INTEGRATED CULTURAL AND MECHANICAL WEED MANAGEMENT FOR ORGANIC WINTER SQUASH SYSTEMS

By

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ABSTRACT

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The Midwest region is a leader in cucurbit production in the U.S., but weed management is a major constraint to successful production, especially for organic growers. Mechanical weed control is a primary tool for managing weeds in cucurbits, but low selectivity, and excessive soil disturbance limit successful adoption. One approach to improving selectivity of mechanical cultivation tools is to select for varieties that are tolerant to those tools. Traits associated with tolerance to mechanical cultivation may also be valuable for establishment in reduced tillage systems. Field experiments were carried out in central Michigan to evaluate six varieties of *Cucurbita pepo* for their 1) tolerance to mechanical cultivation, and 2) ability to establish in a reduced tillage system. Laboratory and greenhouse testing was done to determine which seedling traits may be correlated to field performance. Results confirmed cultivar differences in tolerance to cultivation tools, with inconsistent results across runs of the experiments. No clear cultivar differences in tolerance to reduced tillage were detected. Cultivars varied in traits that may contribute to tolerance to both reduced tillage and cultivation including emergence timing, early vigor, root:shoot partitioning and anchorage force. Germination rate of seeds subjected to aging was well correlated with emergence in reduced tillage systems. Anchorage force of seedlings was positively correlated with tolerance to flextine cultivation. Our results suggest that choosing existing and developing new cultivars tolerant to physical weed control tools, untilled soil, and organic management may improve organic squash production in Michigan.

This thesis is dedicated
to my god Daughter and flower baby Esmeria Wolf Costella-your grace
and fortitude in being born, learning to walk, and cultivating a taste
for full sours in the last two years has inspired
me at every trial and tribulation of my
graduate career.

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KEY TO ABBREVIATIONS

AA	Accelerated aging
RT	Reduced till/tillage
CT	Conventional till/tillage
C. pepo	*Cucurbita pepo*
KBS	Kellogg Biological Station
HTRC	Horticulture Teaching and Research Center
Root:Shoot	Root to shoot ratio
ST	Strip till/tillage

CHAPTER ONE: Cultivars and Traits for Improved Resilience in Organic Squash Production: A Review.

Organic winter squash producers in the Midwest face unique weed and soil management tradeoffs. Physical disturbance of the soil through tillage and cultivation is often the primary tool available to organic growers for weed management and crop establishment (Luna et al. 2012; Lowry and Brainard, 2017). While tillage and cultivation can be effective tools for field preparation and weed management, they entail ecological, biological, and economic tradeoffs including loss of organic matter, damage to soil structure (Arshad et al. 1999), and increased risk of erosion (Karlen et al. 1994; Triplett and Dick, 2008). The choice to reduce soil disturbance through adoption of no-till or strip till systems, or by reducing the use of mechanical cultivation can improve soil characteristics in the long run (Mahboubi et al. 1993). However, shifts in management to reduce soil disturbance can result in economic loss due to poor establishment and increased weed pressure (Luna et al. 2012). Identification of new approaches to minimize economic and biological tradeoffs associated with soil and weed management in organic squash production will be the broad goal of this literature review. Specific objectives include 1) summarizing and synthesizing the current state of knowledge of ecological weed management, with an emphasis on integrated approaches of physical and cultural control; 2) discussing the costs and benefits associated with RT systems in winter squash; and 3) highlighting the potential role that cultivar choice and seedling traits can provide in improving both soil and weed management in organic winter squash production. We argue that choosing existing and developing new cultivars tolerant to physical weed control tools, untilled soil, and organic management may improve organic squash production in Michigan.

Organic food, which offers a price premium to vegetable growers of any scale, continues to grow in demand in the Midwest region. Michigan is ranked as one of the top ten states for certified organic sales in the US and shows projected increases for production (USDA, NASS 2016). The Midwest region is also a leader in cucurbit production accounting for 40% of the conventionally grown cucurbits in the US (USDA-NASS, 2013). Over 600 acres of organic squash were harvested in Michigan in 2016 at a value of over $1,700,000 (USDA, NASS 2016). At the same time, Michigan has hot humid summers that can foster weeds, pests, and disease and complicate organic production.

Weed Management Challenges and Opportunities in Squash Production

Weed management in winter squash is especially difficult due to the growth habit and morphology of the crop (Miville and Leroux 2018). Sprawling vines of non-bush or semi bush squash complicate weed management between rows and lateral roots that grow close to the soil surface make root damage a potential outcome of any soil disturbance (Miville and Leroux 2018). If left uncontrolled, weeds emerging during the critical weed free period of the first four weeks can lead to yield losses of up to 100% in related cucurbit corps (Dittmar and Boyd 2015). In no-till and RT systems the influence of weeds on a cucurbit crop can be equally dramatic (Rapp et al. 2004). Rapp et al. (2004) conducted a study analyzing the effect of tillage, residue and herbicides on weed suppression and yield of pumpkin. This study found that plots that were disk tilled and not weeded for the rest of the growing season resulted in weed densities 21 times that of plots that were disked and treated with herbicides. A comparison of yield found that the conventionally tilled treatment with no weed control had nearly half the mature fruit yield of plots that were disked and herbicide treated. Studies of weed management impacts specifically for organic squash production are less common. However, a survey of organic producers in the

United States found that weed management and the high expense of weed control labor was the most important challenge faced by respondents (MDA M. Moynihan 2007; Organic Farming Research Foundation 2004) Cultural and mechanical options in organic *Cucurbita pepo* (acorn and delicata squash) will be necessary for better weed control in the Midwest region.

Cucurbits in Michigan are affected by both pre and post emergence broadleaf and grass weeds. It is likely that species effecting the crop may vary widely depending on management system. For instance, Frost et al. (2017) suggests that in long term reduced till systems there was an increase in the seed persistence of large crabgrass (*Digitaria sanguinalis),* along with a persistence of Powell amaranth (*Amaranthus powellii*), and a decrease in broadleaves compared to conventional systems. Other long-term studies of conventional versus organic row crop systems demonstrate that changes in species composition, weed biomass, and species density can occur (Menalled et al. 2001). For example, Menalled et al. (2001) found in a six-year trial of a corn, soy, and wheat rotation in Western Michigan that organically managed systems that underwent full width tillage, were dominated by red clover (*Trifolium pretense*), quackgrass (*Elytrigia repens*) and common lambsquarters (*Chenopodium album*). While monocot and dicot species were similar in the conventional systems, the no-till system was dominated by annual grasses such as large crabgrass (*Digitaria sanguinalis*). Little research currently exists on the weed makeup of organic cucurbit systems in Michigan specifically.

Most curcurbit growers rely on a combination of herbicides and/or mechanical cultivation to manage weeds. Both forms of weed control have significant tradeoffs in winter squash systems. While mechanical cultivation can be effective at earlier stages of growth, later cultivation can damage roots which limits water and nutrient uptake (Dittmar and Boyd 2015). For conventional producers, standard herbicides used in Michigan include halosulfuron, ethalflurialin, and clomazone (Zandstra 2017). Halosulfuron is the only pesticide registered for post emergence control of broadleaf weeds in winter squash and pumpkins, but it can be injurious to the crop if weather and soil moisture are not ideal and is especially damaging to *C. pepo* (Zandstra 2017; Starke et al. 2006). Clomazone is only recommended for processing squash and pumpkin leaving fresh market cucurbit crops without a reliable option for post emergence chemical control of broadleaf weeds. Among pre-emergence herbicides, both ethalflurialin and clomazone are unreliable for control of many broadleaves and entail risks of crop damage (Zandstra 2017; Brown and Masiunas 2002; Grey 2000) To make up for this lack of chemical weed control options, many producers supplement herbicide use with some form of mechanical cultivation or hand weeding (Brainard unpublished).

Organic growers' heavy dependence on mechanical cultivation and hand weeding, may negatively impact desirable soil characteristics (Hamza and Anderson 2005). According to a 2014 survey of Michigan organic farmers (Lowry and Brainard 2017), approximately 46% of winter squash growing respondents used row crop cultivators to control weeds and 19% used rototillers. The mean number of total tillage operations was 6.6 with 3.5 used for field preparation and the remaining for mechanical cultivation. While specific data for hand weeding in organic squash in Michigan is not available, several studies have shown that the cost of hand weeding in organic crops such as tomato, soybean, and other row crops is significantly higher in organic systems compared to conventional (Archer et al. 2007; Hillger et al. 2006; Mcbride and Greene 2009). The extra cost of labor on organic farms can offset the premium offered for organic crops and discourage growers from converting conventional systems to organic ones.

Ecological Weed Management. The theory of ecological weed management suggests that a multi-tactic approach to controlling weeds in agricultural systems has the potential of reducing the use of herbicides while improving crop yields (Liebman et al. 2001). The tactics used in ecological weed management include cultural and physical controls. Cultural control is the deliberate alteration of the production system by management or practice, that results in the reduction of weeds (and other pests) or the ability of those weeds to injure crops (Ashdown, 1977). One needs to implement biotic and ecological knowledge into management decisions for cultural controls to be effective. For example, knowledge of the identity and preferred habitat of seed predators may suggest habitat management practices to promote predation and decrease the weed seed bank over time (Brust and House 1988). Physical control is the direct action of killing a weed by physical means such as cultivation. Some examples of cultural control can include crop sowing time, crop spacing, using living mulches, and crop cultivar choice (Barberi 2003). All ecological weed control practices benefit from an understanding and exploitation of ecological phenomena in order to reduce the fitness, survival, fecundity of weed species. These phenomena could include: competition, allelopathy, herbivory, disease, and soil disturbance (Liebman and Dyke 1993a; Wyse 1994). The balance one must strike in ecological weed management is the maximizing of stress on the weed and the minimizing of stress on the crop (Liebman et al. 1997). In order to strike this balance, ecophysiological research is needed to identify mechanisms of crop-weed resource competition. Measurements that inform these mechanisms may be the rate of growth, resource conversion, and allocation processes (Berkowitz 1988; Kropff and Lotz 1993). By taking advantage of these naturally occurring phenomena, and integrating them with direct curative approaches, weeds may be successfully managed in both the short and long term.

The optimal choice of weed management tactics may be informed through quantification of relative responses of weed and crop growth to biological, physical, and chemical factors

(Liebman et al. 1997). For example, Wells et al. (2014) investigated how cultural practices such as planting time and row spacing effected soil moisture, weed density, and crop responses such as lodging in organic no-till soybean production. The use of a high-density cereal rye cover crop in no till soy was identified previously as an effective tool at decreasing weed cover in the region of study (Wells et al. 2013). In this example of cultural control, the team exploited the fact that soybeans had the ability to emerge and grow successfully in these conditions. The weeds in this system were either unable to grow at all or germinated and died under the mulch which reduced the weed seed bank over time. This tactic made the no-till organic system more economically sustainable to potential organic no-till growers. However, loss of soil moisture and lodging were identified as potential trade-offs to weed suppression gained from high residue production. The loss of soil moisture during cover crop growth due to transpiration and the increase of lodging could outweigh the benefits gained by no-till if not addressed through this multi factor experimental design. The study was able to determine how cultural practices such as planting time and row spacing, could be optimized to maintain weed suppression and balance tradeoffs.

In addition to differences between crop and weed response to management, differences in weed tolerance (ie. "sustain the presence of weeds with little or no yield loss compared to weed-free control treatments" (Liebman and Gallandt 1997)) among crop types and cultivars can also be exploited to improve weed management. Differences among crop cultivars in their tolerance to weeds, as expressed by yield, has been described for several crops including carrot *Daucus carota* (William and Warren 1975) soybean (*Glycerine max*) (Callaway and Forcella 1993) and winter wheat (Wicks et al. 1986). It is important that varieties selected for their ability to tolerate weeds are able to maintain yield and quality characteristics that can be affected by the presence of weeds (Millar et al. 2007). Millar et al. (2007) developed a study comparing the effect of different levels of weed pressure on five soy bean (*Glycerine max*) varieties' yield, protein, and oil content. The study did not find significant cultivar by weed pressure effects on overall yield. However, there was a significant interaction of high and medium weed pressure on the important seed quality characteristics of oil and protein percentage.

Ideally, ecological weed management optimizes many cultural control choices in order to sustain a multi tactic approach to control weeds and maintain or improve agronomic characteristics of the crop. For example, Beres et al. (2010) worked to develop a sustainable management package to improve both the productivity and weed competitiveness of winter wheat in Canadian prairies. The study included factors of cultivar, seeding rate, and timing of herbicide application. This factorial experiment found that integrated practices of best agronomic management and cultivar choice resulted in greatest yield and survival stability.

Physical Weed Control and Selectivity. Selectivity is a critical concept in weed management, involving suppression of weeds while limiting damage to crops (Rasmussen 1990). The physical destruction of weeds through tillage and cultivation are the primary forms of weed control for organic growers. Traditionally, mechanical weed control tools have been crude with a high rate of expected crop injury or low selectivity. Mechanical cultivation with low selectivity usually means more passes will be necessary to achieve the desired amount of weed control or higher rates of crop damage are expected. Increasing the number of cultivation passes means an increase in ecological and economic tradeoffs. Such tradeoffs include a possible decrease in crop quality, such as an increase of forked carrots (Ascard and Mattsson 1994); degradation of soil structure, (Grandy and Robertson 2006; Franzluebbers 2002) and increased risk of soil erosion (Karlen et al. 1994; Triplett and Dick 2008).

Although in-row mechanical cultivation often entails low selectivity, improvements over the last twenty years in mechanical and biosystems engineering have opened up possibilities for improving the selectivity of these tools (Kouwenhoven, 1997; Perez-Ruiz et al. 2014). Private and public research efforts are making strides in both camera guided systems and the precision of tool action, to decrease the amount of crop loss and the need for additional hand weeding (Van Der Weide et al. 2008). A less studied, but potentially equally important approach for improving selectivity involves integration of cultivation tolerant crops (Bueren et al. 2011; Gallandt et al. 2017; Hitchcock-Tilton, 2018). The identification of more efficient and selective cultivation and tillage tools, coupled with crop phenotypes that are tolerant to mechanical injury may be helpful in reducing tradeoffs of weed control in organic systems.

In Row Weed Control Machines. Primary tillage prior to crop planting, and between row mechanical cultivation following crop emergence have historically been the primary forms of mechanical weed management for squash farmers. Between row cultivation is common, because it has limited potential to damage crops. In contrast, in-row cultivation can cause crop damage or mortality due to the close proximity of crop and weed (Bond et al. 2003; Van Der Weide et al. 2008; Gallandt et al. 2017). In order for in row weed control to be selective, management choices should be made to promote a differential in crop and weed size or morphology at the time of post emergence cultivation. Some management choices could include creating a stale seed bed so that weed and crop do not emerge simultaneously (Melander et al. 2005); deeper seed placement and pre-emergence cultivation that can give the crop a growth advantage (Van der Weide et al. 2008); and transplanting to promote a greater crop-weed size differential (Bleeker et al. 2002).

In addition to creating a size differential between crops and weeds, in-row tool selectivity may be improved by selection and calibration of appropriate tools and guidance systems. In-row cultivation machines have been designed to function for a specific range of crop, weed and soil conditions. Understanding the acceptable range for any given tool is key to achieving the highest level of tool efficacy (Mohler 2001). Most in-row cultivation tools work best to control weeds at the white thread or cotyledon stage (Van Der Schans et al. 2006). Many weeds can easily re-root after cultivation if more complex root systems are allowed to develop. While fruiting crops such as squash, with rapid early growth and long-life cycles are appropriate for in-row cultivation, crops such as carrot, onion, or leafy greens that are more sensitive to damage may be more challenging. Weather and soil conditions (e.g. moisture) are both difficult to manage for mechanical cultivation and key to improving selectivity in mechanically cultivated systems (Kurstjens et al. 2000; Kurstjens 2002; Terpstra and Kouwenhoven 1981; Cirujeda and Taberner 2004). Soil conditions cannot be so wet that weeds will not desiccate when uprooted or buried, while conditions that are too dry or crusted will make it difficult to uproot weeds, or result in shattered soil clods in which weeds can survive. Some in-row tools or tool combinations may be better able to cope with such sub-optimal soil conditions than others, but limited information is available to better match tools and conditions to improve selectivity.

The finger weeder is a cultivation tool designed to uproot or bury weeds within the crop row by a set of spinning rubber “fingers”. The tool is made up of two rubber disks with fingers protruding around its circumference. Attached to each disk is a metal drive wheel with metal pins that stick into the ground and drive the fingers through the crop row in a spinning fashion. The smaller diameter to the drive disk allows the fingers to move through the crop row faster than the ground speed of the tractor wheel. As the fingers spin through the crop row they tear weeds from the ground sheering them at the soil surface or burying them.

As with all mechanical cultivation machinery, finger weeders have the highest efficacy and selectivity within a certain range of crop, weed and soil conditions, and their selectivity depends on proper calibration of speed, depth and tip distance. It is generally recommended that finger weeders be run at relatively high speeds (Ascard and Bellinder 1996). Kouwenhove (1998) found the finger weeder to be most effective at speeds above 10km/h. Crop plants should be well rooted at the time of cultivation and have between 1-6 true leaves. Any smaller and crops are likely to be uprooted, any larger and the possibility for damage increases (Van der Schans and Bleeker 2006). Appropriate ranges of crop, weed, and soil conditions for the finger weeder were investigated by Bleeker et al. (2002). The study included factors of direct seeded (onion and sugar beet) transplanted crops (leek and lettuce) and cultivation in two types of soils (sand and clay). They determined that the finger weeder had its highest efficacy in sandy soils rather than clay and in transplanted crops rather than direct seeded. Transplanted crops are easier to cultivate because of the larger differential between crop and weed. While sandy soils are preferable for tools that uproot, because weeds can easily be pulled completely from the soil. These conclusions are consistent with other general knowledge that a friable soil is ideal for cultivation (Gallandt et al. 2017). However, the importance of soil texture may depend on whether the finger weeder is set to kill weeds to uproot or to bury. When burying is the primary mode of action it was found by Baerveld and Ascard (1999) that a finer particle size of soil was more effective at killing weeds than a courser size.

The flextine cultivator is a blind cultivation tool designed to uproot weeds between and within the crop row in pre and post emergence applications. Blind cultivation refers to the fact the tool is designed to select smaller plants growing randomly in the field (the weed) over relatively larger plants emerging in rows (the crop). The tool consists of a set of mounting bars that extend behind the tractor. Attached to the three to six mounting bars are staggered flexible metal tines (up to 25 tines per toolbar foot) (Bowman 1997). The tool is designed to run as level as possible to the soil surface so that tines move through the soil at the same depth. As the machine is run through the crop rows the tines shake and vibrate through the soil and uproot small weeds and shatter soil clods where other newly germinated weed seeds are. The depth and angle of the tines of the flextine harrow are adjustable and their optimal settings can vary depending on the farming system. It is generally agreed that the tines are most effective when set at an angle in which the tines are pointed forward (Van Der Schans and Bleeker 2006)

The selectivity of the flextine harrow is highly dependent on the conditions in which it is used. The flextine is strictly recommended for control of white thread stage weeds (Van Der Schans and Bleeker 2006; Van Der Weide 2008). The potential for crop damage from the flextine cultivator means a well-established crop and a weakly rooted weed is necessary to improve selectivity. Management of the cropping system that allows for this differential in crop/weed establishment and morphology is the most important criteria for flextine efficacy (Rasmussen 1990). While increased speed can be a relatively simple adjustment for increasing weed death, relatively high speeds will also lower selectivity (Rassmussen et al. 2008). The maximum working speed recommended for the flextine is between 8-10km/hr (Fogelberg 2007; Rasmussen 1992). Soil moisture will greatly influence the efficacy of the flextine harrow. Higher soil moisture will allow for weeds to be more easily uprooted (Kurstjens et al. 2000). However, tines will be more restricted from vibration in wet soils meaning they will have less range of motion and will kill fewer weeds. For example, Kurstjens (2002) found that as soil moisture decreased from 16% to 5%, weed mortality increased from 36% to 91%.

In summary, mechanical cultivation machines can be useful tools. However, their potential for usefulness is only possible if they are used in the conditions for which they are designed. A better understanding of the optimized cultural controls of weeds and the correct set up and calibration of the equipment would do much to improve the efficacy of these tools for the farmers that rely on them.

Costs and Benefits of Reduced Tillage Systems for Squash

Reduced Till (RT) Organic Systems. A major criticism for organic systems is the use of soil disturbance as the primary form of weed control. Tillage and mechanical cultivation require inputs of petroleum, cause an increase in CO_2 release, and can compromise soil health (Ball and David, 2007). Research into ways of reducing soil disturbance while maintaining yield and quality of high value horticulture crops will be important in further adoption of more sustainable organic production.

RT systems can involve many different types of management. From no-till (NT) systems; where the soil is never tilled or cultivated, to various forms of targeted tillage including strip tillage (ST) and permanent bed systems where primary tillage is limited in space or time compared to conventional tillage systems. For the purpose of this review we have included literature of a wide variety of management styles that attempt to reduce the amount of tillage in the agricultural system.

RT systems can offer many benefits to both organic and conventional cropping systems. RT systems can improve soils through increased sequestration of carbon (Lal and Kimble 1997) and improve soil water infiltration and holding capacity (Franzluebbers 2002; Mahli and O'Sullivan 1990). Other biological and ecological benefits of RT include the suppression of certain diseases by reducing soil splash (Wang and Ngouajio 2008) and an increase in beneficial insects and earthworms by providing a more hospitable habitat for dwelling and reproduction (Luna and Staben 2002; Overstreet et al. 2010).

RT can be executed successfully when a thorough understanding of the specific crop responses such as yield, quality, and weed suppression is understood. Plant responses that benefit or hinder reproduction may include growth and allocation processes (Liebman, et al. 1997). Growth analysis of soybean under tilled and no-till systems (e.g. Yusuf et al. 1999) helps to explain how alterations in plant development compensatory growth allow no till soy to attain similar yields to tilled soy. Yusef et al.'s initial research into crop response to tillage has informed further applied research into nutrient placement and subsequent accumulation in the crop in no-till systems (Farmaha 2012). These subsequent studies informed by basic research into crop response offers valuable information to growers on best management practices for a wide scope of their production needs.

Cucurbita pepo and its relatives are economically important crops that have been shown in previous studies to perform relatively well in RT systems. Pumpkin and winter squash have been identified as potentially important crops for RT systems with weed-suppressive mulches, because of the lack of registered herbicides for the crop (Harrelson et al. 2007). RT systems in which pumpkins are grown on mulch are also attractive to growers because of cleaner fruits that don't contact the soil surface (Walters et al. 2008). Rapp et al. (2004) studied differences in pumpkin yield between strip tilled, conventionally tilled, and no tilled treatments. The study found little and no significant differences in the number and weight of mature fruits produced across treatments in both years. In herbicide treated RT summer squash, Walters and Kindhart (2002) found no difference in yields between conventional and strip tillage for two years. However, in the third year, strip tillage marketable yields exceeded those of the conventionally tilled treatment. Walters and Young (2008) evaluated squash that was no-till transplanted into an herbicide treated rye cover crop and observed stunting of plants and reduction in yield up to 50% compared to tilled treatments. These reductions in plant growth and subsequent yield were proposed to be a result of lowered soil temperatures.

Although RT systems have several important potential benefits, several key challenges remain before they will be widely adopted by organic producers. When tillage is not used to kill weeds throughout the growing season an increase in the weed pressure can be expected. A lack of organic herbicides and the challenge of mechanical cultivation in the presence of surface residue, can make weed pressure an even greater source of stress on crops (Bond and Grundy 2001). In addition to weed pressure, interference from the growth or decomposition of cover crops (Luna et al. 2012), lack of aeration, and relatively colder soils in RT systems create several challenges for successful crop establishment (Mohler 2001). Indeed, many studies have shown reduced stand establishment in RT compared to CT systems. Knavel and Morrison (1977) showed a significant stand reduction in NT grown cucumber compared to a conventionally tilled treatment. Rapp et al. (2004) found that NT pumpkins were less vigorous at early stages of growth then conventionally tilled plots. This difference in vigor was suggested to be a result of cool soil temperatures.

In order to address these constraints of RT systems, and increase successful adoption, new strategies are needed. These may include greater reliance on targeted tillage (e.g. strip tillage), development of alternative fertilization such as placement of nitrogen cover crops closer to the root zone (Lowry et al. 2017) and weed management strategies such as better high residue management (Brainard et al. 2013), and identification of crop cultivars and traits that are well adapted to the often-challenging edaphic conditions associated with RT.

Potential Role of Cultivar Choice and Plant Breeding for Addressing Weed and Soil Management Constraints

Efforts have been made in recent years to identify traits suited to a variety of growing environments and systems rather than focusing exclusively on high input and/or conventional systems (Wollenweber et al. 2000). Screening varieties in organic systems and breeding varieties specifically for performance in organic systems is a concept gaining legitimacy in the breeding community (Bueren et al. 2011).

Breeding for Weed Management. Plant breeding's contribution to weeds and weed management have largely focused on crop tolerance to herbicides (Regnier et al. 1990). Research into herbicide tolerance has resulted in the development of herbicide tolerant crops including-most notably—tolerance to glyphosate (Gianessi et al. 2007). The use of herbicides in vegetable production systems have allowed for a reduction in tillage and weed management costs which undoubtedly have brought benefits to the conventional farmer. However, the over-reliance on herbicides on many farms has contributed to the evolution of herbicide resistant weeds and other environmental and human health costs (Gianessi et al. 2007; Wechsler 2017). This decrease in the efficacy of herbicides makes physical and cultural weed control all the more important.

To a lesser extent, the selection of weed-competitive and weed-suppressive cultivars have also been a research topic of interest (Bond and Grundy, 2001). Plant architecture traits such as height have been positively correlated with weed competition in several species (Murphy et al. 2008; Garrity et al.1992; Christensen 1995). In addition, certain cultivars of wheat have been shown to have significant allelopathic ability to reduce the dry weight of the weed rye grass (Wuet al.1999). Less research has been conducted evaluating the potential for breeding to improve weed suppressive ability of vegetable crops.

The need for crop tolerance to mechanical cultivation has been identified as a priority for organic research (Bueren et al. 2011; Gallandt et al. 2017) but very little research has been directed to this type of tolerance by the breeding community. Murphy et al. (2008) in a trial comparing wheat varieties for their tolerance to mechanical cultivation found differences among varieties in their yields and weed suppressive ability but did not detect any cultivation by genotype interactions. Studies in carrots showed that commercially available carrot cultivars vary in their tolerance to cultivation tools but the traits that confer that tolerance are still unclear (Hitchcock Tilton 2018). Research has shown that crop tolerance to mechanical cultivation tools that uproot crops may be related to the crop tolerance to lodging or herbivory (Gallandt et al. 2017). Traits that may confer tolerance to cultivation include greater anchorage force, stem strength or increased branching or the root system (Pinera-Chavez et al. 2016; Strauss and Agrawal 1999) Further investigation is needed to understand what effects mechanical cultivation has on crops, and what traits are may be associated with tolerance to those effects. If a cultivation by genotype interactions are identified, opportunities may exist for the use or development of cultivars that are more productive in mechanically cultivated environments.

Breeding for Reduced Tillage Systems. RT management of pumpkin and winter squash has been shown to be successful in systems where herbicides are available (Walters et al. 2008). However, the increase of crop stress in RT organic systems suggests that the development of varieties that are better adapted to RT systems could increase crop productivity and adoption of the practice (Trethowan et al. 2012)

RT organic systems can vary widely from farm to farm and some changes in disease (Wang and Ngouajio 2008) and pest communities (Luna and Staben 2002) can improve growing conditions for certain crops. Water availability can also be improved for certain crops under certain environments in RT (Hoyt and Konsler 1988). However, according to a recent meta analysis of genotype by tillage interactions (Herera et al. 2013) in RT wheat and corn, plants consistently face denser soil, suboptimum soil contact, and lower soil temperature then conventional systems. These conditions are all thought to be negatively correlated to crop establishment and emergence (Ball and David 2007). In addition to these conditions, crops also may face increased physiological stress from pest and disease pressure along with potential injury from aggressive cultivation machines (Hal and Cholick, 1989). Because of the unique challenges of RT organic system there is a need for varieties bred for resilience to those conditions.

Seedling development and biomass partitioning characteristics such as early seedling vigor, rapid leaf production and increased partitioning to roots influence the ability of crops to quickly emerge and establish in an RT system (Ball and David; Joshi, et al. 2007). Cornish and Lymbery (1987) found significantly reduced population and early growth rate of wheat in untilled systems. A strong relationship was shown in an increase in soil strength and a reduction in root growth. Plants with this reduction in root growth were shown to have lower above ground biomass. It was suggested by this relationship that strong early root growth was important for subsequent shoot growth and photosynthetic capacity in RT systems.

Previous research suggests that genetic differences in root:shoot partitioning and root morphology may be important for successful establishment, but evidence is mixed and inconclusive (Koevoets et al. 2016, Jung and McCouch, 2013). While little attention has been paid to identifying valuable traits for breeding vegetable crops for RT systems, progress has been made in small grain crops. Emergence, establishment, and seedling vigor have been found to be important selection criteria for small grains grown in no-till systems (Trethowan et al. 2005;

Joshi et al. 2007). However, it is unclear how partitioning of root to shoot is related to seedling vigor and subsequent establishment in RT cropping systems. Cui et al. (2002) used seedling growth rate and germination rates in rice to quantify seedling vigor. They then further analyzed seedlings in order to identified quantitative trait loci for seedling vigor and physiological traits that were correlated with seedling vigor. They found significant and positive correlations of seed weight and initial seedling dry weight suggesting relatively high seed reserves could result in more vigorous seedlings. Cui et al. (2002) also found a negative correlations of root activity (expressed as oxidized α-naphthylamine (μg) minus auto-oxidized α-naphthylamine in the control per g of fresh root per h.) with seedling vigor. Suggesting roots may compete for carbohydrates with shoot parts of the plant in early stages of growth. Kumar et al. (2012) found that in maize, highly branched root systems improve water and nutrient uptake. Similarly, Baligar et al. (2001) found that higher root density and longer roots are important for improved nutrient uptake and an increase in dry matter yield in RT systems. However, selection for some root traits and partitioning may come at a cost. Selection for root:shoot ratio and specific root system architecture may compromise yields or other important morphological traits (Koevoets et al. 2016). Further research is needed to understand what types of seedling morphology and partitioning are best suited to productivity in RT vegetable systems.

Early seed and seedling vigor are extremely important for improving establishment in RT systems. Seedling vigor is defined as a “property determined by the genotype and modified by the environment which governs the ability of a seed to produce a seedling rapidly in soil and the extent to which the seed tolerates a range of environmental factors” (Perry 1972). Early vigor has been shown to be well correlated with weed competitiveness and yield stability in RT wheat (Watt 2005). Developments in breeding wheat for vigor characteristic have made progress in recent years. Rebetzke et al. (2004) defined early vigor in wheat as greater relative seedling leaf area (SLA). SLA is described as having potential to increase weed competitiveness, water use efficiency and grain yield making a high SLA phenotype ideal for RT production systems. In their study Rebetzke et al. (2004) discovered a large genetic variation in SLA that would be useful in in future research into breeding for more vigorous lines of wheat. The study also found a high heritability in leaf width and a strong genetic correlation with total leaf area and above ground biomass. It was suggested that these traits are likely to produce greater genetic improvement in early vigor. It is important to remember that increases in vigor may have tradeoffs because of how plants allocate resources and develop morphologically. In the case of breeding for SLA Wilson et al. (2015) discovered that breeding for an increase in SLA could have the effect of decreasing transpiration efficiency (TE) which could have implications for plant biomass and potential yield. (Condon et al. 2004). Therefore, further breeding projects had to be undertaken to combine the selection of both TE and SLA. This is just one of the many examples for how selection for characteristics of vigor can have multiple physiological and morphological tradeoffs that must be addressed.

Challenges to organic RT squash may differ widely from those confronted by grains grown in conventional RT systems where breeding efforts are currently focused. While breeding efforts focused on the improvement of *C. pepo* specifically for organic RT may still lie years in the future, screening varieties and investigating traits associated with increased performance in these systems could assist in future breeding efforts.

Summary and Thesis Outline

Organic squash is an important and growing crop for Michigan. However, growers rely heavily on soil disturbance for field preparation and weed control, resulting in potentially important negative effects on soil health (Lowry and Brainard, 2017). As the adoption of organic and RT agricultural practices increases, so should the improvement of cultural and mechanical tools for weed control. Ecological weed research has progressed to a point where the understanding of the lifecycle of both weeds and crops can be used to make real world recommendations to growers on how to manage weeds by choosing appropriate cultural practices. However, further research is needed to understand how best to integrate cultural practices to reduce dependence on physical soil disturbance for weed control. We argue that improved understanding of the growth and morphology of *C. pepo* varieties may improve their productivity in mechanically cultivated and RT systems. The development of crop cultivars better adapted to systems that utilize mechanical cultivation and RT is a potentially valuable research goal for improving the sustainability of organic production.

The primary goals of the work presented in this thesis are to evaluate tolerance of six *Cucurbita pepo* cultivars in their response to RT management and CT mechanically cultivated organic systems and to evaluate traits associated with that tolerance. In Chapter 2, results from germination chamber studies evaluating differences in seed and seedling characteristics are presented. Field experiments evaluating tolerance of cultivars to RT are then described, and correlations between traits and field performance evaluated. In Chapter 3, results from greenhouse studies are used to characterize cultivar differences in seedling partitioning and anchorage force. Results are then presented from field studies evaluating cultivar differences in seedling tolerance to mechanical cultivation tools. Finally, greenhouse and field studies are compared to determine the relation of seedling traits in the greenhouse to seedling performance in the field.

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CHAPTER TWO: Assessment of Cultivars of *C. pepo* Tolerance to Reduced Tillage Systems.

Abstract

Reduced tillage (RT) in vegetable systems has been shown to have environmental benefits. However, vegetable crops face challenges in RT systems, including denser soil, suboptimum soil-seed contact and changes in disease and pest pressure. This project's objectives were to evaluate if tolerance to RT existed among six varieties of acorn and delicata squash (*Curcurbita pepo*). We then sought to determine which cultivars were most tolerant and to identify possible traits associated with that tolerance. Germination rate and seedling analysis were performed using the standard and accelerated aging (AA) germination test. Seed and seedling traits were characterized using WinSeedle and WinRHIZO Image Analysis Systems to digitally analyze seed and seedling size, root morphology and root:shoot partitioning. In both 2017 and 2018, a field experiment was conducted to compare emergence and early growth of the same six cultivars under conventionally tilled and no-till (NT) conditions. We found significant differences in seed and seedling traits, as well as field performance across cultivars in all of our experiments. In our field experiments, cultivar emergence and biomass accumulation were reduced in NT compared to conventional tillage in both years. However, we did not detect clear differences in cultivar tolerance to NT (no significant tillage by cultivar interactions). Correlation analysis between cultivar characteristics and field performance suggest that 1) the AA germination test is a better indicator of field performance under untilled conditions than standard germination testing and 2) seed projected area was also well correlated with above ground biomass at 30 days in 2017 In contrast, we did not detect clear relationships between root partitioning or root morphology on field performance or tolerance to NT conditions.

Nomenclature.

Winter squash, *Cucurbita pepo*

Key Words.

Reduced tillage, squash, seedling vigor, emergence, *Cucurbita pepo*, seedling testing

Introduction

Reduced tillage (RT) systems, including no-till (NT) and strip till (ST) can improve soils in organic production systems compared to conventional tillage (CT), which for the purpose of this paper includes primary tillage (e.g. moldboard plow or rototill) across the entire field. However, several key challenges remain before RT can be widely adopted. RT systems may increase sequestration of carbon (Lal and Kimble 1997) and improve soil physical properties resulting in improvements in water infiltration and holding capacity (Franzluebbers 2002; Mahli and O'Sullivan 1990). However, RT can diminish the availability of nutrients from cover crops or organic fertilizers (Dou et al. 1994). In addition, when tillage is not used to kill weeds, an increase in weed pressure, and changes in weed community composition can be expected and surface residue can interfere with efficient weed management (Bond and Grundy 2001; Brainard et al. 2013). RT systems are associated with positive (Luna and Staben 2002) and negative (Bailey and Lazarovits, 2003) shifts in insect and disease communities relative to CT.

Reduced tillage effects on crop establishment. Soil conditions in RT systems create several challenges for successful crop establishment. For example, according to a recent review of studies examining genotype x tillage interactions in wheat (*Triticum aestivum)* and maize *(Zea mays)*, plants consistently face denser soil, suboptimum soil-seed contact, changes in disease and pest pressure, and lower soil temperature in RT systems compared to CT (Herrera et al. 2013). In vegetable cropping systems, RT has generally been shown to result in lower soil temperatures, and higher soil moisture, which may be beneficial or detrimental to crop establishment, depending on the specific crop and climate (Haramoto and Brainard, 2012; Licht and Al-Kaisi, 2005; Overstreet and Hoyt, 2008; Hoyt and Konsler, 1988). In temperate climates, these conditions are often negatively correlated with crop establishment and emergence (Herrera et al.

2013). Indeed, many studies have shown reduced stand establishment in RT compared to CT systems, particularly for warm season crops including most members of the Curcurbitaceae family. For example, Knavel et al. (1977) showed a significant stand reduction in RT cucumber (*Cucumis sativas*) compared to CT. Similarly, Rapp et al. (2004) found that for pumpkin *(Curcubita pepo*), RT resulted in less vigor at early stages of growth compared to CT, although no differences in final yields were detected. This difference in early vigor was suggested to be a result of cooler soil temperatures.

While previous research has shown negative effects of edaphic factors on crop establishment in responses to RT systems, positive or negative shifts in insect and disease communities may also influence crop establishment. For example, in potato production systems, tillage has been shown to bury and kill slugs that may damage the tuber (Leake 1999). The same can be said for other soil dwelling pests such as cutworms and wireworms (Leake 2003). Conversely, RT systems may promote beneficial insects such as predators due to the protective environment offered by residue left on the soil surface (Luna and Staben 2002; Pullaro et al. 2006) which could reduce crop pests. Similarly, RT systems change plant pathogen dynamics, resulting in either positive (Wang and Ngouajio 2008) or negative (Bailey and Lazarovits, 2003) effects on crop disease, depending on the cropping system.

One approach to improving crop establishment in RT systems, is to identify cultivars and associated traits that are well adapted to the generally more challenging edaphic conditions of RT. Traits that are thought to improve adaptation to RT systems include those that allow for optimization of emergence and establishment. This could include broad (Trethowan et al. 2005) or more specific resistance or tolerance to diseases and pests (Okubara et al. 2009). RT systems often have increased organic matter in the upper soil profile which can create differences in distribution of nutrients. Therefore, in order to improve uptake and use of nutrients in RT, it may be important for crops to have deeper rooting or improved root distribution to counteract this effect (Reynolds et al. 2007; Watt et al. 2005). Emergence, establishment, and seedling vigor have also been found to be important selection criteria for small grains grown in NT systems (Trethowan et al. 2005; Joshi et al. 2007). Adaptation and biomass partitioning that optimize the rate of leaf production and maturation (Kumudini 2008) could improve vigor up to the point of closing canopy when vegetative growth is most important (Dao and Nguyen 1989). Seedling development and morphological characteristics such as early seedling vigor, and more rapid root growth relative to shoot at the seedling stage may be important in determining successful establishment of crops grown in an RT system (Joshi, et al. 2007; Watt et al. 2005). Cornish and Lymbery (1987) found significantly reduced population and early growth rate of wheat in NT compared CT systems. They found a strong negative correlation between soil strength and root growth that they suggested resulted in a reduction in subsequent shoot growth. Their work suggests that breeding for roots with greater ability to penetrate compact soils might be beneficial for establishment under RT.

Traits that can promote early vigor may also involve significant tradeoffs. Kumudini (2008) found that across four varieties of wheat, tillering was greater in NT systems compared to CT, and was positively correlated with a decrease in harvest index that in some years lead to lower yields. Vigorous vegetative growth beyond the establishment phase may increase internal competition for assimilates and divert energy away from the marketable portion of the crop. These tradeoffs should not be ignored when analyzing traits that improve plant survival and stand in RT and NT systems.

Previous research suggests that genetic differences in root:shoot partitioning and root morphology may be particularly important for successful establishment in RT systems (Koevoets et al. 2016, Jung and McCouch, 2013). While little attention has been paid to understanding the importance of root traits for successful establishment of vegetable crops in RT systems, progress has been made in small grain crops. For example, Hall and Cholick (1989) found a cultivar by tillage interaction in the yield for wheat grown in tilled and untilled plots. Based on past research that showed the inhibition of root growth of hard red spring wheat in NT, Hall and Cholick (1989) suggested that certain cultivars' ability to with stand this inhibitory effect may have contributed to the cultivar by tillage interaction. Watt et al. (2005) later showed that vigorous genotypes had faster growing root systems, greater number of branched (lateral) roots, and longer root lengths relative to non-vigorous genotypes. The vigorous genotypes then showed higher leaf area than the non-vigorous type until around 48 days from sowing. Although yield data was not taken for Watt et al.'s study, previous research such as that of Pietola (2005), found that a reduction in early root growth was followed by a grain yield reduction of barley and oats in two consecutive years of RT.

How early partitioning to root versus shoot tissue is related to seedling vigor and subsequent yield in winter squash is unclear. Cui et al. (2002) evaluated correlations between physiological and morphological traits (total amylase activity, α-Amalase activity, reducing sugar content, root activity, and seed weight) and seedling vigor traits (germination rate, total dry weight of seedling, shoot dry weight, root dry weight, and max shoot length) in rice. They found a negative correlation between root activity (defined as: oxidized α-naphthylamine (μg) minus auto-oxidized α-naphthylamine in the control per g of fresh root per h.) and seedling vigor. Their results suggest that assimilates going to roots may interfere with seedling vigor. However, root qualities and root health more generally have been shown to be important in a plant's ability to withstand environmental stresses related to RT, including reduced nutrient availability. For example, Kumar et al. (2012) showed in maize growth chamber experiments that highly branched roots systems may improve water and uptake of nutrients including nitrates, phosphorous, and potassium. Higher root density and longer roots can also improve nutrient uptake and increase yield in NT silage corn (Baligar et al. 1998).

Although selection for root traits may be beneficial for crop establishment in RT systems—other things equal—this approach may entail tradeoffs that limit its practicality. For example, selection for greater partitioning to roots, or greater root branching may be associated with reduced partitioning to shoots, or loss of other morphological traits that are important for maintaining growth and yield in RT systems (Koevoets et al. 2016). For example, selection for greater partitioning to roots may improve nutrient uptake but result in less shoot development over time (Watt et al. 2005). The result of less leaf area in plants could result in a reduced suppression ability of crop to weed (Callaway and Forcella 1993). This tradeoff may be particularly problematic in RT systems, where weed competitiveness is sometimes cited as a major breeding goal (Kumar and Ladha, 2011). Further research is needed to understand what types of seedling morphology and partitioning are best suited to productivity in RT vegetable systems.

The importance of seedling vigor in crops grown in RT systems. Early seed and seedling "vigor" are extremely important for improving establishment in RT systems. However, definitions of vigor, and methods for evaluating vigor, differ considerably across studies. In general, seedling vigor is defined as a "property determined by the genotype and modified by the environment which governs the ability of a seed to produce a seedling rapidly in soil and the extent to which the seed tolerates a range of environmental factors" (Perry 1972). Seed and seedling vigor testing include a wide variety of procedures, such as the quantification of seed size and viability (e.g. tetrazolium testing); germination and early seedling growth under standardized moisture and temperature conditions ("standard vigor testing"); and germination and early growth following accelerated aging of seeds. These tests are intended to provide low cost predictions of field performance including but not limited to stand establishment, yield, and tolerance to stress (Baalbaki et al. 2009). Seed size is one of the simplest tests shown to have a correlation to field emergence and establishment in wheat (Lafond and Baker 1986). Seedling vigor based on time to emergence, seedling dry weight, and stage of plant development at 28 days in field conditions has been shown to vary by genotype in wheat (Lafond and Baker,1986) and to be positively correlated with stand establishment and subsequently higher yields of RT soybean and RT canola (Elliott et al. 2007; Kaya et al. 2016). Early vigor, as measured by time to emergence in the field, has been shown to be well correlated with weed competitiveness and yield stability in RT wheat (Amram et al. 2015).

There are several standardized screens of seedling vigor developed by the Association of Official Seed Analysts (AOSA), shown to be more effective than the standard germination test in predicting field establishment (Baalbaki et al. 2009). Vigor tests that require a period of aging by exposure to high heat and moisture such as controlled deterioration (CD) and accelerated aging (AA) are positively correlated with emergence in the field for several species including sunflower (Anfinrud and Schneiter 1984), tomato (Alsadon et al. 1995) and eggplant (Demir et al. 2005). Seedling vigor tests such as the AA and CD tests, have also been shown to have better correlation with field establishment then standard germination in a number of cucurbit species including cucumber (*Cucumis sativus* L.) (Demir and Mavi 2010) and melon (*Cucumis melo* L.)

(Mavi and Demir 2007). Additional studies have shown that these tests are also good indicators of field establishment of melon, cucumber, and watermelon (*Citrullus lanatus* Thunb.) in stressful environments (Mavi et al. 2010). For example, Mavi and Demir (2007) used the AA (following Hampton and TeKrony, 1995) and CD tests (following ISTA, 2003) to predict relative seedling emergence in melon under cool and warm conditions in the field. After seeds had undergone CD/AA treatments they were germinated using the paper towel roll method. The same seed lots were sown in fields in early April for a low temperature emergence test and in August for a high temperature emergence test. Results of this study found that emergence under stressful field conditions was much better correlated to vigor testing using AA/CD methods, compared to standard vigor testing. However, few if any studies have evaluated alternative seedling vigor tests as predictors of field performance in RT organic systems.

Performance of Cucurbita pepo in RT systems. *Cucurbita pepo* and its relatives are economically important crops that have been shown in previous studies to perform relatively well in RT systems. For example, Rapp et al. (2004) found little or no difference in yields between ST, CT, and NT pumpkins in Central NY. Similarly, in herbicide treated summer squash grown in Southern Illinois, Walters and Kindhart (2002) found no difference in yields between CT and ST for two years. However, in the third year, ST marketable yields exceeded those of the CT treatment. Such shifts to higher yields in the third year of production may be due to changes in soil quality characteristics that occur in the transition from CT to RT systems such as micro and macro biological activity, increases in soil organic matter, and improvements infiltration and aeration (Stubbs et al. 2004). In contrast, Walters and Young (2008) observed stunting and yield losses of up to 50% in zucchini (*C. pepo*) that was no-till transplanted into an herbicide treated rye cover crop. These reductions in plant growth and subsequent yield were proposed to be a result of lowered soil temperatures.

Despite the importance of cucurbit crops, relatively little information is available on cultivar differences in tolerance to stresses imposed by RT, or the traits associated with that tolerance, especially in organic production systems. Leavitt et al. (2011) showed a decrease in yield, up to 75%, of zuchini grown in an organic rye vetch mulch compared to a no-cover control. However, only one variety was used in the study. Likewise, most studies comparing RT to CT in cucurbit crops, examined only one variety (Walters and Young 2008; Rapp 2004; Leavitt et al. 2011). Therefore, the primary objectives of our research were to 1) Assess which cultivars of Acorn and Delicata squash (*C. pepo* turbinate and *C. pepo*) are most tolerant to stress associated with RT systems, (2) and identify traits associated with that tolerance. We hypothesized that: 1) *C. pepo* cultivars will vary in their time to emergence, percent emergence, and biomass in both CT and RT (no till with surface residue) systems; 2) the relative performance (emergence and early growth) of *C. pepo* cultivars will vary with tillage (ie. cultivar by tillage interactions will be significant); and 3) cultivar tolerance to reduced tillage will be predictable based on seed and seedling characteristics determined in the laboratory including seed size, tolerance to accelerated aging and root morphology at the seedling stage.

Materials and Methods

Seed and Seedling Vigor Testing. Laboratory testing for the seeds used in all field experiments was performed in the winter and spring of 2018 in order to determine seed and seedling characteristics of the cultivars. Separate experiments were conducted for seeds that were germinated under optimal temperature (25° C) and moisture conditions (Experiment 1) and those that were subjected to accelerated aging through high heat and humidity (Experiment 2).

Cucurbita pepo cultivars and seed source. Six *Cucurbita pepo* cultivars were chosen to represent a range of genotypes that are commercially available for organic production, and commonly grown in the Midwest region (Table 2.1). A single seed lot was chosen for each cultivar and used in all field and laboratory experimentation. The cultivars chosen were Honey Bear, Taybelle, Tuffy, Jester, Delicata JS, and Sugarbush. Among these, Honey bear, Jester, and Sugarbush were hybrid cultivars, while, Taybelle, Tuffy and Delicata JS were open pollinated. Tuffy, Delicata JS and Sugarbush were all organic certified seed. However, organically certified seed was not available for Jester, Honey Bear, and Taybelle cultivars, so untreated, conventionally grown seeds were used.

Figure 2.1: Images of tested varieties: a-Sugarbush; High Mowing 2019, b-Delicata JS; Johnny's 2019, c-Jester; Johnny's 2019, d-Tuffy; Johnny's 2019, e-Honey Bear; Johnny's 2019, f-Taybelle; Vesey's 2019.

Seed dry weight. Seed dry weight prior to germination testing was determined for all seed lots following the procedure outlined by the International Seed Testing Association (1999). In brief, four replications of 50 seeds of each cultivar were crushed into pieces no larger than 4 mm across using a mortar and pestle. The samples were then weighed, dried for 24 hours at 60 C and weighed again so that an estimate of dry weight per seed could be calculated

Experiment 1. Seedling morphology analysis for unaged seed. Seedling vigor analysis on un-aged seed was performed in January 2018 (Table 2.2). Using the six cultivars of *C. pepo*, seeds were tested for their seed size and morphological characteristics. Eight replications of ten undamaged seeds were weighed. Seeds were then scanned using an Epson Perfection V700 2.80A scanner (Seiko Epson Corporation, Suwa, Nagona Prefecture, Japan). The projected area, length and width of scans of these 80 individual seeds of each cultivar were then determined using WinSeedle 2008a Image Analysis System (Regent Instruments, Inc., Quebec, Canada). Each of the 8 replications of 10 seeds of each cultivar were placed in a tray so that no seeds were touching each other or the side of the tray. Seeds were kept in order from 1-10 so that seed dimensions could be correlated with the characteristics of their associated seedlings following germination. The following methods used for germination were adjusted from the Association of Official Seed Analysts, Seed Vigor Testing Handbook (Baalbaki 2009). A numbered line was drawn on #38 30.48cm x 45.72 cm germination paper 6.35 cm from the top of the paper. Numbers 1-10 were written on each germination paper along with the seed lot and replication number in order to keep track of each seed identity. The ten scanned seeds were then placed on the paper and covered with a piece of blotter paper. The sheets were then dampened with three times their weight in deionized water, rolled into a tube of approximately 3 cm in diameter, secured with a piece of string and placed vertically in a 1.5-quart size plastic container so that each seed's radical would be facing down. The 48 rolls of seeds (6 cultivars x 8 rolls) were placed randomly in 4 containers (12 rolls per container) in a complete random design. Each container was covered with a gallon size plastic bag, secured with a rubber band, and placed in a light free germination chamber set to 26° C).

At 38, 61, and 88 hours from placement in the germination chamber, each roll was removed from the germination chamber, and each seed evaluated for radical emergence and lateral root appearance. When lateral roots had emerged from more than 50% of the seedlings within a replication, seed husks were removed, and seedlings were scanned using the same procedure as described above for un-germinated seeds. Using this criterion, all seeds from a given cultivar were scanned over the course of three days. After scanning, seedlings and non-germinated seeds were counted to determine the germination percentage, bulked by replicate, dried at 60° C for three days and weighed.

Analysis of Scanned Images. Scanned seedling images from both Experiment 1 and Experiment 2 were analyzed following the same procedure. First, the Gnu image manipulation program (2.10.8, URL https://www.gimp.org) was used to separate the scanned seedling images into shoot and root scans, with root tissue identified as starting at the top of the first lateral root. Root images were then analyzed using the WinRHIZO Reg 2016 Image Analysis System (Regent Instruments, Inc., Quebec, Canada) to determine the number of root tips, forks, and crosses for each seedling. The measurement of root tips, forks and crosses is a way of estimating the number of lateral roots. Crosses are representative of when two roots intersect one another while a fork is representative of one root growing out of another. Shoot and root scans of each seedling were analyzed separately for projected area using WinSeedle 2008a Image Analysis System (Regent Instruments, Inc., Quebec, Canada). The root shoot ratio of each seedling was then estimated as the ratio of the projected area of the root divided by the projected area of the shoot.

Experiment 2: Accelerated aging (AA) procedure. Seedling vigor analysis was performed on aged seed in April of 2018 (Table 2.2). The same seed lots and cultivars were used for the AA test as previously described for Experiment 1. The AA and germination procedures were replicated in time during four separated intervals because of limited incubator capacity for accelerated aging. Therefore, experimental units were arranged in a RCBD with 4 replicates (blocks) in time, with each replicate consisting of fifty seeds of each cultivar placed first in a growth chamber for accelerated aging, and then transferred to a different growth chamber for germination. The same two growth chambers were used for each replicate in time, with each replicate initiated shortly after completion of the previous replicate (Table 2.2).

Prior to initiation of aging, for each replicate, 50 seeds of each cultivar were scanned and analyzed as described previously for Experiment 1. Once seeds were scanned the AA process was initiated using the methodology of Mavi and Demir (2007). In brief, clear plastic boxes (11 x 11 x 4 cm) and wire mesh trays (10 x 10 x 3 Hoffman Manufacturing, Inc Corvallis) specifically designed for AA testing (Baalbaki 2009) were used for the experiment. Trays and boxes were washed and sterilized with isopropyl alcohol before every use. For each cultivar, the fifty scanned seeds were placed in a single layer on the wire mesh tray being sure to keep the seeds in order from 1-50 so the seedlings could be tracked back to their initial seed size. The plastic box was then filled with 40 ml of deionized water and sealed with parafilm. For each replicate, all 6 boxes were placed in random order inside an incubation chamber set to 40 ± 0.1° C and aged for 96 hours.

Analysis for AA seed. Following AA treatment, the 50 aged seeds of each cultivar, were transferred to 5 separate paper roles, each containing 10 labeled aged seeds, and tested for seedling vigor at 26° C by the paper roll method as described previously for Experiment 1. Seedlings were pulled from the germination chamber between 2 and 7 days after placement in paper rolls when more than 50% of seedlings began to develop lateral roots or when one seedling was growing past 25.4 cm which began to constrict growth and complicate scanning procedures. For some cultivars, germination was greatly inhibited by the AA process with occasionally only one seed germinating per roll. In these cases, the seed often germinated early on in the process and had grown significantly larger than other scanned seedlings. Seedlings were scanned using the same procedure as previously described for Experiment 1. After scanning, seedlings and non-germinated seeds were counted to determine the germination percentage, bulked by replicate, dried at 60° C for three days. After drying seeds and seedlings were weighed separately.

Trial Design and Experimental Treatments. Field experiments were conducted in 2017 and 2018 comparing the emergence and early growth of 6 cultivars of *C. pepo* under no-till vs tilled soil conditions (Table 2.3). In 2017, the trial was conducted within a larger winter squash trial, on organically certified land at the Kellogg Biological Station (KBS) in Hickory Corners, Michigan (42.4° N, -85.4° W). The soil type was a Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs). The field was in winter wheat and frost seeded red clover in the summer of 2016. In August of 2016 the field was disked and a rye-vetch cover crop mix was seeded at 63 kg/ha rye and 23 kg/ha vetch. In May, the rye-vetch cover crops were flail mowed. In 2018 due to severe pest pressure at the Kellogg Biological Station site, the trial was conducted at the Michigan State University Horticulture Teaching and Research Center (HTRC) in Holt, MI (42.7° N,-84.5° W) on a Marlette fine sandy loam (mesic Oxyaquic Glossudalfs). The field had been planted to winter wheat in the fall of 2017 and harvested in July of 2018.

In both years, plots were arranged in a split plot design with tillage as the main plot factor and squash cultivar as the sub plot factor (Table 2.3), with main plots arranged in a randomized complete block design with six replications. In 2017, the tillage factor had two levels: 1) conventional tillage (CT) accomplished with a chisel plow and field cultivator following rye-vetch termination; or 2) no tillage (NT) with the rye-vetch cover crop residue left on the soil surface. The subplot factor had six treatments, corresponding to the 6 *C. pepo* cultivars (Table 2.1). In 2018, the tillage main plot factor consisted of 1) tillage accomplished with a Hiniker 6000 strip tiller; and 2) no tillage with wheat residue left on the soil surface. In 2017, main plots measured 3 m x 5.5 m, and sub plots consisted of one 3 m long row of squash. In 2018, main plots measured 18 m x 0.76 m, with subplots consisting of one 3 m long row of squash

Field Management. In early September of 2016, winter rye and hairy vetch were drilled at 25.4 kg/ha and 20 kg/ha respectively into both the tilled and RT treatments (Table 2.4). Mowing and tillage occurred in late April. The tillage treatment consisted of one pass with a chisel plow and two passes in May of 2017 with a field conditioner. Before the last field conditioning all treatments were fertilized with 98 kg/ha actual N of non-gmo soybean meal (Zeeland Farm Services Inc, Zeeland, Michigan) containing 7% Nitrogen (400 kg/ha fertilizer) by broadcasting over plots. Squash varieties were seeded in June of 2017, 5 cm apart in each row with a 30 cm break from the end of one row to the beginning of the next. No weed management was used.

The second year's trial began in July of 2018. Wheat that had been planted in the fall of 2017, was harvested in early July, leaving wheat stubble as residue in all plots. Tillage was accomplished with a Hiniker 6000 strip tiller equipped with a row cleaner, shank, offset disks and a rolling basket that created a residue-free tilled strip approximately 25 cm wide and 25 cm deep. Plots were seeded with one squash row per plot at a distance of 76 cm between rows. Squash was seeded 5.0 cm apart in row at a depth of 2.5 cm. Weed density was very low, so no subsequent weed management occurred. However, in several plots, volunteer wheat density was high, so spot applications of clethodim (0.14 kg ai/ha) were applied to minimize competition between the wheat and squash.

Data collection. Each year of this trial, phenotypic data was taken on time to emergence, number of emerged plants, and shoot biomass at 29 and 37 days from planting, in 2017 and 2018 respectively. In 2017, starting six days after seeding, plots were monitored daily for seedling emergence, and the number of newly emerged seedlings was recorded. A seedling was considered emerged if both cotyledons were above the soil surface. Emergence data was taken for seven days. At 13 days from planting seedlings were thinned to approximately 15 cm spacing to minimize intraspecific competition. At 23 days from planting they were thinned again to approximately 30 cm spacing. At 29 days from planting all above ground biomass was collected for each plot. The number of survived plants per plot was recorded. The samples were dried in a 60°C oven for one week and weighed.

In 2018, emergence data began to be recorded at four days from seeding and continued for seven days. At 37 days from planting all above ground biomass was collected. Plants were collected by cutting them at the soil line with clippers and storing them in brown paper bags. The number of survived plants per plot was recorded. Samples were dried in a 60°C oven for one week and weighed.

Statistical Analysis. Statistical analysis was performed using Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). Assumptions of normality and equal variance were evaluated using PROC MIXED, PROC UNIVARIATE and Levene's test. Where needed, data were transformed using ln,square root, or arcsin transformation to improve normality and equal variance assumptions. The effect of cultivar on seed and seedling characteristics were analyzed using PROC GLIMMIX procedures in SAS with cultivar treated as a fixed effect, and replicate as a random effect. For field experiments, the effects of tillage and cultivar on seedling performance were analyzed separately for each year using PROC GLIMMIX producers in SAS, with cultivar and tillage treated as fixed effects, and replicate as a random effect. Correlation analyses between seed and seedling characteristics and field performance were conducted separately using the PROC CORR to determine the Pearson's correlation coefficients.

Results and Discussion

Seed and Seedling Vigor from Experiments 1 and 2.

Seed size and germination rate. Both the dry weight and projected area of seeds varied considerably between cultivars (Table 2.5). Honey Bear had the greatest dry weight while Tuffy and Delicata had the least seed dry weight. In terms of projected area, Taybelle had the largest seeds, while Tuffy had the smallest, with a projected area of approximately 67% of the largest seeds. Percent germination of seeds also differed by cultivar (Table 2.5). For unaged seeds, the germination percentages were within a relatively high range of 95-98% for Honey Bear, Jester, and Taybelle. Rates were significantly lower for Tuffy, Delicata and Sugarbush with germination rates as low as 69% for Delicata. The germination rates for aged seedlings differed from those that were not aged with Jester, Taybelle, and Sugarbush having the highest rates and Tuffy, Delicata and Honey Bear having the lowest. Seeds that were aged had much lower germination rates than normal germinated seedlings.

Seedling size and root shoot partitioning. Seedling dry weights differed significantly by cultivar (Table 2.6). For unaged seedlings, Honey Bear had the highest dry weight per seedling and Delicata and Tuffy had the lowest. For aged seedlings, Jester had the highest dry weight per seedling while Delicata had the lowest. Young (4-5 day old) seedlings emerging from unaged seeds also varied considerably in their projected area (Table 2.7). Taybelle had the largest total projected area, while Jester, Delicata, and Sugarbush did not vary from one another and were the smallest (approximately 66% of the largest seedlings). Differences in the root:shoot (R:S) ratio also existed among cultivars (Table 2.7). For unaged seedlings, Tuffy had the largest R:S and Delicata had the lowest R:S with half that of Tuffy. For aged seedlings, fewer differences in total projected area were detected. Again, Tuffy had the highest R:S (1.4, with all other cultivars having R:S of less than 1.0).

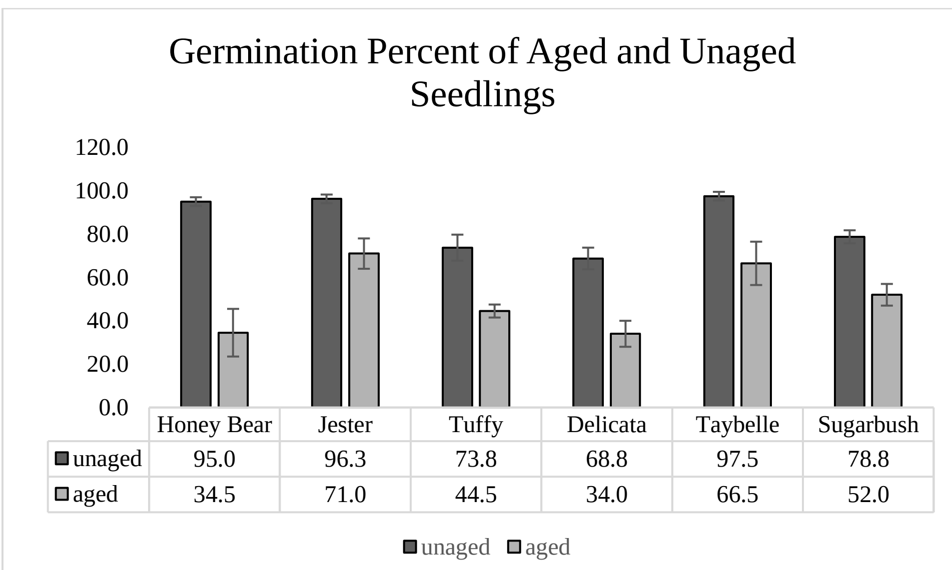
Seedling root morphology. Cultivars also differed in the morphology of their roots at 2-7 days after placement in the growth chamber (Table 2.8). For unaged seedlings, Tuffy, Delicata, and Taybelle had the largest number of root tips, with approximately 50% more tips than Sugarbush and Jester. Similar trends were evident for root forks, with Tuffy having significantly higher numbers of root forks than the other varieties. For aged seedlings, Tuffy also stood out as having greater root tip and fork numbers compared to all other cultivars.

Relationships between seedling characteristics. Seed size was weakly positively correlated with shoot, root, and total projected area of unaged seedlings at 4-5 days from planting (Table 2.9). This suggests that larger seedlings result from larger seeds because of seed reserves. This correlation of seed size to seedling vigor is in agreement with Nerson (2002) who showed that muskmelon (*Cucumis melo*) grown from smaller seeds resulted in lower germination rates, emergence and seedling growth.

The total projected area of seedlings was strongly positively correlated with root tip and fork number (Table 2.9). In addition, we detected a weak and positive correlation between total projected area and the root shoot ratio, suggesting that in general, larger seedlings partitioned more to roots than smaller seedlings. However, this correlation was much stronger for some cultivars than others. For example, total projected area and root shoot ratio were positively correlated (0.5 coefficient) for cultivar Sugarbush but uncorrelated for cultivar Delicata. This result suggests that morphology (production of more lateral roots) was more dependent on the relative size of seedlings and their rate of development rather than distinctive differences in how varieties put energy into different morphological traits. These correlations are generalizations across all cultivars and may not hold true for individual cultivars.

Aged and unaged seedlings cannot be directly compared statistically because of differences in the timing of germination. However, our results suggest some important differences between cultivars that may be worth further investigation (Figure 2.2). Germination was severely decreased for some varieties with aging. The greatest loss of germination percentage was Honey Bear with a difference in germination between aged and unaged seeds of 61%. The low germination of Honey Bear also may have had an effect of making total projected area relatively high (Table 2.7). The majority of Honey Bear did not germinate and there was a longer period of waiting for the germinating seedlings to develop then for other cultivars. Other cultivars, such as Jester, appeared to be much less adversely impacted from the aging process.

Figure 2.2: Germination Percent of Aged and Unaged Seedlings.



Such difference in mean germination percentage from aged to unaged seeds could indicate that some varieties are more tolerant to stress related to RT than others. Mavi and Demir (2007) found that the germination percentage of AA treated melon seeds was better correlated with the emergence of seeds grown in a range of stressful environments including stress inducing low temperatures and deep seeding. For a variety such as Honey Bear the germination of aged seeds were less than half that of unaged seeds. While for a variety such as Jester differences in germination rate of aged and unaged seeds was only around 25%. The test suggests that the seed lot and variety of Jester was slower to deteriorate than others. This slower deterioration whether related to seed quality or genotype may be related to some of the measurements of field performance observed in the same seed lot in 2018. Because RT systems are often characterized by poor soil seed contact, lower soil temperatures and higher soil moisture than CT systems, seeds in RT may undergo longer periods of deterioration before germination is possible. For this reason, the selection of seed lots and genotypes that are slower to deteriorate may be useful in combating the issues with emergence and establishment found in these systems.

Effects of tillage and cultivar on field performance of six C. pepo varieties.

Weather. In 2017, using a base temperature of 15 C, cumulative growing degree days (GDD) from the time of planting to the time of emergence was between 20.2 to 57.3 GDD (Table 2.10). Total rainfall at the completion of emergence data collection was 6.9 mm. At the point of biomass collection, the total GDD were 172.7 GDD, and total rainfall accumulation was 113.5 mm. In 2018 planting occurred later with higher mean temperatures. Growing degree days at the time of emergence data collection was 20.2 °C to 70.3 °C. Total rainfall at the completion of emergence data collection was 2.8 mm. At the point of biomass collection in 2018 total growing degree days were 199.4 °C. The total accumulated rainfall in 2018 was 88.6 mm.

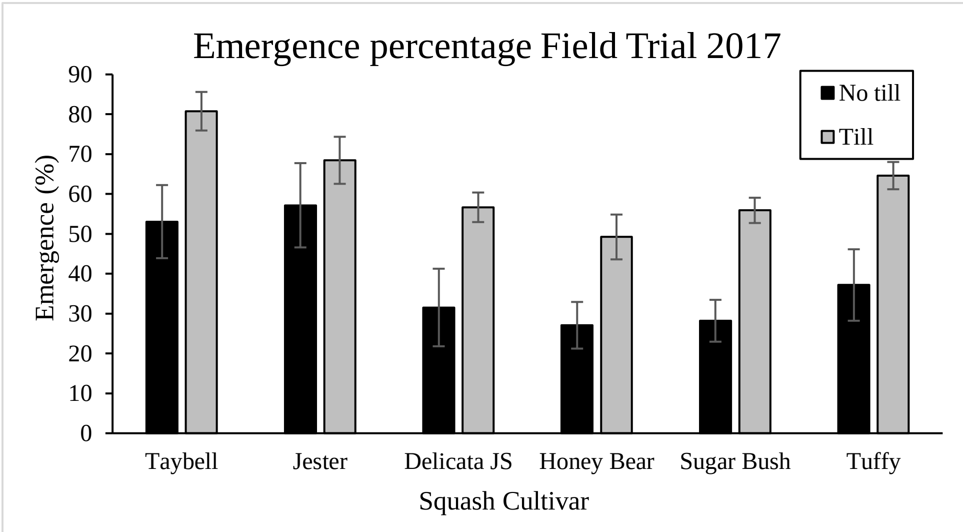
Tillage and cultivar effects on early growth. In both years, the number of days to emergence varied by cultivar, but not by tillage (Table 2.11). Taybelle emerged faster than all other cultivars in both years. In 2018, Honey Bear and Jester emerged more slowly than all other cultivars, with emergence delayed approximately two days from that of Taybelle which was the fastest to emerge. Final plant density also differed by cultivar, but no significant tillage or cultivar by tillage interactive effects were detected (Table 2.11). Taybelle and Jester had the highest final density in in 2017 while Taybelle had the highest final density in 2018.

Dry weight per plot and per plant was affected in both years by tillage and cultivar, but no significant tillage by cultivar interaction was detected (Table 2.11). On the per plot basis in 2017, dry weight was highest for Jester and lowest for Delicata, Sugarbush, and Tuffy, while in the same year the cultivars with the highest per plant dry weights were Honey Bear, Jester and Taybelle and the lowest were Delicata and Tuffy. In 2018 Taybelle had the highest dry weight per plot and Tuffy had the lowest, while on a per plant basis Taybelle and Sugarbush were the highest and Tuffy was the lowest.

Differences in cultivar response to tillage. One of the goals of this research was to assess which cultivars of Acorn and Delicata squash (*C. pepo* turbinate and *C. pepo*) were most tolerant to stress associated with RT systems. Unfortunately, in many cases, high variability in cultivar responses in our field study limited our statistical power to detect cultivar by tillage interactions. Therefore, although we cannot conclude with confidence that these cultivars differed in their tolerance to RT, we can speculate (with somewhat lower confidence), that there are important differences between the responses of these cultivars to tillage. In 2018, the marginally significant ($P = 0.106$) tillage x cultivar interaction for dry weight per plant suggests that cultivars differ in their tolerance to RT. Specifically, the cultivars Jester and Delicata appear to be more tolerant to reductions in growth in RT than Taybelle, Honey Bear, Sugarbush or Tuffy (Figure 2.4). While

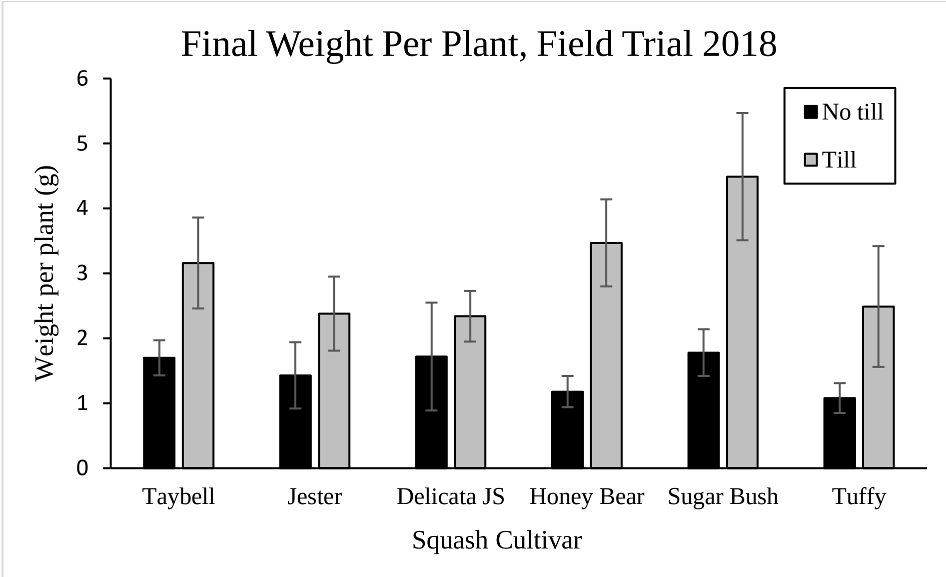
Sugar Bush, Honey Bear and Tuffy suffered reductions of 50% or more under RT, no effects of tillage on dry weight per plant were detected for Delicata or Jester.

Figure 2.3: Emergence percentage field trial 2017.



Although our results suggest that Jester and Delicata were more tolerant of RT conditions in 2018, the reasons for that tolerance are unclear. In measurements that approximate vigor under field conditions such as days to emergence, percent emergence, and final plant density, Delicata and Jester did not consistently rank higher than other varieties (Table 2.11). This result contrasts with Watt et al. (2005) who found that more vigorous (quicker to emerge) wheat was able to maintain growth in unplowed soil despite the possibility of roots encountering blocky soil peds that restricted normal root growth. Differences in other traits that might improve performance under RT, were also not apparent for these two varieties. For example, neither Jester nor Delicata were noteworthy in their partitioning to roots (Table 2.7), nor in their root morphology (Table 2.8).

Figure 2.4: Final weight per plant field trial 2018.



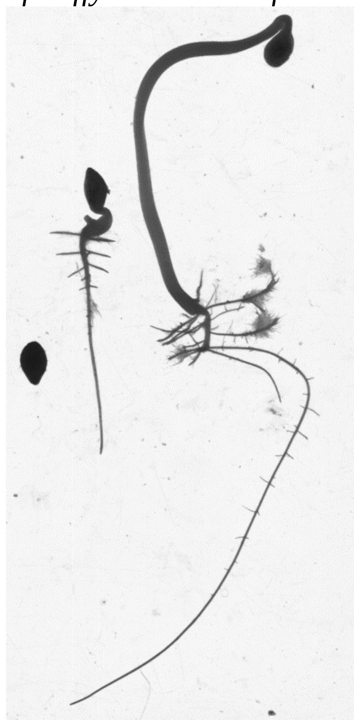
The correlation of seedling characteristics to field performance. Because the number of data points used to evaluate correlations between seed and seedling characteristics and field performance was very low, our analysis had low power to detect meaningful relationships. Nonetheless, results from correlation analysis (Table 2.12) suggest some relationships worth investigating in future studies.

Perhaps the most interesting result of the correlation analysis, was the positive correlation between germination percentage in lab testing and emergence under field conditions. In particular, we found that the percentage germination of seeds that had been subjected to accelerated aging was strongly positively correlated with emergence under field conditions in both years. In contrast, the correlation between standard germination testing and field emergence was weaker, especially under untilled conditions. This result is consistent with previous research on other *Cucurbita* species done by Demir and Mavi (2010).

A second interesting result from our correlation analysis was the positive relationship between seed size (projected area) and dry weight per plant under field conditions in both tilled and untilled plots in 2017 (Table 2.12). This result is consistent with studies in other crops demonstrating that large seeded cultivars often perform better under challenging field conditions (e.g. Lafond and Baker 1986).

Although we did not detect any correlations between root morphological traits and measures of field performance, this was likely due in part to high variability in root morphological traits given variation in the time of germination and stage of development at scanning. In aged experiments there was as much as a five-day difference when seedlings were scanned based on the time of development. There was also much more variation in stages of development within germination rolls (Figure 2.5). Future studies evaluating these potential relationships will require greater replication and adjustments in sampling protocol to reduce variability.

Figure 2.5: Stages of development at of Tuffy at the time of scanning.



Summary and Conclusions

The primary objective of this research was to evaluate *C. pepo* responses to RT organic systems, and to determine if those responses differed between cultivars of *C. pepo.* In addition, we wished to determine if seed and seedling characteristics evaluated under controlled conditions were associated with tolerance to RT systems. If cultivars of *C. pepo* have different responses to the RT environment, there could be potential for farmers to choose varieties better suited to RT organic systems. Organic growers are already demanding both seeds produced in organically managed systems (Hubbard et al. 2016) and varieties that are adapted to their conditions (Hultengren et al. 2016). Varieties adapted to organic RT systems must not only be productive in RT systems but meet all other current agronomic and market standards. Differences in how cultivars respond could also lead to further research by squash breeders into selection for RT adaptive cultivars. Our results demonstrated strong differences across cultivars in their morphology and growth in both lab and field conditions. These differences suggest that genetic diversity within commercially available *C. pepo* cultivars may be exploited to improve performance under diverse field conditions. However, large variability in field conditions from year to year in terms of soil, weather, and management, coupled with inadequate replication limited the power of our testing to detect cultivar differences in tolerance to RT conditions.

Results from our correlation analyses suggest that seed size and vigor as determined by accelerated aging test are potentially useful predictors of field performance under stressful conditions associated with RT. Although we did not detect any correlations between root traits and field performance, this likely was due in part to inadequate replication and suboptimal sampling methods. Future research tracking the traits and fates of individual plants is need to more fully evaluate the potential importance of root traits in conferring tolerance to RT.

APPENDIX

Table 2.1: Cultivar descriptions and sources.

Cultivar	Type	Seed Source	Days to Maturity
Honey Bear F1	*Cucurbita pepo var. turbinate*	Johnny's	85
Taybelle	*Cucurbita pepo var. turbinate*	Vesey's	72
Tuffy (organic)	*Cucurbita pepo var. turbinate*	Johnny's	90
Jester F1	*Cucurbita pepo var. pepo*	Johnny's	95
Delicata JS (organic)	*Cucurbita pepo var. pepo*	Johnny's	100
Sugar Bush F1 (organic)	*Cucurbita pepo var. turbinate*	High Mowing	90

Table 2.2: Schedule of major lab procedures and data collection events for seed vigor testing.

	Experiment 1 (Unaged Test)		Experiment 2 (Accelerated Aging Test)							
			Run 1		Run 2		Run 3		Run 4	
Event	Date	DAS[1]	Date	DAS	Date	DAS	Date	DAS	Date	DAS
Seeds are scanned and weight is recorded	1/24	0	4/5	-5	4/10	-5	4/13	-5	4/19	-5
Accelerated aging begins	NA	NA	4/5	-5	4/10	-5	4/13	-5	4/19	-5
Seeds are placed in germination chamber	1/24	0	4/10	0	4/15	0	4/18	0	4/24	0
Seedlings are removed from germ chamber, scanned, and placed in dryer	1/28-1/29	4-5	4/14	4	4/17-4/20	2-5	4/23-4/25	5-7	4/26-4/29	2-5

[1] Days after seeding (placement in germination chamber)

Table 2.3: Field Trial Design and Treatments of Field Experiment.

	Main-plot	Sub-plot
Trt	Tillage	Cultivar
1	None	Honey Bear
2	None	Taybelle
3	None	Tuffy
4	None	Jester
5	None	Delicata JS
6	None	Sugar Bush
7	Tilled	Honey Bear
8	Tilled	Taybelle
9	Tilled	Tuffy
10	Tilled	Jester
11	Tilled	Delicata JS
12	Tilled	Sugar Bush

Table 2.4: Schedule of major field operations and data collection events for cultivar response to tillage, 2017 and 2018.

	2017		2018	
Event	Date	DAS	Date	DAS
Tilled treatment	6/12	-8	8/1	-5
Squash planted	6/20	0	8/6	0
Emergence begun	6/26	6	8/9	3
Emergence concluded	7/2	12	8/16	10
Plants are thinned to 15 cm spacing	7/3	13	NA	NA
hand weeding 15 cm around plants	7/4	14	NA	NA
Plants are thinned to 30 cm spacing	7/13	23	NA	NA
Final squash counts and biomass	7/19	29	9/12	37

Table 2.5: Seed weight, projected area and germination percentage of unaged and aged seeds, for six C. pepo cultivars.

Cultivar	Dry Weight/seed		Projected Area		Percent germination Unaged		Percent germination Aged	
	----------mg---------		--------mm²-------		---------------------%------------------------			
Honey Bear	100.6	a	80.6	b	95.0	a	34.5	a
Jester	92.8	b	74.4	c	96.3	a	71.0	b
Tuffy	65.6	d	55.3	e	73.8	b	44.5	a
Delicata	63.7	d	63.4	d	68.8	b	34.0	a
Taybelle	90.5	b	83.7	a	97.5	a	66.5	b
Sugarbush	81.7	c	79.0	b	78.8	b	52.0	ab
	-----------------------------------Significance (P-value)--------------------------------							
Cultivar	***		***		***		**	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

Table 2.6: Dry weight per seedling.

Cultivar	Dry weight per seedling			
	Unaged		Aged	
	-------(estimate of mg/seedling)--------			
Honey Bear	85.2	a	61.9	abc
Jester	73.9	b	74.5	a
Tuffy	47.8	d	45.0	cd
Delicata	50.2	d	41.1	d
Taybelle	73.6	b	69.6	ab
Sugarbush	63.5	c	53.1	bcd
	--------Significance (P-value)-----------			
Cultivar	***		*	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

Table 2.7: Mean shoot, root and total projected area, and the ratio of root and shoot projected area of seedlings emerging from aged or unaged seeds of six cultivars of C. pepo at beginning of lateral development (2-7) days after planting.

	Total		Root		Shoot		RSR[a]	
Cultivar	Unaged	Aged	Unaged	Aged	Unaged	Aged	Unaged	Aged
Honey Bear	479 ab	788 a	248 b	342 a	231 a	447 a	1.0 c	0.7 c
Jester	348 c	384 b	191 c	172 cd	157 bc	212 b	1.2 b	0.9 b
Tuffy	438 b	398 b	285 ab	231 ab	154 c	166 b	1.9 a	1.4 a
Delicata	343 c	336 b	157 c	154 cd	184 b	182 b	0.9 c	0.9 bc
Taybelle	522 a	394 b	291 a	190 bc	231 a	204 b	1.3 b	0.9 b
Sugarbush	342 c	311 b	195 c	134 d	147 c	177 b	1.3 b	0.7 c
	---------------------------------Significance (P-value)-------------------------------------							
Cultivar	***	***	***	***	***	***	***	***

[a] RSR=Projected root area divided by projected shoot area

b Honey Bear n = 69

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

Table 2.8: Mean shoot, root and total projected area, and the ratio of root and shoot projected area for six cultivars of C. pepo at 2-7 days after planting.

Cultivar	Tips				Forks				Crosses			
	Unaged		Aged		Unaged		Aged		Unaged		Aged	
	---#/plant---											
Honey Bear	31.2	b	23.4	ab	37.9	b	24.7	b	2.3	b	1.6	ab
Jester	24.5	c	26.3	b	26.0	c	21.1	c	2.1	b	1.2	b
Tuffy	39.7	a	33.5	a	57.9	a	38.8	a	5.2	a	2.2	ab
Delicata	36.3	a	20.8	b	35.8	b	22.0	bc	2.3	b	1.0	b
Taybelle	38.7	a	26.9	b	35.7	bc	23.5	bc	2.4	b	1.1	b
Sugarbush	27.4	bc	22.2	b	32.2	bc	18.7	bc	2.2	b	1.3	ab
	--------------------------------------Significance (P-value)---------------------------------------											
Cultivar	***		*		***		***		***		+	

[a] RSR=Projected root area divided by projected shoot area

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

Table 2.9: Correlation (Pearson Correlation Coefficients) between seed/seedling characteristics from germination chamber studies.

Seed/Seedling Characteristic		Age (DAS)	Data points (n)	Correlations Corr	Seed SPA	Seedling Size/Vigor Unaged Seed RPA	SPA	TPA	Root characteristics RSR	TN	FN	CN
Seed projected area		0	480	Pearson Correlation Coefficients P-value	1	0.185 **	0.231 ***	0.217 ***	-0.090 +	-0.108 *	-0.147 **	-0.169 **
Seedling Size/Vigor (normal) Unaged seeds												
	Root projected area (RPA)	4-5	369	PCC P-value		1	0.730 ***	0.954 ***	0.523 ***	0.712 ***	0.735 ***	0.603 ***
	Shoot projected area (SPA)	4-5	370	PCC P-value			1	0.901 ***	-0.109 *	0.646 ***	0.578 ***	0.425 ***
	Total projected area (TPA)	4-5	369	PCC P-value				1	0.284 ***	0.733 ***	0.716 ***	0.565 ***

Table 2.9: (cont'd).

Root characteristics								
	Root shoot ratio (unaged) (RSR)	4-5	369	PCC	1	0.289	0.368	0.363
				P-value		***	***	***
	Tip number (TN)	4-5	369	PCC		1	0.834	0.649
				P-value			***	***
	Fork number (FN)	4-5	369	PCC			1	0.781
				P-value				***
	Cross number (CN)	4-5	369	PCC				1
				P-value				

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

Table 2.10: Temperature, degree days (base 15 C) and rainfall, 0-30 days after planting (DAP) squash, 2017 and 2018.

	2017				2018			
DAP	Mean Temp	Cumulative Growing Degree Days	Daily Growing Degree Days	Rain (mm)	Mean Temp	Cumulative Growing Degree Days	Daily Growing Degree Days	Rain (mm)
	----------------------C----------------			----mm---	-------------------C-------------------			---mm----
1	17.3	2.3	2.3	1.0	22.1	7.1	7.1	0.0
2	16.3	3.6	1.3	0.0	21.1	13.2	6.1	0.0
3	22.3	10.9	7.3	29.0	21.9	20.2	6.9	0.0
4	19.6	15.5	4.6	13.0	21.7	26.9	6.7	0.0
5	19.0	19.5	4.0	0.0	22.1	33.9	7.1	0.0
6	15.7	20.2	0.7	0.0	23.1	42.0	8.1	0.0
7	15.2	20.4	0.2	0.5	22.4	49.4	7.4	2.8
8	15.4	20.8	0.4	0.0	21.8	56.2	6.8	0.3
9	21.8	27.7	6.8	0.0	22.3	63.5	7.3	0.0
10	22.9	35.6	7.9	2.5	21.8	70.3	6.8	0.0
11	23.6	44.1	8.6	3.6	21.2	76.5	6.2	0.0
12	21.9	51.0	6.9	0.0	21.8	83.3	6.8	4.1
13	21.3	57.3	6.3	0.3	18.0	86.3	3.0	0.0
14	21.1	63.4	6.1	0.0	17.9	89.2	2.9	0.0
15	19.9	68.3	4.9	0.0	17.4	91.6	2.4	0.0
16	20.9	74.2	5.9	0.0	21.5	98.1	6.5	14.2
17	22.8	82.0	7.8	0.0	23.7	106.8	8.7	0.0
18	23.6	90.6	8.6	39.1	25.2	117.0	10.2	0.0
19	20.2	95.8	5.2	0.0	24.7	126.7	9.7	18.0
20	18.8	99.6	3.8	0.0	20.8	132.5	5.8	0.5

Table 2.10: (cont'd).

21	21.7	106.3	6.7	6.4	17.0	134.5	2.0	0.0
22	24.3	115.6	9.3	2.8	19.3	138.8	4.3	0.0
23	24.2	124.8	9.2	6.9	23.1	146.9	8.1	8.1
24	24.1	133.8	9.1	8.6	25.0	156.9	10.0	18.8
25	21.5	140.3	6.5	0.0	25.2	167.1	10.2	9.1
26	20.4	145.7	5.4	0.0	25.6	177.7	10.6	0.0
27	20.9	151.6	5.9	0.0	26.2	188.9	11.2	9.4
28	20.0	156.6	5.0	0.0	18.9	192.9	3.9	0.5
29	21.9	163.6	6.9	0.0	16.2	194.1	1.2	0.0
30	24.2	172.7	9.2	0.0	15.3	194.4	0.3	0.0
31					14.0	193.4	0.0	2.5
32					14.7	193.1	0.0	0.3
33					16.2	194.3	1.2	0.0
34					16.9	196.2	1.9	0.0
35					18.2	199.4	3.2	0.0

Table 2.11: Effect of main effects on mean days to emergence, emergence percentage, plant number and dry weight for 6 C. pepo cultivars under two tillage systems, 2017 and 2018.

	Days to emergence		Emergence Percentage		Final Plant Density		Dry weight 30-40 DAP Per plot		Dry weight 30-40 DAP Per plant	
	2017	2018[a]	2017	2018	2017	2018	2017	2018	2017	2018
	---------days--------		---------%--------		---------#/plot--------		---------------------grams----------------------			
Cultivar main effects										
Taybelle	8.5 c	7.4 c	66.9 a	73.4 a	10.0 a	41.8 a	77.6 ab	98.9 a	7.6 a	2.4 a
Jester	9.5 ab	9.0 a	62.8 a	57.7 b	10.3 a	32.9 b	81.2 a	60.2 bc	8.0 a	1.9 bc
Delicata JS	9.8 a	8.3 b	44.1 bc	42.7 c	8.4 b	24.3 c	44.1 c	46.5 bc	5.3 b	2.0 bc
Honey Bear	9.5 ab	8.9 a	39.2 c	39.0 c	7.9 b	22.3 c	66.8 abc	54.5 bc	8.3 a	2.3 abc
Sugar Bush	9.2 b	7.9 b	42.1 bc	44.1 c	8.1 b	25.2 c	55.8 c	71.5 b	6.8 ab	3.1 a
Tuffy	9.3 b	8.4 b	50.9 b	39.0 c	8.7 b	22.1 c	50.8 c	36.7 c	5.7 b	1.7 c
Tillage main effects										
Full width tillage	9.1	8.3	62.6 a	48.2	9.3	27.6	79.7 a	81.7 a	8.4 a	3.1 a
Reduced tillage	9.6	8.3	39.4 b	50.5	8.4	28.8	45.1 b	41.1 b	5.4 b	1.5 b
Significance										
Cultivar (C)	***	***	***	***	***	***	***	***	**	*
Tillage (T)	NS	NS	**	NS	NS	NS	+	*	+	**
C x T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS[d]

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant

a Days to emergence in 2018 is underestimate, since some plants emerged after last count which occurred 10 DAP.

b Each plot is equal to three meters of row

c Thinning was done in 2017 but not in 2018 and thus final plant densities should not be compared directly.

d. P-value 0.106. See Figure 2.3.

Table 2.12: Correlation between measures of seed and seedling vigor and field performance. Pearson correlation coefficients are shown (n=6).

		Field Performance									
		Days to Emergence		Emergence Percentage				Dry weight per plant 30-40 DAP			
				Tilled		Untilled		Tilled		Untilled	
Seed/Seedling Characteristics	code	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Seed projected area	SDPA	-0.495	-0.245	0.132	0.658	0.173	0.412	0.792 +	0.618	0.853 *	0.396
Seed germination											
Unaged seeds	PG	-0.469	0.104	0.385	0.764 +	0.568	0.474	0.951 **	0.128	0.974 **	-0.109
Aged seeds	PGA	-0.582	-0.227	0.793 +	0.843 *	0.884 *	0.742 +	0.506	-0.022	0.488	0.262
Seedling projected area											
Roots (unaged)	RPA	-0.707	-0.311	0.460	0.127	0.212	0.374	0.313	0.048	0.215	-0.486
Roots (aged	RPAA	0.084	0.454	-0.345	-0.244	-0.272	-0.352	0.424	-0.009	0.379	-0.768 -
Shoots (unaged)	SPA	-0.351	-0.182	0.118	0.347	0.026	0.339	0.404	0.040	0.550	-0.026
Shoots (aged	SPAA	0.178	0.483	-0.496	-0.073	-0.321	-0.361	0.591	0.209	0.607	-0.449

Table 2.12: (cont'd).

Total (unaged)	TPA	-0.659	-0.307	0.376	0.258	0.158	0.426	0.410	0.051	0.416	-0.345
Total (aged)	TPAA	0.143	0.486	-0.447	-0.149	-0.310	-0.369	0.538	0.123	0.529	-0.601
Root shoot ratio											
Unaged	RSR	-0.334	-0.138	0.310	-0.265	0.123	0.025	-0.143	-0.057	-0.356	-0.499
Aged	RSRA	-0.134	-0.056	0.383	-0.282	0.212	0.073	-0.424	-0.545	-0.532	-0.536
Root morphology											
Tip number (unaged)	TN	-0.319	-0.490	0.312	-0.210	-0.072	0.263	-0.512	-0.327	-0.468	-0.144
Tip number (aged)	TNA	-0.336	0.013	0.489	-0.053	0.392	0.179	-0.005	-0.377	-0.162	-0.646
Fork number (unaged)	FN	-0.046	-0.062	-0.031	-0.630	-0.294	-0.248	-0.453	-0.224	-0.581	-0.625
Fork number (aged)	FNA	-0.032	0.110	0.105	-0.483	-0.048	-0.180	-0.309	-0.410	-0.446	-0.763 +
Cross number (unaged)	CN	-0.041	0.015	0.106	-0.535	-0.084	-0.192	-0.421	-0.323	-0.583	-0.644
Cross number (aged)	CNA	0.045	0.285	-0.143	-0.583	-0.215	-0.415	-0.090	-0.127	-0.296	-0.865 *

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LITERATURE CITED

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CHAPTER THREE: Improving Selectivity of Physical Weed Control in Winter Squash: Cultivation Tolerant Varieties and Traits.

Abstract

Physical weed control (PWC) is an important tool for managing weeds in organically produced cucurbit crops, but low selectivity at early growth stages limits successful adoption. One approach to improve selectivity of PWC is to use varieties that are tolerant to forces applied by PWC tools. Field experiments were carried out during the 2017 and 2018 growing seasons in southwest Michigan to test whether commercially available *Cucurbita pepo* (acorn and delicata squash) varieties varied in their tolerance to mechanical disturbance from flextine and finger weeders, and to identify traits associated with that tolerance. For each tool, separate experiments were conducted in a split plot design, with PWC (hand weeding vs flextine or finger weeding) as the main plot factor, and *C. pepo* variety (Taybelle, Jester, Delicata, Honey Bear, Sugarbush and Tuffy) as the subplot factor. Pre- and post- counts of weeds and crops were used to calculate the efficacy and selectivity of the tools. In separate greenhouse studies, seedling traits including root:shoot ratio (RSR), projected area, and anchorage force were evaluated to gain insight into variation in traits associated with tolerance to PWC. Varietal differences in tolerance to both flextine cultivation and finger weeding were evident in several site-years, although results were variable and inconsistent. Cultivars also differed in their biomass and their tolerance to uprooting (anchorage force) at ten days after seeding in the greenhouse. Tolerance to flextine cultivation under field conditions was positively correlated with plant size at the time of cultivation and to plant anchorage forces determined in the greenhouse. However, tolerance to finger weeding was not well correlated with seedling traits. Several cultivars including Taybelle and Jester were consistently tolerant to both tools, with survival rates of at least 80% and no detectable adverse effects on plant weight. These results suggest that growers can improve weed

control through the selection of commercially available varieties that are tolerant to PWC tools, and that plant breeders may improve this tolerance through selection for PWC tolerant traits.

Nomenclature.

Acorn and Delicata squash, *Cucurbita pepo*

Key Words.

Mechanical cultivation, weed control, cultural control, selectivity, winter squash

Introduction

Weed management is a major constraint to successful production of both organic and non-organic vegetable crops. According to a 2013 national survey of farmers, performed by Oregon State University and Oregon tilth, weed management is the most significant obstacle preventing transition to organic production (Brown et al. 2017). The cost and accessibility of labor for hand weeding can be a limiting factor in weed control on organic farms (Sortori et al. 2005) and herbicide resistance on conventional farms is increasing the need for alternative weed control techniques (Green et al. 2016).

Mechanical cultivation is an important tool for weed management in both conventional and organic winter squash systems. In conventional systems cultivation is important because of limitations in post emergence herbicides for winter squash and pumpkin crops (Zandstra 2017; Starke et al. 2006). In organic systems cultivation is important in order to limit the expense of hand labor. It was found by Lowry (2015) that approximately 26% of Michigan organic winter squash growers use row crop cultivators to control weeds. The importance of mechanical cultivation for Midwest vegetable growers means that cultivation systems must continue to improve.

Mechanical cultivation and the importance of selectivity. Selectivity is a critical concept in mechanical weed management, involving suppression of weeds while limiting damage to crops (Rasmussen 1990). Poor selectivity of cultivation machines can result in the death or yield reduction of the crop in addition to inadequate weed control (Gallandt et al. 2017). Especially in fresh market vegetable systems, mechanical cultivation can negatively affect the quality of the crop (Ascard and Mattsson 1994). The potential damage of the crop by the cultivator is a greater risk when using tools that are designed to kill weeds within the crop row because of proximity of weed to crop. Because in-row cultivators act on the crop in a similar manner to the weed it is important for there to be precision and consistency of the crop and the cultivator (Kurstjens et al. 2004).

Mechanical cultivation machines were designed for optimized function within a range of environments, crop types and crop stages. In order for in row weed control to be effective, management choices should be made to promote a differential in crop and weed size or morphology at the time of post emergence cultivation. Several approaches may improve the selectivity of mechanical cultivation. The approach most under the control of the grower is the precision of field preparation and planting in order to achieve a consistent action of the tool on the crop and weed (Mohler et al. 2001). Stale seed bedding and pre plant cultivation also help ensure that weeds and crop do not emerge simultaneously (Melander et al. 2005). Deeper seed placement coupled with pre-emergence cultivation can also give the crop a growth advantage (Van der Weide et al. 2008). Crops can be transplanted so they are significantly larger than weeds at time of cultivation (Bleeker et al. 2002). Of equal importance, tools must be designed to take advantage of differences in size between crop and weed. Lastly, the grower must understand the correct set up and calibration of implement settings such as working depth and working speed which can affect the selective potential of the implement being used (Vanhala et al. 2004)

Understanding the acceptable range of crop, weed, and environment for any given tool is key to achieving the highest level of tool efficacy (Mohler 2001). Most cultivation machines that are designed to destroy weeds within the crop row are best used to control weeds at the white thread or cotyledon stage (Van Der Schans et al. 2006). Weather and soil conditions/moisture are both difficult to manage for mechanical cultivation and key to improving selectivity in mechanically cultivated systems (Kurstjens et al. 2000; Kurstjens 2002; Terpstra and Kouwenhoven 1981; Cirujeda and Taberner 2004). The myriad of logistical challenges associated with mechanical weed control make an integrated approach to weed management on the farm that much more important.

Mechanical cultivator manufacturers have also improved the efficacy and selectivity of their cultivators through precision agriculture technology and improved design of traditional in row weeders. Fennimore et al. (2010) found that camera guided mechanical weed control had a higher net return in lettuce then the standard herbicide weed control strategy. However, Melander et al. (2015) found that the best quality intra-row flextine, finger and torsion weeders to have superior efficacy in vegetable systems compared to the intelligent weeder the Robovator (F. Poulsen Engineering, Denmark).

Improvement of selectivity through cultivation tolerant crops. Another approach for improving selectivity of mechanical cultivation is the identification and use of cultivation tolerant crop cultivars. Very little of this work has been done in the area of vegetable and has been explored to a small extent in grains with variable results (Rassmussen et al. 2009). For example, recent research in wheat has shown some varieties are more tolerant to cultivation than others, thus building the case for selecting for tolerant genotypes (Murphy et al. 2008). Similarly, Hitchcock-Tilton (2018) found differences in survival rate of carrot cultivars when the torsion weeder and flextine harrow were used.

The improvement of crop tolerance to mechanical cultivation has shown potential importance in improving selectivity, but relatively little research has been done to identify and select for the traits associated with that tolerance. Identification of traits associated with cultivation tolerance may be helpful for development of new varieties that are better suited for vegetable production systems that do not rely on herbicides. Hitchcock-Tilton (2018) observed a positive relationship between carrot root size and tolerance to the torsion weeder along with carrot shoot size and tolerance to the finger weeder. Although recent reviews on breeding for organic systems have highlighted the importance of breeding for cultivation tolerance (van Bueren et al. 2011), no research on cucurbit species' tolerance to cultivation has been performed.

Traits associated with tolerance to cultivation. For improved selectivity of the weed over the crop by the cultivation machine, the crop must be able to tolerate more force from the cultivation machine than the weed (Kurstjens 2004; Gallandt et al. 2017). The potential forces of the cultivation machine on the crop and weed include: burial, uprooting and severing (Mohler 2001; Gallandt et al. 2017). For example, Kurstjens et al. (2004) demonstrated that crop anchorage forces that exceeded those of associated weeds were critical in determining selectivity of a harrow that functioned primarily by uprooting. To attain greater crop tolerance to these forces, the importance of maintaining a size difference between the crop and the weed is often emphasized (Van Der Weide et al. 2008). Resistance to uprooting is suspected to be related to the tensile strength of the root system (Ennos and Fitter 1992) or to stem strength (Piñera-Chavez et al. 2016 ;Gallandt et al. 2017). Other crop traits, such as anchorage forces, offer the possibility of selecting for carrot survival even when the seedling is at the same development stage as the competing weed (Fogelberg and Dock Gustavsson 1998). Traits that resist the force of cultivation must be studied further in order to understand which are important for specific tool and crop combinations and to evaluate potential trade-offs with other desirable traits.

Root system morphology and partitioning may be especially important for resistance to the damage caused by mechanical cultivation. To avoid uprooting, the root system must resist both vertical and rotational forces (Ennos and Fitter 1992). By distributing the force of the machine across many lateral roots and a strong tap root, plants may not only be capable of withstanding uprooting but recovering more quickly from damage associated with root pruning. At early stages of growth when cultivation is most important, carbon allocation to roots could be most valuable in resisting uprooting. Yet, a crop that consistently allocates more carbon to roots rather than harvested plant parts throughout the crop's life may adversely affect yield (Koevoets et al. 2016). More research is needed to understand the potential tradeoffs of selecting for larger or stronger root systems.

Further research is needed to understand how plant traits associated with size, carbon allocation, and root morphology may assist in improvement of the selectivity of mechanical cultivators. Therefore, the primary objectives of our research were to determine which cultivars of Acorn and Delicata squash are most tolerant to mechanical cultivation and to identify traits associated with that tolerance. We hypothesized that: 1) Squash cultivars vary in important seedling characteristics relevant to selectivity including early vigor, root:shoot partitioning, root morphology and anchorage force; 2) squash cultivars vary in their tolerance of finger weeders and flextine cultivators in the field; and 3) cultivar tolerance to mechanical cultivation in the field is positively correlated with seedling characteristics including seedling size, root:shoot ratio, and anchorage force.

Materials and Methods

Greenhouse Experiment Treatments and Design. An experiment to evaluate seed and seedling characteristics, seedling morphology, partitioning and anchorage force was carried out in February of 2018 at the Michigan State University (East Lansing, Michigan) research greenhouses. The greenhouse experiment was a randomized complete block design with variety as the only factor and five replications of each treatment. Each replication consisted of twenty seeds of each seed lot and each seed was planted into its own pot.

Cucurbita pepo cultivars and seed source. Six *Cucurbita pepo* cultivars were chosen to represent a range of genotypes that are commercially available for organic production, and commonly grown in the Midwest region (Table 3.1). A single seed lot was chosen in each year for each cultivar. and used in all field and laboratory experimentation. The cultivars chosen were Honey Bear, Taybelle, Tuffy, Jester, Delicata JS, and Sugar Bush. Among these, Honey Bear, Jester, and Sugar Bush were hybrid cultivars; Taybelle, Tuffy and Delicata JS were open pollinated. Tuffy, Delicata JS and Sugar bush were all organic certified seed; Jester, Honey Bear, and Taybelle were conventionally grown and untreated.

Greenhouse Management. The greenhouse was set to 24° C a 12 hour light cycle throughout the experiment. Watering occurred by hand using a watering wand. One fertigation occurred once five days after planting with Peters Professional 20-20-20 at a rate of approximately 360 ppm N (2017, ICL Fertilizers, Dublin, Ohio). Seedlings were arranged on one bench oriented North/South with all pots touching one another (Table 3.2)

Uprooting Force and Seedling Biomass. Using the six cultivars of *C. pepo,* five replications of twenty seeds of each cultivar were characterized for size. Undamaged seeds for each replication were first selected by visual examination. Each replication of 20 seeds was then weighed. Those same seeds were then scanned using an Epson Perfection V700 2.80A scanner (Seiko Epson Corporation, Suwa, Nagona Prefecture, Japan). The projected area, length and width of scans of these seeds were then determined using WinSeedle 2008a Image Analysis System (Regent Instruments, Inc., Quebec, Canada). In order to scan each replication of 20 seeds of each cultivar was placed in a tray so that no seeds were touching each other or the side of the tray. Seeds were kept in order from 1-20 so that seed dimensions could be correlated with seedling characteristics. After seeds were assigned their identifying number they were sown at 2.5 cm depth in 0.47 L cups filled with potting mix made up of one part sand to four parts vermiculite by volume. Cups were arranged in a randomized complete block design.

Fourteen days after planting, 10 plants of each cultivar were mechanically uprooted, and the force needed to pull seedlings completely out of the ground was recorded, using a Shimpco FGE-100XY digital force gauge (Nidec-Shimpo America Corporation, Glendale Heights, IL). In order to uproot each plant, a tripod was placed directly above each seedling. The Shimpco force gauge was hung from the tripod. Attached to the hook of the gauge was a small metal pully (Figure 3.1). A nylon cord was run through the pully and attached to one end was large binder clip with two pieces of plexiglass measured 7.5 x 3.8cm placed inside to avoid tearing the leaves from the seedling. The clip was placed over the two or three leaves of the seedling and the chord was pulled until the seedling either broke or was uprooted completely from the potting mix.

Figure 3.1: Uprooting procedure.

On the same day of the force gauge uprooting, the remaining 10 plants of four replications were carefully uprooted for further analysis. Seedlings were cut at the soil surface, the leaves and stems were placed in a labeled plastic bag with one damp paper towel to prevent wilting. The roots were carefully washed in trays filled with water in three stages to remove sand and vermiculite.

After washing, roots and shoots were prepared for scanning. Leaves were detached from the primary stem so they would lay flat on the scanner. Scanning was performed using the Epson Perfection V700 2.80A scanner (Seiko Epson Corporation, Suwa, Nagona Prefecture, Japan). Each seedling's leaves were scanned by laying the material flat on a tray without any of the parts touching one another or the edge of the scanner. Roots of each seedling were laid flat on the scanner with roots arranged in a way to minimize overlap. Once roots and leaves were scanned they were analyzed using WinRHIZO Reg 2016 Image Analysis System (Regent Instruments, Inc., Quebec, Canada) to determine the projected area of the seedling parts.

Statistical Analysis. Statistical analysis was performed using Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). Assumptions of normality and equal variance were evaluated using PROC MIXED, PROC UNIVARIATE and Levene's test. Where needed the data were log, square root, or arcsin transformed to meet assumptions of normality and equal variance. The effect of cultivar on seed and seedling characteristics in the greenhouse studies was analyzed using PROC GLIMMIX procedures in SAS with cultivar treated as fixed effect, and replicate as a random effect. Correlation analyses between seed and seedling characteristic were conducted separately using the PROC CORR procedure of SAS to determine the Pearson's correlations coefficient. Distributions of data points were calculated using PROC UNIVARIATE in SAS.

Field Experiment Treatments and Design. In 2017 and 2018, a series of field experiments were conducted to evaluate the tolerance of 6 cultivars of *C. pepo* to mechanical cultivation (Table 3.1). Separate experiments were conducted to evaluate cultivar tolerance to cultivation with an Einbock Tined AEROSTAR 150 cultivator (model # 50015 (Einbock, Dorf an der Pram) (Experiment 1) and cultivation with and the Kult Kress small finger weeder with medium hardness mounted to the Kult Kress Argus steerable tool bar (K.U.L.T. - Kress LLC, Gordonville, PA) (Experiment 2). Both experiments were repeated twice in 2017 and twice in the 2018 growing seasons. All trials were conducted at the Kellogg Biological Station in Hickory Corners, Michigan (42.401963° N, -85.375465° W). The soil type was a Kalamazoo loam (fine-loamy, mixed, mesic Typic Hapludalfs). The organic certified field was in winter wheat and frost seeded red clover in the summer of 2016.

Both trials had a split plot design, with cultivation as the main plot factor and squash variety as the sub plot factor (Table 3.3). Main plots were arranged in a randomized complete block design with 4 replicates for the first trials in 2017 and five replicates for the remaining trials. The cultivation factor consisted of two levels: 1) cultivation with either a Einbock flextine cultivator (Experiment 1) or Kult-Kress finger weeder (Experiment 2) or 2) a hand weeded control (Table 3.3). The squash variety factor included 6 levels, corresponding to the 6 cultivars of *C. pepo*.

Field Management. In early May of 2017 and 2018 the red clover cover crop planted the previous year was chisel plowed for both Experiment 1 (Table 3.4) and Experiment 2 (Table 3.5). Soil samples were taken in both years to determine if additions of Phosphorous or Potassium were needed. The levels of P and K were found to be within the acceptable range and were thus not fertilized. For the second round of trials in 2017 and 2018, each trial was rototilled to destroy squash plants and weeds prior to field preparation. Each trial was fertilized at a rate of 50 kg/ha actual N of non-gmo soybean meal (Zeeland Farm Services Inc, Zeeland, Michigan) containing 7% Nitrogen (700 kg/ha fertilizer). Fertilizer was broadcast by hand and incorporated using a John Deere 980 field cultivator (John Deere and Company, Moline, Illinois). Both early season trials began in June of 2017 and 2018. The second round of flextine and finger weeder trials began in July of 2017 and 2018. Squash seed was planted by hand at a distance of 38 cm between row and 15 cm in row (Table 3.4; Table 3.5). The first flextine trial in 2017 had four rows per plot while all subsequent trials were reduced to three. rows All finger weeder trials in 2017 and 2018 had two rows per plot.

The entire experimental area of all trials was flextine cultivated one time before crop emergence before initiation of the experimental treatments. The number of days after seeding (DAS) for this first pre emergence weeding varied between 1-5 days depending on weather conditions (Tables 3.4 and 3.5). The flex tine cultivator was run at a standard speed of approximately 6 km/hour. The tines were set at an angle approximately 90 degrees when run in the field. The depth of tines when moving through the field was approximately 13 mm.

Cultivation treatments (hand weeding vs flextine or finger weeding) were initiated between 13 and 19 DAS (Table 3.4 and 3.5). Flextine cultivation was performed using the Einbock Tined AEROSTAR cultivator (model # 50015;Einbock, Dorf an der Pram) and the Kult Kress small finger weeder with medium hardness mounted to the Kult Kress Argus steerable tool bar (Model # ;K.U.L.T. - Kress LLC, Gordonville, PA) . Prior to initiation of the cultivation treatments, each tool was calibrated using squash in an adjacent field that had been planted on the same day as squash in experimental plots. For the flextine cultivator, down pressure, tip angles and speed were adjusted such that the entire width of the soil surface was worked to a depth of approximately 2.5 cm. Flex tine tips were tilted forward at a 100-degree angle relative to the ground. For the finger weeder, the rubber tips were overlapped when being run in the field by approximately 2.5 cm; down pressure for the finger weeders was adjusted using the toolbar gauge wheels and the spring on the floating arm such that the fingers nearest the row were flattened and penetrated the soil to a depth of approximately 1.3 cm. During calibration, tractor speed was adjusted so that no more than approximately 10% of the most tolerant cultivar was noticeably damaged by the tools. In some experiments, because of a low number of plants due to pest damage, this was difficult to accomplish and a higher percentage of plants were killed. Speed was also determined by the rate of weed death. Faster speeds tend to result in greater in row weed death. So, speeds were increased until the rate of crop death had reached our threshold Using this criterion, tractor speeds ranged from approximately 5-8 km/hr for the flextine cultivator and 3-5 km/hr for the finger weeder. Within each run of the experiment, the speed was kept constant for all cultivars and replicates. However, the speed varied between runs due to differences in soil conditions and crop stage at the time of cultivation. On the same day of every post emergence cultivation the weed free control was weeded by hoeing directly around plants and between rows with hand held stirrup hoes.

Data Collection. In each trial, data on time to emergence, size of plants at cultivation, plant survival from cultivation, and final stand count per plot were collected (Table 3.4, Table 3.5). Yield data were also collected for the final flex tine cultivated trial in 2018. Emergence was recorded daily by marking the number of seedlings that had emerged in a plot each day up to 15 days after planting. A seedling was considered emerged when both cotyledons were above ground. Because of pest pressure in 2017 in the second finger weeder trial, emergence was not recorded. One day before cultivation, five plants per plot were sampled to determine the height and leaf number of each variety at the time of cultivation. Sub sample plants were labeled with foot stakes so the same plants could be measured for each cultivation. Only leaves that were mature enough to be opened were counted. The height of plants was recorded using a set of 6-inch digital calipers (Harbor Freight Tools, Calabasas, California). Calipers were calibrated with a standard before each replication. For each plant the zero point of the caliper was at the soil surface. The caliper was then opened till the end point reached the tallest point of plant.

Data collection of stand counts and weed counts were taken before and after each post emergence cultivation. For each cultivated plot, counts were taken within 24 hours of cultivating. Before cultivation the number of living plants were counted and recorded for each plot. The day following cultivation, live plant counts were repeated for each plot. Weed counts were conducted for each cultivated plot at the same time as plant counts. Weed counts were conducted by placing a 0.25 m^2 pvc quadrats randomly in two rows of each cultivated plot. The placement of this quadrat was then marked with a wooden foot stake. Counts of any rooted weed that fell within the quadrat was counted and recorded. The following day the weed counts were repeated in the same location.

At 28 to 35 DAS, all above ground biomass was collected from each plot. The surviving plants were counted for both cultivated and non-cultivated plots. The plants were then cut at the soil surface, bagged and dried in a 60°C oven for one week, weighed and discarded. Root analysis was done on a three-plant sub sample from each plot to determine differences in root characteristics. The three plants were chosen at random in each cultivated and non-cultivated plot. To preserve root morphology each plant was carefully excavated using hand trowels and a garden spade. Each root sample was placed in a gallon size plastic bag wrapped in a wet paper towel. Each root was then washed using the same protocol as the greenhouse experiment. Evaluation of each fresh plant root mass included measurements with digital calipers of the stem diameter and tap root diameter. After measurements were taken each sample was dried in a 60°C oven for three days. The samples were then weighed and discarded.

For the final flextine trial in 2018, six plants per plot were not harvested at 30 DAS and allowed to grow to maturity in order to gather yield data. After early biomass collection, all plots were hand weeded one time to reduce weed competition. Eighty-one days after planting, yield data and above ground biomass was collected. Fruits were graded into marketable (mature, undamaged, appropriate size) and non-marketable (immature, damaged, too small). The number of fruits and fresh weights were recorded for each plot. A three-plant sub sample was also collected to determine the above ground biomass of each plot upon fruit maturity. The three samples were dried at 60°C for one week their dry weights were recorded and the samples were discarded.

Statistical Analysis. Statistical analysis was performed using Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). Assumptions of normality and equal variance were determined using PROC MIXED, PROC UNIVARIATE and Levene's test. Where needed the data were transformed using log or square root procedures to improve normality and equal variance assumptions. The effects of tillage and variety on seedling performance in the field were analyzed separately for each year using PROC GLIMMIX procedures in SAS, with variety and tillage treated as fixed effects, and replicate as a random effect. Correlation analyses between seed and seedling characteristic and field performance were conducted separately using the PROC CORR procedure of SAS.

Results and Discussion

Greenhouse Seedling Experiments.

Seed Characteristics. Significant differences were found in seed characteristics including seedling fresh weight and projected area of seeds (Table 3.6). Honey Bear, Taybelle, and Sugarbush had the highest projected area and Tuffy had the lowest. Cultivars also differed in their seed fresh weight (Table 3.6) with Honey Bear having the highest fresh weight and Delicata having the lowest seed fresh weight, nearly half that of the greatest.

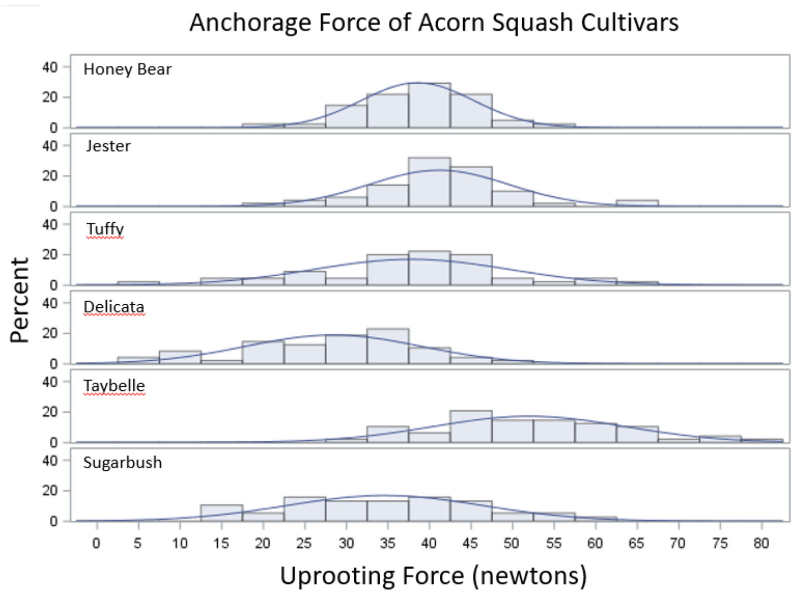
Seedling Dry Weight and Anchorage Force. Seedling dry weight at 13 DAS differed by cultivar, with Taybelle having the highest seedling dry weight and Delicata having the lowest (Table 3.7). The ranking of cultivars by anchorage force followed a similar trend, with Taybelle requiring the most force to uproot and Delicata requiring the least force.

Seedling projected area and root shoot partitioning. Total projected area (PA) for seedlings at 13 days after planting differed significantly between cultivars (Table 3.8). Taybelle was among the cultivars with the highest PA while Delicata was among those with the lowest PA. Differences in root projected area also differed between cultivars, with Taybelle, Sugarbush, and Tuffy among those with the highest projected root areas and Delicata among those with the lowest. Similarly, leaf projected area differed with Taybelle having one of the highest leaf areas and Delicata and Honey Bear being among the lowest. Differences in the root to leaf ratio were not significantly different across cultivars.

Variation of seedling characteristics and their relationships to one another. Under controlled greenhouse conditions, we found several consistent differences between cultivars in traits potentially important for cultivation tolerance. Taybelle was always among the cultivars with the greatest seed projected area, greatest dry weight, anchorage force, and seedling projected area. In contrast, Delicata was always among the cultivars with the lowest fresh weight, dry weight, anchorage force and seedling area. This suggests relation of seed size to seedling size and seedling size to anchorage force potential. This finding would be consistent with Guerro-Campo and Fitter (2001) who found a correlation of seed size to root size in a study of over 300 species. This finding suggests that the selection of larger seeded varieties may improve the uprooting force of plants in the field.

Characterization of anchorage force distributions that any given can also be helpful in selecting for more tolerance to uprooting. Varieties with a greater variance in anchorage force may be less consistent in the field in their ability to resist uprooting, resulting in greater crop mortality for a given uprooting force and hence lower selectivity of tools that uproot (Kurstjens et al. 2004;

Figure 3.2: Distributions of uprooting force for six cultivars.



Gallandt et al. 2017). For example (Figure 3.2) Honey Bear and Tuffy have similar mean anchorage forces yet Honey Bear has much smaller variance. This suggests that Honey Bear would have fewer plants vulnerable to death by cultivation tool than Tuffy even though they have similar mean anchorage forces.

Flextine Cultivation Experiment (Experiment 1).

Weather. In 2017, using a base temperature of 15°C for the first flextine trial, cumulative growing degree days (GDD) at the time of emergence ranged from 20.8 to 119.6 (Table 3.9). Total rainfall at the completion of emergence data collection was 28.45 mm. At the point of biomass collection, the total GDD were 165.3. Total rainfall accumulation was 52.58 mm. The second flextine trial of 2017, GDD at the time of emergence ranged from 47.3 to 98.1. Total rainfall at the completion of emergence data collection was 3.3 mm. At the point of biomass collection, the total GDD were 181.8. Total rainfall accumulation was 40.89 mm. In 2018, flextine trial three at the time of emergence data collection were between 36.8 to 77.1. Total rainfall at the completion of emergence data collection was 33.78 mm. At the point of biomass collection in 2018 total GDD were 159.7. The total accumulated rainfall was 104.65 mm. For the second flextine trial of 2018, GDD at the time of emergence data collection was between 23.8 to 106.4. Total rainfall at the completion of emergence data collection was 32 mm. At the point of biomass collection in 2018 total GDD were 217.2. The total accumulated rainfall was 82.55 mm. The total amount of rainfall in 2018 was nearly twice that of 2017 rainfall. 2017 also had significantly lower rainfall during the time of trials then the previous four years. The differences between 2017 and 2018 could have had a major effect on plant response from year to year.

Time to emergence, height and leaf stage at time of cultivation. Across four trials of the flextine there were fairly consistent differences between cultivars in the mean days to emergence (Table 3.10). In most trials, Taybelle was among the fastest to emerge, and Delicata and Jester among the slowest.

The height at the time of the first flextine cultivation also varied by cultivar for all flextine trials (Table 3.10). In trial one, Taybelle and Honey Bear were the among the tallest varieties while Jester, Delicata, and Tuffy were among the shortest. For trial two, three, and four Taybelle was one of the tallest varieties and Delicata was the shortest. Similar trends existed for leaf stage. Differences between cultivars in leaf number at the time of cultivation were less consistent; no differences were detected in trial one and leaf data was not collected for trial two.

In trials three and four, Taybelle was always among the cultivars with the most leaves at the time of cultivation and Honey Bear among those with the fewest leaves.

Effect of flextine cultivation and cultivar choice on plant survival. Few differences between cultivars in plant survival were detected in the four trials in 2017 and 2018 (Table 3.11). The only case where cultivars differed in their survival following flextine cultivation occurred in trial four. In that case, Delicata had lower survival compared to all other varieties. However, it should be noted that high variability in trials one and two limited our power to detect differences. In addition, insufficient data was available for all but two cultivars to evaluate cultivar survival in trial three.

Effect of flextine cultivation and cultivar on final biomass and stand count at 30 DAP. Above ground biomass per plant differed by cultivar in all four trials (Table 3.12). However, no effects of flextine cultivation on weight per plant (compared to the hand weeded control) were detected in trials one, three or four. In all trials, Taybelle was among the cultivars with highest dry weight per plant, and Delicata and Tuffy were among those with the lowest. In trial two there was an interaction between flextine and cultivar, but this interaction did not reflect a clear difference in cultivar tolerance to cultivation.

Dry weight per plot did differ among cultivars for flextine trials. With Taybelle consistently having amongst the greatest dry weight per plot. However differences in cultivar tolerance to flextine cultivation was not detected (Table 3.13).

Cultivars varied in final stand count in all four flextine trials (Table 3.14). For trial one, there were significant differences for cultivar and flextine. However, there was no significant interaction. Hand weeded Taybelle and Jester were among the cultivars with the highest final stand counts while flextined Honey Bear and flextined Sugarbush were among those with the lowest final stand count. For trial two, only cultivar was a significant effect with Jester having the highest final stand count and Sugarbush the least. For trial three, cultivar was again the only significant effect. Taybelle had the highest stand count and Delicata, Honey Bear, Sugarbush, and Tuffy had the least. Trial four had a significant cultivar effect and interaction of cultivar and finger weeder. This interaction appears to reflect differences in cultivation effects on Sugarbush compared to Taybelle. The reasons for this difference are not clear but may reflect hoeing damage sustained by Sugarbush rather than any differences in cultivar tolerance to the flextine cultivator itself.

Effect of cultivar and flextine cultivation on yield. Yield was only collected for the fourth flextine trial (Table 3.15). The only significant effect for total and marketable yield was cultivar, with no flextine or flextine bycultivar interaction detected. Total yield was greatest for Taybelle with no significant differences in the remaining varieties. Taybelle also had the greatest marketable yield per plot, with more than four times that of the Honey Bear which had the lowest marketable yield. There was a significant cultivar by flextine interaction in the yield of non-marketable fruit as represented in cull percentage (Table 3.15). This interaction reflects the fact that hand weeded Sugarbush had a higher cull percentage than flextined Sugarbush.

Finger Weeder Experiment (Experiment 2).

Weather. In 2017, the first finger weeder trial had GDD at the time of emergence from 36 to 101.8 with a base temperature of 15°C (Table 3.16). Total rainfall at the completion of emergence data collection was 29.46 mm. 209.4 the point of biomass collection, the total GDD were 123.2. Total rainfall accumulation was 71.37 mm. The second finger weeder trial of 2017 did not have emergence data recorded. However, emergence was considered concluded at the time of the first cultivation which was 18 days after planting. At that time, the total cumulative growing degree days were 123.2. Total rainfall at the completion of emergence data collection was 3.3 mm. At the point of biomass collection, the total GDD were 194.8. Total rainfall accumulation was 28.19 mm. In 2018, finger weeder trial one GDD at the time of emergence data collection were 54.9 to 83.7. Total rainfall at the completion of emergence data collection was 17.53 mm. At the point of biomass collection total GDD were 211.8. The total accumulated rainfall was 32.77mm. For the second finger weeder trial of 2018, GDD at the time of emergence data collection was between 40.6 to 92.6. Total rainfall at the completion of emergence data collection was 33.27 mm. At the point of biomass collection in 2018 total GDD were 211.2. The total accumulated rainfall was 82.55 mm.

Time to emergence, height and leaf stage at the time of finger weeding. Mean days to emergence differed significantly by cultivar in trials one, three, and four of the finger weeder experiment (Table 3.17). For trial one, Taybelle had one of the least days to emergence with 8.4 and Sugarbush had one of the most at nearly 10. Emergence data was not collected for the second trial of 2017 due to pest pressure. In trial three Taybelle and Tuffy had among the least days to emergence. The remaining four varieties were not significantly different from one another. In trial four Taybelle once again had the shortest days to emergence and Jester, Delicata, and Honey Bear had among the greatest. Size and leaf stage at the time of cultivation differed significantly for most of the finger weeder trials (Table 3.17). For trial one Delicata and Sugarbush were significantly shorter than the rest of the varieties tested. In trial two and four Taybelle was the tallest cultivar and Delicata remained the shortest. In trial three the tallest variety was Taybelle while the remaining varieties were not statistically different from one another. In trial one Taybelle also was among the cultivars with the greatest leaf number while Jester and Sugarbush were two of the least. For trial two Taybelle, Jester, Honey bear and Tuffy all had the greatest number of leaves at cultivation while Delicata and Sugarbush had the least. Differences in leaf number were not significant for trial three. In trial four Taybelle had the greatest number of true leaves while the remaining varieties did not differ significantly.

Effect of finger weeder cultivation and cultivar choice on plant survival. Cultivars differed in their tolerance to the first finger weeding in trial one and trial two in 2017 (Table 3.18) but did not differ in trials three or four (Table 3.18), nor in their tolerance to the second cultivation for any of the trials (data not shown). For trial one, Taybelle was among the cultivars that were most tolerant of finger weeding, while Sugarbush and Honey Bear were among the least. For the first cultivation of trial two, Honey Bear had lower survival than all other cultivars other than Sugarbush.

Effect of finger weeder cultivation and cultivar on final biomass and plant number at 30 DAP. Cultivars differed in dry weight per plant in trials one, three, and four (Table 3.19). However, the effect of finger weeder and the interaction of finger weeder and cultivar were not significant for final dry weight per plant for any of the finger weeder trials. In trials one, three, and four, Taybelle was among the cultivars with the highest dry weight per plant. For trial one and four Delicata was always among the cultivars with the lowest dry weight per plant.

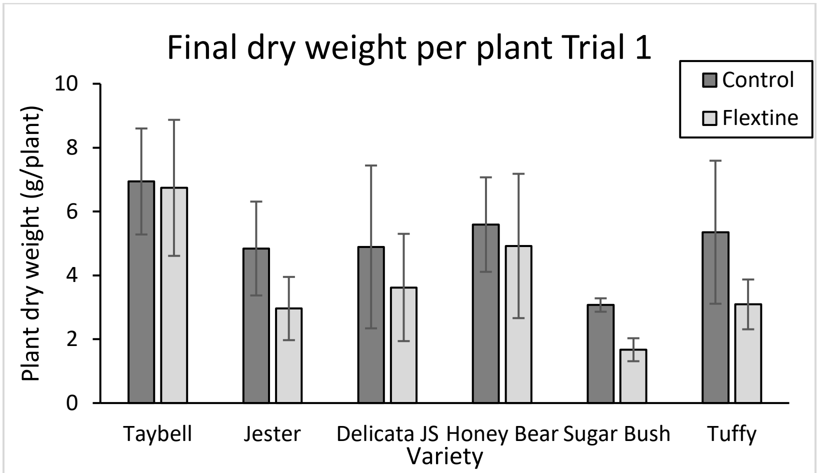
Dry weight per plot (Table 3.20) was significant for the cultivar effect in all four finger weeder trials. The effect of finger weeder was only significant for trial three, and there were no cultivar by finger weeder interactions detected. For trial one, Taybelle had the highest dry weight per plot. Delicata, Honey Bear, Sugarbush, and Tuffy had the lowest dry weight per plot. In trial two Taybelle and Jester had the highest dry weight per plot and the remaining cultivars had the lowest. In trial three, hand weeded Taybelle had one of the highest per plot dry weights. Delicata, Honey Bear, and finger weeded Sugarbush and Tuffy had among the least dry weight per plot. There were significant differences in the cultivation response for Taybelle and Sugarbush but not for the remaining cultivars. In trial four Taybelle once again had the highest per plot dry weight. Delicata, Honey Bear, Sugarbush and Tuffy had the least.

There was a significant effect of cultivar on final plant number for three of the fourfinger weeder trials (Table 3.21). There was no significant effect of finger weeder on final plant number in trials one, three or four. In trial two the effect of the finger weeder was significant statistically but too few plants remained at the end of the trial for results to be meaningful (data not presented). There was no significant interaction of finger cultivation and cultivar for any of the finger weeder trials. Taybelle and Jester had the highest final stand count in trials one, three, and four with no significant differences in the remaining cultivars

Differences in C. pepo cultivars' response to mechanical cultivation. Tolerance to mechanical cultivation was expected to be most apparent by the ability of some cultivars to survive cultivation more than others. This difference in response to cultivation was not apparent in the majority of the trials and cultivations that occurred for finger weeder and flex tine weeder. These differences in tolerance to cultivation were also not consistent across different cultivation tools with Delicata being the most susceptible to flex tine cultivation while Sugarbush and Honey Bear were the most susceptible to the finger weeder. These inconsistencies make it difficult to make recommendations of one variety over another based on our results. It is common for growers to use multiple mechanical cultivation machines for a specific crop and this it may not be useful knowledge to recommend a variety based on tolerance to one or the other. On the other hand, several cultivars including Taybelle and Jester were consistently tolerant to both tools, with survival rates of at least 80% and no detectable adverse effects on plant weight. Our results also suggest that tool recommendations may be tailored to specific varieties. For example, growers wishing to produce Delicata, may want to avoid flextine cultivation, and rely more heavily on fingerweeding. Alternatively, less aggressive settings for flextine cultivation could be used, but greater handweeding costs may be required to maintain adequate weed control to avoid yield loss.

Tolerance to cultivation can also be defined by other cultivar responses including the final yield, biomass at 30 days, and the stand count at 30 days. In the one trial where crops were taken to yield, the lack of cultivar by cultivation interaction on marketable yield suggests that practical impacts of differences in cultivation tolerance were minor. However, the interaction of cultivar by cultivation in the cull percentage suggest that cultivation effected the number of marketable fruits differently for different cultivars. For example, flextined Honey Bear had a cull percentage several times that of Taybelle and Sugarbush. This suggests that Taybelle and Sugarbush may have had improved tolerance to the flex tine weeder.

Figure 3.3: Dry weight per plant.



One of the key challenges with our field experiments was high variability in cultivar establishment and growth due to factors outside of our control, including variation in field moisture and pest injury, particularly from 13-lined ground squirrels. This variability limited our ability to detect differences in cultivar tolerance to mechanical cultivation. While a statistically significant cultivar by cultivation interaction was only shown for final stand count in flextine trial four, certain trends may be worth further investigation with greater replication and/or control over field variability. For example, although only marginally significant (P= <.05, dry weights of Sugarbush appeared to be more adversely affected by flextine cultivation than that of Taybelle in trial 1 (Figure 3.3).

The only statistical interaction for yield was found in the percentage on non-marketable fruit or cull percentage. While quantitative categories of culls were not taken, the majority of non-marketable fruit was identified as such because it was either too small or had reached full size but had not reached full maturity. It should be noted that the number of immature fruits or culls for any given variety did not appear to be closely related to the days to maturity of the cultivar (Table 3.1). Suggesting that some other quality of the cultivars was influencing the different responses to treatments.

Seedling characteristic and their relation to field results. Because of the relatively low number of sampling points in field experiments there was little power to adequately evaluate correlations between field performance and plant traits. There were however, some significant correlations worth further investigation (Table 3.22). In particular, flex tine survival was positively correlated to both anchorage force and plant dry weight. This suggests that the primary mode of action for the flex tine cultivator is uprooting and that a resistance to uprooting force increased plants' ability to survive flextine cultivation. In contrast, we did not detect a correlation between finger weeder survival and anchorage force, suggesting that the primary force of the finger weeder in our trials was not uprooting. This can be seen most readily for the cultivar Delicata, which had the lowest anchorage force among all cultivars, but was more tolerant of finger weeding than Honey Bear in Trial 2. We also had expected Honey Bear to have a relatively higher survival rate because of its lower variance of anchorage forces compared to other varieties such as Tuffy (Figure 3.2). This did not prove to be the case for the finger weeder where Honey Bear ranked among the lowest for some finger weeder trials.

Summary and Conclusions

The primary objective of this research was to determine if there were differences across *C. pepo* cultivars in their response to finger weeder and flextine cultivation and if there were traits associated with that tolerance. While some differences in tolerance were observed, results were inconsistent across cultivation treatment and trials. More investigation is needed to characterize effects of cultivation on crop yield and quality. Although flextine cultivation caused both increased mortality and reduced plant growth of *C. pepo*. However ,the lack of significant damage from the finger weeder suggests that further investment in research into selecting cultivars that are resistant to its potential damaging effect may not be justified. Correlations between anchorage force and seedling size and tolerance to flextine cultivation suggest that further trialing of these cultivars across seed lots may be useful. Future research should further investigate the heritability of these traits of anchorage force and whether or not breeding for such a trait would result in a significant gain in yield or other measurements of productivity.

APPENDIX

Table 3.1: Cultivar descriptions and sources.

Cultivar	Type	Seed Source	Days to Maturity
Honey Bear F1	*Cucurbita pepo var. turbinate*	Johnny's	85
Taybelle	*Cucurbita pepo var. turbinate*	Vesey's	72
Tuffy (organic)	*Cucurbita pepo var. turbinate*	Johnny's	90
Jester F1	*Cucurbita pepo var. pepo*	Johnny's	95
Delicata JS (organic)	*Cucurbita pepo var. pepo*	Johnny's	100
Sugar Bush F1 (organic)	*Cucurbita pepo var. turbinate*	High Mowing	90

Table 3.2: Schedule of major lab operations and data collection events.

Event	Date	DAS
Seeds are scanned and planted	2/8/2018	0
12h lights are set up and seeds are fertilized	2/12/2018	4
Plants are uprooted washed and stored for analysis	2/21/2018	13
Seedlings are scanned	2/22/2018	14

Table 3.3: Treatments for Field Experiment 1 and 2.

	Main-plot	Sub-plot
Trt	Cultivation	Cultivar
1	None*	Honey Bear
2	None	Taybelle
3	None	Tuffy
4	None	Jester
5	None	Delicata JS
6	None	Sugar Bush
7	Cultivated**	Honey Bear
8	Cultivated	Taybelle
9	Cultivated	Tuffy
10	Cultivated	Jester
11	Cultivated	Delicata JS
12	Cultivated	Sugar Bush
*	Handweeded	
**	Fingerweeder (Exp x-y) or Flextine weeder (Exp x-y)	

Table 3.4: Schedule of major field operations and data collection events for cultivar response to flextine cultivation, 2017 and 2018.

	2017				2018			
	Flextine Trial 1		Flextine Trial 2		Flextine Trial 3		Flextine Trial 4	
Event	Date	DAS	Date	DAS	Date	DAS	Date	DAS
Cover crop incorporated	4/25	-37	NA	NA	5/4	-26	NA	NA
Soil samples taken	5/5	-27	NA	NA	5/4	-26	NA	NA
Field fertilized	5/30	-2	7/5	-6	5/28		7/9	-1
Field "finished"	5/30	-2	7/5	-6	5/28	-2	7/9	-1
Squash planted	6/1	0	7/11	0	5/30	0	7/10	0
Flextine cultivation (0) pre-emergence (all plots)	6/6	5	7/14	3	6/4	5	7/14	4
Emergence counts initiated	6/7	6	7/18	7	6/7	8	7/13	3
Emergence counts concluded	6/19	18	7/24	13	6/15	16	7/24	14
Pre-cultivation (1) squash size data	6/19	18	7/24	13	6/14	15	7/24	14
Pre-cultivation (1) weed and stand counts	6/19	18	7/24	13	6/14	15	7/24	14
Flextine cultivation (1) treatment	6/19	18	7/25	14	6/14	15	7/25	15
Post-cultivation (1) weed and stand counts	6/20	19	7/26	15	6/15	16	7/25	15
Handweeded non cultivated plots (1)	6/19	18	7/25	14	6/14	15	7/25	15
Pre-cultivation (2) squash size data	NA	NA	8/1	21	6/25	26	7/31	21
Pre-cultivation (2) weed and stand counts	NA	NA	8/1	21	6/25	26	7/31	21
Flextine cultivation (3) treatment	NA	NA	8/2	22	6/25	26	8/1	22
Post-cultivation (2) weed and stand counts	NA	NA	8/3	23	6/26	27	8/2	23
Handweeded non cultivated plots (1)	NA	NA	8/2	22	6/25	26	8/1	22
30-40 DAT squash plant counts and biomass	6/30	29	8/10	30	6/28	29	8/7	28
Yield and quality assessment	NA	NA	NA	NA	NA	NA	9/27	79

Table 3.5: Schedule of major field operations and data collection events for cultivar response to finger weeder cultivation, 2017 and 2018.

	2017				2018			
	Finger Trial 1		Finger Trial 2		Finger Trial 3		Finger Trial 4	
Event	Date	DAS	Date	DAS	Date	DAS	Date	DAS
Cover crop incorporated	4/25	-44	NA	NA	5/4	-38	NA	NA
Soil samples taken	5/2	-37	NA	NA	5/3	-39	NA	NA
Field fertilized	6/6	-2	7/13	-1	6/7	-4	7/11	-1
Field "finished"	6/6	-2	7/13	-1	6/7	-4	7/11	-1
Squash planted	6/8	0	7/14	0	6/11	0	7/12	0
Flextine cultivation (0) pre-emergence (all plots)	6/19	11	NA	NA	6/14	3	7/13	1
Emergence counts initiated	6/12	4	NA	NA	6/18	7	7/16	4
Emergence counts concluded	6/21	13	NA	NA	6/25	14	7/24	12
Pre-cultivation (1) squash size data	6/21	13	8/1	18	6/25	14	7/25	13
Pre-cultivation (1) weed and stand counts	6/21	13	8/1	18	6/25	14	7/25	13
Finger cultivation (1) treatment	6/22	14	8/2	19	6/28	17	7/26	14
Post-cultivation (1) weed and stand counts	6/22	14	8/3	20	6/29	18	7/27	15
Handweeded non cultivated plots (1)	6/22	14	8/2	19	6/28	17	7/26	14
Pre-cultivation (2) squash size data	6/28	20	8/8	25	7/5	24	7/31	19
Pre-cultivation (2) weed and stand counts	6/28	20	8/8	25	7/5	24	7/31	19
Finger cultivation (2) treatment	6/29	21	8/9	26	7/6	25	8/1	20
Post-cultivation (2) weed and stand counts	6/30	22	8/10	27	7/7	26	8/2	21
Handweeded non cultivated plots (1)	6/29	21	8/9	26	7/6	25	8/1	20
30-40 DAT squash plant counts and biomass	7/10	32	8/18	35	7/10	29	8/14	33

Table 3.6: Projected area and fresh weight of seeds.

Cultivar	Projected area of seeds		Seed fresh weight	
	------------- mm² ----------		--------------mg------------	
Honey Bear	7.4	a	112.5	a
Jester	6.9	b	101.3	b
Tuffy	5.2	d	73.0	d
Delicata	6.3	c	68.8	e
Taybelle	7.7	a	99.4	b
Sugarbush	7.5	a	87.3	c
	--------------------Significance (P-value)----------------			
Cultivar	***		***	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.7: Seedling dry weight and anchorage force.

Cultivar	Dry Weight		Anchorage Force	
	---------mg---------		---------N--------	
Honey Bear	127.3	c	38.5	bc
Jester	137.0	bc	41.1	b
Tuffy	143.0	bc	37.8	bc
Delicata	107.3	d	28.4	d
Taybelle	194.8	a	52.0	a
Sugarbush	147.3	b	34.6	c
	----------Significance (P-value)-------			
Cultivar	***		***	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.8: Mean leaves, root and total projected area, and the ratio of root and leaves projected area of seedlings emerging from six cultivars of C. pepo at 13 days after planting.

Cultivar	Total		Root		Leaves		RLR[a]
	----------	----------	----------	cm^2	----------	----------	----------
Honey Bear	47.9	cd	10.5	cd	37.4	c	0.3
Jester	55.1	bc	11.3	bc	43.8	bc	0.3
Tuffy	59.2	b	12.9	ab	46.3	bc	0.3
Delicata	47.1	d	8.9	d	38.2	c	0.2
Taybelle	69.6	a	14.6	ab	55.0	a	0.3
Sugarbush	62.3	ab	13.0	ab	49.2	ab	0.3
	----------	----------	----------	Significance (P-value)	----------	----------	----------
Cultivar	***		***		***		NS

a RSR=Projected root area divided by projected shoot area

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.9: Temperature, degree days (base 15C) and rainfall, 0-30 days after planting (DAP) squash, 2017 and 2018.

	2017								2018							
	Flex Tine Trial 1				Flex Tine Trial 2				Flex Tine Trial 3				Flex Tine Trial 4			
DAP	Mean Temp	Daily Growing Degree Days 15 C	Cumulative Growing Degree Days 15 C	Rain (mm)	MT	DGDD	CGDD	RN	MT	DGDD	CGDD	RN	MT	DGDD	CGDD	RN
1	16.9	1.9	1.9	0.0	24.2	9.2	9.2	0.0	25.5	10.5	10.5	26.9	22.0	7.0	7.0	0.0
2	18.5	3.5	5.4	0.0	23.2	8.2	17.4	20.3	23.3	8.3	18.8	0.3	21.8	6.8	13.8	0.0
3	23.2	8.2	13.6	0.5	20.4	5.4	22.8	4.1	16.9	1.9	20.7	0.0	25.0	10.0	23.8	0.0
4	18.1	3.1	16.7	0.8	19.5	4.5	27.3	0.0	18.4	3.4	24.1	0.0	23.9	8.9	32.7	0.0
5	16.9	1.9	18.6	0.0	21.6	6.6	33.9	0.0	17.8	2.8	26.9	13.5	25.2	10.2	42.8	0.0
6	17.2	2.2	20.8	0.0	21.7	6.7	40.6	0.0	17.4	2.4	29.3	0.5	26.6	11.6	54.4	0.3
7	18.0	3.0	23.8	0.0	21.7	6.7	47.3	0.0	15.9	0.9	30.2	1.0	22.6	7.6	62.0	6.9
8	20.9	17.4	29.8	0.0	23.6	8.6	55.9	0.0	21.6	6.6	36.8	0.0	20.2	5.2	67.2	0.3
9	22.3	14.1	37.1	0.3	25.4	10.4	66.3	0.0	21.7	6.7	43.4	0.0	21.0	6.0	73.2	0.0
10	26.2	23.1	48.2	0.0	23.8	8.8	75.1	0.0	20.8	5.8	49.2	0.0	22.5	7.5	80.7	0.0
11	26.6	24.7	59.8	0.0	23.7	8.7	83.8	0.3	17.7	2.7	51.9	27.2	21.7	6.7	87.4	0.0
12	26.9	24.7	71.7	0.0	24.3	9.3	93.2	3.0	19.8	4.8	56.7	6.6	20.3	5.3	92.7	12.4

Table 3.9: (cont'd).

13	25.2	22.2	81.9	0.0	19.9	4.9	98.1	0.0	19.3	4.3	61.0	0.0	20.9	5.9	98.7	8.9
14	23.7	6.3	90.7	18.0	19.3	4.3	102.4	0.0	19.6	4.6	65.6	0.0	22.7	7.7	106.4	3.3
15	23.2	9.1	98.8	0.0	22.1	7.1	109.4	0.0	19.5	4.5	70.1	0.0	22.4	7.4	113.8	1.5
16	24.5	1.4	108.3	0.0	23.6	8.6	118.1	0.0	22.0	7.0	77.1	0.0	20.4	5.4	119.2	0.0
17	21.9	0.0	115.2	6.9	20.5	5.5	123.6	0.0	25.5	10.5	87.6	0.0	19.2	4.2	123.4	0.0
18	19.3	0.0	119.6	3.3	19.5	4.5	128.1	0.0	25.9	10.9	98.4	3.6	19.0	4.0	127.4	0.0
19	18.2	0.0	122.8	0.3	21.1	6.1	134.1	0.0	28.1	13.1	111.6	0.0	19.3	4.3	131.7	0.0
20	17.9	11.6	125.7	1.0	20.8	5.8	139.9	0.0	20.0	5.0	116.6	0.0	20.9	5.9	137.6	0.0
21	23.1	14.0	133.7	0.0	21.1	6.1	146.0	0.0	18.3	3.3	119.8	2.8	19.4	4.4	142.1	0.0
22	21.3	19.9	140.1	2.3	23.3	8.3	154.3	0.0	18.6	3.6	123.4	7.9	22.4	7.4	149.4	0.0
23	18.6	18.6	143.7	5.1	22.1	7.1	161.4	0.0	17.9	2.9	126.3	0.0	22.9	7.9	157.4	7.1
24	16.8	16.8	145.4	0.0	16.6	1.6	163.1	0.3	19.9	4.9	131.3	6.4	22.4	7.4	164.8	0.0
25	15.9	15.9	146.3	0.3	18.7	3.7	166.7	10.7	19.4	4.4	135.7	0.5	23.1	8.1	172.9	0.3
26	15.6	4.0	146.9	0.0	18.1	3.1	169.8	0.5	19.7	4.7	140.4	0.0	26.7	11.7	184.7	0.5
27	17.1	3.1	149.1	0.0	18.7	3.7	173.5	1.8	19.9	4.9	145.3	0.0	22.9	7.9	192.6	0.0
28	22.9	3.0	157.0	0.0	18.3	3.3	176.8	0.0	21.5	6.5	151.8	0.0	25.0	10.0	202.6	0.0
29	23.3	4.7	165.3	14.0	19.9	4.9	181.8	0.0	22.9	7.9	159.7	7.6	22.4	7.4	210.0	41.1
30	-	-	-	-				0.0					22.2	7.2	217.2	0.0

Table 3.10: Mean days to emergence and height and leaf number 1 day before first flextine cultivation for 6 C. pepo cultivars, 2017 and 2018.

	Mean days to emergence				Height (mm)				Leaf stage			
	2017		2018		2017		2018		2017		2018	
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
	-----------------Days from planting------				-------------------------mm--------------------				--------------------Leaf number---------			
Cultivar												
Taybelle	14.4 b	7.1 c	10.4 b	7.0 d	53.4 a	51.2 a	35.0 a	58.1 a	2.1	NA	1.7 a	2.8 a
Jester	15.3 a	8.2 b	11.9 a	8.3 a	34.1 b	40.4 b	26.2 b	54.4 ab	1.1	NA	1.3 ab	2.0 bc
Delicata JS	15.3 a	9.0 a	11.6 a	7.8 b	31.5 b	28.0 c	17.4 c	37.6 c	1.8	NA	1.2 bc	2.2 b
Honey Bear	15.0 a	8.4 ab	12.2 a	7.5 bc	56.4 a	38.5 b	26.6 b	55.3 ab	1.6	NA	0.9 c	1.9 c
Sugarbush	15.3 a	7.8 bc	11.1 ab	7.3 cd	41.5 ab	41.3 b	28.6 ab	48.7 ab	1.2	NA	1.4 ab	2.1 bc
Tuffy	15.0 a	8.3 b	11.3 ab	7.9 ab	37.8 b	42.2 b	26.8 b	51.6 b	1.6	NA	1.4 ab	2.1 b
Significance	--------------------------------P-value--------------------------------											
Cultivar	*	***	*	***	**	***	**	***	NS	NA	**	*

'+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.11: Survival after first and second flex tine cultivations 2017 and 2018.

	2017		2018		
	Trial 1	Trial 2	Trial 3	Trial 4	
	1st cultivation	1st * cultivation	1st cultivation	1st cultivation	
Cultivar	-----------------------	---------------%---------------	-----------------------		
Honey Bear	62.0	84.3	NA	95	a
Jester	74.3	81.0	71.6	96.8	a
Tuffy	71.3	88.9	NA	94	a
Delicata JS	79.9	76.3	NA	86.6	b
Taybelle	89.1	81.7	90.5	95.8	a
Sugarbush	65.8	85.6	NA	97	a
Significance	-----------------------	-------------P-value-------------	-----------------------		
Cultivar	NS	NS	NS	*	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

* Data is not normal and could not be fixed with transformation.

Table 3.12: Shoot dry weight per plant of 6 C. pepo cultivars at 28 to 30 DAP, 2017 and 2018 for flextine trials.

Cultivar	2017						2018			
	Trial 1		Trial 2				Trial 3		Trial 4	
			Hand-weeded		Flextined					
	---gm/plant---									
Honey Bear	149.4	b	93.3	bcde	110.6	abcd	48.2	b	153.4	bc
Jester	110.2	b	95.8	bcde	110.3	abc	51.9	b	182.3	ab
Tuffy	119.2	b	65.2	cde	65.2	cde	42.0	b	111.1	c
Delicata JS	120.3	b	51.0	de	50.5	e	43.4	b	155.6	bc
Taybelle	192.7	a	134.9	a	139.2	ab	73.7	a	212.3	a
Sugarbush	64.1	b	111.1	abc	68.9	cde	57.8	ab	202.7	a
Significance	---Significance (P-value)---									
Cultivar (C)	*		***				*		**	
Flextine (F)	NS		NS				NS		NS	
C x F	NS		*				NS		NS	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.13: Shoot dry weight per plot of 6 C. pepo cultivars at 28 to 30 DAP, 2017 and 2018 for flextine trials.

Cultivar	Trial 1		Trial 2		Trial 3		Trial 4	
	-grams/12 meters--		-------------grams/7.5 meters---------					
Honey Bear	51.0	b	25.8	c	13.0	b	73.6	c
Jester	72.4	b	79.2	a	27.9	b	136.4	b
Tuffy	52.6	b	17.3	c	6.8	b	37.9	d
Delicata JS	53.6	b	15.7	c	8.9	b	106.2	bc
Taybelle	123.5	a	53.4	b	52.9	a	182.0	a
Sugarbush	21.2	b	12.7	c	15.2	b	76.4	c
Significance	------------------------Significance (P-value)--------------------							
Cultivar (C)	**		***		***		***	
Flextine (F)	NS		NS		NS		NS	
C x F	NS		NS		NS		NS	

'+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.14: Final Stand Count for 6 C. pepo cultivars for flextine trial in 2017 and 2018.

	2017								2018			
	Trial 1				Trial 2		Trial 3		Trial 4			
Cultivar	Hand-weeded		Flex						Hand-weeded		Flex	
	---plants/plot---											
Honey Bear	10.8	bcd	8.5	cd	5.0	cd	7.0	c	10.6	def	12.6	def
Jester	22.3	a	16.8	ab	20.5	a	11.6	b	21.8	bc	18.6	bc
Tuffy	15.8	d	8.3	b	7.5	bc	4.5	c	7.0	f	11.4	def
Delicata JS	15.8	b	13.5	bc	8.2	bc	5.4	c	20.6	bc	17.6	bcd
Taybelle	21.8	a	13.3	bcd	9.0	b	19.1	a	28.0	a	21.8	ab
Sugarbush	10.3	cd	7.8	cd	3.0	d	6.7	c	8.2	ef	14.8	cde
Significance	--------------------------------Significance (P-value)--------------------------------											
Cultivar (C)	***				***		***		***			
Flextine (F)	+				NS		***		NS			
C x F	NS				NS		NS		**			

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.15: Yield summary for Flextine trial four.

Cultivar	Total Yield		Marketable Yield		Cull/Non-marketable yield Hand-weeded		Flex-tine	
	-------------kg/plot-----------				--------------%--------------			
Honey Bear	1.70	b	0.71	c	55	a	65	a
Jester	2.66	b	2.01	b	7	bc	35	ab
Tuffy	1.79	b	1.22	bc	33	abc	33	abc
Delicata JS	2.48	b	1.47	bc	38	ab	56	a
Taybelle	3.97	a	3.69	a	0	c	12	bc
Sugarbush	2.46	b	1.55	bc	67	a	7	bc
Mean	2.51	b	1.78		33		35	
Significance	----------------------Significance (P-value)-----------------							
Cultivar (C)	***		***		*			
Flextine (F)	NS		NS		NS			
C x F	NS		NS		*			

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.16: Temperature, degree days (base 15 C) and rainfall, 0-32 days after planting (DAP) squash, 2017 and 2018 for finger weeder trial.

	2017								2018							
	Finger Weeder Trial 1				Finger Weeder Trial 2				Finger Weeder Trial 3				Finger Weeder Trial 4			
DAP	Mean Temp	Daily Growing Degree Days	Cumulative Growing Degree Days	Rain (mm)	MT	DGDD	CGDD	RN	MT	DGDD	CGDD	RN	MT	DGDD	CGDD	RN
1	20.9	5.9	5.9	0.00	19.5	4.5	4.5	0.00	19.3	4.3	4.3	0.00	25.0	10.0	10.0	0.00
2	22.3	7.3	13.2	0.25	21.6	6.6	11.1	0.00	19.6	4.6	8.9	0.00	23.9	8.9	18.9	0.00
3	26.2	11.2	24.4	0.00	21.7	6.7	17.8	0.00	19.5	4.5	13.4	0.00	25.2	10.2	29.1	0.25
4	26.6	11.6	36.0	0.00	21.7	6.7	24.5	0.00	22.0	7.0	20.4	0.00	26.6	11.6	40.6	6.86
5	26.9	11.9	47.9	0.00	23.6	8.6	33.1	0.00	25.5	10.5	30.9	0.00	22.6	7.6	48.2	0.25
6	25.2	10.2	58.1	0.00	25.4	10.4	43.5	0.00	25.9	10.9	41.8	3.56	20.2	5.2	53.4	0.00
7	23.7	8.7	66.8	18.03	23.8	8.8	52.3	0.00	28.1	13.1	54.9	0.00	21.0	6.0	59.4	0.00
8	23.2	8.2	75.0	0.00	23.7	8.7	61.0	0.25	20.0	5.0	59.9	0.00	22.5	7.5	66.9	0.00
9	24.5	9.5	84.5	0.00	24.3	9.3	70.3	3.05	18.3	3.3	63.2	2.79	21.7	6.7	73.6	12.45
10	21.9	6.9	91.4	6.86	19.9	4.9	75.3	0.00	18.6	3.6	66.8	7.87	20.3	5.3	78.9	8.89
11	19.3	4.3	95.7	3.30	19.3	4.3	79.6	0.00	17.9	2.9	69.7	0.00	20.9	5.9	84.9	3.30
12	18.2	3.2	98.9	0.25	22.1	7.1	86.6	0.00	19.9	4.9	74.6	6.35	22.7	7.7	92.6	1.52
13	17.9	2.9	101.8	1.02	23.6	8.6	95.2	0.00	19.4	4.4	79.1	0.51	22.4	7.4	100.0	0.00

Table 3.16: (cont'd).

14	23.1	8.1	109.9	0.00	20.5	5.5	100.7	0.00	19.7	4.7	83.7	0.00	20.4	5.4	105.4	0.00
15	21.3	6.3	116.2	2.29	19.5	4.5	105.2	0.00	19.9	4.9	88.6	0.00	19.2	4.2	109.6	0.00
16	18.6	3.6	119.8	5.08	21.1	6.1	111.3	0.00	21.5	6.5	95.1	0.00	19.0	4.0	113.6	0.00
17	16.8	1.8	121.6	0.00	20.8	5.8	117.1	0.00	22.9	7.9	103.1	7.62	19.3	4.3	117.9	0.00
18	15.9	0.9	122.5	0.25	21.1	6.1	123.2	0.00	24.2	9.2	112.2	0.00	20.9	5.9	123.8	0.00
19	15.6	0.6	123.1	0.00	23.3	8.3	131.5	0.00	28.3	13.3	125.5	0.00	19.4	4.4	128.3	0.00
20	17.1	2.1	125.2	0.00	22.1	7.1	138.6	0.00	27.3	12.3	137.8	0.00	22.4	7.4	135.7	7.11
21	22.9	7.9	133.2	0.00	16.6	1.6	140.2	0.25	24.1	9.1	146.9	4.06	22.9	7.9	143.6	0.00
22	23.3	8.3	141.5	13.97	18.7	3.7	143.9	10.67	24.6	9.6	156.4	0.00	22.4	7.4	151.1	0.25
23	22.2	7.2	148.7	0.51	18.1	3.1	146.9	0.51	27.2	12.2	168.6	0.00	23.1	8.1	159.2	0.51
24	21.8	6.8	155.5	0.25	18.7	3.7	150.7	1.78	27.6	12.6	181.2	0.00	26.7	11.7	170.9	0.00
25	21.9	6.9	162.4	8.13	18.3	3.3	154.0	0.00	20.3	5.3	186.5	0.00	22.9	7.9	178.8	0.00
26	22.4	7.4	169.8	2.54	19.9	4.9	158.9	0.00	19.1	4.1	190.6	0.00	25.0	10.0	188.8	41.15
27	21.9	6.9	176.7	0.00	20.6	5.6	164.6	0.00	20.3	5.3	195.8	0.00	22.4	7.4	196.2	0.00
28	22.9	7.9	184.6	0.00	21.4	6.4	171.0	0.00	21.7	6.7	202.5	0.00	22.2	7.2	203.4	0.00
29	23.1	8.1	192.6	0.00	20.2	5.2	176.2	11.68	24.3	9.3	211.8	0.00	22.8	7.8	211.2	0.00
30	19.5	4.5	197.1	8.64	19.3	4.3	180.5	0.00	-	-	-	-	-	-	-	-
31	19.2	4.2	201.3	0.00	22.0	7.0	187.5	0.00	-	-	-	-	-	-	-	-
32	23.1	8.1	209.4	0.00	22.3	7.3	194.8	0.00	-	-	-	-	-	-	-	-

Table 3.17: Mean days to emergence and height and leaf number 1 day before first cultivation with finger weeder for 6 C. pepo cultivars, 2017 and 2018.

	Time to emergence								Height (mm)								Leaf stage							
	2017				2018				2017				2018				2017				2018			
	Trial 1		Trial 2		Trial 3		Trial 4		Trial 1		Trial 2		Trial 3		Trial 4		Trial 1		Trial 2		Trial 3		Trial 4	
Cultivar	--------------Days from planting--------								----------------------mm------------------								---------------Leaf number--------------							
Honey Bear	9.2	bc	NA		9.6	b	9.1	c	41.0	a	39	ab	41.9	b	31.8	b	1.7	bc	1.3	ab	1.4		1.6	b
Jester	9.0	abc	NA		9.5	b	8.9	c	40.6	a	40	ab	37.1	b	27.7	bc	1.6	c	1.5	a	1.5		1.4	b
Tuffy	8.9	ab	NA		8.8	a	8.2	bc	45.2	a	35	b	40.6	b	25.1	bc	2.1	ab	1.5	a	1.8		1.7	b
Delicata JS	8.8	ab	NA		9.5	b	9.2	c	28.1	b	26	c	29.9	b	16.6	c	1.8	abc	1.1	b	2.0		1.4	b
Taybelle	8.4	a	NA		8.6	a	7.5	a	50.0	a	46	a	57.8	a	44.5	a	2.1	a	1.5	a	1.9		2.6	a
Sugarbush	9.7	c	NA		9.2	ab	7.9	b	37.7	ab	35	b	33.0	b	29.4	bc	1.5	c	1.1	b	1.3		1.5	b
Significance	--P-value--																							
Cultivar	*		NA		*		***		*		***		*		**		*		*		NS		***	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.18: Percent survival of 6 C. pepo cultivars following first and second finger weeding, 2017 and 2018.

	2017		2017		2018	2018
	Trial 1		Trial 2		Trial 3	Trial 4
	1st cultivation		1st cultivation		1st cultivation	1st cultivation
Cultivar	--------------------	----------	----------%	----------	----------	----------
Honey Bear	72.8	b	38.0	b	93.3	88.9
Jester	87.3	ab	80.4	a	94.9	94.7
Tuffy	80.2	ab	76.7	a	99.0	67.1
Delicata JS	79.7	ab	90.0	a	100.0	71.6
Taybelle	94.2	a	80.2	a	96.3	82.7
Sugarbush	68.9	b	66.5	ab	99.0	78.7
Significance	----------	----------	----------Significance (P-value)	----------	----------	----------
Cultivar (C)	+		*		NS	NS

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.19: Final dry weight per plant for finger weeder trials.

	2017				2018			
Cultivar	Trial 1		Trial 2		Trial 3		Trial 4	
	--------------------g/plant--------------------							
Honey Bear	104.9	b	144.6		94.4	b	303.1	bc
Jester	100.4	b	100.6		136.6	ab	294.6	bc
Tuffy	85.9	bc	48.2		121.3	ab	228.5	cd
Delicata JS	75.2	c	59.5		88.2	b	172.1	d
Taybelle	125.5	a	102.9		169.8	a	451.3	a
Sugarbush	78.6	bc	87.6		140.6	ab	348.1	ab
Significance	--------------------Significance (P-value)--------------------							
Cultivar (C)	**		NS		+		***	
Finger (F)	NS		NS		NS		NS	
C x F	NS		NS		NS		NS	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.20: Final dry weight per plot for finger weeder trials.

	2017		2018							
					Trial 3					
	Trial 1		Trial 2		Hand-weeded		Finger		Trial 4	
Cultivar	--kg/6 meters-		------------------kg/5 meters-----------------------							
Honey Bear	1.96	c	0.13	b	0.36	d	0.26	d	2.16	c
Jester	2.68	b	0.62	a	2.18	ab	1.68	b	4.94	b
Tuffy	1.78	c	0.06	b	0.65	cd	0.52	d	1.43	c
Delicata JS	1.61	c	0.15	b	0.16	d	0.16	d	1.34	c
Taybelle	3.29	a	0.89	a	2.96	a	1.55	bc	7.01	a
Sugarbush	1.45	c	0.19	b	1.39	bc	0.29	d	2.08	c
Significance	------------------------Significance (P-value)------------------------									
Cultivar (C)	***		***		***				***	
Finger (F)	NS		NS		*				NS	
C x F	NS		NS		NS				NS	

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.21: Final Stand Count for 6 C. pepo cultivars for finger weeder trials in 2017 and 2018.

	2017		2018	
	Trial 1	Trial 2	Trial 3	Trial 4
Cultivar	------------------------------------	-plants/plot-	------------------------------------	
Honey Bear	18.8 b	NA	3.2 b	6.9 b
Jester	27.1 a	NA	14.2 a	16.8 a
Tuffy	20.4 b	NA	5.1 b	6.2 b
Delicata JS	21.4 b	NA	1.9 b	8.2 b
Taybelle	26.7 a	NA	13.8 a	16.0 a
Sugarbush	18.5 b	NA	4.9 b	5.7 b
Significance	------------------------------	Significance (P-value)	------------------------------	
Cultivar (C)	***	NA	***	***
Finger (F)	NS	NA	NS	NS
C x F	NS	NA	NS	NS

+ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001; NS nonsignificant; NA not applicable

Table 3.22: Correlations of seedling characteristics and survival after mechanical cultivation.

		Size at the time of Cultivation		Seedling characteristics from Lab/Greenhouse Studies			
		Leaf Number	Height	Anchorage Force Force	Shoot Dry Weight	Leaf Proj Area	Root:Leaf Ratio
Flextine survival	Coeff	0.762	0.419	0.845	0.920	0.505	0.454
	P-value	0.004	0.084	0.034	0.009	0.307	0.366
	N	12	18	6	6	6	6
Finger weeder survival	Coeff	0.099	0.135	0.350	0.316	-0.145	-0.061
	P-value	0.695	0.592	0.497	0.542	0.784	0.909
	N	18	18	6	6	6	6

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