Development and evaluation of interspecific Cucurbita maxima x Cucurbita moschata hybrids for processing squash

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DEVELOPMENT AND EVALUATION OF INTERSPECIFIC
*CUCURBITA MAXIMA* X *CUCURBITA MOSCHATA*
HYBRIDS FOR PROCESSING SQUASH

BY

JACOB URETSKY
B.S., University of Massachusetts, Amherst, 2004

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

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in
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This thesis has been examined and approved.

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ABSTRACT

DEVELOPMENT AND EVALUATION OF INTERSPECIFIC

*CUCURBITA MAXIMA X CUCURBITA MOSCHATA*

HYBRIDS FOR PROCESSING SQUASH

by

Jacob Uretsky

University of New Hampshire, May, 2012

Interspecific hybrids derived from crosses of *Cucurbita maxima* Duch. x *C. moschata* Duch. have been utilized for taxonomic studies and introgression of advantageous traits, as well as for cultivation for fresh market squash. The present study involves the development and evaluation of *C. maxima* x *C. moschata* F₁ hybrids for use as processing squash. Crosses were performed in the summers of 2009, 2010, and 2011 between inbred bush *C. maxima* breeding lines and vine *C. moschata* cultigens, and the resulting progeny were evaluated in the greenhouse and field in 2010 and 2011 for phenological growth traits, fruit traits, and fruit fresh and dry weight yields.

Compatibility between species was variable, but the *C. maxima* line NH65 was compatible with several *C. moschata* cultigens in terms of the ability to set fruit and produce abundant, viable seed.

In interspecific hybrids utilizing the *C. moschata* fresh market cultivar 'Waltham Butternut' (WBN), internode lengths resembled the bush parent (<2.0 cm) for the first three internodes but increased rapidly between nodes 4 and 7 and exceeded those in the vine parent (>17.0 cm) for several nodes. In hybrids between NH65 and the *C. moschata*
processing cultivars ‘Long Island Cheese’ (LIC) and ‘Dickinson Field’ (DF). Internode lengths did not exceed 5.0 cm until nodes 8 and 10, respectively, and mature internode lengths in these hybrids resembled, but did not exceed, those in the vine parents.

All interspecific hybrids observed were highly gynoecious. In the greenhouse, hybrids utilizing WBN produced precocious pistillate flowers below node 5, but pistillate flowering commenced later in hybrids 65xLIC (node 10) and 65xDF (node 14). Early flowering and fruit set in hybrids utilizing WBN resulted in suppressed vegetative growth in the field. The hybrids 65xLIC and 65xDF exhibited prolific leaf canopy formation prior to fruit set.

Fruit fresh weight in DF (12.1 kg) was significantly greater than that of the interspecific hybrids 65xLIC (4.1 kg) and 65xDF (7.1 kg), but mesocarp percent dry weight was higher in 65xLIC (9.3%) and 65xDF (7.7%) than in DF (5.6%). Additionally, fruit fresh weight yield in DF (48.1 mt/ha) was greater than in 65xDF (32.0 mt/ha) but similar to 65xLIC (46.6 mt/ha), and mesocarp dry weight yield in 65xLIC (3.9 mt/ha) was 50% greater than in DF (2.6 mt/ha).
INTRODUCTION

The New World genus *Cucurbita* is composed of 12-14 species, including five domesticated species, three of which, *C. pepo*, *C. moschata*, and *C. maxima*, are of global economic importance (Robinson and Decker-Walters, 1996). According to the FAO (2011), over 22 million mt of pumpkins, squash, and gourds were harvested from 1.6 million ha globally in 2009. In the U.S. in 2010, 37,000 ha yielded over 480,000 mt of pumpkins and 296 thousand mt of squash (USDA, 2011).

In recent decades, F1 hybrid cultivars have increasingly gained prominence in cultivated cucurbits, particularly in *C. pepo* (Robinson, 2000). In squash used for pie stock processing, however, high-yielding open-pollinated varieties such as ‘Golden Delicious’ (*C. maxima*) and ‘Long Island Cheese’ (*C. moschata*), but primarily ‘Dickinson Field’ (*C. moschata*), still dominate production (Loy, 2004). Hybrid varieties must yield at least as much as standard open-pollinated cultivars, as well as offer additional qualities such as disease resistance, for adoption by the squash processing industry.

Interspecific *C. maxima* x *C. moschata* hybrids that produce seedless fruit when intercropped with pollen-donor cultivars may offer unique combinations of traits attractive to growers and processors. Advantages of interspecific squash hybrids may include: 1) efficient semi-bush growth habit; 2) wide adaptability; 3) resistance to biological and/or environmental stress; 4) improved eating quality; 5) increased vigor; 6) nutritionally beneficial carotenoid profiles; 7) higher yields.
The present study involves the development and evaluation of bush *C. maxima* x vine *C. moschata* F$_1$ hybrids for cultivation as processing squash. Objectives are:

1) Identification of *C. maxima* x *C. moschata* crosses that yield abundant, well-filled seed.

2) Comparative evaluation of interspecific hybrids, parental lines and/or standard processing cultivars for growth traits, including main stem length, internode length, leaf initiation rate, leaf area, and flowering habit.

3) Comparative evaluation of interspecific hybrids, parental lines, and standard processing cultivars for fruit characteristics, including fresh and dry weights of fruit and mesocarp dry weight, soluble solid content, color, and subjective evaluation of eating quality.

4) Yield comparison between interspecific hybrids and standard processing cultivars.
CHAPTER I. LITERATURE REVIEW

The Genus *Cucurbita*

*Cucurbita* spp., of the family Cucurbitaceae or cucurbits, are generally characterized by vining growth habits, large and lobed leaves, monoecious flowering, and hard-rind fruits, but significant variation exists within and between species, all of which are diploid and share a basic chromosome number $x = 20$ (Robinson and Decker-Walters, 1996; Whitaker and Davis, 1962). The cultivated *Cucurbita* species most likely arose from mesophytic wild ancestors, though xerophytic wild species exist (Singh, 1990). The fruit of *Cucurbita* spp. are commonly classified according to use. Summer squash refers to fruit that are harvested and eaten when immature, while winter squash are fruit harvested at maturity and often stored prior to consumption. In North America, *C. pepo* cultivars are typically used as summer squash, and *C. pepo*, *C. moschata*, and *C. maxima* cultivars are grown for winter squash. Gourds and pumpkins usually refer to ornamental cultivars of *C. pepo*, although some *C. maxima* cultivars are also grown for ornamental use. Furthermore, squashes cultivated for the processing industry are often referred to as processing squash or pumpkin, and include open-pollinated *C. maxima* cultivars such as ‘Golden Delicious’ (*C. maxima*), but most commonly, large round-fruited *C. moschata* cultivars, such as ‘Dickinson Field’ and ‘Long Island Cheese.’

*Cucurbita moschata* Duch.

*Cucurbita moschata* is the least cold tolerant of the domesticated *Cucurbita*, having originated in the tropical lowlands between northern South America and Mexico.
(Nee, 1990; Robinson and Decker-Walters, 1996; Sanjur et al., 2002), and particularly in present-day Colombia, where landraces exhibit abundant variation and some primitive traits (Nee, 1990; Wessel-Beaver, 2000a). Although \textit{C. moschata} is closely related with and morphologically similar to \textit{C. argyrosperma} (Merrick, 1990; Nee, 1990; Wessel-Beaver, 2000b), the two species were most likely domesticated from distinct wild species, and the wild progenitor of \textit{C. moschata} remains unknown (Nee, 1990; Sanjur et al., 2002). \textit{Cucurbita moschata} is characterized by a vining, prostrate growth habit and tan, green, or mottled fruit with angular, flared peduncles. Horticultural groups of \textit{C. moschata} include cheese, crookneck, and butternut types, all of which are cultivated as winter squash in North America, butternut being most common for fresh market sales. Additionally, calabazas are \textit{C. moschata} squash principally cultivated in the tropics for harvest of the mature fruit. Open-pollinated cultivars of \textit{C. moschata}, particularly strains of ‘Dickinson Field,’ currently dominate the pumpkin processing industry because of high fresh weight yields, exceptional flesh color, and suitable consistency of cooked flesh.

\textbf{Cucurbita maxima Duch.}

\textit{Cucurbita maxima} subsp. \textit{maxima} is thought to have arisen in the temperate central South American lowlands from the wild species \textit{C. andreana} Naud. and was cultivated in Peru as early as 4000 BP (Nee, 1990; Robinson and Decker-Walters, 1996; Sanjur et al., 2002; Whitaker, 1981). \textit{C. maxima} and \textit{C. andreana} closely resemble one another and readily hybridize, producing fertile F\textsubscript{1} offspring (Nee, 1990; Robinson and Decker-Walters, 1996; Whitaker and Bemis, 1964). Thus, \textit{C. andreana} is often classified as \textit{C. maxima} subsp. \textit{andreana} (Robinson and Decker-Walters, 1996). In North America,
C. maxima is most commonly grown as a winter squash; however, landraces are still cultivated in South America for harvest of immature fruit as summer squash (Nee, 1990). *Cucurbita maxima*, which includes the kabocha, hubbard, delicious, marrow, turban, banana, and giant show pumpkin horticultural types, is the most cold-tolerant of the domesticated cucurbits (Robinson and Decker-Walters, 1996). The *C. maxima* growth habit is often vining and prostrate, but compact forms exist, including some extreme bush phenotypes (Broderick, 1982; Denna and Munger, 1963; Loy and Broderick, 1990). The round, oval, or oblate fruit are smooth or slightly ribbed and possess rinds ranging from white to grey-blue to green to pink to bright orange. *Cucurbita maxima* peduncles are corky and unflared, and blossom scars vary in size from ~2.0 cm in kabochas to several cm in turban cultivars and are either flush with the rind or protruding. *Cucurbita maxima* squash, particularly kabocha types, are prized for exceptional eating quality.

**Interspecific hybridization in Cucurbita**

Inquiry into hybridization between *Cucurbita* species began in the mid-19th century (Naudin, 1856) and was revisited in the 1890s by other investigators, possibly prompted by morphological diversity within species mistaken for chance outcrossings (Bailey, 1890 and 1920; Pammel, 1893). More deliberate research involving interspecific hybridization in *Cucurbita* did not occur, however, until the 1920s, with attempts to define barriers between species (Castetter, 1930; Erwin and Haber, 1929; Van Eseltine, 1936). More recently, crosses between squash species, including wild and cultivated species, have been used to introgress advantageous traits, such as the bush growth habit (Cho et al., 2003; Rhodes, 1959; Robinson and Decker-Walters, 1996) and disease
resistance (Andres and Robinson, 2002; Vaulx and Pitrat, 1980; Oliveira et al., 2003; Rhodes, 1959; Robinson and Decker-Walters, 1996; Washek and Munger, 1983).

The literature indicates low compatibility between cultivated species, and combining ability between parental lines of different species is difficult to predict (Robinson and Decker-Walters, 1996). Even in crosses that yield viable seed, embryos are often underdeveloped and misshapen. Bud pollination (Hayase, 1961) and heterozygosity in parental lines (Wall and York, 1959) have been suggested to improve fruit set in interspecific crosses, and embryo culture techniques (Kwack and Fujieda, 1987; Wall, 1954) may facilitate germination of interspecific F₁ progeny. While some C. pepo x C. moschata crosses yield viable seeds (Bailey, 1920; Castetter 1930; Erwin and Haber, 1929), the highest compatibility between cultivated species is observed in C. maxima x C. moschata crosses and reciprocals (Castetter, 1930; Erwin and Haber, 1929; Whitaker and Bohn, 1950). Results reported by Erwin and Haber (1929), Castetter (1930), and Hayase (1956) suggest greater compatibility when C. maxima is the pistillate parent. Individuals in interspecies F₁ populations are highly self-sterile, and parthenocarpic fruit set must be induced by donor pollen (Castetter, 1930; Erwin and Haber, 1929; Mendonça et al., 2006; Robinson-Decker-Walters, 1996; Whitaker and Bohn, 1950). The development of breeding lines resulting from interspecific crosses requires backcrossing to one parent species or the other, and BC-line phenotypes tend to drift toward the recurrent parent.

Of course, if one aims only at producing an F₁ generation, parthenocarpy poses no problem for squash cultivation or fruit set, and may contribute to higher yields of fruit mesocarp, the economically important squash product. Reports describing C. maxima x
C. moschata F₁ hybrids are mostly anecdotal, but plants are described as vigorous and highly gynoecious with leaf shape intermediate to parental lines and vines resembling the C. moschata parent (Castetter, 1930; Erwin and Haber, 1929; Robinson et al., 1978; Whitaker and Bohn, 1950). Although the plants are highly sterile, fruit set can be induced in the presence of pollen donors. Fruit are said to be high-yielding, possessing rinds with the general appearance of the C. maxima parental line and peduncles resembling the C. moschata parent. Fruit flesh has been described as of very good quality (Robinson et al., 1978), similar to flesh of C. maxima (Whitaker and Bohn, 1950).

Japanese breeders have developed C. maxima x C. moschata F₁ hybrids on a commercial basis, most notably ‘Tetsukabuto,’ a cross between ‘Delicious’ (C. maxima) x ‘Kurokawa No. 2’ (C. moschata) (Robinson and Decker-Walters, 1996). ‘Tetsukabuto’ was introduced to Brazil in 1960 and Brazilian plant breeders have since released similar C. maxima x C. moschata hybrids (Mendonça et al., 2006), but details documenting the development of commercially produced interspecific cultivars are scant. Interspecific squash cultivars have become the most popular squash type grown in the Brazilian state of Minas Gerais, where it is called abóbora híbrida (Mendonça et al., 2006). Researchers have assessed yield potential (Iacuzzo and Costa, 2009; Mendonça et al., 2006), eating and keeping quality (Iacuzzo and Costa, 2009), and carotenoid content (Azavedo-Meleira and Rodriguez-Amaya, 2007) in such hybrids. These studies indicate that interspecies hybrids are fairly prolific in terms of fresh weight yields, but it is unclear if eating quality is acceptable by North American standards. Other studies have shown C. maxima x C. moschata hybrids to be viable rootstock for melon grafts, an increasingly important method of melon and watermelon production. Use of interspecific Cucurbita rootstocks
in melon cultivation has resulted in improved disease control, extreme temperature
tolerance, excess/deficit moisture tolerance, salt tolerance, beneficial flowering habit, and
more efficient nutrient uptake (reviewed by Davis et al., 2008 and Lee, 1994).

**Crop Yield**

The ultimate function of any crop plant is the photosynthetic fixation of carbon
and subsequent partitioning of photosynthate to the harvested plant organs. Plant growth
can be defined as net carbon fixation or biomass accumulation and may be measured
using various methods, each emphasizing different aspects of plant development. For
instance, crop growth rate (CGR) and relative growth rate (RGR) both define carbon
fixation in terms of dry matter accumulation by time, but CGR measures dry matter
accumulation by land area, while RGR references previously acquired biomass (Fageria
et al., 2006).

Since photosynthesis produces over 90% of plant dry matter, several means for
assessing crop growth account for photosynthetic efficiency in terms of leaf area. For
example, leaf area ratio (LAR) conveys plant dry weight per leaf area, and similarly, net
assimilation rate (NAR) expresses dry mass change over time per leaf area. Lastly, leaf
area index (LAI), a calculation of leaf area per land area, is indicative of light
interception by the crop canopy (Watson, 1958). Brown (1984) points out that changes in
leaf biomass may follow different patterns from total plant biomass in crop plants, since
high early rates of leaf initiation are followed by leaf senescence and slower rates of leaf
production during development of reproductive organs. This is particularly true in crops
with determinate growth habit, such as cereals, in which vegetative growth completely
ceases during grain development. Consequently, early canopy formation is imperative
for high crop yields. In indeterminate crops such as squash that set fruit for an extended
time period, canopy maintenance, as well as production of new leaves, may be of great
importance in determining yield capacity.

In many crop plants, changes in planting density influence LAI such that an
optimal planting density results in rapid acquisition of a critical LAI, translating to
optimal light interception (~95%), and consequently, photosynthesis and productivity.
When planting density exceeds a critical LAI, mutual shading between leaves can result
in reduced productivity (Fageria et al., 2006).

Each of the previously discussed methods for evaluating crop growth account for
total, or biological, yield. Of course, the objective in cultivating crop plants is optimizing
yield of the economically valuable plant components, as opposed to the biological yield.
Harvest index (HI), the ratio of harvested yield to biological yield, has historically been
the most important measure of crop productivity and criterion for breeders, particularly in
cereal grains (Fageria et al., 2006; Gifford and Evans, 1981).

**Yields in Cucurbita**

As noted by Loy (2004), HI may not suitably measure squash productivity for
several reasons: 1) Squash plants are indeterminate and can set multiple fruit non-
simultaneously; 2) Senescence of vegetative organs during reproductive development
may distort HI values; 3) Dry matter is partitioned variably between fruit mesocarp and
seeds in squash, but economic value is derived from the fruit mesocarp, while the seeds
are often a waste product; 4) Fruit fresh weight truly determines economic value in
squash. In squash, proper plant spacing is foremost in exploiting the yield potential of a
given genotype, and past studies reporting squash yields are difficult to interpret, since
plants may not have been grown at optimal density. Also, yield studies infrequently consider percent dry matter of the fruit mesocarp, which is negatively correlated with fresh weight yield (Loy, 2004, 2006).

For example, Hutchins and Croston (1941) reported that yields of nine C. maxima squash cultivars planted at a density of 2.7 m x 2.7 m (0.14 plants/m²) ranged from 8.1 mt/ha in ‘Arikara’ to 48.3 mt/ha in ‘Mammoth Chili,’ but the authors do not indicate the use of guard rows or report dry weight yields. In fact, Culpepper and Moon (1945) reported higher percent dry matter in ‘Arikara’ (10.5%) compared with ‘Mammoth Chili’ (7.5%), one of the lowest ranking squash in terms of total solids.

Several studies in C. maxima have shown that the highest yields occur at plant densities resulting in production of one fruit per plant. Broderick (1982) reported that yields increased in the bush cultigen ‘Autumn Pride’ with increasing plant density, and the highest density of 2.2 plants/m² yielded 74.8 mt/ha, compared with 61.5 mt/ha at 1.1 plants/m² and less than 60 mt/ha at lower densities. Also, dry weight yields in ‘Autumn Pride’ were higher than the control C. maxima vine cultigen ‘Blue Hubbard’ at the highest densities.

Buwalda and Freeman (1986) recorded yields of 30 and 46 mt/ha, depending on sowing date, in the C. maxima cultivar ‘Delica’ planted at a density of 2.2 plants/m² with plants producing between 1.0-1.3 fruit each. In a related study assessing the effects of phosphorus and potassium fertilizers on ‘Delica’ squash, yields reached approximately 40 mt/ha (Buwalda et al., 1987). A similar Australian study showed yield increases concurrent with increases in plant density, but maximum yields of marketable fruit (18.1 mt/ha at 0.6-1.8 plants/m²) were lower than reported in the New Zealand studies, possibly
because acceptability was rated on stringent Japanese import standards (Botwright et al., 1998).

**Carbon Partitioning**

Crop productivity partially depends on the manner and efficiency of assimilate partitioning to the harvested plant organs, often described as the source-sink relationship, in which plant organs where assimilate is produced are classified as sources, and those where assimilate is utilized as sinks (Wardlaw, 1990; Wareing and Patrick, 1975; Wien, 1997). In fact, the most significant improvements in crop yields have resulted from optimizing source-sink relationships, as quantified by harvest indices (Gifford and Evans, 1981). Competition between potential sinks is influenced by organ type, size, and number, or ‘sink strength’ (Evans, 1975), but physiological control of photosynthate partitioning is not well-understood, and most data describing such processes are empirical (Wien, 1997).

While leaves act as the primary source organs in plants, and reproductive and storage organs as sinks, the character of particular organs may change depending on crop species, growth stage, and economic value. For instance, during the vegetative growth stage, the developing leaves, stems, and roots act as the main carbon sinks, with mature leaves providing photosynthate. However, at the onset of the reproductive phase of growth, vegetative growth slows, and often ceases, and the reproductive organs act as the dominant photosynthetic sink (Wien, 1997).

In cereal crops, the developing grain acts as the major sink, as well as the economically important plant organ. In squash, though, the reproductive organs are comprised of the economically important mesocarp, as well as seed, the latter often a
waste product. The developing seeds act as a major photosynthetic sink, often taking precedence over mesocarp development and or affecting the remobilization of sugars from the mesocarp to the developing embryo. Depending on seed yield and squash type, squash seeds can comprise between 5-40% percent of total plant dry weight (Loy, 2004). In acorn squash (C. pepo) possessing ~10% mesocarp dry matter (DW), similar to that of processing cultivars (Culpepper and Moon, 1945; Lana and Tischer, 1951; Loy, 2004), seeds can account for 15-25% of the entire fruit DW (Loy, 2004). While squash mesocarp is primarily composed of carbohydrates (Phillips, 1946), squash seeds contain approximately 50% lipids and 35% proteins (Jacks et al., 1972). Seeds require greater photosynthate requirements per unit DW compared with mesocarp, since one gram of the fundamental photoassimilate glucose, corresponds to 0.826 g carbohydrates, 0.404 g nitrogenous compounds, and 0.330 g lipids in plant tissues (Penning de Vries, 1975). Thus, in the acorn squash described above, the seed may contain one-third of the stored energy of the entire fruit. Furthermore, developing seeds exert the greatest sink strength in squash, and carbohydrate stored as mesocarp starch may be remobilized to the seed in situations of limited photosynthate (Loy, 2004).

**Plant Architecture**

Differences between bush and vine phenotypes determine the architectural framework in squash plants, affecting stem morphology, rates of leaf initiation and canopy formation, and leaf morphology and leaf area, all traits influencing yield. Traditional North American squash cultivars exhibit vining, prostrate growth, often with one primary shoot and several secondary shoots and/or lateral branches and axial tendrils (Whitaker and Davis, 1962). The compact, non-branching bush-type C. pepo cultivars
grown for summer squash are the major exception to this rule, but recently, breeders have exploited both bush and heterozygous bush-vine growth habits in developing new squash and pumpkin varieties (Loy, 2004, 2010). In squash, the bush trait is expressed as reduced internode lengths, but traits associated with the bush growth habit include a thicker main stem with reduced tendril production, upright canopy and cupped leaf morphology, higher pistillate to staminate flowering ratio, and earlier flowering (Broderick, 1982; Denna and Munger, 1963; Loy, 2010).

A single bush (Bu) gene governs compact versus vining growth habit in C. pepo (Paris and Kabelka, 2009; Shifriss, 1947). Likewise, Wu et al. (2007) showed monogenic inheritance in a C. moschata bush mutant. On the other hand, Singh (1949) proposed a digenic model for inheritance of the bush phenotype in C. maxima based on evaluation of progeny resulting from a C. maxima vine x bush cross. While individuals homozygous for the Bu allele(s) often maintain shortened internode lengths with slight elongation in later growth, bush-vine heterozygotes resemble the bush parent in early growth followed by successively longer internode lengths in later growth, a phenomenon described as ‘reversal of dominance’ by Shifriss (1947). In a study comparing lengths of individual internodes in bush, vine, and bush x vine hybrids, Denna and Munger (1963) showed that while mature internode lengths in C. maxima heterozygotes were similar to those in the vine parent, mature internode lengths in C. pepo subsp. ovifera heterozygotes were intermediate to those in bush and vine plants. Exogenous application of gibberellic acid has resulted in internode elongation in plants possessing the bush genotype, suggesting that bush alleles in squash act by suppressing production or reception of gibberellins (Broderick, 1982; Denna, 1963).
In squash cultivation, bush and bush-vine (semi-bush) growth habits offer several advantages compared with vine-type plants, primarily in allowing for higher-density planting. Broderick (1982) compared growth traits in ‘Blue Hubbard,’ a vine-type *C. maxima* cultivar and ‘Autumn Pride,’ a bush-type *C. maxima* developed at UNH. His results highlight the distinction between growth traits in individuals and populations. For instance, individual bush plants showed reduced rates of leaf initiation with increasing plant density, particularly in later growth stages. On the other hand, higher plant densities allowed for rapid canopy formation, resulting in high LAI in early growth and increased fruit fresh weight and dry weight yields. Additionally, earlier flowering and fruit set reduced overall vegetative growth in the bush cultivar, contributing to more efficient partitioning of photosynthate than in the vine cultivar (Loy and Broderick, 1990).

**Heterosis in Squash**

Heterosis, or hybrid vigor, refers to the increase in biomass, growth rate, and/or fertility in progeny resulting from hybridization. The phenomenon was described by Darwin (1876) regarding numerous plant species: “To my surprise, the crossed plants when fully grown were plainly taller and more vigorous than the self-fertilized ones.” The genetic basis for heterosis, however, was identified in the early 20th century in studies involving maize (*Zea mays*), in which researchers found that vigor was restored in F1 hybrids when inbred lines were crossed (Birchler et al., 2010). Heterosis may result from gene complementation and masking of deleterious recessive alleles through heterozygosity and is most pronounced in outcrossing species in which inbreeding depression is particularly evident. Inbreeding depression is of small significance in
Cucurbita (Castetter, 1930; Erwin and Haber, 1929; Haber, 1929; Whitaker and Bohn, 1950), but reduced vigor has been shown in self-pollinated populations of *C. pepo* (Borghi et al., 1973; Chekalina, 1976), *C. maxima* (Chekalina, 1976), and *C. moschata* (Cardoso, 2004). Cardoso (2004) observed reductions in fruit weight, length, and seed number between the *S₀* and *S₁* generations, but no subsequent loss of vigor was evident. On the other hand, Chekalina (2004) found reduced vigor after several generations of selfing.

While heterosis in squash may not be of the magnitude of outcrossing species such as maize, researchers have shown hybrid vigor in winter squash. Hutchins and Croston (1941) compared ten *C. maxima* *F₁* hybrids and parental lines and observed heterosis in several hybrids for total fruit yield and individual fruit weight; however, dry weight yields were not assessed. In another study involving 15 *C. maxima* hybrids, Hayase and Ueda (1956) observed heterosis in most hybrids for fruit number per plant, fruit weight, and days to maturity. Also, Korzeniewska and Niemirowicz-Szczytt (1993) showed heterosis in some hybrids, particularly for fruit dry weight, in a diallel cross involving nine *C. maxima* inbred lines, but data were inconsistent.

Anecdotal accounts of *C. maxima* x *C. moschata* interspecific hybrids describe cultivars as being highly vigorous (Castetter, 1930; Erwin and Haber, 1929; Robinson et al., 1978; Whitaker and Bohn, 1950). Lacuzzo and Costa (2009) recorded significantly higher yields and high DW in the interspecific hybrid ‘Tetskabuto’ compared with five winter squash cultivars, but specific cultivar names were not provided.
**Parthenocarpy and Sterility in Cucurbits**

Parthenocarpy has been exploited in the cultivated cucurbits, primarily in production of gynoecious cucumber (*Cucumis sativus* L.) cultivars. In cucumbers, parthenocarpic fruit set has been an important trait for greenhouse production of gynoecious cultivars that tend to be higher yielding than monoecious or andromonoecious cultivars (Ponti, 1976). Researchers have also attempted to select for parthenocarpy in summer squash (*C. pepo*) for greenhouse production and for field production with row covers or in conditions unsuitable for pollinator species (Nijs and Zanten, 1982; Robinson, 1993; Robinson and Reiners, 1999). In cucumber, the parthenocarpic trait is controlled by an incompletely dominant gene *Pc* or *P* (Pike and Peterson, 1969; Xie and Wehner, 2001) in conjunction with additional modifying genes and environmental conditions (Ponti, 1976). No alleles specific to parthenocarpy have been identified in *Cucurbita* (Paris and Kabelka, 2009), and genotypic differences for percent parthenocarpic fruit set appear to be quantitative (Robinson and Reiners, 1999).

Triploid seedless watermelons resulting from hybridization between 4x and 2x breeding lines are also an important example of parthenocarpy in cucurbits (Maynard and Elmstrom, 1992). Similarly, sterility of commercial interspecific squash hybrids results in development of seedless fruit, as long as a pollen source is present for inducing fruit set. In South American commercial squash production, interspecific squash hybrids are inter-planted with pollinator cultivars at a 9:1 to 4:1 ratio (Mendonça *et al*., 2006).

Seedless fruit may offer several advantages in squash production: 1) photosynthate normally partitioned to developing seeds may be available for dry matter accumulation in fruit flesh; 2) seedless fruit may have less of an inhibitory effect on
development of additional fruit compared with fruit containing abundant seeds (Stephenson, 1981; Stephenson et al., 1988); 3) seedless fruit may have less of an inhibitory effect on the development and/or maintenance of vegetative organs. On the other hand, species not prone to development of parthenocarpic fruit may show increased or total fruit abscission in the absence of seeds (Stephenson, 1981).

**Eating Quality**

Numerous studies have shown a positive correlation between eating quality and mesocarp DW and soluble solids in winter squash (Corrigan et al., 2006; Culpepper and Moon, 1945; Harvey et al., 1997). In kabocha squash, mesocarp DW between 20-30% is considered acceptable for fresh eating (Harvey et al., 1997). In squash grown for processing, however, color and consistency of fruit mesocarp are of foremost importance (Lana and Tisher, 1951). Generally, squash containing 9-11% mesocarp DW has favorable processing consistency, as measured by the degree of spreading of squash puree using a consistometer, and mesocarp DW is positively correlated with consistency (Culpepper and Moon, 1945; Lana and Tisher, 1951). Cultivars possessing tan or orange rinds are desirable compared with green-fruited cultivars, since green rinds can impart undesirable color to the processed product (Culpepper and Moon, 1945; Lana and Tisher, 1951; Whitaker and Bohn, 1950).

An important consideration in assessing squash cultivars is that mesocarp DW values are negatively correlated with fresh weight (FW) yields (Loy, 2004, 2006). The relatively low DW in acceptable processing squash allows for utilization of cultivars unacceptable for fresh eating but that are exceptionally high yielding. For example, the processing cultigen ‘Dickenson Field’ yields between 44-82 mt/ha (M. Babadoost,
personal communication), but maximum yields in ‘Delica,’ a high DW C. maxima cultivar, have been reported between 30-40 mt/ha (Buwalda and Freeman, 1986; Botwright et al., 1998).

During squash fruit development, mesocarp dry matter accumulation peaks between 30-40 days after pollination (Culpepper and Moon, 1945; Irving et al., 1997), but may decrease in the absence of additional photosynthate due to respiratory losses (Irving et al, 1997) or remobilization to developing seeds (Loy, 2004). Also, increases in soluble solids in harvested squash results from the metabolism of starch into simple sugars (Irving et al., 1997; Loy, 2006), and increases in mesocarp carotenoid content imparting intense flesh color occur after peak dry matter is reached (Harvey et al., 1997; Hopp et al., 1960; Noseworthy and Loy, 2008). Processing squash could potentially be harvested as soon as mesocarp DW reached ~10%, as long as fruit rinds were of desirable color and processing occurred prior to conversion of starch to sugar or the remobilization of starch reserves to seeds. Also, since flesh color intensifies after harvest, the dry matter, sugar, carotenoid relationship should be considered in assessing processing cultivars.
CHAPTER II. MATERIALS AND METHODS

Plant Materials

Interspecific Crosses

*C. maxima x C. moschata* crosses were performed during the summers of 2009, 2010, and 2011 by controlled hand pollination using standard squash pollination protocol (Whitaker, 1960). Staminate and pistillate flowers were tied off one day pre-anthesis (DPA) to prevent pollen contamination, and pollinations were performed between 6:00 A.M.-8:00 A.M. on the morning of anthesis. In some cases, bud pollinations were performed 1 DPA (Hayase, 1961). Multiple pollinations were attempted when initial pollinations failed to set fruit, and self-pollinations and intraspecific crosses were performed in respective species for maintenance of parental lines and as controls. The date, time, and weather conditions were recorded for each pollination, and flowers were tagged to indicate pollination date and pollen parent. Additional pistillate flowers were removed from plants with developing fruit.

Although some reciprocal crosses were attempted, *C. maxima* lines were utilized as pistillate parents for several reasons: 1) Previous studies indicate greater success with crosses made in the *C. maxima x C. moschata* direction (Castetter, 1930; Erwin and Haber, 1929; Hayase, 1956; Korakot, et al., 2010), including greater seed number per fruit and bigger embryos (Bemis and Nelson, 1963), than in the reciprocal; 2) *C. maxima x C. moschata* crosses allowed for greater efficiency in performing pollinations, since *C. moschata* lines outnumbered *C. maxima* lines; 3) Late pistillate flowering in *C. moschata*
lines increased the likelihood of incomplete seed fill and or non-viable seeds in reciprocal crosses; 4) Large-scale commercial production of interspecific F₁ hybrid seed might utilize ethephon (2-chloroethyl-phosphonic acid) applications to induce gynoecy in bush C. maxima lines (Robinson et al., 1970; Rudich et al., 1969).

The Cucurbita maxima parental cultigens were large-fruited bush processing inbred lines developed at the University of New Hampshire, including NH65, NH245-10, ‘Bush Pink Banana’ (NHBPB), and ‘Autumn Pride’ (AP). Early pollination attempts primarily utilized NH245-10 and NHBPB as pistillate parents because hybrid fruit phenotypes were anticipated to be most acceptable. In the summer of 2009, various vine C. moschata cultigens were utilized as potential parents, including ‘Waltham Butternut’ (WBN), ‘Ponca,’ ‘Musque de Provence’ (MDP), ‘New Hampshire Baby Butternut’ (NHBBN), ‘Long Island Cheese’ (LIC), NH1511-11, ‘Dickinson Field’ (DF), and a ‘Dickinson Field’-type breeding line SC936 (Rupp Seeds, Inc., Wauseon, OH).

Adverse weather conditions resulted in late flowering in most C. moschata lines, so WBN was the pollen parent for the majority of hybrids. Based on preliminary observations of fruit set, seed yield and hybrid plant phenotype, crosses in subsequent field seasons utilized NH65 as the primary pistillate parent and the C. moschata processing cultigens LIC, DF, and/or SC936 as pollen parents.

**Seed Harvesting**

Fruits resulting from crosses were harvested approximately 55-60 days after pollination (DAP), unless plants senesced beforehand, in which case the fruit was harvested at senescence. Harvested fruit were stored in a greenhouse at 18-26 °C until ~65 DAP to ensure complete seed fill. Seeds were extracted, cleaned, and dried at 30 °C,
and the number of seeds containing embryos was noted for each cross. Additionally, mean seed weight and embryo weight were recorded for each interspecific cross, as well as for parental lines and several intraspecific crosses.

**Seed Germination**

Scarification of seeds by scoring the seed coat opposite the micropyle was observed to enhance germination in plug trays and in the field. In the fall of 2011, percent germination of scarified and non-scarified seeds was compared among the interspecific hybrids 65xLIC and 65xDF and parental cultigens NH65 (C. maxima), ‘Long Island Cheese’ (C. moschata), and ‘Dickinson Field’ (C. moschata). Seeds were sown in 50-cell plug trays containing soilless potting medium (Pro-Mix BX, Griffin Greenhouse Supplies, Tewksbury, MA) in a split-plot design consisting of three replicates. Main plots contained 20 seeds of each cultigen, while sub-plots consisted of 10 scarified or non-scarified seeds. Greenhouse temperatures were maintained at approximately 23 °C. Seeds were sown 16 November, 2011, and observations for percent germination were conducted one week and two weeks after sowing.

**Greenhouse studies**

**Spring, 2010**

A comparative study of main stem length, internode length, leaf initiation, leaf area, and flowering habit in interspecific hybrids and parental cultigens was performed in the greenhouse in the spring of 2010. Two hybrids, NH245-10 x ‘Waltham Butternut’ (245xWBN) and ‘Bush Pink Banana’ x WBN (BPBxWBN), were selected according to fruit set, seed yield per fruit, seed fill, available seed, and anticipated phenotype. Seeds were sown 1 February, 2010 in 50-cell plug trays containing soilless potting medium and
transplanted into 8.7 L plastic nursery pots on 17 February after expansion of the first true leaf. Pots were arranged in a randomized complete block design consisting of six one-plant replicates. All plants were pruned to a single main stem and trellised. Greenhouse temperatures were maintained at 17°C nights and 25°C days, and plants were irrigated using trickle irrigation and were constantly fertilized with soluble fertilizer (15-5-15) at a rate of 250 ppm.

Measurements were taken from each plant, except where noted. Anthesis of flowers was recorded daily until 50 days after transplant (DAT), beginning at commencement of flowering, and observations included the sex and node location of each flower. Leaf number was recorded once weekly during early growth stages and twice weekly in later growth, beginning 5 DAT. Main stem length, from the cotyledons to the shoot apex was measured twice weekly beginning 21 DAT. Measurements of leaf number and main stem length ceased at 38 DAT upon onset of fruit development.

Internode lengths and leaf area were measured at 56 DAT. Leaves were harvested from each plant in three sets, those initiated 0-12 DAT, 13-24 DAT, and 25-34 DAT, to account for potential leaf variation within plants and varying degrees of leaf senescence. Leaf fresh weight was measured, as well as fresh weight of a leaf sample of 19.63 cm² area extracted with a core-sampler. Fully senesced leaves were traced, and leaf area was estimated from traced samples. Cumulative leaf area was estimated as leaf number x (FW_{total}/FW_{sample}) x 19.63 cm².

Spring, 2011

A comparative study of plant growth traits similar to that in the spring of 2010 was performed in the spring of 2011, but with interspecific hybrids NH65 x ‘Long Island
Cheese' (65xLIC) and NH65 x 'Dickinson Field' (65xDF) and parental cultigens. Seeds were sown 17 January, 2011 in plug trays and seedlings were transplanted into nursery pots 3 February and arranged in a randomized complete block design consisting of six one-plant replicates. Fertility management and environmental conditions matched those in Spring 2010.

Measurement of growth traits commenced 14 DAT. Additional traits evaluated included main stem diameter and petiole diameter at node 10. Cumulative leaf area was determined at anthesis of the first female flower, approximating canopy development at first fruit set, and accounted for all unfurled leaves. Individual leaf areas were estimated as leaf length from petiole to tip multiplied by leaf width at the basal lobes (Fig. 1) (Loy and Broderick, 1990).

Figure 1. Leaf areas were estimated as leaf length from petiole to tip by leaf width at the basal lobes.
Field Studies

Summer/Fall, 2010

In the summer of 2010, the interspecific hybrid 245xWBN and processing cultivars ‘Dickinson Field’ (DF, open-pollinated *C. moschata*) and Rspl232 (‘Golden Delicious’-type, *C. maxima* bush-vine *F₁* hybrid, Rupp Seeds, Inc., Wauseon, OH) were grown in the field at Kingman Research Farm in Madbury, NH for a comparative growth study. Four other interspecific hybrids were observed in non-replicated plots, including APxWBN and 65xWBN and small populations of 245xLIC and 65xLIC.

Seeds were sown in 50-cell plug trays containing soilless potting medium on 21 May and transplanted on 2 June at first true leaf stage into raised beds covered with black plastic mulch. Plants were spaced at 0.9 m within row and 2.4 m between rows (0.5 plants/m²) in a completely random design consisting of six eight-plant replicates, each with two guard plants. Soil type was a Charlton fine sandy loam broadcast with 56.1 kg N and K₂O each per ha. Plants were irrigated using drip irrigation and fertilized weekly for four weeks through the irrigation system with soluble fertilizer at a rate of 2.8 kg N and K₂O/ha. Pest control included one application of carbaryl followed by injection of imidacloprid through the drip irrigation for striped cucumber beetle (*Acalymma vittatum*), and one application of chlorothalonil and myclobutanil at onset of symptoms of powdery mildew. Weed control was achieved with mechanical cultivation between raised beds and spot application of paraquat.

Growth Traits. Data were collected on initiation of pistillate and staminate flowers at anthesis, leaf initiation on the main stem, and branching habit. Fruit were
tagged at date of pollination, and six randomly selected fruit per plot were harvested between 55-60 DAP or at plant senescence.

**Fruit Harvest.** At harvest, fruit were evaluated for the following characteristics: fruit fresh weight (FW), dry weight (DW), percent soluble solids content (%SSC), mesocarp thickness, and flesh color. Cooked fruit was rated subjectively for texture, dryness, sweetness, and overall flavor.

Fruits were divided into mesocarp, placenta, and seed components, and placental tissue FW and seed FW were determined separately. Mesocarp FW was calculated as

\[ \text{FW}_{\text{fruit}} - \text{FW}_{\text{seed+placenta}} \]

For mesocarp DW determination, a mesocarp sample of approximately 5-9 g was extracted using a cork borer with a diameter of 1.5 cm on the side opposite the ground spot midway between the peduncle and blossom scar. The rind and placental tissues were removed from the sample. Mesocarp samples were weighed before and after drying at 68°C for at least 48 hrs., and total mesocarp DW was calculated as

\[ (\text{DW}_{\text{sample}}/\text{FW}_{\text{sample}}) \times \text{total mesocarp FW} \]

A similar mesocarp sample, frozen and thawed, was used to determine percent soluble solids content using a refractometer. Mesocarp color was rated relative to a Roche Yolk Colour Fan ranging from light yellow (#1) to dark orange (#15).

Mean percent DW of placental tissue and seeds for cultigens was determined by extracting approximately 45-50 g of placental tissue and seeds from each fruit, combining, and weighing before and after drying at 68°C for at least 48 hrs. Dry weights of the placenta and seeds were calculated for each fruit as mean %DW x individual FW.
**Summer/Fall, 2011**

A comparative yield study was performed at the Kingman Research Farm, Madbury, NH in the summer of 2011 among the interspecific hybrids 65xLIC and 65xDF and standard open-pollinated processing cultivars ‘Dickinson Field’ (DF, *C. moschata*) and ‘Golden Delicious’ (GD, *C. maxima*). Non-replicated plots of two parental lines, NH65 (*C. maxima*) and ‘Long Island Cheese’ (LIC, *C. moschata*), and two interspecific hybrids, 65xWBN and 245xWBN, were also observed.

Plants were seeded in 50-cell plug trays containing soilless potting medium on 27 May and transplanted 10 June at the first true leaf stage. Poor germination of GD necessitated re-sowing on 10 June and transplanting on 20 June. Plants were arranged in a randomized complete block design consisting of four sixteen-plant replicates per cultigen. To account for vigorous vine growth, each data plot contained two eight-plant rows surrounded by two guard rows on either side and four guard plants per row (Fig. 2).

![Figure 2](image)

Figure 2. Sixteen-plant data plots in the summer of 2011 consisted of two eight-plant rows with four guard plants surrounded by two guard rows on either side. O = data plant; X = guard plant.
In-row spacing was 0.6 m and between-row spacing was 2.4 m (0.7 plants m⁻¹). Unhealthy or dead plants were replaced during early growth to help ensure uniform plots. Cultural methods were similar to those followed in the summer of 2010, but included one application of endosulfan for control of squash vine borer (*Melittia cucurbitae*) and two mixed applications of chlorothalonil and myclobutanil for powdery mildew control.

**Growth Traits.** Four plants per replicate were chosen at random for weekly measurements of main stem length, branching habit, and leaf initiation on the main stem. Also, petiole length, canopy height, area of the fourth and tenth leaves on the main stem, and area of leaves on mature plants were recorded. Leaf area was estimated using the procedure described for the greenhouse in 2011 (Fig. 1).

**Fruit Harvest.** Fruits were tagged at pollination and harvested at approximately 55-60 DAP or plant senescence. All fruit were harvested per plot and weighed to determine fresh weight (FW) yields. A subsample of four randomly selected fruit per plot was evaluated for total FW, percent soluble solids content (%SSC), flesh thickness, flesh color, and FW and DW were determined for mesocarp, placenta, and seed fruit components as described for the field in 2010. Fruit were also evaluated for blossom scar diameter and degree of ribbing, rated subjectively from no ribbing (0) to excessive ribbing (5). Additionally, the diameter of the peduncle at the base and 2.5 cm from the base was determined in four randomly selected fruit from each cultigen.

Mesocarp fresh weight and dry weight yields were calculated using values measured in the four-fruit subsamples selected from each plot. Mesocarp fresh weight yields were calculated as \( \left( \frac{FW_{\text{Mesocarp}}}{FW_{\text{Fruit}}} \right) \times FW_{\text{Total}} \). Dry weight yields were calculated as mesocarp %DW \times mesocarp fresh weight yield.
Data analysis

An ANOVA was performed for all data where applicable, and Tukey’s multiple comparison test was performed among treatment means.
CHAPTER III. RESULTS

Interspecific Hybridization

Field, 2009

Attempts to hybridize *C. maxima* x *C. moschata* in the field in 2009 indicated compatibility was largely dependent on the *C. maxima* pistillate parent (Table 1). Only 5 of 32 pollinations of NH245-10 resulted in fruit set, and in most cases, NH245-10 flowers pollinated with *C. moschata* pollen abscised within one week. On the other hand, ‘Bush Pink Banana’ (NHBPB) showed far greater percentage of fruit set in interspecific crosses, with 26 of 39 pollinations resulting in developing fruit. Two additional *C. maxima* cultigens, NH65 and ‘Autumn Pride’ (AP), exhibited good fruit setting ability in crosses with *C. moschata*; however, limited population sizes of both cultigens made preliminary data inconclusive (Table 1).

![Normal C. maxima embryo (left) and an underdeveloped, malformed C. maxima x C. moschata F₁ embryo (right).](image)

Figure 3. A normal *C. maxima* embryo (left) and an underdeveloped, malformed *C. maxima x C. moschata* F₁ embryo (right).
Table 1. Number of pollinations, number of set fruit, number of fruit containing seeds, mean seeds per fruit, and number of seeds in individual fruit in bush *C. maxima* × vine *C. moschata* crosses performed in the field in 2009. *Cucurbita maxima* cultivars included NH65, NH245-10, ‘Bush Pink Banana’ (NHBPB), and ‘Autumn Pride’ (AP). *Cucurbita moschata* cultivars were ‘Waltham Butternut’ (WBN), ‘Long Island Cheese’ (LIC), SC936 (Rupp Seeds, Inc., Wauseon, OH), butternut breeding line NH1511-11, ‘New Hampshire Baby Butternut’ (NHBBN), ‘Ponca,’ and ‘Musque de Provence.’

<table>
<thead>
<tr>
<th></th>
<th>C. maxima</th>
<th>C. moschata</th>
<th>No. of Pollinations</th>
<th>Fruit Set</th>
<th>Fruit w/ Seed</th>
<th>Mean Seeds/Fruit</th>
<th>Range Seeds/Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHBPB</td>
<td>WBN</td>
<td>9</td>
<td>8</td>
<td>3</td>
<td>189</td>
<td></td>
<td>145,207,216</td>
</tr>
<tr>
<td></td>
<td>LIC</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>211</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SC936</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH1511-11</td>
<td>14</td>
<td>8</td>
<td>4</td>
<td>120</td>
<td></td>
<td>65,115,145,156</td>
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<tr>
<td></td>
<td>NHBBN</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ponca</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>39</td>
<td>26</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH245-10</td>
<td>WBN</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>218</td>
<td></td>
<td>213,223</td>
</tr>
<tr>
<td></td>
<td>LIC</td>
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<td>2</td>
<td>1</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SC936</td>
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<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NH1511-11</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>165</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NHBBN</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ponca</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>MDP</td>
<td>2</td>
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<td>0</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH65</td>
<td>WBN</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>101</td>
<td></td>
<td>88,115</td>
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<td>NHBBN</td>
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<tr>
<td></td>
<td>LIC</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>5</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>WBN</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>133</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Seeds contained thin or underdeveloped embryo.
One fruit rotted.

In crosses involving NH65 and NH245-10, most mature fruit contained seed, whereas in NHBPB, only 9 of 26 fruit contained seed, and one of four set fruit in AP contained seed (Table 1). Fruit resulting from interspecific crosses often contained well-
developed seed coats lacking embryos, and many seeds had liquid endosperm but lacked well-developed embryos (Fig. 3).

The mean number of seeds per fruit in crosses utilizing NHBPB ranged from 58 seeds in NHBPB x ‘New Hampshire Baby Butternut’ crosses to 211 seeds in NHBPB x ‘Long Island Cheese’ (LIC) crosses, but each of these crosses only produced one fruit with seeds. Three fruit resulting from NHBPB x ‘Waltham Butternut’ (WBN) crosses contained an average of 189 seeds, and individual NHBPB x WBN fruit contained between 145-216 seeds. Four fruit resulting from crosses between NHBPB and the butternut-type breeding line NH1511-11 contained an average of 120 seeds, ranging from 65-156 seeds in individual fruit (Table 1).

Of the four fruit derived from NH245-10 as the pistillate parent, one fruit contained 85 seeds with underdeveloped embryos (x LIC), and one fruit contained 165 well-developed embryos (x NH1511-11). The cross NH245 x WBN yielded two fruits containing 213 and 223 seeds.

In two NH65 x WBN crosses, fruits had 88 and 115 seeds. The one fruit resulting from a NH65 x LIC cross contained only 11 filled seeds, and one AP x WBN fruit contained 133 seeds with underdeveloped embryos.

Five crosses were performed in the field in 2009 between bush C. maxima lines and the vine C. maxima cultivar ‘Pink Banana Jumbo’ (PBJ) performed in the field in 2009 all produced fruit containing well-filled seeds. In three crosses utilizing NHBPB as the pistillate parent and PBJ as the staminate parent, individual fruit contained 182, 198, and 280 seeds, and fruit from one reciprocal cross contained 330 seeds. Lastly, one fruit resulting from pollination of PBJ by NH245-10 contained 426 seeds.
Field, 2010

Field crosses in 2010 utilized NH65, NH245-10, and AP as seed parents. ‘Long Island Cheese’ (LIC) and ‘Dickinson Field’ (DF) were used as pollen parents because of their known utility for pie stock and based on observations of vegetative and fruit phenotypes in small populations of C. maxima x LIC F1 hybrids. Crosses to NH65 and AP consistently set fruit, whereas percentage fruit set was low in crosses with NH245-10, regardless of the C. moschata parent. In NH245-10, only two fruits were obtained from over twenty pollinations: one resulting from self-pollination and one from a cross with LIC. Although AP fruit produced from interspecific pollinations appeared normal, few seeds were obtained from each cross. In interspecific crosses involving NH65, most fruit contained abundant well-filled seeds.

Field, 2011

Attempts at C. maxima x C. moschata hybridization in the field in 2011 were consistent with 2010 observations. Crosses between NH65 and C. moschata cultigens resulted in a high percentage of fruit set, and all set fruit contained filled seeds (Table 2). Eight of nine attempts at pollinating NH65 with LIC resulted in fruit containing an average of 163 ± 79 seeds. In crosses to the ‘Dickinson Field’-type breeding line SC936, NH65 set 22 of 23 fruit that averaged 217 ± 82 seeds per fruit. The cross NH65 x DF resulted in 3 fruit containing 269 ± 60 seeds, and NH65 crossed with the C. moschata breeding line M3X produced 3 fruit with 152 ± 34 seeds. The only pollinations that failed to set fruit in NH65 x SC936 and NH65 x DF crosses were performed one day pre-anthesis.
Table 2. Number of pollinations, number of set fruit, and mean seeds per fruit in *C. maxima* x *C. moschata* crosses performed in 2011.

<table>
<thead>
<tr>
<th>Pistillate Parent</th>
<th>Staminate Parent</th>
<th>No. of Pollinations</th>
<th>No. Set Fruit</th>
<th>Mean Seeds/Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65 †</td>
<td>⊙</td>
<td>1</td>
<td>1</td>
<td>251</td>
</tr>
<tr>
<td>BH ‡</td>
<td>1</td>
<td>1</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>LIC ‡</td>
<td>9</td>
<td>8</td>
<td>163 ± 79</td>
<td></td>
</tr>
<tr>
<td>SC936 ‡</td>
<td>23</td>
<td>22</td>
<td>217 ± 82</td>
<td></td>
</tr>
<tr>
<td>DF ‡</td>
<td>4</td>
<td>3</td>
<td>269 ± 60</td>
<td></td>
</tr>
<tr>
<td>M3X ‡</td>
<td>3</td>
<td>3</td>
<td>152 ± 34</td>
<td></td>
</tr>
<tr>
<td>NH245-10 ‡</td>
<td>SC936</td>
<td>13</td>
<td>10</td>
<td>105 ± 64</td>
</tr>
<tr>
<td>NHBPB †</td>
<td>SC936</td>
<td>3</td>
<td>2</td>
<td>127 ± 54</td>
</tr>
<tr>
<td>LIC</td>
<td>⊙</td>
<td>1</td>
<td>1</td>
<td>560</td>
</tr>
<tr>
<td>SC936</td>
<td>⊙</td>
<td>1</td>
<td>1</td>
<td>529</td>
</tr>
<tr>
<td>DF</td>
<td>⊙</td>
<td>1</td>
<td>1</td>
<td>654</td>
</tr>
</tbody>
</table>

*All set fruit contained seeds.*

*Values ± SD.*

*’Blue Hubbard,’ open-pollinated *C. maxima* cultivar.*

*‘Long Island Cheese’ (LIC), ‘Dickinson Field’ (DF), and SC936 are vine *C. moschata* processing cultivars.*

*Breedling line resulting from a three-way *C. moschata* cross.*

In attempts to cross NH245-10 with *C. moschata*, only SC936 pollen resulted in fruit set, and ten of thirteen pollinations produced fruit containing 105 ± 64 seeds (Table 2). Fruit set did not occur in three NH245-10 x LIC crosses and one NH245-10 x DF cross. Two of three NHBPB x SC926 crosses resulted in fruit containing 88 and 165 seeds (Table 2).

One NH65 self-pollination and one cross to the vine *C. maxima* open-pollinated cultivar ‘Blue Hubbard’ (BH) yielded fruit containing 251 seeds and 197 seeds, respectively. Also, one fruit each from self-pollinations of LIC, SC936, and DF contained 560, 529, and 654 seeds, respectively.
Embryo Weight

Variability in individual seed weights among crosses were reflected in embryo weights (Table 3). In 2010, mean seed weights in fruit of self-pollinated *C. maxima* parental lines NH65 (245 mg) and NH245-10 (271 mg) were significantly higher than in LIC (179 mg) and DF (152 mg). Embryo weights averaged 178 mg in NH65® and 176 mg in 245-10®, compared with 141 mg in LIC® and 114 mg DF®. Seed weights in interspecific crosses involving NH65 were significantly less than in NH65® due to small embryos in NH65 x LIC (92 mg) and NH65 x DF (114 mg). Seeds from one NH245-10 x LIC cross averaged 199 mg, but mean embryo weight in that cross was 94 mg. Embryos in the interspecific crosses NH65 x DF (114 mg) and NH245-10 x LIC (94 mg) were relatively small compared with total seed size, and exhibited high coefficients of variation for embryo size (Table 3).

Table 3. Mean weights of individual seeds and embryos in *C. maxima*, *C. moschata*, and *C. maxima* x *C. moschata* crosses performed in the field in 2010.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>No Fruit</th>
<th>Seed Wt. (mg)</th>
<th>Embryo Wt. (mg)</th>
<th>C.V. Embryo Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65®</td>
<td>1</td>
<td>245 ± 14 d</td>
<td>178 ± 13 c</td>
<td>0.073</td>
</tr>
<tr>
<td>NH245-10®</td>
<td>1</td>
<td>271 ± 26 d</td>
<td>176 ± 20 c</td>
<td>0.113</td>
</tr>
<tr>
<td>LIC®</td>
<td>1</td>
<td>179 ± 8 bc</td>
<td>141 ± 6 b</td>
<td>0.043</td>
</tr>
<tr>
<td>DF®</td>
<td>1</td>
<td>152 ± 10 ab</td>
<td>114 ± 9 ab</td>
<td>0.079</td>
</tr>
<tr>
<td>NH65 x LIC</td>
<td>7</td>
<td>142 ± 16 a</td>
<td>92 ± 13 a</td>
<td>0.140</td>
</tr>
<tr>
<td>NH65 x DF</td>
<td>6</td>
<td>179 ± 32 bc</td>
<td>114 ± 33 ab</td>
<td>0.288</td>
</tr>
<tr>
<td>NH245-10 x LIC</td>
<td>1</td>
<td>199 ± 30 c</td>
<td>94 ± 32 a</td>
<td>0.342</td>
</tr>
</tbody>
</table>

*The number of fruit from which seed was derived. For crosses with multiple fruit, seed was bulked before sampling.

*Values (±SD) represent means of 10 seeds and embryos per cultigen.

*C. maxima* bush processing lines.

*C. moschata* vine processing cultivars ‘Long Island Cheese’ (LIC) and ‘Dickinson Field’ (DF).

Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).
In 2011, seed weight in self-pollinated fruit of *C. moschata* cultigens LIC (158 mg), DF (148 mg), and SC936 (132 mg) was significantly lower than in *C. maxima* (Table 4). Seeds in one self-pollinated fruit of NH65 averaged 211 mg. A fruit from a NH65 x ‘Blue Hubbard’ cross contained the largest seeds at 278 mg. Mean seed weights in four interspecific crosses, NH65 x LIC (226 mg), NH65 x DF (180 mg), NH65 x SC936 (196 mg), and NH245-10 x SC936 (217 mg), were not significantly different than in the self-pollinated *C. maxima* line NH65. As in 2010, comparative embryo weights between crosses reflected differences observed for seed weights, with the exception of fruit resulting from the NH245-10 x SC936 cross, which contained relatively large seeds (217 mg) but small embryos (111 mg) (Table 4).

Table 4. Mean weights of individual seeds and embryos in *C. maxima*, *C. moschata*, and *C. maxima* x *C. moschata* crosses performed in the field in 2011.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>No. Fruit</th>
<th>Seed Wt. (mg)</th>
<th>Embryo Wt. (mg)</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65®</td>
<td>1</td>
<td>211 ± 14 cd&quot;</td>
<td>146 ± 14 de</td>
<td>0.095</td>
</tr>
<tr>
<td>NH65 x BH&quot;</td>
<td>1</td>
<td>278 ± 21 e</td>
<td>214 ± 15 f</td>
<td>0.069</td>
</tr>
<tr>
<td>LIC®'</td>
<td>1</td>
<td>158 ± 13 ab</td>
<td>123 ± 11 bc</td>
<td>0.090</td>
</tr>
<tr>
<td>DF®'</td>
<td>1</td>
<td>148 ± 15 ab</td>
<td>107 ± 11 ab</td>
<td>0.106</td>
</tr>
<tr>
<td>SC936®'</td>
<td>1</td>
<td>132 ± 12 a</td>
<td>99 ± 11 a</td>
<td>0.114</td>
</tr>
<tr>
<td>NH65 x LIC</td>
<td>8</td>
<td>226 ± 34 d</td>
<td>165 ± 25 e</td>
<td>0.150</td>
</tr>
<tr>
<td>NH65 x DF</td>
<td>3</td>
<td>180 ± 20 bc</td>
<td>123 ± 16 bc</td>
<td>0.134</td>
</tr>
<tr>
<td>NH65 x SC936</td>
<td>22</td>
<td>196 ± 22 cd</td>
<td>136 ± 22 cd</td>
<td>0.160</td>
</tr>
<tr>
<td>NH245-10 x SC936</td>
<td>10</td>
<td>217 ± 34 d</td>
<td>111 ± 22 ab</td>
<td>0.203</td>
</tr>
</tbody>
</table>

*The number of fruit from which seed was derived. For crosses with multiple fruit, seed was bulked before sampling.

1Values (±SD) represent means of 10 embryos per cultigen.

'C. maxima' bush processing line.

"'Blue Hubbard,' open-pollinated vine *C. maxima*.

'Long Island Cheese' (LIC), 'Dickinson Field' (DF), and SC936 are *C. moschata* vine processing cultigens.

1Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).
Germination and Seedling Development

Seed germination and seedling development were monitored in seeds sown for transplants used in greenhouse and field studies. Early observations indicated lower percent germination in some interspecific hybrids compared with parental cultigens. However, scarification of seed coat ends opposite the micropyle with a razor blade improved germination in interspecific hybrids and some parental lines, and germination in scarified interspecific F1 seeds approached 100% in seeds germinated in plug trays for use in field studies.

In most interspecific hybrids, seedling growth appeared normal compared with parental cultigens (Fig. 4). In some hybrids, however, seedlings exhibited abnormal early growth. While malformed plants often assumed normal growth after several weeks of development, these plants lacked vigor and/or were shaded by surrounding plants with normal growth.

Figure 4. Seedlings of two interspecific hybrids and parental cultigens. Clockwise from top left: NH65, ‘Long Island Cheese,’ ‘Dickinson Field,’ 65xDF, and 65xLIC.
A germination test comparing non-scarified and scarified seeds in interspecific hybrids and parental lines was performed in the fall of 2011. Percent germination among cultigens and between scarification treatments was significantly different (Table 5). At one week after sowing, percent germination in hybrid 65xLIC was 13% without scarification and 30% with scarification, and percent germination was 43% in non-scarified seeds and 100% in scarified seeds in 65xDF. In LIC, percent germination in non-scarified and scarified seeds was 53% and 90%, respectively. Percent germination was 80% without scarification and 100% with scarification in NH65, and 93% without and 87% with scarification in DF (Table 5).

Scarification treatments in cultigens did not significantly affect germination percentage two weeks after sowing, with the exception of LIC, in which 70% non-scarified and 97% scarified seeds germinated. Percent germination in 65xLIC was 60%
in non-scarified and 70% in scarified seeds. All other treatments showed complete or near-complete germination two weeks after sowing (Table 5).

**Greenhouse, 2010**

**Main Stem Length**

In the spring of 2010, NH245-10 x ‘Waltham Butternut’ (245xWBN) and ‘Bush Pink Banana’ x ‘Waltham Butternut’ (BPBxWBN) were grown alongside parental cultigens in the greenhouse. Main stem length in the vine parent WBN was significantly longer than either bush parent for the duration of the study, reaching 261.8 cm at 38 days after transplant (DAT) compared with 35.8 cm in NHBPB and 83.0 cm in NH245-10. Differences in main stem length between bush parents resulted from more rapid main stem elongation in NH245-10 compared with NHBPB beginning approximately 30 DAT (Fig. 5).

![Figure 5. Mean main stem length (cm) in ‘Bush Pink Banana’ (NHBPB), NH245-10, and ‘Waltham Butternut’ (WBN) parental cultigens by days after transplant (DAT). Values (±SD) represent means of six plants.](image-url)
Although interspecific hybrids resembled bush parents in early growth (below node 6), main stem lengths were similar to the vine parent as early as 21 DAT in BPBxWBN (Fig. 6) and 27 DAT in 245xWBN (Fig. 7). At 38 DAT, mean main stem length was 275.7 cm in BPBxWBN (Fig. 6) and 228.0 cm in 245xWBN (Fig. 7).

Figure 6. Mean main stem length (cm) in ‘Bush Pink Banana’ (NHBPB), ‘Waltham Butternut’ (WBN), and the interspecific hybrid BPBxWBN by days after transplant (DAT). Values (±SD) represent means of six plants.
Internode Length

Differences in main stem length between cultigens were primarily due to differences in internode length. Internode length in the vine parent WBN increased steadily from 3.2 cm at internode 2 to 15.6 cm at internode 8, after which internode lengths stabilized (Table 6; Figs. 8-9). In the bush parent NHBPB, internode 2 was 0.5 cm, and successive internode lengths increased until node 17, at which point internode lengths stabilized between 4.0-4.5 cm (Table 6; Fig. 8). While internode lengths in the bush parental line NH245-10 were not significantly different from NHBPB in early growth (Table 6), more rapid internode elongation commencing at internode 10 resulted in later internode lengths similar to WBN (Table 6; Fig. 9).
Table 6. Representative internode lengths (cm) in the interspecific hybrids BPBxWBN and 245xWBN and parental cultigens.5

<table>
<thead>
<tr>
<th>Internode Number</th>
<th>Cultigen</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NHBPB</td>
<td>0.5 a</td>
<td>0.9 a</td>
<td>1.1 a</td>
<td>1.5 a</td>
<td>2.4 a</td>
<td>3.9 a</td>
<td>4.4 a</td>
</tr>
<tr>
<td></td>
<td>NH245-10</td>
<td>0.3 a</td>
<td>0.5 a</td>
<td>0.6 a</td>
<td>0.9 a</td>
<td>1.6 a</td>
<td>9.5 b</td>
<td>15.9 b</td>
</tr>
<tr>
<td></td>
<td>WBN</td>
<td>3.2 d</td>
<td>6.7 c</td>
<td>12.4 b</td>
<td>15.6 b</td>
<td>15.3 b</td>
<td>16.0 c</td>
<td>16.6 b</td>
</tr>
<tr>
<td></td>
<td>BPBxWBN</td>
<td>0.9 b</td>
<td>2.0 b</td>
<td>11.5 b</td>
<td>23.5 d</td>
<td>21.7 c</td>
<td>17.3 c</td>
<td>14.6 b</td>
</tr>
<tr>
<td></td>
<td>245xWBN</td>
<td>1.3 c</td>
<td>2.3 b</td>
<td>11.8 b</td>
<td>19.6 c</td>
<td>17.6 b</td>
<td>15.2 c</td>
<td>15.7 b</td>
</tr>
</tbody>
</table>

5Values represent means of six plants.
1Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

Internode lengths in interspecific hybrids resembled those of the bush parental lines during earliest growth, but later internode lengths resembled those in the vine parent after a period of successive elongation (Table 6; Figs. 8-9). Internode length in BPBxWBN reached 2.0 cm at internode 4. Successive internode lengths increased rapidly and exceeded those in the vine parent at internode 7, measuring 20.7 cm compared with 14.2 cm in WBN. The maximum internode length in BPBxWBN was 23.5 cm at internode 8 (Table 6; Fig. 8). Internodes in BPBxWBN remained longer than those in WBN until internode 14, when internodes shortened and stabilized at lengths similar to WBN (Table 6; Fig. 8).

Internode length in 245xWBN was 1.3 cm at internode 2, significantly greater than 0.3 cm in the bush parent NH245-10 (Table 6). Similarly to BPBxWBN, successive internodes elongated rapidly after node 4 and surpassed the vine parent at internode 7, measuring 18.1 cm (Fig. 9). The maximum internode length was 19.6 cm at internode 8. Internode lengths decreased at node 10 and remained similar to those in the vine parent for the remainder of the study (Table 6; Fig. 9).
Figure 8. Successive mean internode lengths in the interspecific hybrid BPBxWBN and parental cultigens. Values represent means of six plants.

Figure 9. Successive mean internode lengths in the interspecific hybrid 245xWBN and parental cultigens. Values represent means of six plants.
Leaf Initiation

In 2010, rates of leaf initiation on plant main stems were not significantly different among four of five cultigens observed, with 17.0-19.5 leaves at 37 DAT. The extreme bush line NHBPB exhibited a slower rate of leaf initiation than the other cultigens grown, with only 14.8 ± 0.8 leaves on the main stem at 37 DAT (Table 7).

Table 7. Number of leaves on the main stem at select days after transplant (DAT) in the interspecific hybrids BPBxWBN and 245xWBN and parental cultigens grown in the greenhouse in 2010. 

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>12 DAT</th>
<th>20 DAT</th>
<th>24 DAT</th>
<th>30 DAT</th>
<th>37 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHBPB</td>
<td>3.3 ± 0.5</td>
<td>6.0 ± 0.0</td>
<td>7.8 ± 0.8</td>
<td>10.8 ± 0.8</td>
<td>14.8 ± 0.8</td>
</tr>
<tr>
<td>NH245-10</td>
<td>3.7 ± 0.5</td>
<td>6.7 ± 1.4</td>
<td>8.5 ± 1.5</td>
<td>12.7 ± 1.9</td>
<td>17.0 ± 3.0</td>
</tr>
<tr>
<td>WBN</td>
<td>3.7 ± 0.8</td>
<td>6.7 ± 0.8</td>
<td>9.7 ± 1.2</td>
<td>13.5 ± 1.1</td>
<td>19.5 ± 1.7</td>
</tr>
<tr>
<td>BPBxWBN</td>
<td>3.8 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>9.3 ± 0.8</td>
<td>13.7 ± 0.5</td>
<td>18.0 ± 1.8</td>
</tr>
<tr>
<td>245xWBN</td>
<td>3.2 ± 0.4</td>
<td>6.0 ± 0.0</td>
<td>8.2 ± 0.8</td>
<td>12.3 ± 1.0</td>
<td>18.0 ± 1.3</td>
</tr>
</tbody>
</table>

Values (±SD) represent means of six plants.

Leaf Area

With the exception of NHBPB, cumulative leaf area and average individual leaf area of leaves initiated within 34 DAT were not significantly different among interspecific hybrids and parental cultigens (Table 8). Although the rate of leaf initiation in NHBPB was slower compared with other cultigens (Table 7), large individual leaf areas of 835.2 cm$^2$ resulted in significantly greater cumulative leaf area (10,346.7 cm$^2$) than in WBN (6,875.3 cm$^2$) and 245xWBN (7,510.3 cm$^2$), each with respective individual leaf areas of 405.9 cm$^2$ and 479.7 cm$^2$. Cumulative leaf areas in the interspecific hybrid BPBxWBN (7,731.1 cm$^2$) and the $C. maxima$ bush parent NH245-10 (8,018.4 cm$^2$) were not significantly different from that in NHBPB (Table 8).
Table 8. Cumulative leaf area and mean individual leaf area (cm²) in leaves initiated within 34 days after transplant in the interspecific hybrids BPBxWBN and 245xWBN and parental cultigens.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Cumulative (cm²)</th>
<th>Individual (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBN</td>
<td>6,875.3 ± 520.3</td>
<td>405.9 ± 35.1</td>
</tr>
<tr>
<td>NHBxPB</td>
<td>10,346.7 ± 2561.1</td>
<td>835.2 ± 172.0</td>
</tr>
<tr>
<td>BPBxWBN</td>
<td>7,731.1 ± 1,404.4</td>
<td>482.2 ± 79.2</td>
</tr>
<tr>
<td>NH245-10</td>
<td>8,018.4 ± 1,301.2</td>
<td>524.6 ± 78.8</td>
</tr>
<tr>
<td>245xWBN</td>
<td>7,510.3 ± 576.4</td>
<td>479.7 ± 34.5</td>
</tr>
</tbody>
</table>

Leaf area was estimated gravimetrically using a core sampler of known area.

¹Values (±SD) represent means of six replicates, with the exception of five replicates in WBN and four replicates in BPBxWBN due to early leaf senescence.

²Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

Flowering Habit

Date of commencement of flowering, total pistillate and staminate flowers, and pistillate to staminate flower ratios differed significantly among cultigens grown in the greenhouse in 2010. The vine parent WBN initiated pistillate flowering at 30 DAT and staminate flowering at approximately 45 DAT, whereas bush parents produced staminate flowers prior to pistillate flowers (Table 9). Pistillate flowering commenced at approximately 42 DAT in NHBxPB and 46 DAT in NH245-10, and staminate flowers were first produced at 32 and 33 DAT in NHBxPB and NH245-10, respectively (Table 9). Pistillate flowers appeared as early as 22 DAT in the interspecific hybrid BPBxWBN and 24 DAT in 245xWBN. Staminate flowering commenced at 42 DAT in 245xWBN, and BPBxWBN only produced only one staminate flower on one plant at 44 DAT (Table 9).

Total pistillate and staminate flowers and the pistillate to staminate flower ratio in 245xWBN resembled the vine parent (Table 9). At 50 DAT, WBN produced a total of 7.0 pistillate and 3.7 staminate flowers, and the hybrid 245xWBN produced 6.3 pistillate and 2.7 staminate flowers. The hybrid BPBxWBN was almost entirely gynoecious with

44
Table 9. Commencement of flowering in days after transplant (DAT) for pistillate (P) and staminate (S) flowers and total pistillate and staminate flowers at 50 DAT in the interspecific hybrids BPBxWBN and 245xWBN and parental cultigens.  

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>DAT</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>S</td>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td>WBN</td>
<td>30.0 b</td>
<td>45.5 b</td>
<td>7.0 b</td>
<td>3.7 a</td>
</tr>
<tr>
<td>NHBPB</td>
<td>42.2 c</td>
<td>32.2 a</td>
<td>0.8 a</td>
<td>31.8 b</td>
</tr>
<tr>
<td>NH245-10</td>
<td>46.5 c</td>
<td>33.3 a</td>
<td>1.0 a</td>
<td>30.7 b</td>
</tr>
<tr>
<td>BPBxWBN¹</td>
<td>22.3 a</td>
<td>-</td>
<td>6.3 b</td>
<td>-</td>
</tr>
<tr>
<td>245xWBN²</td>
<td>24.0 ab</td>
<td>42.8 b</td>
<td>6.3 b</td>
<td>2.7 a</td>
</tr>
</tbody>
</table>

*Values represent means of six replicates with the exception of NHBPB and NH245-10, each with five replicates.

¹Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

²One replicate produced one staminate flower at 44 DAT.

³One of six replicates produced zero staminate flowers.

Individual plants producing an average of 6.3 pistillate and zero staminate flowers at 50 DAT. Only one BPBxWBN plant produced one staminate flower. Both bush parental lines generated more staminate than pistillate flowers at 50 DAT with NHBPB plants each producing 0.8 pistillate and 30.7 staminate flowers and NH245-10 plants producing 1.0 pistillate flowers and 30.7 staminate flowers (Table 9).

**Field, 2010**

**Vegetative Growth**

The interspecific hybrid 245xWBN was evaluated in the field in 2010 in a comparative study with the bush-vine ‘Golden Delicious’-type C. maxima F₁ hybrid Rsp1232 and the vine C. moschata processing cultivar ‘Dickinson Field’ (DF). Non-replicated plots of the interspecific hybrids 65xWBN, APxWBN, 245xLIC, and 65xLIC were also observed. The C. maxima cultivar Rsp1232 possessed a thick, succulent main stem with slight or moderate branching and produced an upright canopy, particularly in early growth. Main stems in DF were narrower and harder than in Rsp1232 and
produced appreciable lateral shoots. The canopy produced by DF plants was generally more prostrate than that of Rsp1232.

The interspecific hybrid plants possessed the narrow, dense main stem and prostrate growth habit typical of *C. moschatu* plants but with only few lateral branches. In fact, plots of interspecific hybrid plants failed to achieve full canopy cover at any point during the growing season. Three plants of the interspecific hybrid 245xLIC and one plant of 65xLIC produced highly vigorous main stems with several long lateral branches and full, upright canopies with large leaves.

**Flowering Habit**

There were significant differences in the date of flowering commencement, total number of flowers, and ratio of pistillate to staminate flowers among cultigens grown in the field in 2010 (Figs. 10-11). In Rsp1232, pistillate flower production commenced at 30 DAT when plant main stems averaged 13 nodes. Staminate flowers first reached anthesis at 31 DAT in Rsp1232, when main stems contained 14 nodes. Although Rsp1232 plants sometimes produced the first pistillate flowers below node 10, these flowers infrequently developed to mature fruit. At 50 DAT, ten-plant plots of Rsp1232 averaged of 16 pistillate and 124 staminate flowers (Figs. 10-11).

In the *C. moschata* processing cultigen DF, pistillate flowering commenced at 46 DAT when main stems possessed 26 nodes, and staminate flowering commenced at 42 DAT when main stem had 22 nodes. In many DF plants, the first fruit set occurred at or near node 20. At 50 DAT, 10-plant plots of DF averaged only 3 pistillate flowers and 30 staminate flowers per plot (Figs. 10-11); however, continued flower production resulted in several pistillate flowers per plant and 1-2 fruit/plant.
The interspecific hybrid 245xWBN was highly gynoeccious, with plots producing 70 pistillate and 4 staminate flowers at 50 DAT (Figs. 10-11). The first pistillate flowers in 245xWBN appeared 28 DAT with only 9 nodes on plant main stems. Staminate flowers were produced beginning at 41 DAT (Figs. 10-11). Pollination of early pistillate flowers often resulted in multiple fruits set within the first 5 nodes in hybrid plants. In most cases, crown fruit sets were pruned because premature fruit set can restrict vegetative growth (Loy, 2004) and result in sparse leaf canopies and/or early plant senescence. Pruned plants set two additional fruit between nodes 15-20 at approximately 60 DAT.

![Graph showing number of pistillate flowers by days after transplant (DAT) in the interspecific hybrid 245xWBN and processing cultivars Rsp1232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata). Values represent means of total flowers in six ten-plant plots.](image)

Figure 10. Number of pistillate flowers by days after transplant (DAT) in the interspecific hybrid 245xWBN and processing cultivars Rsp1232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata). Values represent means of total flowers in six ten-plant plots.
Figure 11. Number of staminate flowers by days after transplant (DAT) in the interspecific hybrid 245xWBN and processing cultivars Rspl232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata). Values represent means of total flowers in six ten-plant plots.

**Fruit Traits**

In the summer of 2010, fruit were tagged with the date of anthesis and harvested approximately 55 days after pollination. The *C. moschata* processing cultivar DF produced significantly larger fruit (9.7 kg) than the *C. maxima* processing cultivar Rspl232 (6.1 kg) and the interspecific hybrid 245xWBN (2.9 kg) (Table 12). Also, the mesocarp was thicker in DF (4.63 cm) compared with Rspl232 (4.18 cm) and 245xWBN (3.35 cm) (Table 10).

The round fruit of Rspl232 had bright orange-red rinds with minimal or no ribbing, and DF fruits were oval or acorn-shaped with tan, moderately ribbed rinds. The hybrid 245xWBN (Fig. 12) produced fruit shaped intermediately to the parental lines containing bright orange rinds with yellow mottling and very slight ribbing. Mesocarp
color was bright orange in Rsp1232 and 245xWBN but appeared as a very intense pink-orange hue in DF, similar to that observed in sweet potato (Table 10). Examination of a DF carotenoid extract by thin-layer chromatography revealed that DF carotenoids are largely β-carotene, and perhaps α-carotene (data not shown). The fruit of interspecific hybrids and some fruit of Rsp1232 contained a green layer approximately midway into the mesocarp.

![Figure 12](image)

Figure 12. Fruit of NH245-10 (C. maxima, left), 'Waltham Butternut' (C. moschata, right), and the interspecific hybrid 245xWBN (center) from field plots in 2010.

The percent dry matter of 245xWBN mesocarp tissue was 16.8%, significantly higher than that of Rsp1232 (11.1%) and DF (7.2%). Although percent soluble solids contents (%SSC) were below 10% in all cultigens, Rsp1232 (7.5%) and 245xWBN (8.2%) fruit had significantly higher %SSC than DF (6.5%) fruit (Table 10).

**Biomass Partitioning within Fruit**

Individual fruits were divided into mesocarp, placenta, and seed components for evaluation of biomass partitioning within the fruit (Table 11). Mesocarp in the
interspecific hybrid 245xWBN comprised 96.2% of total fruit dry weight (DW), compared to 71.5% in Rsp1232 and 82.0% in DF. On the other hand, seed only represented 0.6% of fruit DW in 245xWBN, 25.5% of DW in Rsp1232, and 16.4% of DW in DF (Table 11).

Because seed embryos in squash contain high proportions of proteins and lipids, embryo development utilizes about double the photosynthetic energy per gram of weight than that of mesocarp tissue, comprised primarily of carbohydrates (Penning de Vries, 1975; Phillips, 1946). The energy equivalents stored in edible mesocarp and embryo components of fruit differed among cultivars (Table 12). Hybrid 245xWBN allocated 2,007.0 kJ to mesocarp tissue and 18.1 kJ to embryos per kg fruit FW. The C. maxima processing cultivar Rsp1232, which produced an average of 196 g of seed DW per fruit (Table 11), utilized 1267.0 kJ for production of mesocarp tissue and 606.5 kJ for embryo production per kg fruit FW. Lastly, DF allocated 861.2 kJ to mesocarp tissue and 249.5 kJ to embryos per kg fruit FW (Table 12).
Table 10. A comparison of fruit fresh weight (FW) and mesocarp percent dry weight (%DW), percent soluble solids content (%SSC), thickness (MT), and flesh color in the interspecific hybrid 245xWBN and processing cultivars Rsp1232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata).²

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>FW (kg)</th>
<th>%DW</th>
<th>%SSC</th>
<th>MT (cm)</th>
<th>Flesh Color¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rsp1232</td>
<td>6.1 ± 1.0 b</td>
<td>11.1 ± 2.3 b</td>
<td>7.5 ± 0.9 b</td>
<td>4.18 ± 0.29 b</td>
<td>9.7 ± 1.1 a</td>
</tr>
<tr>
<td>DF</td>
<td>9.7 ± 1.9 c</td>
<td>7.2 ± 0.9 a</td>
<td>6.5 ± 1.0 a</td>
<td>4.63 ± 0.28 c</td>
<td>13.6 ± 0.7 b</td>
</tr>
<tr>
<td>245xWBN</td>
<td>2.9 ± 0.5 a</td>
<td>16.8 ± 1.7 c</td>
<td>8.2 ± 1.1 b</td>
<td>3.35 ± 0.35 a</td>
<td>9.5 ± 0.5 a</td>
</tr>
</tbody>
</table>

²Values (±SD) represent means of four randomly harvested fruit from six plots.

¹Mesocarp flesh color was determined using a Roche Yolk Colour Fan with a gradient from light yellow (1.0) to dark orange (15.0).

¹Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).
Table 11. Individual fruit fresh weight (FW), fruit dry weight (DW), mesocarp and seed DW, and percentage of fruit dry weight in mesocarp and seed components in the interspecific hybrid 245xWBN and processing cultivars Rspl232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata).\textsuperscript{a, b, c}

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>FW (kg)</th>
<th>DW (g)</th>
<th>Meso DW (g)</th>
<th>Seed DW (g)</th>
<th>% Meso</th>
<th>% Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rspl232</td>
<td>6.1 ± 1.0 b\textsuperscript{e}</td>
<td>851 ± 228 b</td>
<td>632 ± 210 ab</td>
<td>196 ± 31.9 c</td>
<td>71.5 ± 5.9 a</td>
<td>25.5 ± 5.3 c</td>
</tr>
<tr>
<td>DF</td>
<td>9.7 ± 1.9 c</td>
<td>802 ± 154 b</td>
<td>668 ± 152 b</td>
<td>122 ± 20.9 b</td>
<td>82.0 ± 4.6 b</td>
<td>16.4 ± 4.4 b</td>
</tr>
<tr>
<td>245xWBN</td>
<td>2.9 ± 0.5 a</td>
<td>483 ± 97 a</td>
<td>466 ± 96 a</td>
<td>3 ± 0.3 a</td>
<td>96.2 ± 0.3 c</td>
<td>0.6 ± 0.1 a</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values (±SD) represent mean of four randomly harvested fruit from six plots. 
\textsuperscript{b}FW and DW values include placental tissue. 
\textsuperscript{c}Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

Table 12. Grams dry weight (DW) and energy utilization (kJ) of embryo and mesocarp components of fruit per kg fruit fresh weight (FW) and ratio of embryo DW and kJ consumption to fruit FW in the interspecific hybrid 245xWBN and processing cultivars Rspl232 (C. maxima) and ‘Dickinson Field’ (DF, C. moschata).\textsuperscript{a, b, c}

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>g DW/kg Fruit FW</th>
<th>kJ/kg Fruit FW</th>
<th>Ratio E/Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Embryo\textsuperscript{f}</td>
<td>Mesocarp\textsuperscript{f}</td>
<td>Embryo\textsuperscript{f}</td>
</tr>
<tr>
<td>Rspl232</td>
<td>24.4 ± 2.6</td>
<td>101 ± 22</td>
<td>0.189 ± 0.039</td>
</tr>
<tr>
<td>DF</td>
<td>10.0 ± 2.1</td>
<td>69 ± 8</td>
<td>0.122 ± 0.033</td>
</tr>
<tr>
<td>245xWBN</td>
<td>0.7 ± 0.1</td>
<td>160 ± 16</td>
<td>0.004 ± 0.000</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values (±SD) represent means of four randomly harvested fruits from six plots. 
\textsuperscript{b}Embryo DW determined as 74.2% of seed DW from 170 seed sample of bulked seeds. 
\textsuperscript{c}Energy equivalents for embryo (24.85 kJ/g) and mesocarp (12.55 kJ/g) tissues adapted from Loy (2004).
**Main Stem Length**

Main stem length in the vine *C. moschata* parental cultivars was significantly longer than in the bush *C. maxima* line. At 42 DAT, main stem length in LIC was 275.2 cm, compared with 179.1 cm in DF and 33.4 cm in NH65 (Fig. 13). Both interspecific hybrids, 65xLIC and 65xDF, exhibited main stem lengths resembling that of the bush parent until approximately 25 DAT, after which internode elongation resulted in main stem lengths intermediate to parental lines (Figs. 14-15). At 42 DAT, main stem length was 167.6 cm in 65xLIC (Fig. 14) and 143.6 cm in 65xDF, approaching that of DF (Fig. 15).

![Figure 13. Mean main stem length (cm) in NH65, ‘Long Island Cheese’ (LIC), and ‘Dickinson Field’ (DF) parental cultigens by days after transplant (DAT). Values (±SD) represent means of six plants.](image-url)
Figure 14. Mean main stem length (cm) in NH65, ‘Long Island Cheese’ (LIC), and the interspecific hybrid 65xLIC by days after transplant (DAT). Values (±SD) represent means of six plants.

Figure 15. Mean main stem length (cm) in NH65, ‘Dickinson Field’ (DF), and the interspecific hybrid 65xDF by days after transplant (DAT). Values (±SD) represent means of six plants.
**Internode Length**

Internode lengths in the bush parental cultigen NH65 did not exceed 3.0 cm in the first 15 internodes, although successive internodes elongated steadily from 0.6 cm at internode 2 to 2.9 cm at internode 15 (Table 13; Figs. 16-17). On the other hand, the second internode in the vine parental cultivar LIC was 3.6 cm, and internode length increased in successive internodes until node 11, after which internodes measured between 19.0-20.0 cm (Table 13; Fig. 16). In the vine parental cultigen DF, internode lengths increased from 2.6 cm at internode 2 to 15.9 cm at internode 15 (Table 13; Fig. 17).

Table 13. Representative internode lengths (cm) in the interspecific hybrids 65xLIC and 65xDF and parental cultigens.1

<table>
<thead>
<tr>
<th>Internode Number</th>
<th>NH65</th>
<th>LIC</th>
<th>DF</th>
<th>65xLIC</th>
<th>65xDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.6 a</td>
<td>3.6 c</td>
<td>2.6 b</td>
<td>1.4 a</td>
<td>1.2 a</td>
</tr>
<tr>
<td>4</td>
<td>0.6 a</td>
<td>9.2 c</td>
<td>4.7 b</td>
<td>1.8 a</td>
<td>1.4 a</td>
</tr>
<tr>
<td>6</td>
<td>1.1 a</td>
<td>14.8 c</td>
<td>8.0 b</td>
<td>2.7 a</td>
<td>2.0 a</td>
</tr>
<tr>
<td>8</td>
<td>1.2 a</td>
<td>15.3 d</td>
<td>10.3 c</td>
<td>6.2 b</td>
<td>3.2 ab</td>
</tr>
<tr>
<td>9</td>
<td>1.0 a</td>
<td>16.6 c</td>
<td>11.2 b</td>
<td>10.7 b</td>
<td>4.5 a</td>
</tr>
<tr>
<td>10</td>
<td>1.4 a</td>
<td>18.0 d</td>
<td>11.8 c</td>
<td>15.9 b</td>
<td>7.6 b</td>
</tr>
<tr>
<td>13</td>
<td>2.0 a</td>
<td>19.7 c</td>
<td>14.1 b</td>
<td>18.7 c</td>
<td>13.5 b</td>
</tr>
<tr>
<td>15</td>
<td>2.9 a</td>
<td>19.7 c</td>
<td>15.9 b</td>
<td>19.0 c</td>
<td>15.6 b</td>
</tr>
</tbody>
</table>

1Values represent means of six plants.
1Unlike letters within columns indicate significant difference as determined by Tukey’s test (p=0.05).

Internode lengths in the interspecific hybrid 65xLIC resembled those in the bush parent NH65 for the first 6 internodes, ranging from 1.4-2.7 cm, but increased progressively beginning at internode 7 resulting in significantly longer internodes (6.2 cm) than NH65 (1.2 cm) at internode 8 and internode lengths approaching those of the vine parent by internode 13 (18.7 cm) (Table 13; Fig. 16). In 65xDF, internode lengths averaged 1.2-2.0 cm for the first six internodes, and increased successively between internodes 7-15, after which internode lengths remained similar to the vine parental cultigen DF, between 15.0-16.0 cm (Table 13; Fig. 16).
Figure 16. Successive mean internode lengths (cm) in the interspecific hybrid 65xLIC and parental cultigens. Values (±SD) represent means of six plants.

Figure 17. Successive mean internode lengths (cm) in the interspecific hybrid 65xDF and parental cultigens. Values (±SD) represent means of six plants.
Diameter of the Main Stem and Petioles

The bush parent NH65 exhibited the largest mean main stem diameter at 2.94 ± 0.34 cm, significantly larger than main stems of the vine parents LIC (1.23 ± 0.18 cm) and DF (1.20 ± 0.07 cm) (Table 14). Main stem diameter in the interspecific hybrids was intermediate between parental lines, measuring 1.63 ± 0.10 cm in 65LIC and 1.57 ± 0.11 cm in 65DF (Table 14).

Petiole diameters were not significantly different among parental cultigens and 65xDF, but petioles of 65xLIC were significantly larger at 1.93 ± 0.08 cm diameter.

Table 14. Diameter (cm) of main stems and petioles in the interspecific hybrids 65xLIC and 65xDF and parental cultigens.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Main Stem (cm)</th>
<th>Petiole (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65</td>
<td>2.94 ± 0.34 c</td>
<td>1.61 ± 0.10 a</td>
</tr>
<tr>
<td>LIC</td>
<td>1.23 ± 0.18 a</td>
<td>1.62 ± 0.10 a</td>
</tr>
<tr>
<td>DF</td>
<td>1.20 ± 0.07 a</td>
<td>1.53 ± 0.14 a</td>
</tr>
<tr>
<td>65xLIC</td>
<td>1.63 ± 0.10 b</td>
<td>1.93 ± 0.08 b</td>
</tr>
<tr>
<td>65xDF</td>
<td>1.57 ± 0.11 b</td>
<td>1.68 ± 0.14 a</td>
</tr>
</tbody>
</table>

*Values (±SD) represent means of six plants.

Values (±SD) represent means of four petioles on each of six plants.

Values within columns followed by the same letter are not significantly different according to Tukey's test (p=0.05).

The vine *C. moschata* cultivar 'Waltham Butternut' (WBN) and two *C. maxima* x WBN interspecific hybrids were evaluated in a plot adjacent to the main study. Main stem diameter was 0.91 ± 0.01 cm in WBN, 1.07 ± 0.02 cm in 65xWBN, and 1.13 ± 0.05 cm in 245xWBN. Petiole diameters were 1.34 ± 0.08 cm in 65xWBN and 1.17 ± 0.06 cm in 245xWBN. WBN petioles senesced prior to measurement.
Leaf Initiation

Rates of leaf initiation on the main stem did not differ significantly among cultigens in the spring of 2011, and at 42 DAT, leaf numbers on the main stem ranged from 16.7 ± 2.3 in the interspecific hybrid 65xLIC to 19.0 ± 0.9 in the bush parental cultigen NH65 (Table 15).

Table 15. Leaves on the main stem at select days after transplant (DAT) in the interspecific hybrids 65xLIC and 65xDF and parental cultigens grown in the greenhouse in 2011.5

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>14 DAT</th>
<th>20 DAT</th>
<th>27 DAT</th>
<th>35 DAT</th>
<th>42 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65</td>
<td>4.0 ± 0.0</td>
<td>6.2 ± 0.8</td>
<td>9.0 ± 0.9</td>
<td>13.8 ± 0.8</td>
<td>19.0 ± 0.9</td>
</tr>
<tr>
<td>LIC</td>
<td>3.3 ± 0.5</td>
<td>5.3 ± 0.5</td>
<td>8.8 ± 1.0</td>
<td>14.3 ± 1.0</td>
<td>18.8 ± 1.6</td>
</tr>
<tr>
<td>DF</td>
<td>3.4 ± 0.5</td>
<td>5.0 ± 0.7</td>
<td>7.7 ± 1.1</td>
<td>12.9 ± 1.7</td>
<td>17.5 ± 2.0</td>
</tr>
<tr>
<td>65xLIC</td>
<td>2.2 ± 1.2</td>
<td>4.5 ± 1.4</td>
<td>7.3 ± 2.1</td>
<td>11.3 ± 2.3</td>
<td>16.7 ± 2.3</td>
</tr>
<tr>
<td>65xDF</td>
<td>3.5 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>7.3 ± 0.8</td>
<td>13.5 ± 1.5</td>
<td>18.2 ± 1.8</td>
</tr>
</tbody>
</table>

5Values (±SD) represent means of six plants.

Leaf Area

Early canopy formation is an indicator of potential yield (Brown, 1984), and the developing fruit acts as a major photosynthetic sink capable of altering patterns of plant growth (Loy, 2004; Wien, 1997; Zack and Loy, 1981). Leaf areas were measured at anthesis of the first pistillate flower to reflect potential canopy formation prior to first fruit set (Table 16). The first pistillate flower reached anthesis at 34.3 DAT in the bush parental line NH65, when plants possessed 13.3 leaves with an average individual area of 780.4 cm² and a combined area of 10,455.4 cm². The vine parental cultivar LIC produced the first pistillate flowers at 39.3 DAT, and plants possessed 16.2 leaves with an average area of 847.3 cm² and total area of 13,724.9 cm². The vine parental cultigen DF produced pistillate flowers later than all other cultigens at 50.0 DAT, when plants had 23.0 leaves with an average area 677.0 cm² and combined area of 15,357.5 cm² (Table 16).
The interspecific hybrid 65xLIC produced pistillate flowers at 38.3 DAT, when plants contained 14.0 leaves averaging 813.4 cm$^2$ and a cumulative leaf area of 11,437.2 cm$^2$. The leaf canopy area of hybrid 65xDF was 16,279.0 cm$^2$ with a mean individual leaf area of 895.8 cm$^2$ at 42.3 DAT when pistillate flowers first reached anthesis (Table 16).

Table 16. Days after transplant (DAT), number of leaves on the main stem, average leaf area of individual leaves, and cumulative leaf area at anthesis of the first pistillate flower for the interspecific hybrids 65xLIC and 65xDF and parental cultigens.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>DAT</th>
<th>No. Leaves</th>
<th>Average Leaf Area (cm$^2$)</th>
<th>Cumulative Leaf Area (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65</td>
<td>34.3 a</td>
<td>13.3 a</td>
<td>780.4 ab</td>
<td>10,455.4 a</td>
</tr>
<tr>
<td>LIC</td>
<td>39.3 ab</td>
<td>16.2 ab</td>
<td>847.3 ab</td>
<td>13,724.9 abc</td>
</tr>
<tr>
<td>DF</td>
<td>50.0 c</td>
<td>23.0 c</td>
<td>677.0 a</td>
<td>15,357.5 bc</td>
</tr>
<tr>
<td>65xLIC</td>
<td>38.3 ab</td>
<td>14.0 ab</td>
<td>813.4 ab</td>
<td>11,437.2 ab</td>
</tr>
<tr>
<td>65xDF</td>
<td>42.3 b</td>
<td>18.0 b</td>
<td>895.8 b</td>
<td>16,279.0 c</td>
</tr>
</tbody>
</table>

Values represent means of six plants.

Values estimated as the product of leaf length from petiole to tip by leaf width.

Unlike letters within columns indicate significant difference as determined by Tukey’s test (p=0.05).

Leaf areas were measured on two plants of the interspecific hybrid 65xWBN growing adjacently to the main study. Leaf area at anthesis of the first pistillate flower was appreciably lower in this hybrid (2,192.0 ± 46.6 cm$^2$) compared with 65xLIC and 65xDF. Furthermore, 65xWBN produced pistillate flowers below node 3 at 23.0 DAT, when main stems possessed only 5.0 leaves. In the hybrid 65xLIC, the average first pistillate flower was located at node 10 and in the hybrid 65xDF at node 14.

**Flowering Habit**

The parental cultigens NH65 and LIC and the hybrid 65xLIC were the earliest to flower, producing the first pistillate flowers between 35-39 DAT. Pistillate flowers in the vine parental line DF and hybrid 65DF appeared later, at 42 DAT and 50 DAT.
respectively (Table 17). Staminate flowering was earlier in NH65 (37 DAT) than in LIC (43 DAT) and DF (46 DAT), and staminate flowers did not appear in 65xDF until 50.6 DAT. The hybrid 65xDF produced staminate flower buds, but all failed to reach anthesis (Table 17).

Table 17. Commencement of flowering in days after transplant (DAT) for pistillate (P) and staminate (S) flowers and total pistillate and staminate flowers in the interspecific hybrids 65xLIC and 65xDF and parental cultigens.

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>DAT P</th>
<th>DAT S</th>
<th>Total P</th>
<th>Total S</th>
<th>P:S</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65</td>
<td>35.3 a</td>
<td>36.8 a</td>
<td>3.8 b</td>
<td>16.2 c</td>
<td>0.2</td>
</tr>
<tr>
<td>LIC</td>
<td>39.3 ab</td>
<td>43.5 b</td>
<td>2.7 ab</td>
<td>9.0 b</td>
<td>0.3</td>
</tr>
<tr>
<td>DF</td>
<td>49.9 c</td>
<td>46.3 b</td>
<td>1.3 a</td>
<td>9.5 b</td>
<td>0.1</td>
</tr>
<tr>
<td>65xLIC</td>
<td>38.3 ab</td>
<td>-</td>
<td>1.7 a</td>
<td>0.0 a</td>
<td>-</td>
</tr>
<tr>
<td>65xDF</td>
<td>42.3 b</td>
<td>50.6 c</td>
<td>2.7 ab</td>
<td>1.8 a</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Values represent means of six replicates.

Values within columns followed by the same letter are not significantly different according to Tukey's test (p=0.05).

At 56 DAT, NH65 plants had produced an average of 3.8 pistillate and 16.2 staminate flowers, compared with 2.7 pistillate and 9.0 staminate flowers in LIC and 1.3 pistillate and 9.5 staminate flowers in DF. The hybrid 65xDF produced 2.7 pistillate and 1.8 staminate flowers per plant, and 65xLIC plants produced 1.7 pistillate flowers (Table 17).

The proceeding pictographs depict flowering patterns in representative single plants of the interspecific hybrids and parental cultigens observed in the greenhouse in 2011 (Figs. 18-20). In the C. maxima and C. moschata parental cultigens, staminate flowers were produced at basal nodes, and pistillate flowers at more distal nodes. Basally located staminate flowers, however, did not necessarily reach anthesis before distal pistillate flowers. In one WBN plant, for example, 7 pistillate flowers were produced
between nodes 7-16 prior to the first staminate flowers appeared at nodes 3 and 5 (Fig. 18). Flowers of similar sex generally developed progressively further from the plant base.

In the vine *C. moschata* parental cultivars, plants usually produced one flower per node. On the other hand, the bush *C. maxima* cultivar NH65 produced multiple flowers at several nodes (Figs. 18-20). While most parental cultivars produced pistillate flowers separated by four or five nodes, WBN produced pistillate flowers at several consecutive nodes (Fig. 18). One NH65 plant produced pistillate flowers as frequently as every two nodes, and produced flowers of both sexes from the same node (not shown).

Flowering patterns differed among interspecific hybrids. In 65xWBN, plants produced a set of pistillate flowers from consecutive basal nodes and an additional set from more distal nodes (Fig. 18). The 65xWBN plant depicted in Figure 18 produced two staminate flowers 50 DAT from nodes 8 and 9. The hybrid 65xLIC did not produce staminate flowers, but two pistillate flowers reached anthesis at distal nodes separated by several internodes (Fig. 19). Hybrid 65xDF produced pistillate flowers similarly to 65xLIC, but also generated staminate flowers (Fig. 20). The plant depicted in Figure 20 produced two staminate flowers from consecutive nodes located between pistillate flowers.
Figure 18. Representative flowering habit in the interspecific hybrid 65xWBN and parental cultigens grown in the greenhouse in 2011. Numbers beside pistillate (●) and staminate (○) flowers represent time of anthesis in days after transplant.
Figure 19. Representative flowering habit in the interspecific hybrid 65xLIC and parental cultigens grown in the greenhouse in 2011. Numbers beside pistillate (●) and staminate (○) flowers represent time of anthesis in days after transplant.
Figure 20. Representative flowering habit in the interspecific hybrid 65xDF and parental cultigens grown in the greenhouse in 2011. Numbers beside pistillate (●) and staminate (○) flowers represent time of anthesis in days after transplant.
Field, 2011

Two interspecific hybrids, 65xLIC and 65xDF, and two standard processing cultivars, ‘Golden Delicious’ (GD, vine C. maxima) and ‘Dickinson Field’ (DF, vine C. moschata) were grown in the field in the summer of 2011 and evaluated for several growth traits, including main stem length, branching habit, canopy height, leaf area, and flowering habit. The same phenological observations were made in adjacent plots of the bush parental cultigen NH65 and the vine parent LIC. Flowers were tagged upon anthesis and fruit was harvested between 55-60 days after pollination and evaluated for traits including fresh weight, blossom scar diameter, petiole thickness, degree of ribbing, and mesocarp thickness, percent dry matter, percent soluble solids, and color. Furthermore, fruits were divided into mesocarp, placenta, and seed components for evaluation of photosynthate partitioning in individual fruit.

Main Stem Length

The main stems in plants of the C. maxima processing cultivar GD and parental line NH65 were thick and succulent compared with main stems of the C. moschata cultivars DF and LIC, which were thinner and dense. In the interspecific hybrids, main stems resembled those of the C. moschata cultivars but were slightly thicker.

At 45 days after transplant (DAT) GD main stem length was 280 ± 22 cm, compared with 148 ± 39 cm in DF, 161 ± 42 cm in 65xLIC, and 173 ± 11 cm in 65xDF. Main stem length in the hybrid cultigens resembled the bush parent until 29 DAT, but subsequent elongation of internodes resulted in main stem lengths in 65xLIC and 65xDF approaching those of the vine parents LIC (207 ± 28 cm) and DF at 45 DAT (Figs. 21-22).
Figure 21. Main stem length (cm) in the interspecific hybrid 65xLIC and parental cultigens by days after transplant (DAT) in field plots in 2011. Values (±SD) represent means of four randomly selected plants in four blocks in 65xLIC, six plants in NH65, and four plants in LIC.

Figure 22. Main stem length (cm) in the interspecific hybrid 65xDF and parental cultigens by days after transplant (DAT) in field plots in 2011. Values (±SD) represent means of four randomly selected plants in four blocks in 65xDF and DF and six plants in NH65.
**Lateral Shoot Development**

Figures 23-26 depict representative growth habits of the interspecific hybrids 65xLIC and 65xDF and the vine parental cultigens. The main shoot in the bush parent NH65 was highly dominant, and plants only produced only short lateral shoots (not shown). The vine parent LIC produced about three laterals of substantial length, all located near the base of the plant, and produced several moderately long or short lateral shoots from subsequent nodes (Fig. 23). Branching was more pronounced in DF plants, which produced up to five long lateral shoots from basal nodes, and a few shorter branches approximately midway along the main shoot (Fig. 25).

The interspecific hybrid 65xLIC produced a dominant main stem with one lateral vine of moderate length near the plant base and several short lateral shoots along the length of the main shoot (Fig. 24). Hybrid 65xDF plants produced the most lateral branches of all cultigens observed, with numerous moderately-long to long shoots distributed along the main stem for some distance from the plant base (Fig. 26).
Figure 23. Representative branching habit in ‘Long Island Cheese’ (*C. moschata*) grown in the field in 2011. Numbers indicate nodes per shoot.
Figure 24. Representative branching habit in 65xLIC \((C.\ maxima \times C.\ moschata)\) grown in the field in 2011. Numbers indicate nodes per shoot.
Figure 25. Representative branching habit in 'Dickinson Field' (*C. moschata*) grown in the field in 2011. Numbers indicate nodes per branch.
Figure 26. Representative branching habit in 65xDF (C. maxima x C. moschata) grown in the field in 2011. Numbers indicate nodes per shoot.
**Petiole Length and Canopy Height**

Petiole length and canopy height differed substantially among interspecific hybrids and processing cultivars (Table 18). Petiole lengths measured 67.9 cm in 65xLIC and 71.9 cm in 65xDF, while petiole length in GD and DF measured 44.5 cm and 47.3 cm, respectively. Long petioles in interspecific hybrids translated to canopy heights of 111.2 cm in 65xLIC and 116.4 cm in 65xDF. In processing cultivars, canopy heights were 60.5 cm in GD and 83.5 cm in DF (Table 18).

**Table 18.** Petiole length (cm) and canopy height (cm) in the interspecific hybrids 65xLIC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).  

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Petiole (cm)</th>
<th>Canopy (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>44.5 ± 5.5 a</td>
<td>60.5 ± 2.2  a</td>
</tr>
<tr>
<td>DF</td>
<td>47.3 ± 5.8 a</td>
<td>83.5 ± 6.4  b</td>
</tr>
<tr>
<td>65xLIC</td>
<td>67.9 ± 8.7 b</td>
<td>111.2 ± 14.1 c</td>
</tr>
<tr>
<td>65xDF</td>
<td>71.9 ± 4.2 b</td>
<td>116.4 ± 9.7  c</td>
</tr>
</tbody>
</table>

*Values (±SD) represent mean of four replicates in four blocks arranged in a randomized complete block design.

*Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).*

**Leaf Area**

There were significant differences in mean leaf areas among cultigens; however, leaf areas varied within cultigens depending upon the stage of plant growth (Table 19). For example, area of the 4th main stem leaf in GD was 283.8 ± 66.3 cm² when fully expanded, significantly larger than those of all other cultigens. In DF plants, area of the 4th main stem leaf was 116.4 ± 29.2 cm², and in hybrids 65xLIC and 65xDF area of the 4th leaf was 196.8 ± 22.6 cm² and 231.6 ± 11.5 cm², respectively (Table 19). As vines
began running between plant rows, the area of individual leaves in the interspecific hybrids exceeded those in either processing cultigen, and remained larger for the duration of the growing season (Table 19). Hybrid 65xLIC produced the largest leaves (1,917.6 ± 128.0 cm²), followed by 65xFDF (1,667.7 ± 85.8 cm²). Individual leaf areas of mature plants in the processing cultivars were 880.6 ± 97.5 cm² in GD and 826.1 ± 66.2 cm² in DF (Table 19; Fig. 27). The large leaf size in mature interspecific hybrid plants was comparable to that of the bush parent NH65 (1,603.4 ± 218.8 cm²) (Fig. 27).

Table 19. Estimated areas (cm²) of fully expanded fourth leaves, tenth leaves, and mature leaves on the main stem in the interspecific hybrids 65xLIC and 65xFDF and standard processing cultivars ‘Golden Delicious’ (GD, C. maxima) and ‘Dickinson Field’ (DF, C. moschata).\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>4\textsuperscript{th} Leaf</th>
<th>10\textsuperscript{th} Leaf</th>
<th>Mature Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>283.8 ± 66.3 c\textsuperscript{2}</td>
<td>753.6 ± 116.8 b</td>
<td>880.6 ± 97.5 a</td>
</tr>
<tr>
<td>DF</td>
<td>116.4 ± 29.2 a</td>
<td>411.6 ± 55.8 a</td>
<td>826.1 ± 66.2 a</td>
</tr>
<tr>
<td>NH65 x LIC</td>
<td>196.8 ± 22.6 b</td>
<td>981.3 ± 84.6 c</td>
<td>1,917.6 ± 128.0 c</td>
</tr>
<tr>
<td>NH65 x DF</td>
<td>231.6 ± 11.5 b</td>
<td>1,028.3 ± 39.2 c</td>
<td>1,667.7 ± 85.8 b</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values (±SD) represent means of four randomly selected plants in four blocks arranged in a randomized complete block design.

\textsuperscript{b}Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).
Figure 27. Relative differences in mature leaf size in the interspecific hybrids 65xLIC and 65xDF and parental cultigens NH65 (C. maxima), 'Long Island Cheese' (LIC, C. moschata), and 'Dickinson Field' (DF, C. moschata).
**Flowering Habit**

Pistillate flower production preceded staminate flower production in the *C. maxima* processing cultigen GD and the interspecific hybrids 65xLIC and 65xDF in the summer of 2011. In GD plots, pistillate flowering commenced at 35 DAT and staminate flowers appeared at 40 DAT. Pistillate flowering commenced at 37 and 33 DAT in 65xLIC and 65xDF, respectively. The hybrid 65xLIC initiated male flower buds that all senesced prior to anthesis. In 65xDF, plants produced several staminate flowers after 50 DAT, but the anthers were shriveled and/or lacked pollen. In plots of the processing *C. moschata* cultigen DF, staminate flowers appeared at 43 DAT, whereas pistillate flowering commenced at 51 DAT.

The first pistillate flowers in GD, 65xLIC, and 65xDF were located between nodes 7-9, and in GD, these early pistillate flowers resulted in fruit set near the plant base. On the other hand, the first pistillate flowers senesced within one week after anthesis in 65xLIC and 10-14 days after anthesis in 65xDF. In the interspecific hybrids, fruit that reached maturity developed from pistillate flowers initiated at approximately 45-50 DAT. In DF, fruit set occurred in pistillate flowers reaching anthesis between 50-60 DAT.

**Fruit Harvest**

The fruit of GD was round with an orange-red rind, and DF fruits (Fig. 29) were oval or acorn-shaped with tan rinds. The interspecific hybrid 65xLIC (Fig. 28) produced slightly squat fruit with a bright orange rind and slight yellow mottling. Fruit shape of the hybrid 65xDF (Fig. 29) was variable but was generally oval with a tapered end and a bright orange rind.
Figure 28. Fruit of NH65 (*C. maxima*, left), ‘Long Island Cheese’ (*C. moschata*, right), and the interspecific hybrid 65xLIC (center) from field plots in 2011.

Figure 29. Fruit of NH65 (*C. maxima*, left), ‘Dickinson Field’ (*C. moschata*, right), and the interspecific hybrid 65xDF (center) from field plots in 2011.
Fruit fresh weight in the interspecific hybrids 65xLIC (4.1 kg) and 65xDF (7.11 kg) was significantly lower than in DF (12.1 kg) but higher than GD (2.8 kg). Mesocarp percent dry weight was significantly higher in 65xLIC (9.3%) and 65xDF (7.7%) than in DF (5.6%), but lower than in GD (11.1%). Fruit in hybrids 65xLIC and 65xDF contained 6.3% and 5.8% soluble solids, respectively, compared with 4.8% in DF and 7.7% in GD (Table 20).

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>FW (kg)</th>
<th>%DW</th>
<th>%SSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>2.7 a'</td>
<td>11.1 c</td>
<td>7.7 c</td>
</tr>
<tr>
<td>DF</td>
<td>12.1 c</td>
<td>5.6 a</td>
<td>4.8 a</td>
</tr>
<tr>
<td>65xLIC</td>
<td>4.1 a</td>
<td>9.3 bc</td>
<td>6.3 b</td>
</tr>
<tr>
<td>65xDF</td>
<td>7.1 b</td>
<td>7.7 b</td>
<td>5.8 b</td>
</tr>
</tbody>
</table>

*Values represent means of four randomly selected fruit from four plots arranged in a randomized complete block design.

*Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

The mean fruit fresh weight of a 48-fruit sample of the bush parental line NH65 was 5.6 kg, with 10.0% mesocarp dry matter and 7.8% SSC at -65 DAP. Mean fresh weight of eight LIC fruit was 5.6 kg, with 6.3% mesocarp dry matter, and 5.4% SSC.

Mesocarp thickness was 3.64 cm in 65xLIC and 3.86 cm in 65xDF, compared with 2.91 cm in GD and 5.85 cm in DF, and the degree of ribbing in the fruit of interspecific hybrids was intermediate to the processing cultigens. Mean blossom scar diameter was 1.09 cm in 65xLIC and 0.91 cm in 65xDF, compared with 1.62 cm in GD and 2.08 cm in DF. Lastly, the fruit of interspecific hybrids showed some green pigmentation in the mesocarp, but not significantly more than GD fruit (Table 21).
Table 21. Mesocarp thickness, blossom scar diameter (BS), degree of ribbing (Rib), flesh color, and degree of green flesh in the interspecific hybrids 65xLIC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).\(^2\)

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>MT (cm)</th>
<th>BS (cm)</th>
<th>Rib(^1)</th>
<th>Flesh Color(^3)</th>
<th>Green(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>2.91 a</td>
<td>1.62 b</td>
<td>0.3 a</td>
<td>12.4 a</td>
<td>0.8 ab</td>
</tr>
<tr>
<td>DF</td>
<td>5.85 c</td>
<td>2.08 c</td>
<td>2.8 c</td>
<td>13.9 b</td>
<td>0.0 a</td>
</tr>
<tr>
<td>65xLIC</td>
<td>3.64 b</td>
<td>1.09 a</td>
<td>2.2 b</td>
<td>12.1 a</td>
<td>1.1 b</td>
</tr>
<tr>
<td>65xDF</td>
<td>3.85 b</td>
<td>0.91 a</td>
<td>1.8 b</td>
<td>11.8 a</td>
<td>0.9 b</td>
</tr>
</tbody>
</table>

\(^2\)Values represent means of four randomly selected fruit harvest from four plots arranged in a randomized complete design.

\(^1\)Rated on a scale between 0 = complete absence of ribbing to 5 = deep, narrow, prominent ribbing.

\(^3\)Mesocarp flesh color was determined using a Roche Yolk Colour Fan with a gradient from light yellow (1.0) to dark orange (15.0).

\(^4\)Rated on a scale between 0 = complete absence of green to 5 = thick, saturated green band along the entire fruit circumference.

\(^5\)Values within columns followed by the same letter are not significantly different according to Tukey's test (p=0.05).
The *C. maxima* parental cultigen contained thick, corky peduncles with a basal diameter of 4.00 cm and a diameter of 3.44 at the midpoint. Peduncles in the fruit of the *C. moschata* parental lines were dense and narrow at the midpoint but significantly flared at the base. In LIC, peduncle diameter was 1.28 cm at the midpoint and 3.86 cm at the base, and in DF, peduncle diameter was 1.60 cm at the midpoint and 4.45 cm at the base (Table 22; Fig. 30). Fruit peduncles in interspecific hybrids were hard and dense, and the diameter at the midpoint was intermediate to parental cultigens, while the basal diameter was reduced compared to parental lines. The diameter at the peduncle midpoint was 1.95 cm in 65xLIC and 1.96 cm in 65xDF, and peduncle basal diameter in 65xLIC and 65xDF was 2.97 cm and 3.22 cm, respectively. Flare in the peduncles of 65xLIC (1.52) and 65xDF (1.65), as measured by the ratio of the basal diameter to the midpoint diameter, was significantly less than LIC (3.01) and DF (2.81), but similar to NH65 (1.16) (Table 22, Fig. 30).
Table 22. Peduncle basal and midpoint diameters and peduncle flare in the interspecific hybrids 65xLIC and 65xLIC and parental cultigens.\textsuperscript{5}

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Peduncle Base (cm)</th>
<th>Peduncle Midpoint (cm)</th>
<th>Peduncle Flare\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH65</td>
<td>4.00 bc</td>
<td>3.44 d</td>
<td>1.16 a</td>
</tr>
<tr>
<td>LIC</td>
<td>3.86 abc</td>
<td>1.28 a</td>
<td>3.01 b</td>
</tr>
<tr>
<td>DF</td>
<td>4.45 c</td>
<td>1.60 b</td>
<td>2.81 b</td>
</tr>
<tr>
<td>65xLIC</td>
<td>2.97 a</td>
<td>1.95 c</td>
<td>1.52 a</td>
</tr>
<tr>
<td>65xDF</td>
<td>3.22 ab</td>
<td>1.96 c</td>
<td>1.65 a</td>
</tr>
</tbody>
</table>

\textsuperscript{5}Values represent mean of four randomly selected fruit per cultigen.

\textsuperscript{1}Flare is defined as the ratio of peduncle base diameter to midpoint diameter.

\textsuperscript{2}Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).

**Biomass Partitioning within Fruit**

Non-placental mesocarp tissue comprised 95.7\% of total fruit dry weight (DW) in the hybrid 65xLIC and 95.8\% fruit DW in 65xDF compared with 84.1\% in GD and 89.3\% mesocarp in DF (Table 23). In interspecific hybrids, seeds comprised a small percentage of total fruit biomass, measuring 0.2\% of total DW in 65xLIC and 1.0\% total DW in 65xDF. Seeds accounted for 13.2\% of total DW in GD and 8.5\% of total DW in DF (Table 23). The remaining fruit DW included placental tissue.

In processing cultivars, embryo development utilized a greater proportion of energy supplied by photosynthates (glucose) per kg fruit FW than interspecific hybrids (Table 23). Embryo biomass in GD fruit utilized 279.2 kJ per kg fruit FW compared with 1,281.7 kJ stored in mesocarp tissue. In DF, estimated energy utilization for embryo tissue was 93.2 kJ per kg fruit FW compared with 675.1 kJ for mesocarp tissue. Embryos in the interspecific hybrids accounted for 0.14 g per kg fruit FW in 65xLIC and 0.57 g per kg fruit FW in 65xDF, utilizing 3.6 kJ and 14.3 kJ, respectively, of photosynthetic energy. Mesocarp energy consumption was 1,065.2 kJ per kg fruit FW in 65xLIC and 912.45 kJ per kg fruit FW in 65xDF (Table 24).
Table 23. Individual fruit fresh weight (FW), fruit dry weight (DW), mesocarp and seed DW, and percentage fruit DW in mesocarp and seed components in the interspecific hybrids 65xLIC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>FW (kg)</th>
<th>DW (g)</th>
<th>Meso DW (g)</th>
<th>Seed DW (g)</th>
<th>% Meso</th>
<th>% Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>2.8 ± 0.1 a'</td>
<td>335.6 ± 19.1 a</td>
<td>285.9 ± 25.3 a</td>
<td>40.9 ± 4.8 b</td>
<td>84.1 ± 1.7 a</td>
<td>13.2 ± 1.9 c</td>
</tr>
<tr>
<td>DF</td>
<td>12.1 ± 1.6 c</td>
<td>727.1 ± 85.5 c</td>
<td>653.0 ± 82.1 b</td>
<td>58.9 ± 11.2 c</td>
<td>89.3 ± 0.9 b</td>
<td>8.5 ± 1.2 b</td>
</tr>
<tr>
<td>65xLIC</td>
<td>4.1 ± 0.1 a</td>
<td>364.5 ± 27.4 a</td>
<td>349.9 ± 24.6 a</td>
<td>0.8 ± 1.2 a</td>
<td>95.7 ± 0.6 c</td>
<td>0.2 ± 0.3 a</td>
</tr>
<tr>
<td>65xDF</td>
<td>7.1 ± 0.8 b</td>
<td>549.4 ± 86.6 b</td>
<td>528.1 ± 85.9 b</td>
<td>5.8 ± 3.4 a</td>
<td>95.8 ± 0.5 c</td>
<td>0.2 ± 0.5 a</td>
</tr>
</tbody>
</table>

Values (±SD) represent mean of four randomly harvest fruit from four plots arranged in a randomized complete block design.

Unlike letters within columns indicate significant difference as determined by Tukey’s test (p=0.05).

Table 24. Grams dry weight (DW) and energy utilization (kJ) of embryo and mesocarp components of fruit per kg fruit fresh weight (FW) and ratio of embryo DW and kJ consumption to fruit FW in the interspecific hybrids 65xLIC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Embryo'</th>
<th>Mesocarp</th>
<th>Ratio E/Fruit</th>
<th>Embryo'</th>
<th>Mesocarp</th>
<th>Ratio E/Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>11.2 ± 1.2</td>
<td>102.1 ± 9.4</td>
<td>0.098 ± 0.014</td>
<td>279.22</td>
<td>1281.70</td>
<td>0.167</td>
</tr>
<tr>
<td>DF</td>
<td>3.8 ± 0.6</td>
<td>53.8 ± 1.7</td>
<td>0.063 ± 0.009</td>
<td>93.21</td>
<td>675.09</td>
<td>0.097</td>
</tr>
<tr>
<td>65xLIC</td>
<td>0.1 ± 0.2</td>
<td>84.9 ± 5.5</td>
<td>0.001 ± 0.002</td>
<td>3.56</td>
<td>1065.23</td>
<td>0.003</td>
</tr>
<tr>
<td>65xDF</td>
<td>0.6 ± 0.3</td>
<td>72.7 ± 4.7</td>
<td>0.008 ± 0.004</td>
<td>14.33</td>
<td>912.35</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Values (±SD) represent means of four randomly harvested fruit from four plots.

Embryo DW determined as 74.2% of seed DW.

Energy equivalents for embryo (24.85 kJ/g) and mesocarp (12.5 kJ/g) tissues adapted from Loy (2004).
Fruit Yield

In 2011, DF (107.6 kg) and 65xLIC (102.7 kg) had the highest fresh weight yields per sixteen-plant plot, but yield values were not statistically different from 65xDF (72.4 kg) (Tables 25, 26). However, when GD was removed from statistical analysis, 65xDF fresh weight yields were significantly lower than DF and 65xLIC. The hybrid 65xLIC produced 26.8 fruit per plot, compared with 16.3 in GD, 11.5 fruit in DF, and 10.8 fruit in 65xDF (Table 26).

Mesocarp dry weight yield in 65xLIC exceeded all other cultigens at 8.7 kg per plot and 0.57 kg per plant (Table 25). Dry weight yield in the hybrid 65xDF, on the other hand, was 5.3 kg per plot and 0.34 kg per plant. Plot fruit dry weight yields in the processing cultivars was 5.8 kg in DF and 4.7 kg in GD, while plant fruit dry weight yields were 0.38 kg in DF and 0.30 kg in GD (Table 25).

Table 27 depicts fresh and dry weight yields per hectare for the processing cultivars ‘Golden Delicious’ (GD) and ‘Dickinson Field’ (DF) and the interspecific hybrids 65xLIC and 65xDF planted at a spacing of 0.6 m within rows and 2.4 m between rows (0.7 plants/m²). Fresh weight yields in GD were 20.1 mt/ha, compared with 48.1 mt/ha in DF. The hybrid 65xLIC yielded 46.6 mt/ha, and 65xDF yielded 32.0 mt/ha. The highest mesocarp dry weight yields were in 65xLIC (3.9 mt/ha) compared with 2.1 mt/ha in GD, 2.6 mt/ha in DF, and 2.4 mt/ha in 65xDF.
Table 25. Fresh weight (FW) and dry weight (DW) yield per plot and per plant and mesocarp flesh DW to fruit FW ratio in the interspecific hybrids 65xLlC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).:

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Yield/plot</th>
<th></th>
<th></th>
<th></th>
<th>Meso DW/Fruit FW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FW (kg)</td>
<td>Meso DW (kg)</td>
<td>FW (kg)</td>
<td>Meso DW (kg)</td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>45.6 a</td>
<td>4.7 a</td>
<td>2.9 a</td>
<td>0.30 a</td>
<td>0.103</td>
</tr>
<tr>
<td>DF</td>
<td>107.6 b</td>
<td>5.8 a</td>
<td>6.9 e</td>
<td>0.38 a</td>
<td>0.054</td>
</tr>
<tr>
<td>65xLlC</td>
<td>102.7 b</td>
<td>8.7 b</td>
<td>6.7 bc</td>
<td>0.57 b</td>
<td>0.084</td>
</tr>
<tr>
<td>65xDF</td>
<td>72.4 ab</td>
<td>5.3 a</td>
<td>4.6 ab</td>
<td>0.34 a</td>
<td>0.073</td>
</tr>
</tbody>
</table>

*DW yield calculated using percent mesocarp as percentage total fresh weight and mesocarp %DW derived from four randomly selected fruit from four plots. Mesocarp as %FW = 84.1% in GD, 89.3% in DF, 95.7% in 65xLlC, and 95.8% in 65xDF. Mesocarp %DW = 11.1% in GD, 5.6% in DF, 9.3% in 65xLlC, and 7.7% in 65xDF. Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).*

Table 26. Fruit fresh weight (FW), number of fruit, and number of plants per plot and fruit FW and number of fruit per plant in the interspecific hybrids 65xLlC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*).

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Plot</th>
<th>Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FW (kg)</td>
<td>No. Fruit</td>
</tr>
<tr>
<td>GD</td>
<td>45.6 ± 6.1</td>
<td>16.3</td>
</tr>
<tr>
<td>DF</td>
<td>107.6 ± 22.3</td>
<td>11.5</td>
</tr>
<tr>
<td>65xLlC</td>
<td>102.7 ± 14.4</td>
<td>26.8</td>
</tr>
<tr>
<td>65xDF</td>
<td>72.4 ± 19.2</td>
<td>10.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>FW (kg)</th>
<th>No. Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>2.9 ± 0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>DF</td>
<td>6.9 ± 1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>65xLlC</td>
<td>6.7 ± 0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>65xDF</td>
<td>4.6 ± 1.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Values (±SD) represent means of four plots of fifteen or sixteen plants arranged in a randomized complete block design.*
Table 27. Fresh weight (FW) yield and mesocarp dry weight (DW) yield in mt/ha in the interspecific hybrids 65xLIC and 65xDF and processing cultivars ‘Golden Delicious’ (GD, *C. maxima*) and ‘Dickinson Field’ (DF, *C. moschata*) grown in the field in 2011.\(^2\)

<table>
<thead>
<tr>
<th>Cultigen</th>
<th>Total FW (mt/ha)</th>
<th>Meso DW (mt/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD</td>
<td>20.1 a(^1)</td>
<td>2.1 a</td>
</tr>
<tr>
<td>DF</td>
<td>48.1 c</td>
<td>2.6 a</td>
</tr>
<tr>
<td>65xLIC</td>
<td>46.6 bc</td>
<td>3.9 b</td>
</tr>
<tr>
<td>65xDF</td>
<td>32.0 ab</td>
<td>2.4 a</td>
</tr>
</tbody>
</table>

\(^2\)Values extrapolated from sixteen plant plots arranged in a randomized complete block design and plants spaced at 0.6 m within rows and 2.4 m between rows (0.7 plants/m\(^2\)).

\(^1\)Values within columns followed by the same letter are not significantly different according to Tukey’s test (p=0.05).
CHAPTER IV. DISCUSSION

Compatibility Aspects of Interspecific Hybridization

Previous attempts at *C. maxima* x *C. moschata* hybridization suggested that crosses between some cultigens produce abundant F\(_1\) seed, but a priori identification of compatible genotypes is elusive (Castetter, 1930; Erwin and Haber, 1930; Robinson and Decker-Walters, 1996; Whitaker and Bohn, 1950). In the current study, interspecific compatibility appeared to be primarily determined by the *C. maxima* female genotype, such that *C. maxima* parental lines were either not compatible with *C. moschata* or were compatible with a range of *C. moschata* genotypes (Tables 1-2).

Fruit set percentage was high in crosses utilizing bush *C. maxima* cultigens NH65 and ‘Autumn Pride’ (AP) and lower in crosses involving NH245-10 and ‘Bush Pink Banana’ (NHBPB), with only a small percentage of pollination attempts resulting in fruit set in NH245-10 (Table 1). Although NH65 set fruit and produced viable seed when pollinated by multiple *C. moschata* lines, fruit set in other crosses did not necessarily result in seed production. For example, fruit resulting from crosses utilizing AP as the pistillate parent appeared normal but contained few filled seeds. In some fruit, plump, well-developed seed coats contained liquid endosperm, but no embryos.

A possible explanation for such a phenomenon is a genic imbalance in the developing endosperm. In angiosperms, double fertilization results in syngamy of one sperm cell with the egg, forming the embryo with one maternal and one paternal genome, and fertilization of the polar cells to form the 3n (2 maternal and 1 paternal genomes)
endosperm. Johnston et al. (1980) proposed that successful interspecific hybridization in *Solanum* resulted from crosses in which the maternal to paternal genome ratio in the endosperm was 2:1, as determined by an empirically assigned endosperm balance number (EBN). This 2:1 genome ratio in endosperm appears essential for normal endosperm development in many crop species, possibly due to the expression of ‘imprinted’ genes of mandatory maternal or paternal inheritance (Haig and Westoby, 1991). Reciprocal interspecific crosses in crops such as wheat indicate that embryos may begin forming in ovules with imbalanced endosperm, but the viability of the F₁ seeds depends on the directionality of the imbalance. Crosses resulting in an overabundance of maternally imprinted genes have been shown to produce a limited number of small but viable seeds, while reciprocal crosses with excessive paternally imprinted genes result in rapid endosperm development and large seeds with no embryos (Haig and Westoby, 1991).

Crosses between NH245-10 x SC936, a ‘Dickinson Field’-type breeding line, revealed an exception to the compatibility patterns observed in other crosses involving NH245-10. In the field in 2011, NH245-10 x SC936 crosses resulted in successful fruit set and, furthermore, production of well-filled seed (Table 2). Such deviations from expected results highlight the difficulty in predicting the success of hybridizing *C. maxima* x *C. moschata*.

**Seed Yield**

Seeds of the open-pollinated squash cultivars used for pie stock are produced in isolated plots by natural insect pollinators. The primary advantage of open-pollinated seed is that it is inexpensive for seed companies to produce and, therefore, inexpensive for growers to purchase. The production of F₁ hybrid squash seed is substantially more
expensive than open-pollinated varieties, and utilizes controlled hand-pollinations, or in the case of bush x vine crosses, exogenous application of ethephon to induce gynoecy in bush parental lines (Loy, 2004; Rudich et al., 1969). The increased costs of F₁ seed are offset by variety protection, complementation of beneficial traits, cultigen uniformity, and in some cases, heterosis.

The most widely grown processing squash cultivars in the U.S. are open-pollinated strains of ‘Golden Delicious’ (C. maxima) and ‘Dickinson Field’ (DF, C. moschata), along with large long-necked cultigens of C. moschata. Successful introduction of interspecific F₁ hybrids is dependent upon production of acceptable seed yields by the pistillate C. maxima parent. In the current study, seed yields in some interspecific hybrids were comparable to those one might expect from open-pollinations in ‘Golden Delicious’, which averaged 230 seeds per self-pollinated fruit in consecutive years (data from six fruit). In 2011, fruit resulting from interspecific pollinations involving NH65 contained an average of approximately 200 filled seeds, not substantially different from NH65 fruit set with a C. maxima pollen source (Table 2). On the other hand, seed numbers in self-pollinated fruit of three C. moschata processing cultivars ranged from 529 to 654 in 2011 (Table 2), and one open-pollinated DF fruit harvested in 2010 contained 792 seeds (data not shown).

**Seed and Embryo Size and Seed Viability**

Singh (1949) showed a positive correlation between seed weight and embryo weight in a series of reciprocal C. maxima crosses. Individual seed weights and embryo weights in C. maxima fruit developing from self-pollination or uncontrolled pollination were significantly higher than in C. moschata, and seed weight reflected embryo weight
The seeds of interspecific crosses were typically larger than in *C. moschata* fruit and often approached seed size observed in *C. maxima* fruit. Seed weight in interspecific crosses, however, did not always reflect embryo size, most notably in crosses involving NH245-10 as the pistillate parent. This line produced seeds with particularly large seed coats that lacked or contained small embryos (Tables 3-4). The NH65 x DF cross in 2010 also produced seeds with smaller embryos than would be expected from seed weights (Table 4). In both cases, the coefficient of variation for embryo size was significantly higher than in other crosses, suggesting discrepancies between seed size and embryo size in these crosses resulted from poor or variable embryo development.

In the germination study conducted in the fall of 2011, the percent germination in the 65xLIC hybrid was lower than in other cultigens, even when seed was scarified (Table 5). However, percent germination in scarified interspecific F₁ seed sown in the spring for field transplants approached 100%, and seedling growth appeared normal (Fig. 4). Differences in germination between fall and spring sowing may indicate prolonged dormancy in some interspecific hybrids. Also, greenhouse temperature in the fall of 2011 (~23°C) may have been lower than optimal for germination of interspecific F₁ hybrid seeds.

Seed viability is essential for adoption of interspecific hybrids for processing squash and may require scarification of F₁ seeds to improve germination. Ideally breeding lines should be developed that produce seeds in interspecific crosses with high percent germination and germination rates comparable to open-pollinated processing cultivars in field sowings.
Phenological Growth Traits

In the current study, bush *C. maxima* x vine *C. moschata* F$_1$ hybrids were evaluated for vegetative and reproductive growth traits, as well as fruit characteristics and fresh and dry weight yields. The *C. maxima* parental lines shared several important traits: extreme bush growth habit, large leaf size and tall canopy, minimal branching, low pistillate to staminate flower ratio, and the tendency to produce one fruit per plant.

The *C. moschata* parental cultigens all possessed a vine-type growth habit, but differed in leaf size, branching habit, stem thickness, and flowering pattern. The open-pollinated fresh market winter squash cultivar ‘Waltham Butternut’ (WBN) produced an abundance of pistillate flowers that outnumbered staminate flowers on the main stem. In WBN, pistillate flowering preceded staminate flowering by as much as 17 days in some plants grown in the greenhouse. On the other hand, the open-pollinated processing cultivars ‘Long Island Cheese’ (LIC) and ‘Dickinson Field’ (DF) produced far fewer pistillate flowers than staminate flowers. Pistillate flowers appeared a few days prior to staminate flowers in LIC, but after staminate flowers in DF. In addition to differences in flowering habit, the processing cultigens were generally more vigorous than WBN, with larger leaves and thicker main stems and petioles.

Phenotypes of the interspecific hybrids reflected differences among parental cultigens in expression of the bush or vine growth habit, stem and petiole thickness, and flowering habit.
Bush vs. Vine Growth Habit

Denna and Munger (1963), Zack and Loy (1981), and Broderick (1982) observed that bush and vine growth habits in squash are defined by internode length. Both phenotypes exhibit successive increases in internode length, but the earliest internodes are generally longer in vine genotypes compared with bush genotypes, and internode elongation occurs both earlier in plant growth and more rapidly in vine genotypes compared with bush phenotypes. In squash, plant growth habit relates to the rate of canopy formation at ideal plant spacing for rapid achievement of optimal leaf area indices (Botwright et al., 1998; Broderick, 1982; Buwalda and Freeman, 1986; Cui, 2005; Loy, 2004).

In the current greenhouse studies, the C. moschata parental cultigens exhibited typical vine growth habits, with earliest internodes lengths being 2.5-4.0 cm and successive internode elongation resulting in mature internode lengths between 15.0-17.0 cm by the ninth internode in WBN (Figs. 8-9), 19.0-20.0 cm at internodes 11-12 in LIC (Fig. 16), and 14.0-16.0 cm at internodes 14-15 in DF (Fig. 17). Maximum internode lengths in two WBN plants grown in the greenhouse in 2011 (14.0-15.0 cm) were slightly shorter than those in plants grown in the 2010 study.

Internode lengths in the C. maxima parental lines were typical of bush genotypes. In NH245-10, NHBPB, and NH65, the earliest internodes were less than 1.0 cm in length, and successive internodes elongated incrementally (Figs. 8-9; 16-17). Although NHBPB and NH65 plants did not display accelerated internode elongation in later growth, internodes in NH245-10 plants elongated rapidly after node 10 and particularly after node 12, such that internode lengths approached those of the vine cultigen by node
Such differences in growth habit among \textit{C. maxima} bush parental lines support the evidence of Singh (1949) for a more complex model of inheritance for the bush phenotype in \textit{C. maxima} as compared with that reported in \textit{C. pepo} (Shifriss, 1947).

In hybrids utilizing DF and LIC as pollen parents, internodes followed the pattern observed by Denna and Munger (1963) in their study comparing bush-vine hybrids with parental cultigens in \textit{C. maxima}. Early internode lengths in bush-vine heterozygotes were intermediate to those in parental plants and remained below 4.0 cm, until node 7 in 65xLIC (Fig. 16) and node 8 in 65xDF (Fig. 17), after which internode lengths rapidly increased, approaching those of the vine parent by node 10 in 65xLIC and node 11 in 65xDF.

Hybrids utilizing WBN as the pollen parent possessed unique and unexpected patterns of internode elongation not reported in previous literature. In BPBxWBN and 245xWBN, the earliest internodes were approximately 1.0 cm in length, but internode length increased rapidly beginning at node 5 and exceeded that of the vine parent by node 7 (Figs. 8-9). Internodes lengths decreased to those of the vine parent between nodes 8-11 in 245xWBN (Fig. 9) and remained significantly longer than the vine parent in BPBxWBN until node 14 (Fig. 8). Similar patterns were also observed in two plants each of the interspecific hybrids 245xWBN and 65xWBN in the greenhouse in 2011. In 65xWBN, however, rapid internode elongation did not occur until node 7, and internode lengths exceeded those in WBN at node 9 but did not regress for the remainder of the study (data not shown).
**Branching Habit**

While plants in the greenhouse studies were pruned to the main stem, plants were allowed to assume normal growth in the field. In general, bush *C. maxima* parental cultigens produced one dominant main stem and only short lateral vines. On the other hand, vine *C. moschata* parental cultigens produced prolific lateral shoots that developed into significant vines, sometimes early in plant growth. In the field in 2011, lateral shoots near the plant base began vining as early as 25 DAT in LIC and 29 DAT in DF, developing into significant branches (Figs. 23, 25).

Branching of the main stem in the interspecific hybrids did not occur until later in plant growth than the *C. moschata* parental lines. In hybrids utilizing WBN as a pollen parent, plants contained one prominent main stem and one or two lateral branches of short or moderate length near the plant base. The hybrid 65xLIC produced one exceptionally vigorous main stem and several lateral branches of moderate length near the plant base (Fig. 24), while 65xDF plants produced a vigorous main stem and abundant lateral branches along the length of the main stem, some of which were significant in length (Fig. 26). In fact, while vegetative growth slowed and eventually ceased in *C. maxima* x WBN hybrids and in 65xLIC upon development of fruit, vines in 65xDF remained vigorous for the duration of the growing season.

**Flowering Habit**

*Cucurbita maxima* bush parental cultigens produced fewer pistillate flowers than staminate flowers. The bush lines NH245-10 and NHBPB grown in 2010 produced an abundance of staminate flowers and fewer pistillate flowers than NH65 in 2011, and NH245-10 plants grown in the greenhouse in 2011 produced significantly more staminate
flowers than NH65 plants (data not shown). Also, in the spring of 2010, NH245-10 and NHBPB initiated staminate flowers before pistillate flowers (Table 9), but pistillate flowering preceded staminate flowering in NH65 in the spring of 2011 (Table 17). In NH65, plants produced pistillate flowers every four or five nodes, with the exception of one plant observed in the spring of 2011 that produced pistillate flowers as frequently as every two nodes.

Such discrepancies in flowering ratios and flowering patterns across seasons may be due to differences in day lengths at the time of flower initiation, since plants were transplanted two weeks earlier in 2011 than in 2010. Nitsch et al. (1952) showed that in C. pepo, day length can affect the production of pistillate flowers, with shorter days resulting in a greater proportion of pistillate flowers. The study did not account, however, for differences in light intensity, which may affect internode lengths in squash (Zack and Loy, 1980).

In the greenhouse, the vine cultigen WBN initiated pistillate flowering approximately two weeks prior to staminate flowering (Table 9). Furthermore, WBN produced more pistillate flowers than staminate flowers. The processing C. moschata cultivars LIC and DF produced more staminate than pistillate flowers, and while pistillate flowering preceded staminate flowering in LIC, pistillate flowering appeared after staminate flowers in DF (Table 17).

All of the interspecific hybrids observed were gynoecious or mostly gynoecious, similar to observations by past investigators (Castetter, 1930; Erwin and Haber, 1929; Robinson et al., 1978). In the present study, specific flowering habits in interspecific hybrids differed depending on the C. moschata parent. In hybrids utilizing WBN,
pistillate flowering preceded staminate flowering by over twenty days, occurring early in plant growth and before vine formation sufficient for supporting large fruit loads. Such hybrids produced female flowers on several consecutive nodes, and in the field, these hybrids produced several fruit below node 10. Hybrids utilizing the processing cultigens as staminate parents tended to initiate pistillate flowering much later in plant growth, and set far fewer pistillate flowers than hybrids utilizing WBN.

The early onset of fruit development most likely contributed to the different growth habits observed between interspecific hybrids. Vegetative growth in hybrids utilizing WBN may have been suppressed due to substrate competition between vegetative and reproductive plant components (Loy, 2004), resulting in sparse plant canopies and weak lateral formation. On the other hand, fruit did not set until canopies completely covered the soil surface in hybrids 65xL1C and 65xDF. The result was a plant canopy that could support multiple large fruit and produced enough photosynthate for continued leaf production and leaf maintenance. In the case of 65xDF, profuse vegetative growth may have resulted in a canopy too dense for fruit set at the spacing used (Botwright et al., 1998).

**Expression of the B Gene**

Squash grown for processing possess either the tan rind of *C. moschata* or the orange or red phenotype in some *C. maxima* cultivars to avoid green pigmentation and/or dull color in the processed product (Culpepper and Moon, 1945; Loy, 2011). In squash, the *B* allele, or *B*\textsuperscript{max} in *C. maxima* (Paris and Kabelka, 2009), conditions precocious yellow pigmentation in fruit (Shifriss, 1981, 1990), and results in light-orange to dark red rind color when present in *C. maxima*. Bush parental cultigens utilized in the current
study were homozygous for the $B$ allele and possessed deep orange rinds. Additionally, the *C. maxima* processing cultivars and Rspl232 and ‘Golden Delicious’ grown in the field studies were homozygous for the $B$ allele and exhibited dark orange, almost red, rinds.

Expression of the $B$ allele was clearly evident in interspecific hybrids in female flower buds prior to anthesis (Fig. 31). In hybrids involving WBN, fruit appeared orange with yellow mottling (Fig. 12). The hybrids 65xLIC (Fig. 28) and 65xDF (Fig. 29) possessed orange rinds with no mottling. In the greenhouse, expression of the $B$ gene was highly pronounced in 65xLIC and particularly in 65xDF, resulting in yellow pigmentation in plant stems, petioles, and even leaf blades (Fig. 31).

Figure 31. Strong expression of the $B$ allele for precocious yellow fruit coloration resulted in yellowing of plant stems and petioles in some interspecific hybrids, particularly 65xDF (pictured above) grown in the greenhouse in spring, 2011.
Although over-expression of the B gene and the resulting chlorophyll degradation can reduce photosynthetic rates in crops (Shifriss, 1990), yellow pigmentation in interspecific hybrids in the field was not as severe as in the greenhouse and did not occur in leaf blades. In inbred *C. moschata* populations homozygous for the B allele transferred from *C. maxima*, Shifriss (1986, 1990) observed B gene expression similar to that seen in 65xLlC and 65xDF, with yellow pigmentation in stems and petioles in the field and yellowing of stems, petioles, and leaf blades in the greenhouse.

**Fruit Characteristics**

**Mesocarp Consistency and Color**

Mesocarp consistency and color are of primary importance in assessing the suitability of squash for processing (Culpepper and Moon, 1945; Lana and Tischer, 1951; Whitaker and Bohn, 1950). Consistency is measured by the spread of pureed mesocarp on a plate with concentric rings (Lana and Tischer, 1951), and studies measuring mesocarp consistency and mesocarp percent dry weight (%DW) indicate these two qualities are positively correlated (Culpepper and Moon, 1945; Lana and Tischer, 1951). A deep yellow or bright orange color is desirable in mesocarp flesh in processing (Culpepper and Moon, 1945; Lana and Tischer, 1951). Green pigmentation in the fruit rind or in the mesocarp is considered undesirable and results in dull or off colors in the pureed product (Culpepper and Moon, 1945).

Based on mesocarp %DW values, it is expected that consistency of the interspecific hybrids 245xWBN, 65xLlC, and possibly, 65xDF would be acceptable or exceptional for processing. Mesocarp %DW in the interspecific hybrids 245xWBN (16.8%) and 65xLlC (9.3%) was higher than in the principle processing cultigen.
‘Dickinson Field’ (DF), with mesocarp %DW of 7.2% in 2010 and 5.6% in 2011. Mesocarp %DW was also significantly different in the hybrid 65xDF (7.7%) than in DF. The C. maxima processing cultigens Rsp1232 (Field, 2010) and ‘Golden Delicious’ (GD; Field, 2011) produced fruit mesocarp with %DW of 11.1% (Tables 11,20).

Flesh color in Rsp1232 ranged from pale orange to dark orange in 2010. The hybrid 245xWBN possessed similarly colored flesh, but many fruit contained a solid green layer approximately midway into the mesocarp. This green ring was also observed in some fruit of C. maxima parental cultigens, and in 65xWBN and 245xWBN grown in 2011. The processing cultigen GD and the hybrids 65xLIC and 65xDF had dark orange flesh. Although some fruit in 65xLIC and 65xDF contained a green flesh layer, it was not as pronounced or common as in hybrids involving WBN. The processing cultigen DF possessed a unique, deep pink-orange flesh with no green coloration.

**Fruit Shape and Fruit Size**

Many aesthetic characteristics important in fresh eating squash are unimportant in squash for processing because fruit are only marketed as canned puree. However, fruit shape and consistency of size may be important during harvest, when fruit are pushed into windrows prior to collection (Duane Belle, Rupp Seeds, personal communication). Presumably, round-shaped fruit of similar size are desirable for ease of windrow formation and harvest. Additionally, overly ribbed fruit tend to cling to soil, thus necessitating cleaning of fruit and distorting fresh weight yields. The fruit of the C. maxima processing cultigen GD were minimally ribbed, round and very consistent in size, with a coefficient of variation of fruit fresh weight (FW) of 0.049. Similarly, the hybrid cultigen 65xLIC possessed shallow ribs and a round, but slightly squat fruit shape.
Hybrid 65xLIC produced the most consistently sized fruit, with coefficient of variation of fruit FW of 0.027. The *C. moschata* processing cultigen DF was moderately ribbed, oval-shaped, and had variable fruit size (FW C.V.=0.131) (Fig. 29). Fruit size in 65xDF was variable (FW C.V.=0.116), and fruit shape varied from round to oblong to acorn-shaped and was moderately ribbed (Fig. 29). Fruit in the hybrid 245xWBN were pear-shaped with very shallow or no ribbing, and coefficient of variation for fruit FW was 0.154 (Fig. 12).

**Blossom Scars and Peduncles**

Large blossom scars and peduncle basal diameters might diminish the proportion of edible mesocarp in fruit. *Cucurbita maxima* cultigens possess corky peduncles with moderately wide and non-flared bases. Although *C. moschata* peduncles narrow at the midpoint, significant flaring can result in substantial diameter of the base (Fig. 30; Table 22). The blossom scars of interspecific hybrids were all highly reduced compared with parental cultigens and standard processing cultigens (Table 21).

**Yield**

**Fresh Weight Yield**

Economic, dietary, and culinary value is derived from the entire caryopsis in cereal grains, and crop yields are determined by the dry weight of the grain. In such crops, breeders have worked to increase yields by optimizing grain to biological dry weight ratios, or harvest indices (Donald and Hamblin, 1976; Fageria et al., 2006; Gifford and Evans, 1981). Yield evaluation in squash is different from that of cereal grains for several reasons: 1) squash plants are indeterminate and can set multiple fruit over an extended time period; 2) economic value is derived from the fruit mesocarp in squash.
and several fruit components, including seeds, are waste products; 3) fruit fresh weight may be the most valuable measure of squash yield.

Squash cultigens used for processing produce very high fresh weight yields, and the most important processing cultigen ‘Dickinson Field’ (DF) often produces between 45 and 85 mt fresh weight per hectare (M. Babadoost, Ill. Coop. Ext., personal communication). Fresh weight yields were not significantly different between DF (48.1 mt/ha) and the interspecific hybrid 65xLIC (46.6 mt/ha) in field studies conducted in 2011 (Tables 25-27). The low fruit yield observed in ‘Golden Delicious’ (20.1 mt/ha) reflected the abnormally small fruit size, possibly resulting from a 10-day delay in transplanting and reduced vine growth caused by premature fruit set in many plants.

Fruit yield in the hybrid 65xDF was highly variable, and in this cultigen, many fruit abscised after several days of growth.

Fruit yields of 65xLIC in non-replicated plots in Wauseon, OH were 48.4 mt/ha, but DF was not evaluated, and plots may not have included guard rows (Duane Belle, Rupp Seeds, Inc., personal communication). Furthermore, plant density in the Ohio plots was 0.2 plants/m² compared with 0.7 plants/m² in plots at the Kingman Research Farm. The wide spacing resulted in plants yielding 4 fruit each, while 65xLIC produced approximately 2 fruit per plant at the closer spacing. The large leaves and vigorous main stem observed in 65xLIC might prohibit planting densities higher than 0.7 plants/m², but a closer spacing combined with the longer growing season of the Midwest might contribute to even higher yields.

The excessive vegetative habit of 65xDF may have resulted in reduced pollination or increased shading such that fruit senesced at a higher rate. Reducing plant density,
however, would not necessarily increase yields. Botwright et al. (1998) observed that with higher plant densities of *C. maxima*, pistillate flower and fruit abortion increased, but while a greater proportion of fruit developed to maturity at low densities than high densities, total yield was reduced.

**Dry Weight Yield**

Dry weight yields were significantly higher in 65xLIC than in DF (Tables 25-27). In squash, mesocarp percent dry matter is usually negatively correlated with FW yield, and cultigens that produce squash of good eating quality generally yield less than cultigens of poor eating quality (Loy, 2004, 2006). Thus, it is significant that 65xLIC produced fresh weight yields similar to DF, but significantly higher dry weight yields. Although the value of processing squash to growers is in fresh weight yields, fruit with higher percent dry matter usually have better perceived eating quality (Culpepper and Moon, 1945; Loy, 2004, 2006; Harvey et al., 1997) and might be used for additional purposes other than pie stock. Also, the addition of water to pureed mesocarp may allow for more processed product produced from less land area, as long as the consistency and color of the puree remains adequate.

A number of factors may have contributed to the higher dry biomass yields in the 65xLIC interspecific hybrid including heterosis, the bush-vine growth habit, and efficient partitioning of photosynthate within the fruit.

**Heterosis.** Similarly to previous descriptions of *C. maxima* x *C. moschata* hybrids (Castetter, 1930; Erwin and Haber, 1929; Robinson et al., 1978; Whitaker and Bohn, 1950), the interspecific hybrids 65xLIC and 65xDF were very vigorous in the field compared with parental cultigens and processing cultivars. Such vigor may have been
due to a heterotic effect of interspecific crosses, but the molecular mechanisms underlying heterosis are not understood.

Although inbreeding depression is not well-documented in Cucurbita, several investigators have documented heterosis in C. maxima for fruit yield (Hayase and Ueda, 1956; Hutchins and Croston, 1941; Korzeniewska and Niemirowics-Szczyt, 1993), eating quality (Hayase and Ueda, 1956), and percent dry matter and dry yield biomass (Korzeniewska and Niemirowics-Szczyt, 1993). Additionally, heterosis has been observed for earliness of flowering (Loy, 2011) and fruit yields (Amaya and Ortega; 1996; Grebenščikov, 1975; Lozanov, 1969) in C. pepo summer squash and seed yields in hull-less seeded pumpkins (C. pepo) (Cui and Loy, 2002).

**Plant Architecture.** Rates of leaf initiation on the main stem were not significantly different among cultigens either in the greenhouse or in the field, with the exception of the C. maxima parental NHBPB which showed slower rates of main stem leaf initiation than other cultigens. In the field, the production of lateral shoots in vine-type cultigens resulted in higher rates of total leaf production compared with bush cultigens that produced only short lateral shoots and bush-vine hybrids that produced lateral shoots later in plant growth.

Leaf initiation rate, however, is only one component of plant canopy formation. In a study comparing the productivity of vine (‘Blue Hubbard’) and bush (‘Autumn Pride’) C. maxima winter squash, Broderick (1982) observed that at close spacing, bush plants achieved an optimum leaf area index more rapidly than vine plants. Although new leaves were produced at faster rates in ‘Blue Hubbard’, the tall plant canopy, large leaf size, cupped leaf morphology, and close inter-leaf proximity in the bush cultigen allowed
for rapid and full canopy development with reduced inter-leaf shading. In the vine
cultigen, a low-lying, prostrate canopy, small leaves, and greater inter-leaf spacing
resulted in slow canopy formation. Also, prolific tendrils in the vine cultigen resulted in
clustered vines and petioles, which increased inter-leaf shading and resulted in sections of
bare soil (Broderick, 1982).

The interspecific hybrids 65xLIC and 65xDF possessed the desirable growth traits
of both bush and vine parental cultivgens. In early growth, short internodes (Table 13;
Figs. 16-17), long petioles (Table 18), and large, cupped leaves (Table 19; Fig. 27)
resulted in rapid canopy formation and uniform growth. At approximately 40 DAT, main
stems in 65xLIC and 65xDF plants began to vine between rows, and by 51 DAT, ground
cover was complete in these hybrids. The extreme branching habit in 65xDF (Fig. 26)
resulted in excessive vine growth and mutual shading of leaves, but in 65xLIC, the large
leaf size allowed for complete ground cover, even though plants only produced one
prominent stem (Fig. 24). This pattern of early and uniform bush to vine growth allowed
for effective weed control by black plastic mulch on the raised beds and mechanical
cultivation prior to leaf canopy cover between beds. The rapid canopy fill suppressed
any late-germinating weeds after cultivation.

In plots of the bush parent NH65, plants rapidly developed leaf canopies but did
not completely cover the ground at the between row spacing of 2.4 m and within row
spacing of 0.9 m. On the other hand, the vine processing cultivars GD and DF were
much slower in developing leaf canopies that covered the surface of the ground.
Vegetative growth was restricted in GD due to early onset of pistillate flowers, but in DF,
small leaf size, randomly oriented prostrate vines, and long internodes left soil uncovered for much of the growing season and appeared to result in mutual shading of leaves.

Interspecific hybrids involving WBN had prostrate vines, smaller leaf size and non-uniform growth. Furthermore, these hybrids did not produce the prominent branches seen in vine cultigens and the hybrid 65xDF. Premature fruit set in interspecific hybrids utilizing WBN may have resulted in reduced vine and canopy development because of competition for photosynthate between vegetative and reproductive organs (Wien, 1997). In the field in 2010 and 2011, plots of C. maxima x WBN plants appeared sparse, and bare soil was clearly seen between leaves. Such a growth habit created an environment where weeds could not be controlled between rows with cultivation or spot application herbicides, and in most cases, weeds eventually grew taller than the plant canopy.

**Biomass Partitioning in Fruit.** In squash, the fruit mesocarp is the economically valuable plant part, and seeds are a waste product. Seeds, however, act as a major photosynthetic sink and take precedence over other plant organs for photoassimilate because of high percent dry weight (Loy, 2004) composed of a largely of energy rich lipids and proteins (Jacks et al., 1972; Penning de Vries, 1975). The defining trait of interspecific squash hybrids is the ability to set seedless fruit, and the hybrids evaluated in the current study were characterized by partitioning of only a small fraction of total fruit biomass to the seeds (Tables 11.23). In the C. maxima processing cultivars, the fleshy mesocarp tissue accounted for only 71.5% total fruit DW in Rsp1232 and 84.1% in GD, while the edible mesocarp comprised between 82.0-89.3% of total fruit DW in DF. In the fruit of interspecific hybrids, mesocarp tissue comprised between 95-96% of total DW. Therefore, in interspecific hybrids, a greater proportion of the fruit fresh weight
tonnage purchased by processors from growers could be utilized in the processed product compared with standard cultivars.
CHAPTER V. CONCLUSIONS

1) Identification and/or selection for interspecific compatibility in terms of fruit set, seed production, and seed viability are prerequisite to development of *C. maxima* x *C. moschata* hybrid cultivars. Significant variation was observed in compatibility between *C. maxima* and *C. moschata* cultigens but appeared to be primarily determined by the *C. maxima* female parent. The bush *C. maxima* breeding line NH65 was compatible with a diversity of vine *C. moschata* cultigens.

2) For adoption by commercial growers, germination percentage and germination rates of interspecific F₁ seed must compare with those of open-pollinated cultivars for direct sowing in the field. Seed dormancy and poor germination might be amended by large-scale scarification techniques and/or selection of breeding lines that produce vigorous embryos in interspecific crosses.

3) Crosses between bush *C. maxima* lines and the *C. moschata* cultivar ‘Waltham Butternut’ produced F₁ hybrids with strong vining tendencies, limited lateral vine development, and uneven canopy development. Bush-vine interspecific hybrids utilizing the *C. moschata* processing cultivars ‘Long Island Cheese’ and ‘Dickinson Field’ were bush-like in early growth, resulting in rapid and uniform canopy development at moderately high planting densities (0.7 plants/m²) and allowing for effective weed suppression, photosynthetic efficiency, and support of large fruit loads.
4) In bush *C. maxima* x ‘Waltham Butternut’ crosses, early pistillate flowering and a high pistillate to staminate flower ratio in the *C. moschata* parental cultivar resulted in F₁ hybrid plants with precocious pistillate flowering and early fruit set that suppressed vine and leaf canopy development. The *C. moschata* processing cultivars ‘Long Island Cheese’ and ‘Dickinson Field’ displayed later initiation of pistillate flowers and fewer pistillate than staminate flowers, and in hybrids utilizing these cultivars, canopy development occurred prior to pistillate flowering and fruit set. In developing *C. maxima x C. moschata* interspecific hybrids, vine *C. moschata* breeding lines should be selected with delayed pistillate flower production and low pistillate to staminate flower ratios to allow for adequate vine and canopy formation before fruit set in the F₁ generation.

5) The fruit produced by the *C. maxima x C. moschata* hybrid 65xLIC, possessed several traits advantageous in squash used for processing, including orange rinds and flesh, uniform size, round shape, and reduced blossom scars and peduncle bases. Also, percent dry matter and soluble solids contents in the fruit of 65xLIC and other interspecific hybrids exceeded those in the open-pollinated *C. moschata* processing cultivar ‘Dickinson Field’. The interspecific hybrid 65xDF, however, produced inconsistent, misshapen fruit, and the fruit of 245xWBN developed green pigmentation in the edible mesocarp. Such undesirable traits would most likely dissuade growers and processors of processing squash from adopting these interspecific cultivars.

6) The bush *Cucurbita maxima* x vine *C. moschata* F₁ hybrid 65xLIC produced fruit fresh weight yields comparable to those observed in the open-pollinated *C.
*moschata* processing cultivar ‘Dickinson Field’. Additionally, mesocarp dry weight yields were 50% higher in the hybrid 65xLIC (3.9 mt/ha) compared with ‘Dickinson Field’ (2.6 mt/ha). The high biomass yields may be due to rapid and efficient leaf canopy development in the bush-vine growth habit, efficient partitioning of photosynthate in the fruit to edible mesocarp instead of seeds, heterosis, or a combination of these traits and unidentified factors. Yield trials of interspecific hybrids should be conducted in a variety of climates and plant densities to determine optimal growing conditions for maximum yields.
REFERENCES


