest weight.

Table 2.7. Average bulb weight (g) difference compared to F1 hybrids produced with either Am or AmSp female in 2008.

Pedigree	Average Bulb Weight (g)			Diff. in bulb weight in line vs. F1 (g)	
	DH line	F1 Am A x DH line	F1 AmSp A x DH line	Am female	AmSp A female
DH CU 0666628	105	118		13	
DH CU 0666631	102	108		7	
DH CU 0666630	99	132		33	
DH CU 0666619	97	114	143	16	45
DH CU 0666604	81	107	151	26	69
DH CU 0666627	80	122		43	
DH CU 0666635	72	89		17	
DH CU 0666616	68	106	120	39	52
DH CU 0666600	63	96	123	32	60
DH CU 0666621	50	113	105	63	55
DH CU 0666614	48	95	84	46	35
DH CU 0666601	47	103	141	56	95
DH CU 0666637	46	101		55	

Hybrid trial 2008.

Due to poor field and growing conditions, analysis of the 2008 trials was limited. The 2008 summer experienced heavy rain and sections of the field were periodically flooded. Individual rows that were noted as flooded were eliminated from the data set, thus lowering the number of reps per genotype. It is possible that other sections of the field were flooded for shorter periods of time and not noted, thus reducing yield of those rows, which were not removed. Additionally, the field had noticeable weed problems affecting multiple patches throughout the field. Along with heavy rain there were two hail events that sheared leaves off many of the onions, affecting larger bulbs the most, thus adding another element of variability.

Six of nine double haploid hybrids had a mean row weight greater than both Medeo and Calibra (Table 2.8). Five of nine DH hybrids had a mean bulb weight greater than Medeo and eight of nine DH hybrids had a mean bulb weight greater than Calibra (Table 2.9). Hybrids created using DH CU 0666619, DH CU 0666613, DH CU 0666604 and DH CU 0666614 as males with the AmSp type female had greater row weight and average bulb weight than the controls Medeo and Calibra created with the same female.

Table 2.8. Mean row weight (kg) AmSp type hybrids in 2008.

Pedigree	No.	Row weight	
		Mean (kg)	Std Err Mean
AmSp A Line X DH CU 0666601	3	21.62	1.21
AmSp A Line X DH CU 0666615	4	20.88	2.37
AmSp A Line X DH CU 0666619	3	20.29	0.49
AmSp A Line X DH CU 0666613	3	18.73	2.75
AmSp A Line X DH CU 0666604	3	18.67	1.50
AmSp A Line X DH CU 0666614	3	17.93	0.74
Medeo (AmSp A line derived)	2	17.68	4.25
Calibra (AmSp A line derived)	3	17.11	0.62
AmSp A Line X DH CU 0666600	3	15.96	3.23
AmSp A Line X DH CU 0666616	2	15.93	0.22
AmSp A Line X DH CU 0666621	3	13.35	1.73

Table 2.9. Mean bulb weight (g) of AmSp type hybrids in 2008.

Pedigree	No.	Bulb Weight	
		Mean g	Std Err Mean
AmSp A Line X DH CU 0666604	3	121.0	30.7
AmSp A Line X DH CU 0666614	3	120.0	20.0
AmSp A Line X DH CU 0666616	2	118.6	13.8
AmSp A Line X DH CU 0666613	3	116.4	5.4
AmSp A Line X DH CU 0666619	3	111.4	18.9
Medeo (AmSp A line derived)	2	108.0	10.9
AmSp A Line X DH CU 0666601	3	103.8	9.6
AmSp A Line X DH CU 0666600	3	100.0	18.2
AmSp A Line X DH CU 0666615	4	97.5	9.7
Calibra (AmSp A line derived)	3	93.6	10.9
AmSp A Line X DH CU 0666621	3	84.1	11.3

In the 2008 trial none of the Am type DH hybrids were greater in mean row weight than Crockett and two of the 16 DH hybrids were greater in mean row weight than Safrane (Table 2.10). Four of the 16 AM type DH hybrids were greater than Safrane and 12 of 16 AM type DH hybrids were greater than Crockett (Table 2.11).

Table 2.10. Mean row weight (kg) of Am type hybrids 2008.

Pedigree	No.	Row weight	
		Mean (kg)	Std Err Mean
Crockett (AM A Line derived)	3	17.03	0.19
AM A Line X DH CU 0666601	4	16.81	1.08
AM A Line X DH CU 0666615	3	16.74	0.92
Safrane (AM A Line derived)	4	15.75	1.22
AM A Line X DH CU 0666619	4	14.91	1.82
AM A Line X DH CU 0666614	4	14.59	1.33
AM A Line X DH CU 0666635	3	14.57	1.81
AM A Line X DH CU 0666630	4	14.37	1.76
AM A Line X DH CU 0666613	2	14.11	0.42
AM A Line X DH CU 0666631	4	14.02	0.84
AM A Line X DH CU 0666604	3	13.85	0.41
AM A Line X DH CU 0666600	2	13.55	2.67
AM A Line X DH CU 0666627	3	13.12	1.45
AM A Line X DH CU 0666634	3	12.75	0.43
AM A Line X DH CU 0666637	3	12.11	2.54
AM A Line X DH CU 0666616	3	12.03	1.20
AM A Line X DH CU 0666628	4	11.37	1.06
AM A Line X DH CU 0666621	4	11.31	0.69

Table 2.11. Mean bulb weight (g) of Am type hybrids in 2008.

Pedigree	No.	Bulb Weight	
		Mean (g)	Std Err Mean
AM A Line X DH CU 0666614	4	117.6	16.3
AM A Line X DH CU 0666615	3	112.3	10.5
AM A Line X DH CU 0666635	3	108.7	10.0
AM A Line X DH CU 0666634	3	106.5	12.9
Safrane (AM A Line derived)	4	101.5	16.5
AM A Line X DH CU 0666619	4	101.1	13.6
AM A Line X DH CU 0666601	4	96.2	16.3
AM A Line X DH CU 0666616	3	93.0	24.9
AM A Line X DH CU 0666631	4	92.2	5.2
AM A Line X DH CU 0666600	2	91.4	27.0
AM A Line X DH CU 0666637	3	90.3	7.5
AM A Line X DH CU 0666613	2	87.8	20.0
AM A Line X DH CU 0666627	3	87.1	5.3
Crockett (AM A Line derived)	3	85.2	5.1
AM A Line X DH CU 0666628	4	82.1	5.7
AM A Line X DH CU 0666604	3	81.4	6.5
AM A Line X DH CU 0666630	4	79.9	10.3
AM A Line X DH CU 0666621	4	72.5	7.5

American Spanish type hybrid trial 2009.

The two American Spanish trials located in Oswego, New York and Elba, New York grew very similarly with the average stand counts in Oswego at 11.3267 per foot and in Elba 11.083 per foot. A student t-test showed no significant difference in stand counts between the two locations with a p-value of 0.4488. Therefore, the data from these two trials were combined and analyzed together. However, one row of AmSp A Line X DH CU 0666604 was removed from the data set because when stand counts were taken it was noted that the row had been hit by the tractor and the stand count was noticeable lowered.

The average row weights for all entries across both locations varied from 40.3 kg per entry to 22.92 kg per entry. The two American Spanish controls Medeo and Calibra had the lowest row weight at 28.5 kg and 25.1 kg, respectively. All American Spanish DH hybrids were greater significantly than Calibra and 5 of 6 were greater significantly than Medeo (Table 2.12). The hybrids with the five greatest mean row weight were the five DH derived hybrids whose male parents were 0666619, 0666604, 0666615, 0666600 and 0666613, each of which were significantly greater for row weight than Medeo and Calibra.

Table 2.12. Am Sp type doubled haploid hybrids compared for row weight with AmSp controls Calibra and Medeo in 2009.

Pedigree	No.	Row weight (kg)	p-value different from Calibra	p-value different from Medeo
AmSp A Line X DH CU 0666619	7	40.34	0.000000*	0.000001*
AmSp A Line X DH CU 0666604	6	39.31	0.000000*	0.000005*
AmSp A Line X DH CU 0666600	7	36.99	0.000005*	0.004289*
AmSp A Line X DH CU 0666613	7	36.98	0.000000*	0.000382*
AmSp A Line X DH CU 0666615	7	36.76	0.000002*	0.000842*
AmSp A Line X DH CU 0666601	7	33.28	0.000046*	0.026083
Medeo (AmSp A line derived)	7	28.45	0.008648	
Calibra (AmSp A line derived)	7	22.92		0.008648

Bonferroni corrected alpha for multiple comparisons 0.0007.
* indicates 95 percent confidences of significantly different than the controls.

All of the American Spanish DH hybrids are significantly greater in average bulb weight than Calibra, but none were significantly greater than Medeo (Table 2.13).

Table 2.13. 2009 AmSp type doubled haploid hybrids compared for bulb weight with AmSp controls Calibra and Medeo.

Pedigree	No.	Bulb Weight (g)	p-value different from Calibra	p-value different from Medeo
AmSp A Line X DH CU 0666604	6	125	0.0000004*	0.1088
AmSp A Line X DH CU 0666619	7	124	0.0000004*	0.1299
AmSp A Line X DH CU 0666600	7	118	0.000071*	0.9483
Medeo (AmSp A line derived)	7	115	0.000006*	
AmSp A Line X DH CU 0666613	7	114	0.000008*	0.9792
AmSp A Line X DH CU 0666615	7	113	0.000116*	0.9721
AmSp A Line X DH CU 0666601	7	110	0.000245*	0.4923
Calibra (AmSp A line derived)	7	84		0.000006

Bonferroni corrected alpha for multiple comparisons 0.0007.
* indicates 95 percent confidences of significantly different than the controls.

The traits for color, root growth, neck tightness, firmness and percent weight loss showed no relationship between DH hybrids and the commercial controls. Bulb uniformity was noticeably better for the American Spanish type DH hybrids (Table 2.14). Cleaning was noticeably better in the commercial controls.

Table 2.14. Quality assessment of AmSp type hybrids grown in Elba and Oswego in 2009.

Elba and Oswego	No.	Bulb Uniformity	Color	Root Growth	Neck Tightness	Cleaning	Firmness	Percent Weight loss
AmSp A Line X DH CU 0666600	8	8.3	7.8	8.9	8.6	8.1	8.5	5.04%
AmSp A Line X DH CU 0666601	14	8.7	7.8	8.6	8.9	7.9	8.3	5.50%
AmSp A Line X DH CU 0666604	14	8.5	7.3	7.8	7.5	7.9	7.2	5.71%
AmSp A Line X DH CU 0666613	14	9.0	8.0	8.9	8.9	8.5	8.6	3.49%
AmSp A Line X DH CU 0666615	14	7.6	7.2	8.7	8.3	7.6	8.1	5.82%
AmSp A Line X DH CU 0666619	14	8.7	7.9	9.2	8.9	8.4	8.8	3.92%
Calibra (AmSp A line derived)	12	8.5	8.2	9.3	7.8	8.7	8.6	4.75%
Medeo (AmSp A line derived)	14	7.2	8.3	8.1	7.6	9.1	8.5	4.56%

Visual quality ratings on a scale of 0-10 10 being the best.
% Weight loss of bulbs from October to January grading.

American type hybrid trial 2009.

The stand counts of the American Type hybrids differed in the two locations; in Oswego it was 9.8375 and in Elba it was 11.1893. A student t test showed a highly significant difference between the locations ($p < 0.0001$); therefore, the two locations were analyzed separately.

The row weight means of the American type hybrid trial in Oswego ranged from 30.42 kg to 18.51 kg (Table 2.15). Crockett had the highest row weight, and Safrane had a row weight in the center of the range. All 12 DH hybrids had row weights that were not different significantly from the control Safrane. The row weight for the American type hybrids with the male parents 0666631 and 0666604 were not different significantly from Crockett.

Table 2.15. Am type hybrids compared for row weight with Am type controls Safrane and Crockett in Oswego in 2009.

Pedigree	No.	Row Weight (kg)	p-value different from Safrane	p-value different from Crockett
Crockett (AM A Line derived)	4	29.98	0.0006*	1.0000
AM A Line X DH CU 0666631	4	27.90	0.0147	0.2026
AM A Line X DH CU 0666604	4	25.26	0.5355	0.0041
AM A Line X DH CU 0666615	4	24.98	0.2429	0.0016*
AM A Line X DH CU 0666613	4	24.95	0.4404	0.0040*
AM A Line X DH CU 0666634	4	24.00	0.7162	0.0012*
Safrane (AM A Line derived)	4	23.77	1.0000	0.0006*
AM A Line X DH CU 0666635	4	23.77	0.9802	0.0006*
AM A Line X DH CU 0666630	4	23.66	0.9306	0.0006*
AM A Line X DH CU 0666619	4	23.36	0.7571	0.0010*
AM A Line X DH CU 0666627	4	22.50	0.6911	0.0001*
AM A Line X DH CU 0666601	4	22.20	0.5686	0.0001*
AM A Line X DH CU 0666600	4	21.44	0.3906	0.0000*
AM A Line X DH CU 0666637	4	18.43	0.0134	0.0000*

Bonferroni corrected alpha for multiple comparisons 0.0035.
* indicates 95 percent confidences of significantly different than the controls.

There was a large variation in the average bulb weight in the AM type hybrids in Oswego ranging from 113.1g to 82.5g (Table 2.16). The majority of the DH hybrids were not different significantly than the controls. Hybrids created with DH CU 0666604, DH CU 0666631, and DH CU 0666630, were greater significantly than the control Safrane.

Table 2.16. Am type hybrids compared for bulb weight with Am type controls Safrane and Crockett in Oswego in 2009.

Pedigree	No.	Bulb Weight (g)	p-value different from Safrane	p-value different from Crockett
AM A Line X DH CU 0666604	4	113	0.0001*	0.7375
AM A Line X DH CU 0666631	4	112	0.0000*	0.9902
Crockett (AM A Line derived)	4	111	0.0000*	
AM A Line X DH CU 0666630	4	105	0.0017*	0.1795
AM A Line X DH CU 0666619	4	101	0.0074	0.0788
AM A Line X DH CU 0666635	4	100	0.0262	0.0227
AM A Line X DH CU 0666615	4	98	0.0211	0.0314
AM A Line X DH CU 0666601	4	97	0.0575	0.0086*
AM A Line X DH CU 0666627	4	96	0.0767	0.0060*
AM A Line X DH CU 0666634	4	94	0.1427	0.0026*
AM A Line X DH CU 0666613	4	88	0.7288	0.0001*
Safrane (AM A Line derived)	4	87		0.0000*
AM A Line X DH CU 0666600	4	86	0.8289	0.0001*
AM A Line X DH CU 0666637	4	82	0.5864	0.0001*

Bonferroni corrected alpha for multiple comparisons 0.0035.
* indicates 95 percent confidences of significantly different than the controls.

There were large differences among entries for mean row weight in the Elba trial of the American type hybrids, ranging from 37.5 kg to 28.6 kg. The two controls, Safrane and Crockett, had the lowest yields, at 31.3 kg and 28.4 kg, respectively. The row weight values for all of the DH hybrids were significantly better than the poorer commercial control, Crockett, and 10 of the 12 DH hybrids also were higher significantly for row weight than that of the better commercial control, Safrane (Table 2.17).

Table 2.17. Am type DH hybrids compared for row weight with controls Safrane and Crockett in Elba in 2009.

Pedigree	No.	Row Weight (kg)	p-value different from Safrane	p-value different from Crockett
AM A Line X DH CU 0666619	4	37.53	0.0015*	0.0000*
AM A Line X DH CU 0666600	4	37.35	0.0003*	0.0000*
AM A Line X DH CU 0666615	4	37.04	0.0032*	0.0000*
AM A Line X DH CU 0666634	4	36.85	0.0059	0.0001*
AM A Line X DH CU 0666635	4	36.43	0.0070	0.0001*
AM A Line X DH CU 0666627	4	35.60	0.0184	0.0003*
AM A Line X DH CU 0666601	4	35.20	0.0392	0.0007*
AM A Line X DH CU 0666604	4	35.15	0.0451	0.0009*
AM A Line X DH CU 0666630	4	35.10	0.0509	0.0010*
AM A Line X DH CU 0666631	4	33.43	0.2526	0.0101
AM A Line X DH CU 0666613	4	33.38	0.4115	0.0223
AM A Line X DH CU 0666637	4	31.63	0.6867	0.0671
Safrane (AM A Line derived)	4	31.38	1.0000	0.1276
Crockett (AM A Line derived)	4	28.62	0.1276	1.0000

Bonferroni corrected alpha for multiple comparisons 0.0035
* indicates 95 percent confidences of significantly different than the controls

Average bulb weight ranged from 127.6 to 99.8 kg. Hybrids created with the males DH CU 0666600, DH CU 0666634, DH CU 0666619 and DH CU 0666615 were greater significantly for mean bulb weight than the control Safrane. The hybrids created with the male DH CU 0666600 had greater significantly for mean bulb weight than the control Crockett (Table 2.18).

Table 2.18. Am type DH hybrids compared for bulb weight with Am type controls Safrane and Crockett in Elba in2009

Pedigree	No.	Bulb Weight (g)	p-value different from Safrane	p-value different from Crockett
AM A Line X DH CU 0666600	4	128	0.0073*	0.0017*
AM A Line X DH CU 0666634	4	125	0.0157*	0.0040
AM A Line X DH CU 0666619	4	124	0.0084*	0.0022
AM A Line X DH CU 0666615	4	122	0.0195*	0.0054
AM A Line X DH CU 0666604	4	118	0.0734	0.0229
AM A Line X DH CU 0666613	4	117	0.3323	0.1354
AM A Line X DH CU 0666630	4	116	0.1345	0.0459
AM A Line X DH CU 0666635	4	115	0.0905	0.0304
AM A Line X DH CU 0666601	4	114	0.1619	0.0583
AM A Line X DH CU 0666627	4	110	0.1939	0.0763
Safrane (AM A Line derived)	4	105		0.5940
Crockett (AM A Line derived)	4	102	0.5940	
AM A Line X DH CU 0666631	4	101	0.7953	0.7845
AM A Line X DH CU 0666637	4	100	0.9054	0.5322

Bonferroni corrected alpha for multiple comparisons 0.0035.
* indicates 95 percent confidences of significantly different than the controls.

The traits for color, root growth, neck tightness, firmness and percent weight loss showed no relationship between DH hybrids and the commercial controls. Cleaning, the ability for the dried outer scale to be removed, is noticeably better in the commercial controls. Bulb uniformity is noticeably better for the American type DH hybrids (Table 2.19).

Table 2.19. Quality assessment of Am type hybrids in Elba and Oswego 2009.

Pedigree	No.	Bulb Uniformity	Color	Root Growth	Neck Tightness	Cleaning	Firmness	Percent Weight Loss
AM A Line X DH CU 0666600	14	9.1	7.6	9.9	8.1	7.7	8.5	2.63%
AM A Line X DH CU 0666601	15	8.9	7.9	9.7	9.1	7.8	8.6	2.46%
AM A Line X DH CU 0666604	14	7.7	7.6	9.6	6.6	7.6	7.6	2.33%
AM A Line X DH CU 0666613	16	8.3	7.9	9.6	8.0	7.8	8.6	2.17%
AM A Line X DH CU 0666615	15	7.8	7.5	9.4	7.7	7.7	8.3	2.88%
AM A Line X DH CU 0666619	13	8.6	8.4	9.8	8.2	7.5	8.5	2.52%
AM A Line X DH CU 0666627	14	8.4	8.1	9.6	8.4	7.6	8.3	2.49%
AM A Line X DH CU 0666630	15	8.5	8.0	9.4	7.3	7.5	8.3	2.64%
AM A Line X DH CU 0666631	16	8.4	7.4	10.0	8.8	7.8	8.4	3.01%
AM A Line X DH CU 0666634	15	8.2	8.0	9.7	6.7	7.7	8.1	2.70%
AM A Line X DH CU 0666635	15	7.8	7.7	9.7	6.8	7.5	8.4	2.26%
AM A Line X DH CU 0666637	12	9.7	8.3	10.0	9.2	8.5	9.3	2.71%
Crockett (AM A Line derived)	16	7.6	8.4	9.9	5.9	9.1	8.3	4.01%
Safrane (AM A Line derived)	15	7.4	7.7	9.7	7.7	8.8	8.5	2.53%

Visual quality ratings on a scale of 0-10 10 being the best.
% Weight loss of bulbs from October to January grading.

Discussion

One key aspect leading to the use of doubled haploids in crops such as maize, rice, wheat, and Brassicas is that even though doubled haploids are entirely homozygous they surprisingly exhibit a high level of vegetative vigor (Bohanec, 2002, Bong and Swaminathan, 1995, Kim, 2007). Hybrid vigor in all crops arises from partial or complete dominance, over dominance or both (Comstock and Robinson, 1953). Dominance contributes to hybrid vigor because it masks deleterious sub-lethal alleles, resulting in the more vigorous growth noted in superior hybrids. DH onion lines in our trials were comparable in row weight and bulb weight, when compared with their open-pollinated source lines and were only slightly lower than that of the commercial onion hybrid controls (Tables 2.2, 2.3, 2.4, 2.5). In DH onions lines the striking vigor might be due to a lack of deleterious or sub-lethal alleles, a similar process to the masking of deleterious sub-lethal alleles in hybrids which leads to hybrid vigor. During the first stages of gynogenesis, the plantlets are completely homozygous and masking of deleterious alleles would be completely absent. Genes that are strongly deleterious to vegetative growth and development would kill the plantlets containing them. This would result in recovery of fewer DH plants, but those produced would be free of the most deleterious alleles. In the production of these double haploid lines, 47,000 cultured onions flowers led to 1,100 gynogenic plants (Alan et al., 2003, 2004). This low recovery rate, similar to those in other attempts to produce other DH onion lines (Bohanec, 2002; Kim, 2007), could be another result of a high level of *in vitro* selection.

In vitro selection would not eliminate all deleterious alleles, only those affecting early development of plantlets and their vegetative growth. For example, genetic control of reproduction would not be subject to selection in the creation of DH plantlets because reproductive genes would not necessarily be expressed during gynogenesis. Poor reproductive ability was seen in a number of initial double haploid onion lines produced (Alan et al., 2003, 2004; Bohanec, 2002), such that those lines were not considered for either preliminary trials or for production of DH hybrids. If removal of deleterious alleles increases vegetative vigor to some extent due to masking of deleterious alleles, DH lines free of deleterious genes should be ideal as one parent of a hybrid. Such DH lines would be expected to show considerable broad sense combining ability, the capability to produce hybrids with superior vegetative growth while crossing onto diverse female lines.

In rice, maize and onion, double haploid lines do not reach the same level of vigor as found in hybrids (Bong and Swaminathan, 1995; Kim, 2007; Röber et al., 2005). This could be because vigor in hybrids due to over-dominance in the heterozygous state cannot be fixed in DH lines; if this is the case, yield will not reach that of hybrids (Bong and Swaminathan, 1995).

These results show that hybrids created with DH males have increased vigor, as assessed by row weight and bulb weight, over the related commercial hybrid controls. More than half of the nine American Spanish type hybrids in 2008 were greater for row weight and bulb weight than both hybrid controls with female line in common (Table 2.8, 2.9) and all six in the 2009 trial were greater than both controls only one not being significant. The American type hybrids in 2008 performed comparably to the controls; however, no significant differences were detected due to exceptionally variable field conditions (Table 2.10, 2.11). In the Elba 2009 trial, under more favorable conditions and a higher stand count, both row weight and bulb weight were higher for all the DH hybrid lines, many being significantly higher (Table 2.17, 2.18). In the Oswego 2009 trial, under less favorable conditions and lower stand counts, the DH hybrids performed comparably to the controls in vegetative vigor (Table 2.15, 2.16). Given that experimental hybrids and control hybrids in each set (Am and AmSp) share the same females, the increase in vigor can be attributed to the male double haploid for two possible reasons. One is that hybrids created with double haploids benefit from using the double haploid male. The other case is that the double haploids were created from lines that had been selected for many years in New York and therefore are, better suited to New York onion growing environment than the male inbred lines used to create the commercial lines, and the increased potential comes from this adaptation. Most likely both of these scenarios play a role in the higher level of vigor in the DH hybrids. The high level of vigor seen in the inbred line, attributed to the removal of deleterious genes, lead to the assumption that the more significant effect on vigor is due to the nature of the double haploid. Trials assessing the performance of the double haploid hybrids in locations other than New York could indicate whether or not the increased vigor is solely due to the nature of the double haploid as a hybrid parent.

Many traits in addition to yield and bulb size must be considered in determining the potential of an onion hybrid. The analysis of bulb traits shows no significant differences between the DH hybrids and the conventional hybrids for the traits accessed overall. Uniformity of bulb shape was the quality trait expected to be improved and was rated noticeably higher in DH hybrids versus conventional hybrids. This was expected because shape is genetically controlled; therefore, the completely homozygous DH parent created a more genetically and visually uniform hybrid. It can be assumed that other beneficial traits genetically controlled such as maturity, leaf wax, plant structure, doubling, number of centers, pungency, disease resistance and soluble solid content also would be more uniform in hybrids created with DH parents. However, fixation of traits in DH lines could result in poorer quality if the trait fixed is an unfavorable type of character such as bulb shape, color, and neck thickness, none of which would be affected by selection pressure inherent in the process of generating DH line. For example, one DH line was fixed, and uniform, for an unacceptably elongated bulb shape that was not masked in its hybrids. This one characteristic would eliminate the use of this DH line in commercial breeding, regardless of its vigor, bulb size, or yield. In conventional breeding, inbred lines would be discarded during their development if they possessed any poor bulb characteristics. In handling DH onion lines, similar selection should be applied in initial evaluations of DH lines, in order to eliminate from consideration those DH lines with poor flowering traits resulting in reduced seed set, or characters detrimentally affecting desired plant or bulb characteristics.

Double haploids are increasingly being used in crops such as rice and maize, due to the rapid time and lower costs to generate the DH inbred lines compared to conventionally bred inbred lines, (Bohanec, 2002; Röber et al., 2005) and this usage is considered a major advance in maize breeding and genetics (Röber et al., 2005). Savings exist because of the reduced costs of sub-line production and evaluation costs of lines as they are developed. Once a line is produced it is a fully homozygous finished product, and no additional selection is needed to further refine the line. Generation time is short, therefore, lines can be fixed for gene combination in the shortest amount of time with the lowest genotyping expense. Once lines are produced no maintenance selection is needed. Furthermore, due to their absolute uniformity and homozygosity, it is easier for DH lines to meet the needs for protection under plant variety rights. Onion breeding can benefit from utilizing double haploids in the same manner, especially with regards to generation time, as onions are a biennial crop with a two year life cycle. In some onion lines such as YIXE, doubled haploids are recovered at approximately 5% (Alan et al., 2004) a reasonable frequency rate comparable to that commonly used in maize (Röber et al., 2005).

Future studies will help determine the overall benefit of using DH lines as hybrid parents in onion production. However, the fact that so many of the

DH hybrids tested were superior for row weight and bulb weight creates a larger pool of possible hybrids for secondary selection for quality characteristics, which were not different from the conventional hybrids. Furthermore, in onion breeding, the increased uniformity, reduction in production, and maintenance costs, increased speed of production, and increased yields of DH hybrids warrants their use.

REFERENCES

Alan, A.R., M.A. Mutschler, A. Brants, E. Cobb, and E. D. Earle. 2003. Production of gynogenic plants from hybrids of *Allium cepa* L. and *A. roylei* Stearn. Plant Science 165: 1201-1211.

Alan, A.R., A. Brants, E. Cobb, P. Goldschmied, M.A. Mutschler, and E.D. Earle. 2004. Fecund gynogenic lines from onion (*Allium cepa* L.) breeding materials, Plant Science 167: 1055-1066.

Bohanec, B. 2002. Doubled haploid onions, pp. 145-158 In: H.D. Rabinowitch, L. Currah (eds.), Allium Crop Science: Recent Advances. CABI Publishing, Wallingford, UK.

Bong, B. B. and M. S. Swaminathan. 1995. Magnitude of hybrid vigor retained in double haploid lines of some heterotic rice hybrids. TAG Theoretical and Applied Genetics 90: 253-257.

Boodley, J. W. and Sheldrake, R. 1972. Cornell peat-lite mixes for commercial plant growing. Cornell University Cooperative Extension Division Informational Bulletin 43.3.

Dowkerb, D. and G.H. Gordon. 1983. pp. 220-231. Heterosis and hybrid cultivars in onions. In R. Frankel (ed.) Heterosis. Monographs on Theoretical and Applied Genetics. Springer-Verlag.

Duvick, D.N. 1959. The use of cytoplasmic male-sterility in hybrid seed production. Economic Botany 13:167-195.

Evoor, S., R. Veere Gowda, E. Gangappa and R. Krisna Monohar. 2007. Heterosis for yield, yield components and quality traits in onion (*Allium cepa* L.). Karnataka Journal of Agricultural Science 20:813-815.

Hosfield, G.L., G. Vest and C.E. Peterson. 1977. Heterosis and combining ability in a diallel cross of onions. Journal of American Society of Horticultural Science 102: 355-360.

Jones, H.A. and A. Clarke 1943. Inheritance of male sterility in the onion and the production of hybrid seed. Proccedings American Society of Horticultural Science 43: 189-194.

Jones, H.A. and G. Davis. 1944. Inbreeding and heterosis and their relation to the development of new varieties of onions. USDA Technical Bulletin 874.

Joshi, H. and J. Tandon. 1976. Heterosis for yield and its genetic basis in the onion. Indian Journal of Agricultural Science 46:88-92.

Kim, S., K.S. Yoo and L.M. Pike. 2007. Production of doubled haploid onions (A*llium cepa*) and evaluation of their field performance. Horticulture Environment, and Biotechnology 48:143-147.

Röber, F., G.A. Gordillo, and H.H. Geiger. 2005. In vivo haploid induction in maize—performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica 50:275–283.