QUALITY EVALUATION OF COATED EXTRA-LARGE HULLED SUNFLOWER (*HELIANTHUS ANNUUS*) KERNELS FOR PRECISION PLANTING

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Domestic and export demand for extra-large (XL) in-shell confectionary sunflower seeds (*Helianthus Annuus*) growing; however, a significant proportion of the hybrid seed for planting goes to the snack food market because the extra-large seed is not acceptable to farmers. The extra-large hybrid seed has poor emergence in the field and is not compatible with precision planters. Therefore, the option of coating the hulled sunflower kernels for improved germination and plantability is investigated in this dissertation. Twenty types of kernel coatings have been tested, through collaboration with five seed coating companies and development of our own in-house seed coating capabilities. Coated kernels were tested for germination, seedling vigor, and other indicators of kernel viability. Coated kernels were also tested for plantability using a precision planter test stand. The top-performing coated kernels achieved singulation and post-singulation germination comparable to large planting seed used by farmers. A field trial was conducted in 2017 at Prosper, ND with eight types of coated kernel treatments having zeolite, lime, Polymer A, and Polymer B In-house coating materials each at 30% and 35% build-up levels. Coated kernels produced grain yields up to 55% greater than from XL seeds, and up to 25% greater than large seeds. Live seed emergence of all the coated kernels (93 – 99%) was significantly higher than the XL seeds (88%) and similar or higher than the large seeds (94%). Another small-scale field trial was conducted at Minot, ND, where moisture stressed conditions were observed. Coated kernels showed similar trends to the Prosper location both in terms of live seed emergence and grain yield as compared to XL seeds. Further, an automated image processing method was developed form the RGB images taken with an unmanned aerial vehicle which predicted the emergence counts and a number of multiples in every row of the sunflower field trial at Prosper with R^2 of 0.94 and 0.92. Overall, coated kernels showed significant improvements in achieving plant stand uniformity compared to XL seeds.

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DEDICATION

To my late mother, Joginder Sidhu, who wanted to see me as Dr. Harjot Sidhu, my husband, Dr. Gurjot Dhaliwal for his continuous support and encouragement, and my daughter Olivia, for bringing joy in my life.

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LIST OF DEFINITIONS

Plantability	Seed plantability is the ability of the seed to flow through the planter to allow uniform and consistent plant spacing.
Singulation	Seed singulation is the ability of the planter to plant one seed at a time at a given spacing without any skips and multiples.
Skips	When no seed is planted at the intended planting position.
Multiples	When more than one seeds are planted at the intended planting position.
Crop establishment	Establishment of a good crop stand by attaining uniform emergence as result of maximum seed emergence and evenly spaced seeds in the field.
Sunflower achene/Sunflower seed	Achene is a small dry indehiscent one seeded fruit with a thin wall. For this dissertation, achene and seed terms have been used interchangeably.
Sunflower kernel	Kernel is the fruit of the sunflower surrounded by the pericarp.
Sunflower pericarp	Outer layer of the sunflower achene is called as pericarp.

GENERAL INTRODUCTION

Breeding efforts have steadily increased the size of in-shell confectionary sunflowers (*Helianthus Annuus*) in response to the domestic and export markets, and the demand for larger confectionary sunflower seed is still growing (Lillieboe, 2017); however, farmers are reluctant to plant extra-large seeded hybrids of confectionary sunflower. Use of current extra-large (XL) hybrid confectionary sunflower seed results in skips and doubles during planting because of the inability of the seeds to fit in the current planting equipment, as well as a higher proportion of seed does not emerge upon planting as seedlings are unable to emerge from the tough shell. This significantly reduces the uniformity of the stand and seed yield, and ultimately causes growers to be unwilling to purchase extra-large seeded hybrids.

Because of the abovementioned issues with the extra-large seed, these high-value seeds for planting are sold to the lower cost snack food markets. Therefore, it may be better to remove the hull and then plant the coated kernels. Effective hulling and coating of kernels will eliminate current problems with planting and help ensure that breeding for even larger seeds does not further aggravate the situation. To be fully successful, the hulling process must produce a high yield of coated kernels with excellent germination (>90%). A gentle way to hull the sunflower kernels without impacting the kernels germination using shearing rolls has been developed by Sidhu et al. (2016) by controlling the seed orientation entering the shearing rolls. To further protect the hulled kernels during possible damage inside the planting equipment, or even during transportation and handling operations, kernels must be gently coated to preserve the germination of the hulled kernels. Coating the kernels will further enhance uniformity and vigor of the stand by providing better size uniformity.

Seed coating has been used to improve plantability by increasing the size of sugarbeet (*Beta Vulgaris* L) seeds since the 1970's and turfgrass seeds since the 1980's (Hathcock et al., 1984; Farley, 1978). In recent years, use of seed coating has been expanded to improve seed sowing, seedling emergence, and stand establishment. Advances in seed coating technology offer exciting potential to improve both the physical and physiological properties of the seed.

The private sector owns and controls most of the seed coating technology both in terms of coating equipment and coating materials. In scientific literature, it is very common to outsource the seed coating process to private seed coating companies. There is relatively little independent academic research involving the use of simplified small-scale seed-coating approaches such as laboratory mixers and manual coating (Pedrini et al., 2017). In addition to owing the advances in seed coating technology, these seed coating companies have developed their own tests for evaluating the quality of coated seeds. However, there are no established standards to quantify the quality of the coated seeds. Thus, the collaboration between academia and the seed industry is critical to standardize the quality checks for coated seeds.

In addition to developing the standard quality evaluation tests for coated kernels, it is also important to automate the data collection from the field studies. Image processing methods have been used for the automated stand counts for corn (*Zea Mays* L) (Gnadinger and Schmidhalter, 2017; Varela et al., 2018), automated weed detection in corn fields (Burgos-Artizzu et al., 2011; Berge et al., 2008), seedling vigor of corn (Matthews and Powell, 2011). Ducournau et al. (2004) developed an automated image processing method for monitoring the emergence of sunflower seeds. However, none of the studies addressed the issue of identifying multiple seedlings placed closely, which is an important trait to determine the seed plantability.

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RESEARCH OBJECTIVES

The overall objective of this research project was to evaluate various methods to evaluate the quality of hulled and coated confectionary sunflower kernels. The specific objectives of this research were to:

Objective 1: Development of lab testing protocols to test the germination and plantability of coated sunflower kernels.

Objective 2: Characterize the performance of coated kernels when precision-planted in the field.

Objective 3: Evaluate the effect of various coating materials and build-up levels on the germination and plantability performance of sunflower kernels.

Objective 4: Develop an automatic method using image analysis techniques to automate the plant stand uniformity characterization data from the field of coated sunflower kernels using aerial imagery collected using UAV (unmanned aerial vehicle).

LITERATURE REVIEW

Sunflower seed

Sunflower (*Helianthus Annuus*) seeds are the achenes produced in sunflower heads. Each sunflower head, or inflorescence, comprises of 1,000 to 2,000 individual flowers joined at a common receptacle (Fig. 1.1). The face of the head is comprised of hundreds of disk flowers, which each form into a seed (achene). The seeds are 10-15 mm long and 4 mm broad, cylindrical or drop-shaped. The sunflower seed consists of a hard hull (pericarp) and a kernel, which is the actual seed.

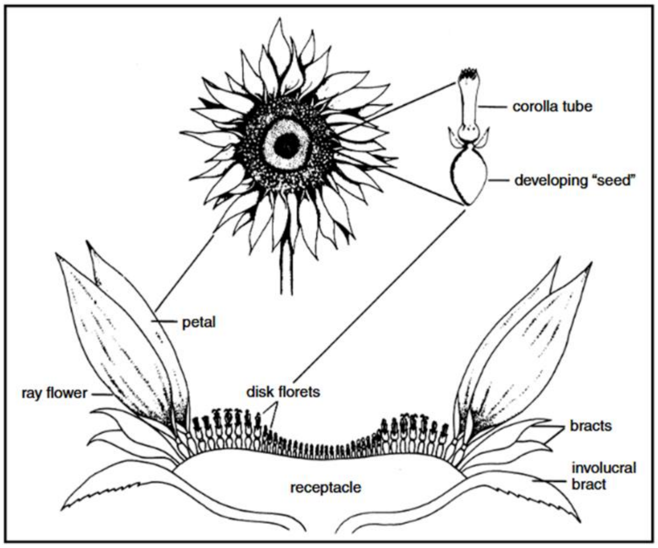

Fig.1.1. Details of a sunflower head (From Berglund, 2007; Fig. 3)

History

Sunflower originated in the U.S., with the southwestern U.S. likely its center of origin. Wild sunflower was used as a food by Native Americans over 4000 years ago (Seiler and Rieseberg, 1997). Sunflower was spread to the other parts of the world, with European countries and Russia being the major producers, following the discovery and settlement of the U.S. (Putt, 1997). Modern sunflower varieties in North American trace much of their lineage back to reintroduced varieties that were developed in Europe and Russia (McClure et al., 2009). Sunflower was not an important agronomic crop in the U.S. until the 1950's. Presently, most U.S. commercial sunflower production is in California, Colorado, Kansas, Minnesota, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas (USDA-NASS, 2017). The production of sunflower grains in the U.S. over the last decade is shown in Fig.1.2.

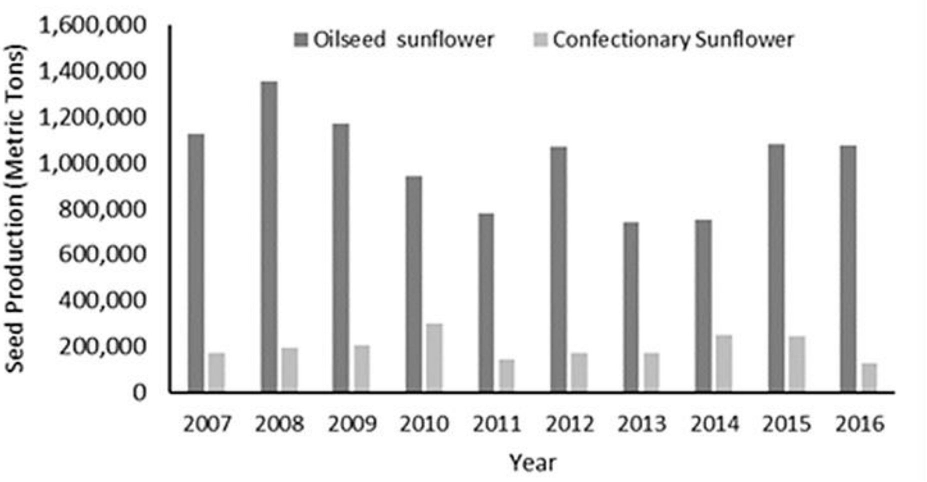

Fig.1.2. Production of oilseed and confectionary sunflower seeds in the U.S. (USDA-NASS, 2017).

Types of sunflower seed

There are two types of sunflower seed: 1) the oilseed type that is grown for vegetable oil, and 2) the confectionary or non-oilseed type grown for the snack food market.

Oilseed

Oil type sunflower seeds contain 40% oil and represent 80-95% of the total sunflower seed production. The hulls (20% of the seed) are black and tend to adhere to the kernels (USDA, 2010). Oil types are grown for their oil, resulting in an oil cake (sunflower meal) which is a popular protein-rich ingredient of livestock feeds (van der Vossen et al., 2007). Discarded seeds from the oil extraction process may be fed whole to livestock (OECD, 2007).

Confectionary

Non-oil or confectionary sunflower contain 30% oil and represent 5-20% of sunflower seed production. The hulls (40% of the seed) are of variable color (black, white, or striped grey/black and white) and are easily removable (USDA, 2010; OECD, 2007; Grompone, 2005). Most of the non-oil seeds are destined for the confectionery market. The smallest seeds (< 0.79 cm) are used as birdseed and pet food (van der Vossen et al., 2007). Seed sizes ranging from 0.71 - 0.79 cm (medium-size seeds), and > 0.79 cm (large-size seeds) in width are used for the edible kernel market, and domestic seed market, respectively. Among the most dramatic trends in the U.S. confection sunflower industry during the past decade has been the expanded demand for large in-shell seeds. Twenty years ago, in-shells comprised approximately 40% of the seeds harvested from the nation's confection sunflower fields; today that percentage is 75-80%, or even higher in a given year (Lilleboe, 2010). The growth of in-shells has been driven by the greater consumer demand for the extra-large seeds (both export and domestic).

Extra-large confectionary hybrid sunflower seeds

The increasing demand for even larger confectionary sunflower seeds resulted in a new category of extra-large confectionary sunflower seeds (seed size > 0.85 cm). While confection growers are economically rewarded for producing the extra-large in-shells, there is, concurrently, a downside: the size of the planting seed. The extra-large confectionary hybrid seeds are larger than their older counterparts. These XL seeds do not fit into the current planting equipment resulting in less consistency in seed placement. These XL seeds also result in the unacceptable germination of < 90% due to the hard hull (Sidhu et al., 2016).

Seed coating

Seed coating technology has been inspired by the pharmaceutical industry for coating medicinal doses. This technology found its application in the agricultural industry in the 1930’s for cereal seeds in Germany. Large-scale applications of seed coating technology in agricultural industry began in the 1960’s in Europe (Ehsanfar and Modarres-Sanavy, 2005). Since then, the practice of seed coating has become the mainstay for many of the horticultural and crop industries worldwide. Seed coating has been used to improve plantability by increasing the size of sugarbeet seeds since the 1970’s and turfgrass seeds since the 1980’s (Hathcock et al., 1984; Farley, 1978). Plantability is the ability of the planter to precisely plant single seed at the given plant spacing without any skips and multipes; skips result due to non-placemnet of seed at the desired spot, and multiples result due to the placement of multiple seeds at the desired spot.

In Agriculture, seed coating is the means of protecting the seed against attack by seed-borne fungi, insects, and destructive influences by unfavorable physical soil and nutritive conditions. Seed coating, in the broadest sense, includes any process for the addition of materials to the seed; in the simplest form, it is the direct application of a material to seeds. The term "coated seed" has been defined as a seed that has been pelleted, tableted, or taped (Roos and Moore, 1975).

Seed coating technology has been majorly owned by the private research sector in regards to both coating equipment and coating materials. Most of the literature coming from the academic world involves a collaboration between seed coating companies. Seed coating technology is comprised of various constituents, which may be introduced onto the seed or they may be applied individually as needed. Coatings can include polymer technology, microbial inoculation, growth regulators, systemic and contact pesticide treatment, and micro and macronutrient applications (Nel, 2013). Each constituent has an important function and can contribute to plant stand establishment problems and to overcome harsh environmental conditions. Higher percent of emerged seeds together with the properly placed seeds duirng planting ensures the success of plant stand establishment. The successful coating can provide various advantages other than protecting the seeds from the physical damage inside the planting equipment. It can protect the seeds during transportation and handling, and during the planting operations while improving the seed appearance and plantability.

Seed coating type

Seed coating types are categorized based on their physical characteristics. Although the nomenclature used in the literature is not consistent, the terminology most used and recognized among industry and academia is based on the weight, size, and sorting properties of the coated seeds. The basic coating treatment is a film coating, where a thin layer of external material (usually < 10% of seed weight) is applied. Where seed weight is increased up to 100–500% (depending on seed morphology), the procedure is described as ‘encrusting’. Where the amount of external material makes it impossible to discriminate the initial seed shape, the process is named ‘coating’ or “pelleting” (Pedrini et al., 2017).

Seed coating equipment

The following three types of equipment standardized in the industry for coating seeds are the rotating pan, fluidized bed, and rotary coater to allow for both automated operations and continuous batch operations.

Rotating pan

The rotating pan was the first machine that was used for seed coating in 1885 (Patent US312041) comprising of a round pan inclined on a rotating motorized pivot. Seeds are placed inside the pan and, while the pan is rotating, liquids are applied with a spray nozzle and powders are added through a hopper or by manual dusting. Rotating pans are mostly used to build-up coating materials on the seed by the gradual addition of materials to increase the build-up (Scott et al., 1997). A lower cost alternative to the rotating pan has been used in some studies too (Hathcock et al., 1984).

Fluidized bed

A fluidized bed which is traditionally used for drying solids was first adapted for seed coating in 1975 (Patent US 3911183). A spray nozzle atomizes the coating material in the form of a liquid or slurry towards the suspended seed mass. This process can be used successfully for film coating on the seed with a very limited application for the creating the build-up on the seeds.

Rotary coater

The rotary coater is comprised of a cylindrical drum, with a concave disk at the base; rotation causes the seed mass to move in a regular flow along drum walls. A smaller rotating disk that is responsible for the atomization and projection of the coating material in liquid or slurry form onto the rotating seed mass. The rotary coater can be used both for the film coating and create build-up applications.

Seed coating materials

The materials used in seed coating are categorized into active ingredients, binders, and fillers**.** The physical and chemical proprieties of the different active ingredients, fillers, and binders provide a variety of possible mechanical and biological outcomes for coatings. Particle size distribution (Scott, 1898), for example, strongly affects the coating behavior; small particles provide higher physical resistance but limited gas and water exchange (Grellier et al., 1999), whereas larger particles increase porosity, but reduce mechanical integrity and coat resilience.

Active ingredients

The most commonly reported active ingredients in coatings include nutrients, fungicides, insecticides, nematicides, predator deterrents, and herbicides.

Binders

Binders are polymers of both natural and synthetic origin that provide adherence and cohesion of material onto the seed and the retention of active ingredients. They are usually applied in liquid form (in water or solvents) and, when dried, the dissolved monomers are rejoined in long polymeric chains forming a continuous film surrounding the seed, binding particles, and chemicals. In the majority of published scientific papers, seed coating has been undertaken with commercial binders of undisclosed composition. However, the most commonly reported binders are methylcellulose, polyethylene glycol, chitosan, polyvinyl alcohol, ethyl cellulose, polyvinyl acetate, and gum Arabic (Pedrini et al., 2017).

Fillers

Coating and encrusting processes require the addition of a bulking agent that allows physical modification. This process is performed with either a single material or a combination of multiple materials. Fillers are usually inert powders, such as bentonite, calcium carbonate, talc, diatomaceous earth, sand, and wood dust.

Sunflower seed hulling

The goal of sunflower hulling has traditionally been for food use applications. Hulling sunflowers for food use is achieved with cracking rollers, hammer mills, bars, discs, or impact hullers (Nag et al., 1983; Miller et al., 1986, Tranchino et al., 1984, Subramanian et al., 1990, Gupta and Das, 1999). The impact huller is commonly used in the sunflower industry where the seed passes through impeller vanes by centrifugal force and impacts a fixed surface, splitting the pericarp and releasing the kernel. For impact hullers, intact kernel content was low at conditions favoring high hulling efficiency (Subramanian et al. 1990; Gupta and Das, 1999). Sidhu et al., (2014) showed that impact hulling resulted in kernel damage of nearly 50%.

Sidhu et al., (2014) evaluated shearing rolls for gentle hulling of sunflower seeds to preserve fragile embryos for future planting. The authors were successful in preserving germination above 90% for the hulled kernels. However, several passes through the rolls were required to achieve a proportion of hulled intact kernels above 80% and increasing the pass number decreased seed germination (Sidhu et al., 2014). In these experiments, no effort was taken to control the seed orientation entering the rolls. In a separate study, the authors took a step further to control the orientation of the seeds entering in the rolls. Hulling was carried out inclining the rolls to 45°, and attaching seeds were entered in the shearing rolls at 45° too, making the seeds to enter in a transverse orientation in the rolls. Seeds were oriented to 45° with the aid of a grooved feeding extension attached to the feed tray at 45°. A 3D schematic of the hulling operation with roll inclination at 45° is shown in Fig. 1.3. This hulling system with controlled seed orientation was able to achieve >85% proportion of hulled intact kernels in just three passes while preserving the germination of hulled kernels > 90%.

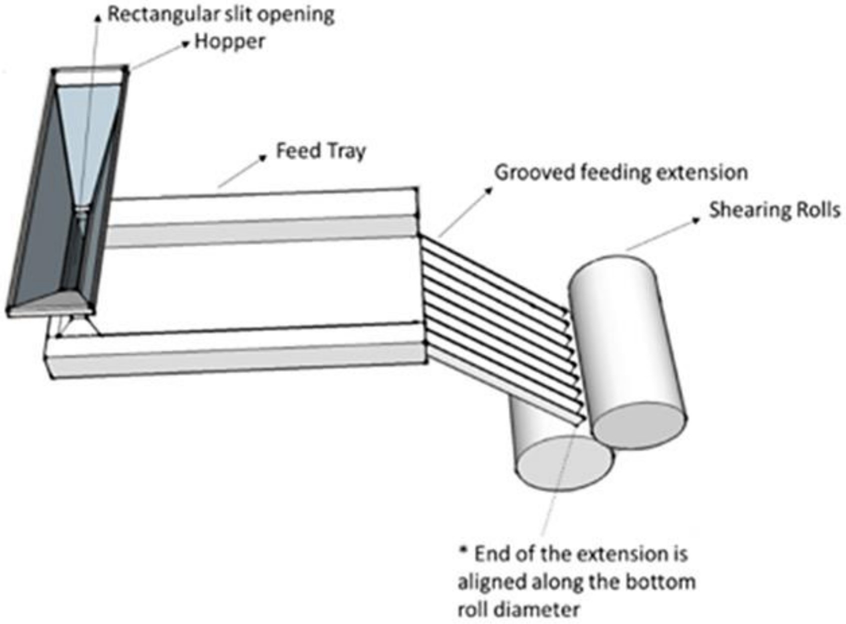

Fig. 1.3. The hulling mechanism set up for confectionary sunflower seeds (From Sidhu et al., 2016; Fig. 3).

Quality assessment of coated seed

Quality control is a parallel process to seed coating as the high-quality seed is essential for all agricultural production. The target of seed coating is to protect the viable kernel with coating agents and other additives while maintaining the seed quality. Ideally, coated seed should perform similarly to the raw seed under similar environmental and field conditions such as climate, soil type, soil moisture, temperature, light etc. The presence of a seed coat can affect the seed performance adversely by delaying radicle emergence and water imbibition (Kelly et al., 1992). No single test is sufficient to evaluate all the attributes that can contribute to both physiological and physical quality of coated seed.

Standard tests to assess the physiological properties of the seeds such as germination, vigor, and storability are well known. However, no standards have been established to evaluate the quality of coated seed. Seed coating companies such as such as Bayer Seed Growth, Incotec, and Summitt seed coating have developed their own tests for quality control of coated seed.

These tests include but are not limited to the following: viability tests, flowability, plantability, and dust control of coated seed (personal communication with Alan Gaul, Iowa State University; June 2016). Standardizing these tests to evaluate the quality of coated sunflower kernels will be of a high value.

Seed viability

Viable seed refers to the seed that is alive and capable of producing normal seedlings under appropriate field conditions. It is very important that the coated seeds are capable of producing plants when sown in the field. Seed must have high viability at the start and end of storage. Seed viability is determined by the standardized germination tests.

Warm germination

Germination is defined as the initiation of active growth by the embryo within the seed that results in rupture of the testa and allows the new seedling to emerge with capabilities of independent growth and development (Barden et al., 1987). The warm germination test is the standard test used to assess the viability of seed lots under ideal conditions. Germination over 90% is desired for coated sunflower kernels (personal communication with Bob Myshak, Red River Commodities; August 2015). For the warm germination test, a representative sample of 25 sunflower seed (3 replications) is planted between double layers of moist germination papers rolled carefully and stored in a plastic tub to prevent moisture loss (Vashisth and Nagarajan, 2010). After storage at room temperature for 7 days, germination counts are recorded and seedlings are classified as being normal or abnormal according to the AOSA (2009) rules. Sidhu and co-authors (2016) found significant differences in germination percent of extra-large sunflower confectionary seeds when germination counts were taken on day 10 instead of at the standard 7 day. This is because of the void space between the sunflower hull and kernel which delays the radicle emergence through the pericarp. More research is needed to standardize the germination count days to account for the late emerging seedlings as some of the coating material can interfere with the seedling emergence rate.

Cold germination

Cold germination tests are conducted to simulate the cold, wet field conditions that early planted summer annual crops experience. These tests simulate early spring field conditions by providing high soil moisture and low soil temperature conditions. In literature, for the testing of sunflower seeds by performing cold germination tests, seeds are typically moistened and chilled at 10°C, followed by covering the seeds with soil, and returning the seeds back to 10°C for seven days without light (Mason et al., 1982). On completion of exposing the seeds to the before mentioned stressed conditions, seeds are moved to room temperature for an additional five to seven days. Seedlings that emerge through the soil are evaluated according to AOSA (2009) rules.

Tetrazolium

Both standard germination and greenhouse tests are the best indicators of seed potential to emerge under field conditions; however, it takes 7-10 days to complete these tests. When a quick test is desired to evaluate seed viability, the tetrazolium (TZ) test can be employed. The Tetrazolium Testing Handbook (Grabe, 1970) reported that tetrazolium test results approximate field emergence under ideal field conditions. German scientist Lakon during the mid-nineteen's (1939-1958) recognized that all living tissues, which respires, are capable of reducing a colorless chemical (2,3,5 triphenyl tetrazolium chloride) into a red colored compound formazen by H transfer reactions catalyzed by the enzyme dehydrogenases (Patil and Dadlani, 2009). Since the tissues within a seed could be at different viability states, only the living parts of viable seed gets stained red. This makes it a very relevant test to check the viability of coated seed to identify the damage caused to the main axis of embryo because of the coating process.

AOSA (2009) standardized the TZ test for sunflower seed. A representative sample of 100 seeds should be tested (2 replications of 50 seed). Seeds are first imbibed overnight on moist media to allow complete hydration of all the seed tissues. Seeds are then placed in 1% tetrazolium solution after cutting it laterally to remove distal end of the cotyledon. Seeds should be allowed to stain overnight at 30-35°C temperature to facilitate the dehydrogenase activity. The stained seeds are then inspected subjectively according to the criteria described by AOSA (2009).

Seed vigor

The seed vigor test is an expansion of the standard germination test. Vigor testing does not only measure the percentage of viable seed in a sample, it also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Seeds may be classified as viable in a germination test which provides optimum temperature, moisture, and light conditions to the growing seedlings; however, they may not be capable of continuing growth and completing their life cycle under a wide range of field conditions. Generally, seeds start to lose vigor before they lose their ability to germinate; therefore vigor testing is an important practice in seed production programs. The use of high vigor coated seed is recommended. The advantage of high-vigor seed is most apparent early in seedling growth and is often associated with an increased rate of emergence and stand establishment (Egli and Tekron, 1995). The procedure for conducting vigor test for sunflower seed has been summarized in the Seed Vigor Testing Handbook, published by

AOSA (2009). The most common method of determining seedling vigor is by taking the dry weight or length of the seedlings (Vashisth and Nagarajan, 2010).

Water imbibition

Germination of seeds begins by imbibing water (Campbell, 1993). The imbibition of water activates the inactive seed by initiating the respiration and biosynthesis which facilitates the seed germination (Mayer and Poljakoff-Mayber, 1974). Bewley and Black (1987) described the seed imbibition process as triphasic. Phase 1 occurs due to the very steep gradient of water potential between the seed and its environment. During phase 2, very little changes occur in the seed water content as seed water potential reaches the equilibrium state with the water. Water uptake again begins during the phase 3 because of call enlargement occurring during the onset of radicle extension, without changing in embryo's water potential. McMohan et al. (2007) ascribed the water abortion phenomena to simulatory hormones such as gibberellin (GA) which activates enzyme systems. The activated enzyme systems, in turn, begin converting the endosperm into nutrients which can be translocated to growing regions of the embryo. Once the GA provides a chemical signal to the aleurone surrounding the endosperm, amylase is produced, and starch can be digested in order to provide the necessary energy for embryo growth, radicle emergence, and plumule development (Campbell, 1993).

The application of the seed coating can alter the water uptake potential of the seed which in turn can affect germination (Taylor 2003). Ideally, water imbibition rate of both uncoated seed and coated seed should be similar. Percent water uptake, commonly used to express seed water potential, is calculated as follows $[W_1\text{-}W_d/W_d]$ x 100, where W_1 and W_d = mass of imbibed and dry seeds, respectively (Turner et al., 2006). For this test, 3 replications of 20 seeds are weighed and put over filter paper, saturated with distilled water, in a petri dish stored in an incubator at 20

± 2°C. Dishes are removed from dishes at predetermined times, and the seeds are drained and surface water removed by blotting between sheets of paper towel. The seeds are then weighed and returned to the incubator.

Accelerated aging

The Accelerated Aging (AA) test is used for determining the storability and longevity of seeds by exposing the seeds to the aging process under high relative humidity and temperature conditions. For sunflower seeds, the accelerated aging test is performed by exposing the seeds to 45°C and 100% relative humidity for up to seven days (Hussein et al., 2011), followed by testing the warm germination of the seeds and classifying the seedlings according to AOSA (2009) rules.

Sunflower seed germination impacts negatively at 45°C, and incubation of seeds at such high temperature results in a progressive reduction of further germination at 25°C and induces abnormal seedling growth (Corbineau et al. 1988, Gay et al. 1991). The work of Corbineau et al. (2002) establish that a sequence of irreversible cellular and metabolic damage is associated with deterioration of sunflower seeds during incubation in water at 45°C.

Dust-off tests

Coated seed can pose several health and environmental hazards due to the dustiness of the coating material. Dust-off is a measurement of the degree to which the applied seed coating material can flake off during seed handling (Platzen, 2010). Dust-off tests are important for coated seed, especially in case of added fungicides, insecticides, and colorants in the coating formulation. A commonly used test protocol involves drawing an air current under slight vacuum through a seed mass that is agitated for a short period, and then weighing the dust retained on a filter. No American standards exist for the dust-off limits; however, these dust-off limits are set in Europe. European limits for dust from coated various seed are presented in Table 1.1:

Table 1.1. Dust-off limits set in Europe.

Seed	Dust-off limit
Maize	0.75 g/100,000 seeds*
Oilseed rape	0.75 g/70,000 seeds
Sugar beet	0.25 g/100,000 seed pellets
Sunflower	0.4 g/75,000 seeds

* Number of seeds correspond to sowing 1ha

Seed singulation/Plantability

Spacing uniformity and the germination after the seed passed through the planter are the most common characteristics used by producers in evaluating planter and seed performance (Liu et al., 2004). Spacing uniformity is commonly measured by the seed singulation. Seed singulation is a planter performance characteristic that ensures that only a single seed is picked up and fed to the planter, enabling precise timing and delivery of seeds to the soil. Knowing seed properties as an input parameter is important as singulation systems can be adjusted for seed density and/or a specific size. The changes may be as simple as an air pressure or vacuum adjustment, or they may involve completely changing the metering disk or plate. Typically, John Deere seed plate (Part Number A52390) consisting of 40 round holes of 0.3 cm diameter and 1 cm spacing, is used for planting confectionary sunflower seeds (Lilleboe, 2008).

Field trial

The field trial is an important check for determining the quality of the seeds under real conditions. Field trials for sunflower seeds are adapted from the sunflower production practices. Berglund (2007) summarizes the production practices for sunflower seeds. Sunflower is adapted to a variety of soil conditions but grows best on well-drained, high water-holding capacity soils with a nearly neutral pH (pH 6.5-7.5). Sunflower has roots of 1.2 m depth and can extract water from below this depth, thus perceived as a drought-tolerant crop. Sunflower planting may begin as early as two weeks before the last killing frost report for the previous year and as late as 100 days before the first killing frost reported for the fall of the previous year, usually ranging from May 1 until late June in the northern Great Plains. Sunflower seeds are harvested about four months after planting.

For confectionary sunflower field trials, the recommended plant population is between 37,000 and 45,000 plants per hectare (Berglund, 2007). Sunflower should be planted 2.5 cm to 5 cm deep in the soil. Additional details on the nutrient requirements and pest management for the sunflower crop is included in the Sunflower Production guide Berglund (2007).

Sunflower plants pass through two main development stages from planting to harvest, and these development stages are classified as vegetative and reproductive stages as developed by Schneiter and Miller (1981). Table 1.2 contains a description of sunflower growth stages. Determining the stage of development is based on using the main branch or head and not branch heads. Generally, sunflower reaches R1 or bloom stage of about 65 to 70 days after planting and maturity about 105 to 115 days after planting (Aiken, 2005).

Remote sensing

Currently available high-resolution satellite-based imaging systems provide exciting opportunities for remote sensing applications in precision agriculture. However, there are several limitations to using satellite acquired imaging for making real-time farming management decisions. Additionally, the spatial resolution of the collected imagery is pretty low, and the quality of the satellite acquired imaging can further be affected by weather conditions. On the other hand, ground-based remote sensing systems are not practical to cover a wide farming area and can be difficult to use under muddy soil conditions. In recent years, imaging systems mounted on unmanned aerial vehicles (UAV's) have shown a promise to overcome above-mentioned challenges and are able to collect high-resolution imagery in real time.

Table 1.2. Description of sunflower growth stages (Berglund, 2007)

Sunflower Stage	Description
VE	Emergence
V1 to n— Vegetative stages	Determined by counting the number of true leaves at least 1.5 inches in length beginning as V-1, etc. If lower leaves have dropped, count leaf scars.
V20	20 True leaves
R1— the beginning of Reproductive stages	The terminal bud forms a miniature floral head rather than a cluster of leaves. When viewed from above, the immature bracts have a many-pointed starlike appearance.
R2	Immature terminal bud < 1 inch above nearest leaf attached to the stem. Disregard leaves attached to the back of the bud
R3	Immature bud > 1 inch above the nearest leaf.
R4	The inflorescence or bud begins to open. When viewed from above, immature ray flowers (on the outer edge of the head) are visible.
R5	Beginning of flowering. Can be divided into sub-stages dependent on the percentage of the head area (interior disk flowers) that has completed or is in flowering; e.g., R 5.3 = 30% of head area completed flowering, R 5.8 = 80%, etc.
R5.5	50% flowered
R6	Flowering is complete and ray flowers (on the outer edge of the head) are wilting.
R7	Back of head has started turning pale yellow.
R8	Back of head is yellow but bracts (behind ray flowers) remain green
R9	Bracts become yellow and brown. Physiological maturity.

Unmanned aerial vehicle (UAV) technology was first implemented in Japan to spray chemicals in an agricultural field in the 1980's (Nonami, 2007). Since then, the use of UAV technology for various precision agricultural applications has risen because it offers an affordable alternative to satellite and ground-based imaging systems to collect high-resolution imagery in real time. Some of the examples of application of UAV technology include crop scouting, weed management, livestock monitoring, frost mitigation, stand count, and fertilizer application (Bryson & Sukkarieh, 2011; Jensen, Baumann, & Chen, 2008; Mancini et al., 2013;

Sato, 2003; Torres-Sánchez et al., 2013; Turner, Lucieer, & Watson, 2011). Images collected using remote sensing technologies are processed using image processing techniques.

Image processing

The basic definition of image processing refers to the processing of the digital image, i.e removing the noise and any kind of irregularities present in an image using the computer. Application of various image processing techniques has been started for the US. Military and Defense during the last four to five decades (Nasrabadi, 2013). Digital image processing became popular in the 1970s due to the availability of inexpensive computers. With emerging powerful personnel fast multicore computers, large size memory devices, graphics software in the 2000s, digital image processing has become the most common form of processing images in real time (Nasrabadi, 2013). Currently, image processing is used for various applications such as remote sensing, medical imaging, forensic studies, textiles, material science, military, film industry, graphic arts, and printing industry (Chitradevi and Srimati, 2014).

The use of image processing techniques in precision agriculture allows researchers and farmers to gain a real-time in-depth understanding of the crop field in terms of stand count, plant diseases, vegetation index, type of weeds, and pests etc. Chahal (2015), and Ugale and Gupta (2016) have summarized various image processing applications in the agricultural sector. No literature has been found on identifying multiple seedlings placed closely during planting. It is challenging to identify multiples as the leaves of those seedlings overlap, making those seedlings count as one during the image analysis.

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PAPER 1: PERFORMANCE OF COATED EXTRA-LARGE HULLED CONFECTIONARY SUNFLOWER KERNELS FOR PRECISION PLANTING

Abstract

Extra-large (XL) confectionary sunflower seeds are too large to plant with current precision planters, and a high proportion of seed does not emerge upon planting. Hulling the XL seeds and then coating the kernels has been proposed to improve the viability and plantability of these seeds. Therefore, the objectives were to evaluate the effect of various coating materials and build-up levels, develop lab methods to measure the viability and plantability of coated kernels, and evaluate the impact of seed lubricants on plantability of coated kernels. In this study, eight types of coating materials were applied to sunflower kernels by seed coating companies, or in-house at build-up levels ranging from 8-50% of build-up levels resulting in 20 different treatments, and the coated kernels were then evaluated for viability and plantability. The pre-planting germination test results ranged from 72 to 92% among all coated kernel treatments; however, germination was reduced by 6 % on average after passage through the planter test stand. The singulation of all the coated kernels improved by up to 24 % compared to the XL seed. Singulation of polymer-coated kernels was comparable to large seed. Addition of lubricant to the coated kernels significantly increased the overall singulation and post-planting germination of the coated kernels by 4% and 3%, respectively. Overall, this study showed that coating of the kernel — together with the use of a seed lubricant — substantially increased singulation compared to XL seeds, while retaining the kernel viability.

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Introduction

Global consumer demand for very large in-shell confectionary sunflower seeds is spurring development of new varieties with even larger seeds (Sidhu et al., 2016; Lilleboe, 2010). However, the poor germination and poor plantability of the extra-large (XL) size fraction of hybrid planting seeds makes that size fraction unpopular among farmers (Lilleboe, 2017). If XL seeds could be made acceptable for use with precision planters, their value would be greatly increased. Hulling the XL seeds and coating the hulled kernels is likely the best solution to achieve acceptable germination and plantability, and will, in turn, add significant value to these seeds. Sidhu et al. (2016) demonstrated the gentle removal of the hull of these XL seeds while achieving kernel germination >90%; however, additional work is required to improve the plantability of the kernels.

The fragile nature of the hulled kernels increases their susceptibility to breakage during handling, shipping, and planting. Coating the kernels prior to planting may protect the kernels inside the planting equipment to preserve the seed viability while improving the plantability. Seed coating was introduced by the vegetable seed industry in the 1930's to increase the seed size and uniformity, and evolved since then to improve sowing, seedling emergence, and stand establishment (Kaufman, 1991; Roos and Moore, 1975). Seed coating also allows the incorporation of insecticides, fungicides, herbicides, nutrients, and biological agents to the seed (Nascimento et al., 1993); thus increasing the effectiveness of these additives.

The private research sector owns the major advances in seed coating equipment and coating materials; there is relatively little independent academic research involving the use of simplified small-scale seed-coating approaches such as laboratory mixers and manual coating (Pedrini et al., 2017). In the recently published literature, the coating process is often outsourced to private seed companies which use rotary coaters and proprietary coatings (Barut, 2008; Taylor, 2001). This collaboration between academia and the seed industry is critical to address the challenges posed to modern mechanized agricultural systems.

Different materials have been used to coat seeds such as diatomaceous earth to improve plantability of tomato and lettuce seeds (Sikhao et al., 2015), chitosan in combination with acetic acid to protect the soybean seeds from pests (Zeng et al., 2012), alkaline phosphatase to improve plant growth for barley seeds (Pilar-Izquierdo, et al., 2012), an inorganic mixture based on clay minerals and a silicate compound to improve plantability of sesame seeds (Barut, 2008), clay loam soil to improve plantability of rice seed (Yoo et al., 2001), and sand to improve germination of lettuce seeds (Sooter and Miller, 1978). However, none of the studies tested the effect of coating build-up level on seed performance.

Seed coating materials can impact the seed viability by altering the seed water status (Taylor, 1992) and oxygen diffusion (Gallardo et al., 2001) by creating a barrier between the seed and the planting environment. Also, the seed viability can be affected by the seed coating process, especially for fragile seeds. In addition to the seed coating process, the seed coating materials and the build-up level can also affect the seed viability and the planting characteristics by physically changing the seed shape and size (Kaufman, 1991). Thus, it is important to accurately evaluate the coated seed quality; however, there is a need to modify the existing standardized tests to assess the viability and plantability of the coated seeds. Furthermore, the addition of a lubricant to aid seed flowability inside the planters is common practice (Platzen, 2010), and thus lubricants should be evaluated together with the coated seeds for plantability tests.

In this study, the effect of various coating materials and build-up levels on the germination and plantability performance of sunflower kernels was examined. This paper also describes the development of lab testing protocols to test the germination and plantability of coated sunflower kernels. Use of seed lubricants to improve the plantability of coated sunflower kernels is also assessed.

Materials and methods

Materials

First generation (F-1) extra-large (XL) hybrid confectionary sunflower seed of the variety “2215” was obtained from Red River Commodities (Fargo, N.D.) which were grown in 2016 in Sacramento, CA. 2,3,5-Triphenyl-2H-tetrazolium chloride for the tetrazolium test was purchased from VWR (Chicago, IL). To conduct the lab germination tests, seed germination paper (30.5 × 45.7 cm) was purchased from Anchor Paper Company (Saint Paul, MN.). The polymer “Seedworx Ag Shine” for in-house coating trials was obtained from AgInnovation (Walnut Grove, CA). The formulations of the six different commercial coating materials are proprietary and identified only by type as discussed in section “*Commercially-applied coating*”.

Hulling and separation

Seed received from Red River Commodities were first cleaned using a Carter Day dockage tester (Seedburo Equipment Co., Des Plaines, IL) to remove foreign particles. Before hulling, the seed moisture content (dry basis) was reduced from 8.3% to 5.7% by drying the seed in a convection oven at 40°C according to the methods of Sidhu et al., (2016). The conditioned seeds were then hulled at a feed rate of 226 ± 7 g min-1 using corrugated rubber shearing rolls (Kamper Fabrication, Ripon, CA) operated at a 1.29 differential roller speed. The seed entered the rolls inclined at 45° at a transverse orientation with the aid of a grooved feed tray. Details of the hulling process and design can be seen in Sidhu et al. (2016). A schematic diagram of the hulling and separation process is presented in Fig. 2.1. The hulling was carried out in three passes in which the gap between the two rolls was 0.21 cm for the first two passes and 0.14 cm for the third pass. Only the inshell seeds were again passed through the shearing rolls during the second and third passes. The inshell seeds after each pass were separated from the mixture of hull, broken kernel, and intact kernel by using a Carter Day dockage tester according to the USDA-GIPSA (2013) methods for sunflower and a gravity table (Model 10-M2, Forsberg Inc., Thief River Falls, MN). The gravity table was operated using a 12-mesh trapezoidal-shaped deck at the following conditions: feed rate of 6 g/s, deck oscillation of 120 cycles/min with an 11 mm amplitude, airflow through the deck mesh of 1.2 m/s, end raise slope of 9°, and side tilt of 4°. The broken kernels were removed from the intact kernels by using an Oregon Seed Blower (Hoffman Manufacturing, Jefferson, OR) according to the method of Sidhu et al. (2014). The intact kernel fraction was further inspected and broken kernels were manually removed.

Coating trials

Commercially-applied coating

Hulled kernels were coated with six types of coating materials and at up to three levels of build-up (amount of coating expressed as weight percent of the uncoated kernel). This resulted in 18 treatments: Gypsum 8%, Gypsum 34%, Gypsum 50%, Zeolite 8%, Zeolite 34%, Zeolite 50%, Lime 8%, Lime 34%, Lime 50%, Pumice 8%, Pumice 34%, Pumice 50%, Cellulose 20%, Cellulose 47%, Polymer A 35%, Polymer B 25% + Talc 5%, Polymer B 35% + Talc 5%, and Polymer B 50% + Talc 5%. These 18 treatments were applied by three different companies in the US. All three companies used a rotary coater to coat the kernels and a fluidized bed to dry the coated kernels. Build-up levels were verified in-house by subtracting the average weight of 100 kernels from the weight of 100 coated kernels.

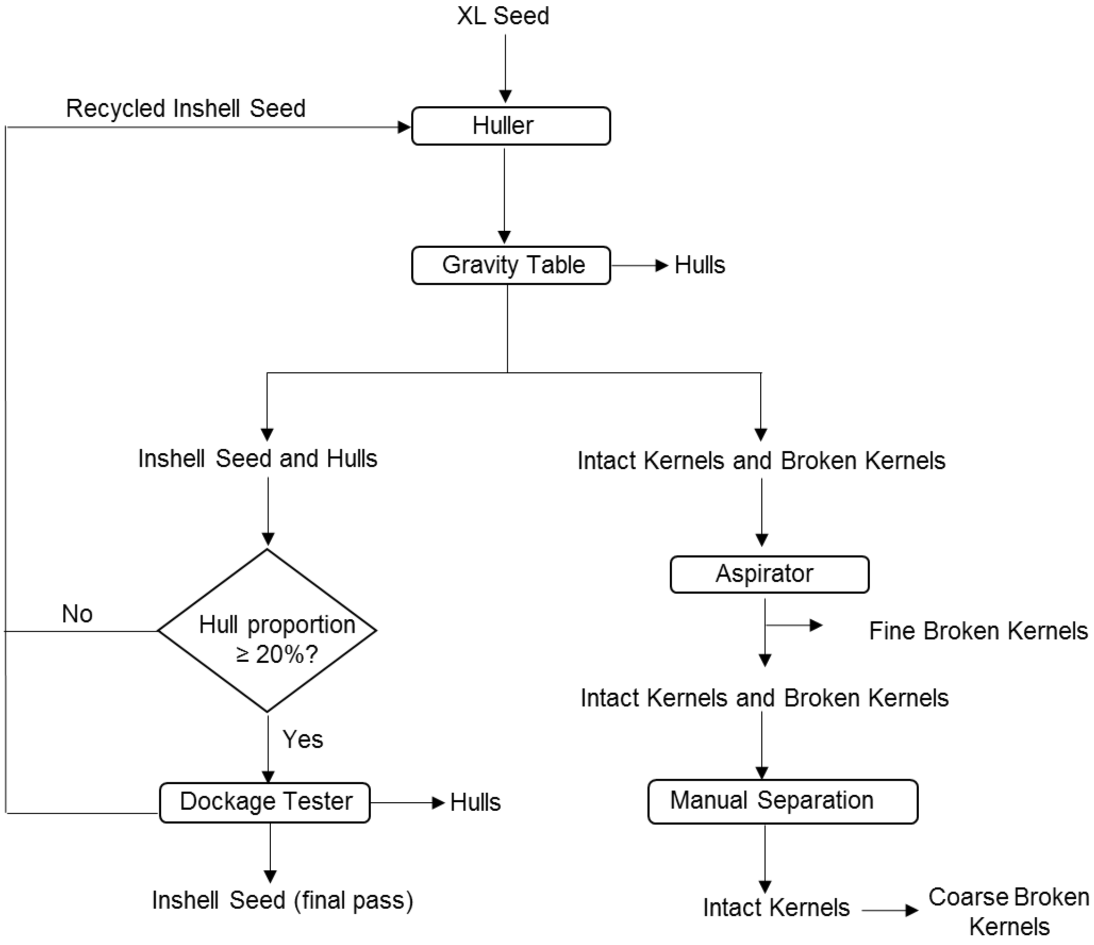

Fig. 2.1. Hulling and separation process of extra-large confectionary sunflower seeds.

In-house coating

A liquid polymer C, Seedworx Ag Shine, was used for in-house coating trials. Hulled kernels (1 kg) were coated with this polymer at 18% build-up using a USC tabletop lab treater (USC, LLC, Shakopee, MN) at 35 Hz speed for 2 min. For a control, 500 g of kernels were hand coated at 16% build-up with the aid of a number 4 paint brush. The coated kernels were dried on a sieve bed at room temperature (24°C ±1) for 3 h with the aid of a 5 blade propeller box fan (Model 3723, Lasko Products Inc., West Chester, PA) at an air speed of 6.5 m/s relative to sieve bed and air direction parallel to and across the top surface of bed. Air speed was measured using a thermo-anemometer (Extech Instruments, Nashua, N.H.). Build-up levels were verified by the method described in section "*Commercially-applied coating*".

Germination and imbibition

Germination and vigor

Germination tests were performed according to AOSA (2009) methods for sunflower seeds with two modifications. One modification was that germination count was taken on the 10th day instead of the standard 7th day to include late-emerging seedlings. The other modification was dipping the seeds in 20% bleach solution for 2 min to inhibit fungal growth during germination, followed by a 2 min rinse with distilled water. The germination test was conducted in triplicate and in each replicate, 25 seeds were placed between two saturated germination papers, rolled carefully, and then stored in an airtight plastic tub to prevent moisture loss. After storage at room temperature (25±1°C) for 10 days, germination counts were recorded and each seedling classified as normal or abnormal according to AOSA (2009). Germination was defined as the proportion of kernels that produced normal seedlings. An adaptation of the method used by Abdul-Baki and Anderson (1973), and Vashisth and Nagarajan (2010) was used to determine average seedling dry weight as a measure of seedling vigor. Ten normal seedlings from each replicate were transferred to an aluminum weighing dish, dried overnight in an oven at 90°C, and weighed.

Tetrazolium test

Seed viability of the samples was also quantified using the tetrazolium test according to AOSA (2009). Fifty seeds of each treatment in triplicate were allowed to imbibe moisture in between two saturated germination papers overnight at room temperature (25±1°C). Each seed was then sectioned longitudinally and incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride in an oven at 20 °C for 2 h. Seeds in which the embryo exhibited red staining were scored as viable.

Water uptake

Water uptake as a function of time was determined by the water imbibition test which was conducted in triplicate. For each replicate, 3 g of seeds were placed in petri dishes lined with a double layer of germination paper saturated with distilled water according to the methods of Kaya and co-authors (2006). Seed weight was recorded at the following time intervals: 0, 2, 4, 6, 8, 12, 16, 18, 24, 32, 48, 60, and 72 h. The water uptake was expressed as the percentage increase in moisture content on a wet weight basis.

Accelerated aging

Accelerated aging was performed by holding the seeds at 45 °C for 48 h and 72 h in sealed buckets at 100% relative humidity (method adapted from Bailly et al., 1997). Seeds were placed on top of wire-mesh trays (14 cm x 14 cm) stacked in groups of 5 trays per 18 L bucket with 5 cm spacing between each tray. Distilled water (50 mL) was added to a 500 mL beaker on the bottom of each bucket. Each treatment consisted of 50 seeds per tray in triplicate with each of the replicate in a different bucket. To determine the loss in viability as a result of accelerated aging, germination tests were conducted as described in section "*Commercially-applied Coating*".

Plantability

A precision planting test stand equipped with a MeterMax and 20/20 SeedSense (Precision Planting, Tremont, IL) was used to determine plantability of the different seed samples. The precision planting test stand was operated with a John Deere seed plate (Part

Number A52390) consisting of 40 round holes of 0.3 cm diameter and 1 cm spacing (Lilleboe, 2008), and the following settings: 457 mm H2O vacuum pressure and seed drop rate of 33 seeds/m. The vertical distance to tube exit and the vertical distance from the tube exit to ground was 143 and 50 cm, respectively. Seeds exited from the bottom of the square seed tube with rearward curvature and were collected in a plastic bucket. Each treatment included two replicates with 1000 seeds/replicate. Plantability for each treatment is reported as seed singulation (%). The seeds that passed through the test stand were then re-evaluated for germination (as described in section "*Germination and vigor*").

Plantability tests were conducted with and without the use of a seed lubricant. Two lubricants were tested: talc (EZ-Slide Talc Powder Seed, Lincoln, NE) and Fluency Agent (Bayer CropScience, Bloomington, IL). Seed lubricant was thoroughly mixed with the seed at a rate of 5 g/1000 seeds in the case of Fluency Agent, and 10 g/1000 seeds in the case of talc.

Statistical analysis

Data was analyzed using analysis of variance (SAS v9.3, SAS Institute Inc., Cary, N.C.), and an F-protected LSD ($P \leq 0.05$) was calculated for comparisons of main effect means.

Results and discussion

Hulling and separation

The fixed process conditions for hulling recommended by Sidhu et al. (2016) for hulling XL confectionary sunflower seeds were used in this study; however, on a bigger batch size of 23 kg instead of 500 g used in the previous study. The intact kernel yield obtained from hulling was 74% after 3 passes. The hulling performance is similar to the results reported by Sidhu et al. (2016). The average time to produce hulled kernels from the inshell seeds, including hulling and separation steps, was 4 h/kg kernels, in which 60% of the time was from the manual separation of broken and intact kernels. This is a limitation which must be overcome for any future industrial production of hulled kernels.

Viability

Germination and vigor

XL seeds resulted in both significantly lower germination of 85% and higher abnormal seedlings of 5% as compared to the large seeds (germination of 90% and 2% abnormal seedlings) as shown in Table 2.1 and Fig. 2.2. This could be linked to the relatively large void space between the hull and the kernel, and a harder shell in XL seeds as compared to the large seeds. Sidhu et al. (2016) reported the void space in flat oriented XL seeds to be > 0.3 cm compared to 0.2 cm in large seeds. XL seeds that outwardly appeared to not germinate were opened to reveal that a seedling had formed, but wrapped around the cotyledon and failed to emerge through the shell. In XL seeds, 58% of ungerminated seeds exhibited this phenomenon; whereas, none of the ungerminated large seeds showed this effect. As mentioned in the introduction, the low germination and high proportion of abnormal seedlings motivated our interest in removing the hull from the kernel for XL seeds. The resulting kernels exhibited germination of 92% and abnormal seedlings of 1%. However, the kernels are expected to be too fragile for storage, transportation, and planting, hence it is important to develop and evaluate a suitable coating for the kernels.

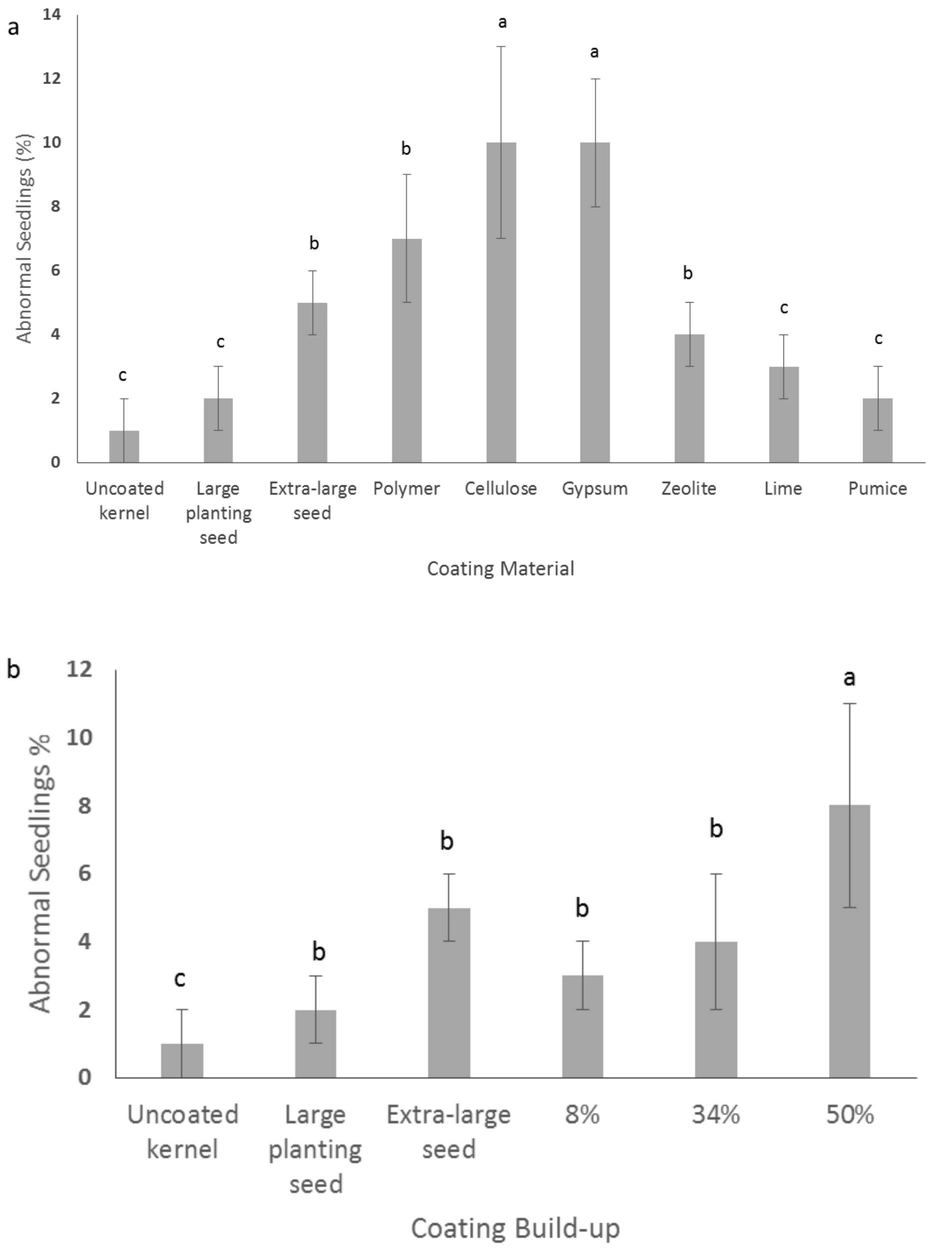

Fig. 2.2. Percent abnormal seedlings as a result of coating material during warm germination for (a) coating material (b) coating build-up level. Means followed by the same letter are not statistically different at P≤0.05

Among all the commercial coated kernel treatments, germination ranged between 72-92% (data not shown). When germination was averaged across the same type of coating material, zeolite-coated kernels had the highest germination (89%), followed by lime and pumice (86%

each), polymer A (85%), polymer B (81%), gypsum (79%), and cellulose (78%) as shown in Table 2.1.

The differences in germination seen with the different coating materials were probably due to these two causes: 1) water and gas transfer properties of the coating materials, and 2) mechanical damage to the kernels during the coating, drying, and/or shipping processes. Seed germination depends on several environmental factors such as water, temperature, light, and gas transfer. Physical properties of the coating material such as permeability and porosity can affect the degree to which these environmental properties affect the water and gas transfer in the seed. Less porous material can induce water and/or oxygen stress in seeds, which affects the metabolic activities required for germination (Gallardo et al., 2001).

Porosity is determined by the packing fraction of the material. The high germination of zeolite (89%) and pumice (86%) coated kernels may be partly explained by the highly porous structure of the coating materials. Zeolite is a microporous crystalline mineral having a porosity of 95% (Polat et al., 2004). Pumice consists of highly porous glassy fragments and exhibits a high porosity of > 90% (Karaipekli et al., 2016). High porosity allows the easy movement of molecules and ions into and out of these structures which aids absorption of water and gases. Even though porosity is a major factor that allows the water and gas exchange, the pores must be connected to allow flow. This ability of water or gas to flow through the material is called permeability. Though limestone has a low porosity of 30%, its pores are well connected to each other (Pipilikaki et al., 2009). This contributes to the high germination (86%) associated with the lime-coated kernels. (Section "*Water uptake*" of this paper further explores the water uptake of the coated kernels).

Table 2.1. Performance of the coated sunflower kernels and controls based on the viability and accelerated aging tests.

				Accelerated Aging Germination	
	Germination	Seedling Dry Weight (g)	Tetrazolium Germination %	Germination % - 48 h	Germination % - 72 h
Uncoated kernel	92a	0.085a	92a	72a	67a
Large planting seed	90a	0.084a	90a	70a	65a
Extra-large seed	85b	0.081b	88b	65b	59b
Effect of Coating Materials [a]					
Polymer A	85b	0.074c	88a	70a	68a
Polymer B + Talc	81c	0.077b	83c	69a	68a
Polymer C – In-house hand coated	91a	0.079b	93a	72a	65a
Polymer C – In-house machine coated	90a	0.082a	91a	70a	62a
Cellulose [a]	78c	0.072d	79c	66b	58b
Gypsum [a]	79c	0.076c	81c	65b	57b
Zeolite [a]	89a	0.078b	89a	69a	59b
Lime [a]	86b	0.080b	88a	68a	57b
Pumice	86b	0.076b	87b	71a	68a
Effect of Coating Build-up % [b]					
8%	88b	0.076c	89a	69a	62b
34%	85b	0.075c	87b	69a	61b
50%	82c	0.071d	83c	67b	59b

Means followed by the same letter within the same column are not statistically significant at $P \leq 0.05$

[a] Results were averaged across all the build-up levels for the same coating material type

[b] Results were averaged across gypsum, zeolite, pumice, and lime within the same build-up levels

Increased coating build-up from 0 to 8% improved the germination, but progressively greater build-up showed no benefit (Table 2.1). For example, increasing build-up further to 34% only resulted in statistically similar germination, and further build-up to 50% resulted in reduced germination, as well as more abnormal seedlings (8%, compared to 3% and 4% abnormal seedlings from 8% and 34% build-up levels, respectively; Fig. 2.2(b)). This might be because of the increased resistance to moisture transfer.

The in-house coated kernels achieved the highest germination and lowest abnormal seedlings among all the coated kernels. The in-house coating and drying methods may have been gentler than the methods used to produce the commercial coating methods. Germination of the commercial coated kernels may also have been reduced by damage caused during shipping and handling. Chaki and co-authors (2011) studied the effects of mechanical wounding on sunflower hypocotyls by pinching with striped-tip forceps and concluded that this triggers oxidative and nitrosative stresses which reduce germination. Similarly, mechanical damage to the kernels might trigger germination-inhibiting stresses in the coated kernels; the stresses induced in mechanical-wounded kernels warrants more research.

Seedling dry weight for the intact kernels (0.085 g) was statistically similar to that of the large seeds (0.084 g) but higher than the XL seeds (0.081 g) as shown in Table 2.1. In-house hand coated kernels resulted in high vigor, as indicated by the highest seedling dry weight of 0.082 g among all the coated samples. Coated kernels with 50% build-up resulted in the lowest seedling dry weight of 0.071 g among the three different levels of build-up, indicating that the emerging seedlings might have been delayed due to the increased resistance to moisture transfer imposed by the thick build-up. The germination results are significantly correlated with the seedling dry weights at the Pearson's correlation coefficient of 0.73 and a p-value of 0.002.

Vashisth and Nagarajan (2012) reported that the sunflower seed with the highest germination had higher vigor too. However, in-house hand-coated kernels and commercial zeolite-coated kernels had relatively low seedling weights, despite high germination. This could be due to seed coat-induced dormancy, or the stresses induced in the seed.

Germination counts were also taken on day 7, in addition to the day 10 results reported in Table 2.1, to evaluate the effect of time on germination (Fig. 2.3). Significant differences were not found between 7 and 10 d for kernels and large seeds. The AOSA standard (2009) states that the sunflower seeds require 7 d to show the full germination potential. However, in contrast to the large seeds, XL seeds showed delayed germination and 10 d more accurately shows the germination potential. The late emergence observed from the XL seed may be due to the harder shell and/or greater void space between the hull and kernel. As a result, the enclosed kernel may require more time to achieve the moisture content needed for the germination process to commence. Germination was not complete at 7 d for coated kernels, except for lime, pumice, zeolite, and gypsum at 8% build-up level. The differences observed between 7 and 10 d with the other coated kernels is likely due to the greater resistance to water uptake caused by the increased build-up.

The tetrazolium (TZ) test was used to check if this test can be used as a fast, simple screening method and as an alternative to the germination test. The Tetrazolium Testing Handbook (Grabe, 1970) reports that the TZ test results approximate the standard germination test results. Actively respiring parts of the seed reduce a colorless chemical (2,3,5 triphenyl tetrazolium chloride) into a red colored compound, formazen, by H transfer reactions catalyzed by the enzyme dehydrogenases (Patil and Dadlani, 2009). While the germination test takes 7-10 days to be completed for sunflower seeds, the TZ test can be finished within 24-48 h.

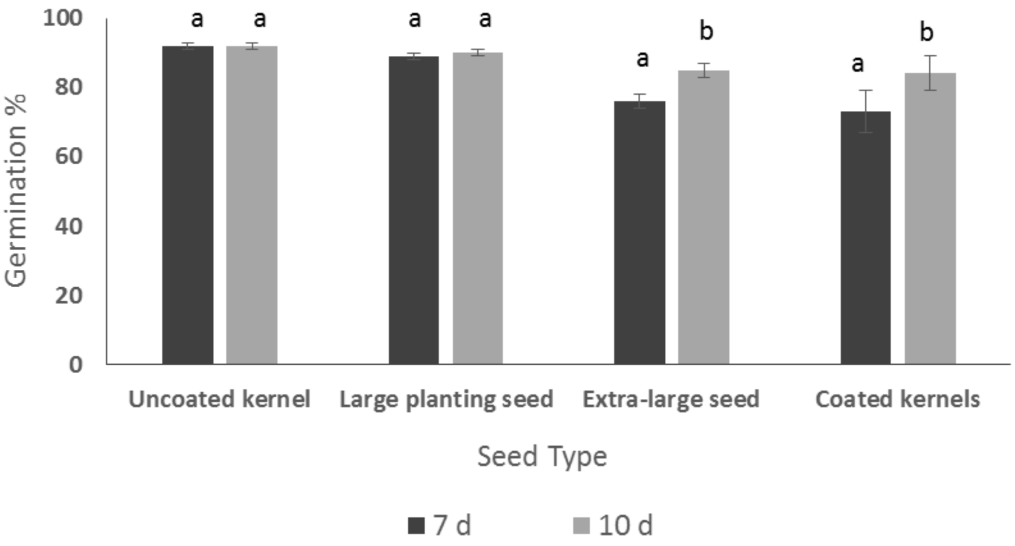

Fig. 2.3. Effect of time taken for the various seed types to germinate. Means followed by the same letter for the same seed type are not statistically different at P≤0.05

Tetrazolium

The TZ test results are significantly correlated with the germination results at the Pearson's correlation coefficient of 0.98 and a p-value of 0.001. The TZ test results for the coated kernels were up to 3% higher than the germination results (Table 2.1); thus, the TZ test may slightly overestimate standard laboratory germination results (Yaklich and Kulik, 1979). However, in our study, the increase in viability resulting from the TZ test could be due to the direct exposure of the kernel tip in the TZ solution; whereas, during germination, the coating material can inhibit the seedlings from emerging.

Water uptake

Seeds absorb water primarily through the seed coat (Achakzai et al. 2014), and the application of seed treatments alter the seed coat physiology, which in turn can affect the water imbibition (water uptake) potential of seeds (Taylor, 2003). Water uptake during the first few hours is crucial for the success of the seed germination process as most of the biochemical and molecular changes are intensified during the first 2-8 h of water uptake (Harb et al., 2012). Fig.

2.4a shows the water uptake when averaged across the same type of coating material for time intervals ranging from 2 to 72 h. The water uptake rank was established during the first 4 h, and then generally maintained during the remainder of the test. Uncoated kernels imbibed the highest amount of water, which was expected because of the lesser barrier between the germplasm and the saturated germination paper. Among all the coating materials, zeolite allowed the highest water uptake at 4 h and then throughout the remainder of the 72 h test period (Fig. 2.4(a)); the water uptake trend of zeolite was statistically higher at 72 h (at p-value ≤ 0.05) than the rest of the coating materials. This is most likely because of the well-connected system of wide pores in the zeolite, which aids in the water uptake (Inglezakis and Zorpas, 2012), as previously mentioned in the *Tetrazolium* section.

All samples displayed the triphasic pattern, as described by Gallardo et al. (2001). The typical triphasic pattern begins with the rapid uptake of water in the first 16 h (Phase I), followed by a lag phase of respiration with little or no water uptake (Phase II) during which the seed is preparing for germination by mobilizing reserves. Uptake of a significant amount of water typically resumes at 32 h (Phase III), which indicates the initiation of germination. An emerging radicle is noted during this growth phase. Zeolite-coated kernels exhibited a triphasic water uptake pattern similar to uncoated kernels and large seeds. In contrast, kernels coated with polymer A, polymer B, gypsum, and cellulose exhibited a triphasic pattern similar to XL seeds, with the phase II prolonged by 4 h relative to large seeds.

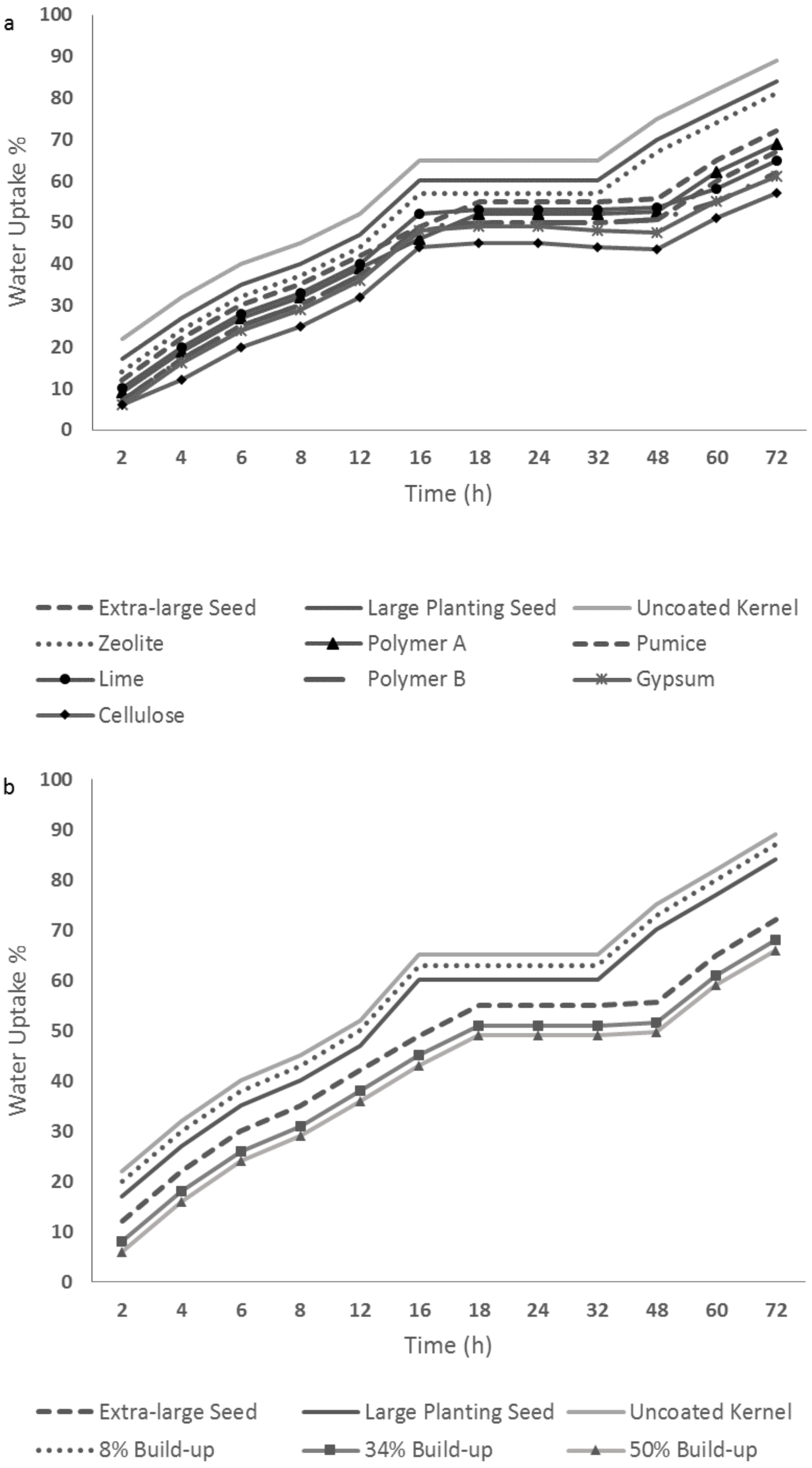

Fig. 2.4. Water imbibition potential of coated kernels as an effect of (a) coating materials and (b) coating build-up level.

Fig 2.4(b) shows the water uptake when averaged across the same build-up levels across the time interval ranging from 2-72 h. Kernels coated with 8% build-up showed a triphasic pattern similar to large seeds, whereas kernels coated with 34% and 50% build-up levels exhibited a tri-phasic pattern similar to XL seeds (Fig. 2.4b) indicating that water uptake decreases as the coating thickness increases. Phase III of the XL seeds and kernels coated at 34% and 50% build-up levels was delayed by 16 h compared to the large seeds, uncoated kernels, and kernels coated at 8% build-up level.

Accelerated aging

Accelerated aging tests have become an increasingly important component of stress tests used in the seed industries to predict long-term storability of seeds. Seeds for planting are expected to retain their capability to produce normal seedlings despite potentially unfavorable storage conditions. In order to better anticipate the field performance under adverse conditions, coated kernels were subjected to an accelerated aging test.

Accelerated aging of our coated kernels at 45°C and 100% relative humidity for 48 h reduced their subsequent germination by 16% (Table 2.1). Other published reports also showed that sunflower seed germination is impacted negatively at 45°C, and incubation of seeds at such high temperature results in a progressive reduction of germination (Corbineau et al. 1988, Gay et al. 1991). The work of Corbineau and co-authors (2002) establish that a sequence of irreversible cellular and metabolic damage is associated with deterioration of sunflower seeds during incubation in water at 45°C.

The coating materials in our study generally helped retain germination compared to the large and extra-large seed, and uncoated kernel (Table 2.1). Cellulose, polymer + talc, and gypsum showed reductions in germination of 12 – 14% as compared to the 20% reduction in germination of large seed, extra-large seed, and uncoated kernel. Furthermore, in the case of gypsum, zeolite, lime and pumice, 34% and 50% build-up levels showed 16% and 15% reduction in germination, respectively, as compared to the 19% reduction in germination with 8% build-up level.

As the accelerated aging time increased to 72 h, the averaged germination of the coated kernels decreased by an additional 7% compared to 48 h (Table 2.1). Commercial coated kernels with polymer A and polymer B + talc material showed only 2% and 1% additional reduction in germination, respectively, from 48 h to 72 h. Overall, polymer and pumice coating materials and 8% and 34% build-ups retained the highest germination after both 48 h and 72 h.

Plantability

Spacing uniformity and the germination after the passage of the seed through the planter are the most common characteristics used by producers in evaluating planted seed performance (Liu et al., 2004). Non-uniform seed spacing during planting has been associated with significant yield loss (Krall et al., 1977; Nielsen, 2001). Seed spacing is determined by the seed singulation system (Koller et al., 2014). ASABE Standard S506 (ASABE, 2011) defines a seed singulation system as a “singulating seed meter” that tests the ability of the planter to pick up single seed to ensure the precise planting time and delivery of the seed to the soil. To obtain a measure of seed singulation, a MeterMax test stand was operated with a John Deere flat disk seed plate. This seed plate is recommended by the National Sunflower Association to plant confectionary sunflower seeds as it aids the pickup of variable-shaped sunflower seeds (Lilleboe, 2008), thus making it a suitable choice for coated sunflower kernels. Singulation and post-planting germination results are summarized in Table 2.2.

The initial singulation test was done without the use of lubricant. Coating the kernels with polymer A and polymer B resulted in singulation similar to large seeds (87%) and much higher compared to XL seeds (68%). The singulation of the kernels coated with polymer A, B, and C (85 – 92%) was significantly higher than the kernels coated with cellulose, gypsum, zeolite, lime, or pumice (72 – 80%). The singulation differences among the coating materials are mainly due to the surface texture, which determines the ability of the seed surface to make a vacuum-seal with the plate opening. A smooth seed surface ensures a strong seal with the plate opening as compared to the rough surface; a rough seed surface results in a poor seal and causes the seed to drop prematurely.

Although the type of coating materials can significantly impact the seed singulation, the level of coating build-up is also important. Kernels coated at the 34% build-up (non-polymer) level resulted in significantly higher singulation of 86% than both the 8% and 50% build-up levels (75% and 76%, respectively). Additionally, the singulation at 34% build-up level was statistically similar to the large planting seed (87%).

Even though both coating materials and build-up levels affect the seed singulation, no trend was found between the seed singulation and the damage caused to the kernels inside the test stand. This damage to the kernels during passage through the planter is reflected in the post-planting germination. As expected, the post-planting germination of uncoated kernel experienced the greatest drop of 24%. Post-planting germination of all coated kernels dropped significantly – with the exception of Polymer B at 34 and 50% build-up levels – when compared with the pre-planting germination ($p \leq 0.05$). Post-planting germination of the coated kernel was often significantly higher than the uncoated kernel, but always significantly less than large and extra-large seed. Although both 34% and 50% build-up levels did not show a significant reduction in post-planting germination, kernels coated at a build-up level of 34% provided the most protection to the kernels as compared to the germination of uncoated kernels. As noted before, polymer B-coated kernels experienced a relatively small reduction in germination, which could be due to the addition of talc to the polymer during the coating process; talc likely also contributed to the exceptionally high singulation of 92% for these kernels. Talc is a seed lubricant which is known to aid the flowability of the seeds inside the planter by making the seed surface smoother. Moreover, talc may cushion the seeds against any impact forces inside the planter to preserve the seed germination.

Talc was not included with any other coated kernels during the initial plantability test; however, the superior results with Polymer B + Talc led us to conduct the second plantability test with seed lubricant added to all treatments. Two types of lubricants, talc and Fluency Agent, were evaluated in this second test. The lubricants were added to the coated kernels prior to the plantability test. Compared to the use of no lubricant, the addition of talc increased the singulation of commercially coated kernels by 3-9% and increased the germination by 7% on average. It is worth noting that seven of the coated samples achieved post-singulation germination comparable to large seed once talc was added. Similarly, the addition of Fluency Agent increased the singulation of coated kernels by 2-6% as compared to the use of no lubricant. Furthermore, the addition of Fluency Agent increased germination by 6% on average, compared to the use of no lubricant. The slight differences between Fluency Agent and talc lubricant on both the singulation and post-singulation germination performance were not significant at $p \leq 0.05$.

Table 2.2. Plantability[a] performance of the sunflower coated kernels and controls resulting from 2 types of tests: 1) without general lubricant addition, and 2) with lubricant addition.

	Singulation %			Germination %			
					Post-planting		
Lubricant type	No lubricant	Talc	Fluency agent	Pre-planting[b]	No lubricant	Talc	Fluency agent
Uncoated kernel	75c	77c	83b	92a	68c	73b	76c
Large planting seed	87a	90a	91a	90a	88a	88a	89a
Extra-large seed	68d	73d	75c	85b	85a	85a	85a
Effect of Coating Materials[c]							
Polymer A	87a	88a	89a	85b	80b	83a	82b
Polymer B + Talc	92a	93a	94a	81c	78b	82a	81b
Polymer C – In-house hand coated	86a	87a	89a	91a	82b	85a	87a
Polymer C – In-house machine coated	85a	86a	88a	90a	84b	88a	88a
Cellulose	80c	84b	86a	78c	73c	75b	76c
Gypsum	78c	83b	84b	79c	75c	79b	78b
Zeolite	77c	79c	82b	89a	80b	87a	85a
Lime	72d	75d	76c	86a	79b	88a	86a
Pumice	77c	80b	82b	86a	82b	84a	85a
Effect of Coating Build-up %[d]							
8%	75c	77d	79c	88a	78b	82a	81b
34%	86a	83b	84b	85b	82b	85a	86a
50%	76c	78c	80b	82c	78b	84a	81b

Means followed by the same letter within the same column are not statistically significant at P≤0.05

[a] Plantability tests were conducted with the John Deere seed plate – Part Number A52390

[b] Pre-planting values are taken from the germination results presented in Table 2.1 for comparison with post-planting germination results

[c] Results were averaged across all the build-up levels for the same coating material type

[d] Results were averaged across gypsum, zeolite, pumice, and lime within the same build-up levels

Conclusions

The coating delayed the water uptake of all of the coated kernels, except for zeolite, which in turn delayed emergence. As a result, the standard AOSA method to test sunflower seed germination was modified to take the germination count on the tenth day instead of the standard seventh day to account for late emerging seedlings. For situations requiring a quick screening test to check the viability of coated kernels, the TZ test was found to be an acceptable alternative and can be completed in 3 days.

Most of the coated kernels resulted in germination which was either similar to or higher than the XL seed, but less than the uncoated kernel. However, the coating is essential for protecting the kernel during planting. Post-planting germination was inferior to that of large and XL seed in all coated kernel; however, with addition of lubricant, the post-planting germination of the following seven types of coated kernels was comparable to large and XL seed: polymer A, polymer B, polymer C – in-house hand coated, polymer C – in-house machine coated, zeolite, lime, and pumice. Furthermore, all of the coatings showed protective effects under the conditions of the accelerated aging test as compared to the XL seed.

Singulation of the all of the coated kernels with the standard seed plate was superior to the XL seed. This is the main benefit of hulling XL seed and coating the kernel. With lubricant addition, the singulation of the following coated kernels was superior to the XL seed and comparable to the large seed: polymer A, polymer B, polymer C – in-house hand coated, and polymer C – in-house machine coated. Overall, with or without lubricant addition, the singulation of all three types of polymer-coated kernels was significantly higher than the kernels coated with cellulose, gypsum, zeolite, lime, or pumice.

Three build-up levels were evaluated for the four mineral coatings. A thinner coating level of 8% did not provide the kernels enough protection inside the planting equipment, and a thicker coating level of 50% appeared to prevent or delay the germination. The kernels coated at 34% build-up level had the highest singulation while preserving the kernel viability. Therefore, to further better the coated kernel performance, it is very important to evaluate the additional build levels over smaller increments ranging from 20 – 40 %. The effect of build-up level should be evaluated for polymer coatings, too.

Acknowledgments

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PAPER 2: COATING THE HULLED KERNELS IMPROVED FIELD PLANTABILITY AND GRAIN YIELD OF EXTRA-LARGE CONFECTIONARY SUNFLOWER ACHENES

Abstract

Extra-large (XL) hybrid confection sunflower achenes are popular among snack food consumers, but these achenes perform poorly with precision planters; therefore, the option of planting coated kernels from XL achenes is evaluated in this study. The objective of this paper was to evaluate the effect of various coating materials and build-up levels on live seed emergence, precision planting, stand establishment, and crop performance under field conditions. Based on the laboratory results on plantability and germination after plantability, four coating materials (polymer A, polymer B, zeolite, and lime) at 30% and 35% build-up levels were selected for the field trails at Prosper, ND. A separate smaller field study was conducted at Minot, ND to verify the performance of coated kernels under different growing conditions. At both Prosper and Minot locations, all the coated kernel treatments resulted in significantly higher live seed emergence and grain yield than XL achenes. Laboratory singulation of all the coated kernels was superior to both XL achenes and large achenes, which contributed to uniform seed spacing in the field for the coated kernels. Coated kernels produced grain yields up to 55% greater than from XL achenes, and up to 25% greater than large achenes at Prosper. Live seed emergence of all the coated kernels (93 – 99%) was significantly higher than the XL achenes

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(88%) and similar or higher than the large achenes (94%) at Prosper. The same trend in coated kernel live seed emergence was also observed when this study was conducted in a previous year at Prosper. Overall, this study showed that coating the hulled kernels substantially increased kernel plantability and crop performance compared to XL achenes and even large achenes. A successful outcome of this study may extend this research to other sunflower seed sizes and possibly other crops.

Introduction

Despite the increasing worldwide demand for very large-seeded varieties of confectionary sunflower (Helianthus annuus L.) achenes in the snack food industry, farmers are reluctant to plant extra-large (XL) size achene fraction of such varieties. Unfortunately, a significant proportion of XL achenes do not emerge upon planting as seedlings are unable to emerge from the tough shell. The XL achenes also cause poor plantability that contributes to non-uniform plant spacing (Lilleboe, 2017). Seed plantability is the ability of the seed to flow through the planter to allow uniform and consistent plant spacing as defined by the American Society of Testing and Materials (ASTA). The XL achenes are not compatible with standard plates in precision planters and this results in skips and doubles, thus poor seed singulation. Both seed singulation and germination/ seedling emergence affect plant spacing, hence uniform stand establishment and subsequent grain yield at harvest. Kandel (2011) reported plant spacing to be the major yield-limiting factor in both confectionary and oil sunflower achenes from surveys of sunflower growers at harvest time.

Sidhu et al. (2016) proposed that hulling the XL achenes and then coating the hulled kernels would achieve more precision sowing, enhanced emergence, and better plant stands. The authors showed that gently hulling one type of XL achene improved kernel germination from 85

% to 92%. Later work showed that coating the hulled kernels improved kernel plantability by 24 % when compared to XL achenes in laboratory studies (Sidhu et al., 2017). Seed coating has been used to improve plantability by increasing the size of sugarbeet seeds since the 1970’s and turfgrass seeds since the 1980’s (Hathcock et al., 1984; Farley, 1978). In recent years, use of seed coating has been expanded to improve seed sowing, seedling emergence, and stand establishment. Advances in seed coating technology offer exciting potential to improve both the physical and physiological properties of the seed. Sidhu et al. (2017) have summarized the benefits of seed coatings for various crops including soybean (*Glycine max* (L.) Merr.), rice (*Oryza sativa* L.), barley (Hordeum vulgare L.), lettuce (*Lactuca sativa* L.), and tomato (*Solanum lycopersicum* L.) for improving germination, plantability, and grain yield.

The effectiveness of various coating materials and build-up levels on coated sunflower kernels was tested under laboratory conditions by Sidhu et al. (2017). The following coating materials were found to be suitable: mineral coatings with polyvinyl alcohol as a binder (zeolite, limestone, pumice), and three different polymers. Furthermore, the authors tested three build-up levels of coating materials (amount of coating expressed as weight percent of the uncoated kernel) of 8, 34, and 50% and found the 34% build-up level to be the most appropriate for preserving kernel germination and improving kernel plantability. These results suggest testing additional build-up levels to 34% in smaller increments to further optimize benefits. However, the performance of these coated sunflower kernels still needs to be evaluated under field conditions.

The objective of this study is to determine whether coated kernels from XL achenes are superior to XL and competitive with large planting achenes when precision-planted in the field. The ideal coating should not interfere with kernel germination and improve kernel singulation for more precise plant spacing. The hypothesis is that optimizing the kernel coating build-up level with the most suitable coating materials can improve achene plantability and seedling emergence. This, in turn, will result in more uniform stand establishment in the field and thus improved grain yield. Four coating materials (Polymer A, Polymer B-In-house, Zeolite, and Limestone) were tested at four build-up levels of 25, 30, 35, and 40% in the laboratory. The treatments that resulted in the best seedling emergence and plantability were selected for the field studies at two different locations.

Materials and methods

Kernel preparation

Extra-large (XL) and large confectionary sunflower achenes of the hybrid "2215" were obtained from Red River Commodities Inc. Fargo, N.D. Achenes were hulled using corrugated rubber shearing rolls according to the method of Sidhu and co-authors (2016). The achene moisture content was adjusted to 5.9% prior to hulling using a convection oven at 40°C according to the method of Sidhu et al. (2014). Hulling was carried out in two passes at a feed rate of 226 ± 7 g/min and 1.29 differential roller speed. The roll gaps were 0.21 cm and 0.14 cm for the first and second pass, respectively. The mixture of hulls, achenes, broken kernels and intact kernels obtained were separated using a gravity table (Model 10-M2, Forsberg Inc., Thief River Falls, MN) and air screen cleaner (Clipper Separation Technologies, Bluffton, IN) as described in Fig. 3.1. The gravity table was operated at the following conditions: feed rate of 6 g/s, deck oscillation of 120 cycles/min with an 11 mm amplitude, airflow through the deck mesh of 1.2 m/s, end raise slope of 9°, and side tilt of 4°.

Kernel coating and treatment

Intact kernels were coated commercially as well as in-house. The coating materials and build-up levels were identified based on the work done by Sidhu et al. (2017). Twelve different coating treatments were obtained commercially with three types of coating materials – polymer A, limestone, and zeolite – using a rotary coater at 25%, 30%, 35%, and 40% levels of build-up. The coated kernels were dried using a fluidized bed. The commercial coating treatments were performed by two different companies in the US.

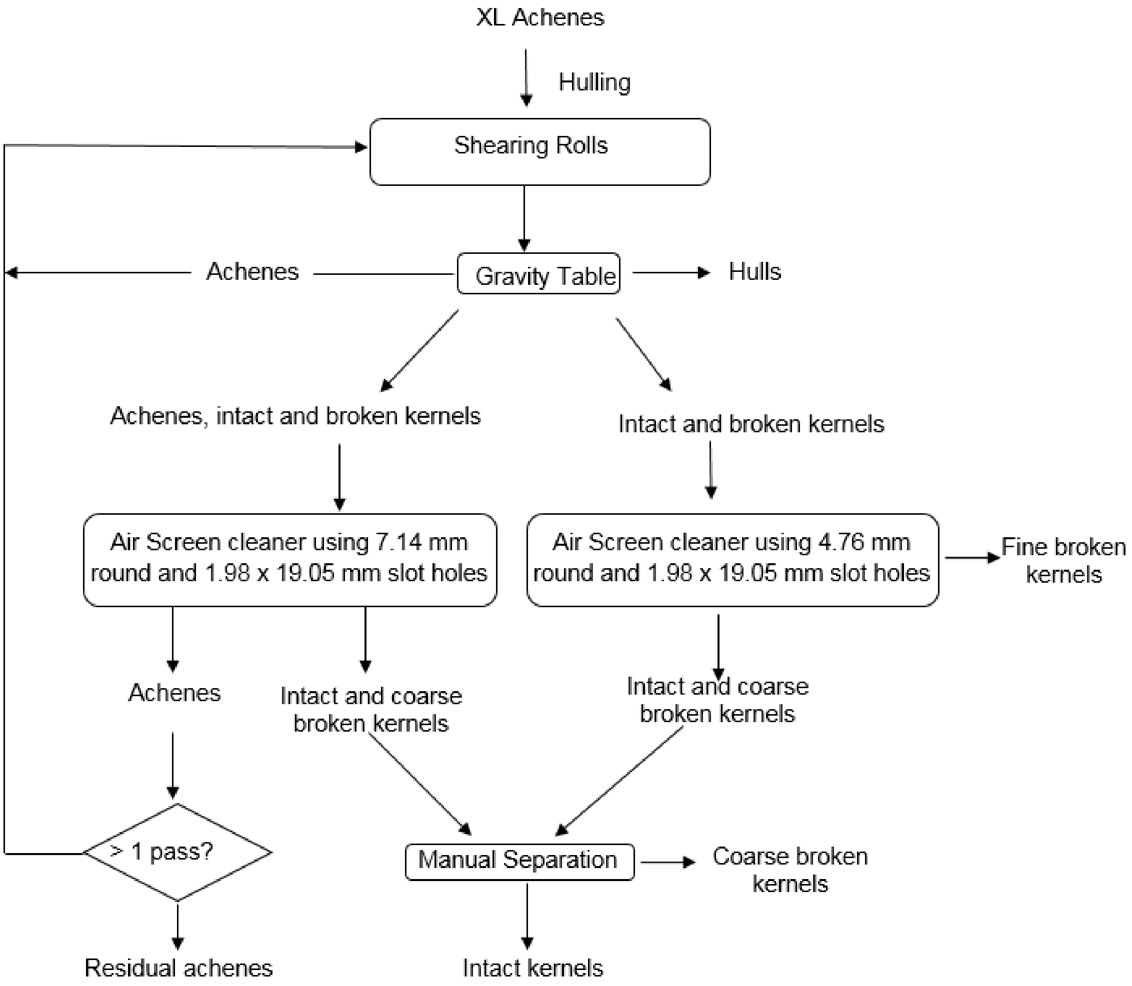

Fig. 3.1. Flowchart of hulling and separation process of extra-large confectionary sunflower achenes.

The liquid polymer B, Seedworx Ag Shine (AgInnovation, Walnut Grove, CA), was used for in-house coating trials. The USC tabletop lab treater (USC, LLC, Shakopee, MN) was used to coat the kernels at 35 Hz drum speed for 2 min. The coated kernels were dried on a sieve bed at room temperature (24°C ±1) for 3 h according to the method of Sidhu et al. (2017).

All of the coated kernel treatments (both commercial and in-house coated kernels) and controls (XL and large achenes) were sent to Syngenta (Minnetonka, MS) for the CruiserMaxx® sunflower seed treatment (3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine). CruiserMaxx® treatment has been widely used by sunflower farmers in the U.S. to protect the sunflower crop from a broad range of insect and disease pests during emergence and seedling establishments.

Lab tests

Germination

Germination tests were performed according to the Association of Official Seed Analysts (AOSA, 2009) methods for sunflower seeds with two modifications described by Sidhu et al. (2017): 1) germination counts were taken on the tenth day instead of the standard seventh day to account for the late-emerging seedlings, and 2) seeds were treated with a 20% bleach solution for 2 min followed by a 2 min rinse with distilled water to inhibit fungal growth during germination. Germination was defined as the proportion of seeds that produced the essential structures associated with the shoot and root (AOSA, 2009). Essential components are at least half of the cotyledon tissue present, the presence of the epicotyl, hypocotyl is healthy and free of cracks, and presence of healthy primary and secondary roots (AOSA, 2009).

Singulation

A precision planting seed-singulator stand equipped with MeterMax and 20/20 SeedSense (Precision Planting, Tremont, IL) was used to determine plantability of the different coated kernel samples according to the method of Sidhu et al. (2017). The seed-singulator was operated with a John Deere seed plate (Part Number A52390) consisting of 40 round holes of 0.3 cm diameter and 1 cm spacing (Lilleboe, 2008), and the following settings: 457 mm H2O vacuum pressure and seed drop rate of 33 seeds/m. Each treatment included two replicates with 1000 seeds/replicate. Plantability for each treatment is reported as seed singulation percentage (%). The seeds were reevaluated for germination after passage through the seed-singulator.

Field trials

A field study was conducted at Prosper, ND (46°58′ N, 97° 4′ W) in 2017, with 10 coated kernel treatments: controls (large planting achenes, and extra-large achenes), commercial coated kernel treatments (Zeolite 30%, Zeolite 35%, Lime 30%, Lime 35%, Polymer A 30%, Polymer A 35%), and in-house coated kernel treatments (Polymer B 30%, Polymer B 35%). A separate field study was conducted at Minot, ND (8°23’N, 101°30’W) with five treatments: controls (large achenes, extra-large achenes) and commercial coated kernel treatments (Polymer A 30%, Polymer A 35%, Zeolite 35%) to test the performance of the coated kernels under moisture stressed conditions. These treatments for the field tests were selected based on the lab germination and singulation test results.

Seeding at Prosper was done on June 1, 2017, using an Almaco SeedPro precision planter (Almaco, Ames, IA). Seeding at Minot was done on June 2, 2017, using a custom built small-plot row-crop planter using Great Plains no-tiller openers and Monosem seed singulation meters (Seed Research Equipment Solutions, South Hutchinson, KS). The experimental design was a randomized complete block design with six and four replicates at Prosper and Minot, respectively. Experimental units (individual plot) consisted of four planted rows spaced 0.76 m apart and 7.6 m in length where 28 achenes were planted in each row at a 4.5 cm depth. The targeted plant population was 48,732 plants/ha.

Emergence data was collected from the center two rows of each experimental unit (plot). Seedling emergence was determined 4, 7, 10, 14, 21, and 28 d post-planting and expressed on a pure live seed basis according to the method of Berti et al. (2008) by correcting the emergence value for the seed germination value as described in section "*Germination*" of "*Results and Discussion*" section. Emergence rate was calculated for the above mentioned time intervals according to the method described by Maguire (1962) as

$$\text{Emergence rate} = \sum(n/t)$$

where n is the number of newly germinated seeds at time t, and time t is the days from planting.

Growth stages were classified into vegetative and reproductive stages according to the methods of Schneiter and Miller (1981). Plant stage for a plot was considered to be when 50% of the plants reached a particular growth stage. Signs of seedling abnormalities, branching, and other plant deformities were noted. All sunflower plots reached stage R9 physiological maturity well before a killing frost. Heads were hand-harvested from the center two rows of each plot, excluding the end plants from each row, by manually cutting the head off the stalk with a hand-clipper. The achenes were threshed with a Hege 125B combine harvester (Kinsley, KS) and Kincaid 8XP combine harvester (Haven, KS) at Prosper and Minot, respectively. Grain yield was determined after harvested grain samples were dried to a uniform moisture level. The test weight and achene size classification of the threshed achenes were done according to the USDA-GIPSA (2013) methods for sunflower seed.

Gravimetric soil moisture content was performed based on the soil sample collected from a 0 to 7.6 cm depth in the seeding furrow on the day of planting. Gravimetric soil moisture content was determined by oven drying 6 replicates of 20 g moist soil samples at 105°C for 24 h according to the methods of Zebath et al. (2008). The gravimetric soil moisture content was 20% and 4.3% at Prosper and Minot, respectively, at planting time. Soil nutrient analysis was performed on the soil sample collected from 0 to 15 cm in the seeding furrow on the day of planting according to the methods of Vendrell and Zupancic (1990), Olsen et al. (1954), and Combs and Nathan (2015). The soil at Prosper was characterized as Perella–Bearden silty clay loam in texture with pH 7.2 containing 50 kg/ha NO3-N, 52 ppm P, 186 ppm K, 4.5 kg/ha S, 2970 ppm Ca, and 880 ppm Mg in the surface soil (0-15 cm). The soil at Minot was characterized as Williams loam in texture with pH 5.8 containing 50 kg/ha NO3-N, 13 ppm P, 370 ppm K, 7 kg/ha S, 990 ppm Ca, and 336 ppm Mg in the surface soil (0-15 cm). Temperature and rainfall data were collected daily from the meteorological station located within 1 km of each field study site (NDAWN, 2017).

Field results from coated kernel treatments planted at Prosper, ND in 2016 are also included in this paper to compare the live seed emergence from multiple years. The methods used to hull the achenes and then coat the kernels were similar to those described above. The following five coated kernel treatments were included in 2016 field study: two controls (large planting achenes and XL achenes), and three commercial coated kernel treatments (Zeolite 35%, Lime 35%, and Polymer A 35%). Seeding was done on June 7, 2016, with an Almaco grain drill planter (Ames, IA). The experimental design was a randomized complete block design with four replicates. Experimental units consisted of three planted rows spaced 0.61 m apart and 7.6 m in length. A total of 50 achenes were planted in each row of each experimental unit at 4.5 cm depth.

Seedling emergence data was collected from the center row of each experimental unit (plot) 21 d post planting. The gravimetric soil moisture content, from 0 to 15 cm in the seeding furrow, was 21% on the day of planting.

Statistical design

Analysis of variance (SAS v9.3, SAS Institute Inc., Cary, N.C.), was used to calculate an F-protected LSD ($P \leq 0.05$) for comparisons of treatment means. The estimated variance of pairwise mean differences and the corresponding degrees of freedom were calculated to estimate the LSD values for comparison of significant main effects and interactions (Carmer et al., 1989).

Results and discussion

Lab tests

Germination

Lab germination tests were performed to examine the effects of both coating materials and coating build-up levels on kernel viability. Sidhu et al. (2017) conducted lab germination tests on coated kernels with build-up levels ranging from 8 to 50% with increments of 14% in build-up levels. Based on that work, a narrower range of build-up levels ranging from 25 to 40% with increments of 5% was chosen for this study, along with the best coating material types identified in the aforementioned study.

The XL achenes resulted in significantly lower germination of 85% as compared to large achenes (90%) and hulled intact kernels (92%) as shown in Table 3.1. Sidhu et al. (2017) reported that 58% of apparent ungerminated XL achenes exhibit a surprising phenomenon in which the hypocotyl/radicle wraps around the cotyledon and is unable to emerge through the pericarp. Thus, hulling the XL achenes improves the germination, while coating protects the fragile embryo from damage during transportation, handling, and planting.

Among all the commercial coating treatments and in-house coating treatments, germination ranged between 80-87% and 87-90%, respectively (data not shown). The significantly higher germination of in-house coated kernels could be attributed to the gentler coating and drying methods as compared to commercial coating methods (Sidhu et al., 2017). Mechanical damage caused to the seed triggers oxidative and nitrosative stresses which reduces germination (Chaki et al., 2011).

Table 3.1. Lab germination and plantability performance of the sunflower coated kernels and controls.

		Plantability	
	Germination %	Singulation %	Post-planting Germination %
Uncoated Intact Kernels	92^{a}†	75^{d}	66^{e}
Large Achenes	90^{a}	88^{a}	88^{a}
Extra-large Achenes	85^{c}	72^{d}	84^{a}
Effect of Coating Materials ‡			
Polymer A	84^{bc}	88^{a}	81^{b}
Polymer B – In-house	89^{a}	86^{b}	85^{a}
Zeolite	85^{b}	$85^{b\ c}$	79^{bc}
Limestone	$84^{b\ c}$	85^{bc}	80^{b}
Effect of Coating Build-up % §			
25%	87^{b}	83^{c}	78^{c}
30%	87^{b}	88^{a}	82^{ab}
35%	86^{b}	90^{a}	83^{a}
40%	83^{c}	84^{bc}	81^{b}

† Means followed by the same letter within the same column are not statistically significant at $P \leq 0.05$

‡ Results were averaged across all the build-up levels for the same coating material type

§ Results were averaged across gypsum, zeolite, pumice, and lime within the same build-up levels

When germination was averaged across the same type of coating material among commercially coated kernels, no significant differences were found between zeolite, polymer A, and limestone as shown in Table 3.1. These results are consistent with the results obtained from the work of Sidhu et al. (2017). Mean germination for polymer B was 89% and was significantly higher than the commercially coating treatments which could be due to the gentler coating and drying methods as previously noted. In contrast, when germination was averaged across the same build-up levels, no significant differences were found between the following levels: 25%, 30%, and 35%, which resulted in germination of 87%, 87%, and 86%, respectively. However, increasing the build-up to 40% significantly reduced the germination to 83% which was 3 to 4% lower than the other coating build-up levels.

Singulation

All four coatings resulted in singulation similar to the large achenes (88%) and much higher compared to the XL achenes (72%) and uncoated kernels (75%) (Table 3.1). When averaged across the same type of coating material, polymer A resulted in the highest singulation of 88% followed by polymer B (86%), and zeolite and limestone (85% each) (Table 3.1). The high singulation of polymer materials could be attributed to an improved vacuum seal created between the plate opening and the coated kernel surface. The polymer creates a much smoother surface as compared to the non-polymer coating materials (Sidhu et al., 2017).

Both 30% and 35% build-up levels were statistically similar, with singulation of 88% and 90%, respectively (Table 3.1). Additionally, singulation at 30% and 35% build-up levels were statistically similar to the large achenes. The build-up of 25% and 40% resulted in statistically lower singulation of 83% and 84%, respectively, compared to the singulation at 30% and 35 build-up levels.

The coated kernels must remain viable after passing through the planter to establish a seedling. Damage to the coated kernels during passage through the planter is reflected in reduced post-planting germination relative to pre-planting germination (Table 3.1). As expected, the post-planting germination of intact kernels dropped by 26% due to the damage caused to the kernel tip inside the planter (Table 3.1). Germination dropped by 4 to 6% for the coated kernels

(Table 3.1). Overall, both Polymer A and polymer B retained the highest germination of > 80% after passing through the planter.

Reduction in germination after the coated kernels passed through the singulator increased from 2 to 9% as the build-up decreased from 40 to 25% (Table 3.1). Even though a 40% build-up level provided the most protection, the singulation was significantly lower than the kernels coated at 30% and 35% build-up levels. Overall, for all the coating material types, both the 30% and 35% build-up levels provided the best overall performance, when considering singulation and post-planting germination results.

Field trials

Field trials were conducted at the Prosper and Minot sites in North Dakota to test the response of the coated kernel treatments under different field conditions. During the study period, both initial soil moisture content and precipitation at Prosper were more favorable than Minot for coated kernels germination and stand establishment. Initial soil moisture content at seeding depth was 11% and 3.5% at Prosper and Minot, respectively, on the day of planting. Growing season precipitation at Prosper was 350 mm and at Minot 187 mm (Table 3.2). The monthly average temperature was within 1°C each month of the growing season at both locations except in September when the average monthly temperature was 2°C cooler at Minot.

Table 3.2. Monthly average air temperature and precipitation for the growing seasons for coated confectionary sunflower kernels at Prosper, ND, and Minot, ND in 2017.

Month	Avg. air temperature (°C)			Precipitation (mm)	
	Prosper, ND	Minot, ND		Prosper, ND	Minot, ND
June	19	18		88	50
July	21	22		50	11
August	19	19		53	70
September	16	14		152	54
October	8	7		7	2
Average	17	16	Total	350	187

Emergence

The XL achenes resulted in lower emergence in the field than the large achenes and all the coated kernel treatments at the Prosper site, as reflected by significantly lower live seed emergence and emergence rate index values (Table 3.3). Additionally, the vegetative and reproductive stage development of the XL achenes was, on average, twelve days behind all the coated kernel treatments and large achenes (data not shown). Sidhu et al. (2017) reported that the XL achenes absorb water less readily due to the large void space between hull and pericarp, and that some seedlings are unable to emerge through the shell, thus reducing germination.

Among all the coated kernel treatments, the high pure live seed emergence range of 93 to 99 % partly reflects the favorable growing conditions at the Prosper site (Table 3.3). Adequate initial soil moisture content of 20% and precipitation of 81 mm during the 21 d emergence period was noted at the Prosper site. No significant differences were found in the emergence rate index among the coated kernel treatments and the large achene treatment.

When averaged across similar build-up levels for all the coated kernel treatments planted in 2017 at the Prosper site, live seed emergence was 4% higher for the 35% build-up level as compared to the 30% build-up level (Table 3.3). Moreover, live seed emergence for the 35% build-up level was statistically higher than for 30% build-up level for each of the four types of coating. Therefore, increasing the build-up from 30 to 35% provides added protection to the embryo during the planting process. The five percent additional build-up level, however, did not provide a statistically significant advantage for the emergence rate index, which was only 1% higher for the 35% build-up level.

Table 3.3. Emergence performance of the coated confectionary sunflower kernels and controls planted using a precision planter at Prosper, ND, and Minot, ND.

	Prosper, ND, 2016	Prosper, ND, 2017				Minot, ND, 2017		
	LSE†	LSE	Singulation %‡	ERI §	Plant Population (plants/ha)	LSE	ERI	Plant Population (plants/ha)
Large Achenes	83^{a} ¶	94^{b}	90^{a}	17^{a}	41,000^{a}	64^{a}	3^{a}	28,000^{a}
Extra-large Achenes	79^{b}	88^{c}	69^{c}	13^{b}	36,000^{c}	40^{b}	2^{a}	16,000^{c}
30% Polymer A	-	94^{b}	89^{a}	16^{a}	39,000^{a}	60^{a}	2^{a}	25,000^{b}
35% Polymer A	83^{a}	98^{a}	91^{a}	18^{a}	40,000^{a}	64^{a}	3^{a}	26,000^{b}
30% Polymer B – IH#	-	95^{b}	85^{b}	16^{a}	41,000^{a}	-	-	
35% Polymer B - IH	-	97^{a}	88^{a}	17^{a}	42,000^{a}	-	-	
30% Zeolite	-	93^{b}	82^{b}	16^{a}	38,000^{b}	-	-	
35% Zeolite	83^{b}	99^{a}	85^{b}	17^{a}	41,000^{a}	-	-	
30% Lime	-	94^{b}	80^{b}	16^{a}	39,000^{ab}	-	-	
35% Lime	82^{a}	99^{a}	83^{b}	17^{a}	40,000^{a}	63^{a}	3^{a}	26,000^{b}

† Live seed emergence

‡ Singulation data retrieved from the Almaco SeedPro precision planter after seeding was done

§Emergence rate index

¶ Means followed by the same letter within the same column are not statistically significant at $P \leq 0.01$

In-house coating type

All coating types resulted in statistically similar live seed emergence when averaged across the same coating material at $P \leq 0.01$ (data deduced from Table 3.3). There were also no statistical differences found in the actual field emergence (data not shown) resulting from all four coating types. Even though polymer B-IH resulted in significantly higher post-planting germination in lab tests compared to other coating material types, it did not provide any advantage in these field trials. Further, singulation results obtained post-seeding (Table 3.3) are in accordance with the laboratory singulation test results as described in section “*Singulation*”.

A separate, smaller field study was conducted at the Minot site to verify the emergence performance of the coated kernels under different growing conditions. Overall, both the live seed emergence and the emergence rate index were much lower than at Prosper (Table 3.3). This lower emergence performance at Minot appears to be to be partly caused by soil moisture limitations which was much lower than at Prosper. Water uptake during the first few hours is critical for the success of the seed germination process as most of the biochemical and molecular changes are intensified during the first 2 to 8 h of water uptake (Harb, 2012). Also, the soil dryness and total precipitation of 45 mm during the 21 d emergence period at Minot was considered inadequate for good germination and seedling establishment of achene and coated kernel treatments.

Despite the low live seed emergence and emergence rate index at the Minot site, the trends are similar to the Prosper site (Table 3.3). All of the coated kernel treatments resulted in live seed emergence significantly higher than the XL achenes, but comparable to the large achenes. This similar trend in the emergence of both favorable and unfavorable environmental conditions supports the effectiveness of all four coating types.

The live seed emergence trends obtained from the 2016 field study at Prosper are also consistent with the 2017 field study results at both Prosper and Minot. The live emergence of the coated kernel treatments (82-83%) was significantly higher than the XL achenes (79%) and statistically similar to the large achenes (83%).

Phenotypic traits

The effect of coating on the phenotypic traits of the sunflower plant, such as plant height, types of branching, and head abnormalities was evaluated on all treatments planted at the Prosper site.

Plant height

All coated kernel treatments and the large achene treatment resulted in statistically similar plant height ranging from 172 – 179 cm (Table 3.4). The plant height of all the coated kernel treatments and large achenes fall within the accepted height range of 160-180 cm for cultivated sunflower. In contrast, the plant height of XL achenes is 18 cm shorter than the coated kernel treatments. Generally, both the genetics and the growing environment determines the plant height (Seiler, 1997). Plant density is one of the major factors that affect the sunflower plant height (Ibrahim, 2012). The shorter plant height resulting from XL achenes appears to be associated with the significantly lower plant population of XL than the large achenes and the coated kernel treatments (Table 3.3).

Stem branching

Sunflower branching is an undesirable trait in commercial sunflower grain production. All the coated kernel treatments in this study showed no branching, except the 30% polymer B treatment in which 4% of plants showed branching (Table 3.4). However, in the case of large achenes and XL achenes, 14% and 21% of plants, respectively, showed branching. Seiler (1997) reported that, apart from genetics and environment, damaged kernels can contribute to branching as well. In this case, branching might have developed due to the injury caused to the seedling during the struggle to come out of the pericarp. The fact that all the coated kernel treatments showed little or no branching supports the conclusion that either the coating treatments provided enough protection to the kernels during planting or the coating materials offered little resistance or stress to the emerging seedling.

Table 3.4. Phenotype traits of the coated confectionary sunflower kernels and controls planted using a precision planter at Prosper, ND. A total of 28 achenes were planted.

Treatments	Avg. plant height (cm)	Branched†	Abnormal heads‡
Large Achenes	179 ^{a}¶	4 ^{a}	6 ^{b}
Extra-large Achenes	158 ^{b}	6 ^{a}	5 ^{b}
30% Polymer A	177 ^{a}	0	8 ^{a}
35% Polymer A	175 ^{a}	0	6 ^{b}
30% Polymer B - IH#	178 ^{a}	1 ^{b}	8 ^{a}
35% Polymer B - IH	174 ^{a}	0	6 ^{b}
30% Zeolite	179 ^{a}	0	9 ^{a}
35% Zeolite	175 ^{a}	0	7 ^{ab}
30% Lime	172 ^{a}	0	8 ^{a}
35% Lime	176 ^{a}	0	9 ^{a}

†Number of branched plants.
‡Plants with abnormal central head
¶ Means followed by the same letter within the same column are not statistically significant at $P \leq 0.05$
# In-house coating type

Abnormal heads

All of the coated kernel treatments and both achene controls large and XL showed some abnormal heads (Table 3.4). This was attributed to damage caused by the sunflower seed maggot (*Neotephritis finalis*) during the reproductive stages R1 to R5 (Schneiter and Miller, 1981). However, despite the abnormality, the plants were allowed to mature to harvest.

Grain yield and quality

Grain yield

Grain yield obtained from the XL achenes was 19% and 14% lower than the large achene treatment at the Prosper and Minot sites, respectively (Table 3.5). The reduced grain yield of the XL achene treatment is associated with lower singulation, lower live seed emergence and increased branching (Tables 3.1, 3.3, and 3.4). Average grain yield of confectionary sunflower in the United States from 2012-2016 was 1804 kg/ha and in North Dakota 1400 kg/ha from 2007 to 2016 (NASS, USDA, 2017). The lower grain yield of XL achenes compared to the national average, and large achenes confirm the need for research to improve emergence and plantability of XL size achenes.

The grain yield of the coated kernels was 2332 kg/ha when averaged over all the kernel coating treatments at the Prosper site (Table 3.5). When averaged across the 30% and 35% build-up levels, polymer IH and lime coated kernels resulted in statistically higher yield than the polymer A and zeolite treatments at the Prosper site. Taking all the coated kernel yields together, the yields at 30% and 35% build-up levels were not significantly different; however, grain yields at 35% build-up level were 6 to 9% higher than the 30% build-up level. These results are in accordance with the live seed emergence (reported in section "*Emergence*") where coated kernel treatments at 35% build-up level resulted in higher live seed emergence than the 30% build-up coated kernel treatments. The additional 5% build-up level provided more protection than 30% build-up level to the embryo during the planting process, thus increased live seed emergence and possibly grain yield. The field emergence is significantly correlated with the grain yield at the Pearson's correlation coefficient of 0.73 and a p-value of 0.002 (data not shown). Polymer B and lime coating types, each at 35% build-up level, resulted in 55% and 50% higher grain yield compared to the XL achenes, respectively, and 25% and 21% higher grain yield than large achenes, respectively.

As expected, the grain yields for all the coated kernel treatments and controls (XL achenes and large achenes) at Minot were substantially lower than the yields at Prosper (Table 3.5). However, all of the coated kernel treatments resulted in grain yields significantly higher than the yield from XL achene treatment and statistically similar to the yield from large achene treatment, pointing to the success of coated kernel treatments even under adverse environmental conditions.

Achene size

Confectionary sunflower achenes are valued based on the seed size. Typically, the higher the seed size, the better it is valued. Achenes with sizes ranging from 0.71 - 0.79 cm (medium-size achenes), 0.79 – 0.87 cm (large-size achenes), and over 0.81 cm (XL-size achenes) are generally used for the edible kernel market, domestic achene market, and export achene market, respectively. Coated kernel treatments from Prosper produced 85 to 91% of large or XL achenes (Table 3.5). In contrast, only 7 to 12% of the harvested achenes from coated kernel treatments at Minot were large or XL. The smaller achene size is attributed to the drier conditions at the Minot.

Test weight

According to U.S. grade requirements for sunflower as stated by Federal Grain and Inspection services, the minimum test weight of harvested sunflower grains should be 31.25 kg/hL (NDSU Extension Service, 2017). Test weight of all the coated kernel treatments and large achene treatment was acceptable (Table 3.5). The unacceptable test weight of XL achenes

Table 3.5. Harvest profile of the coated confectionary sunflower kernels and controls planted using a precision planter at Prosper, ND and Minot, ND in 2017.

	Grain Yield (kg/ha)	Test Weight (kg/hL)	Achene Size (%)		
			> 0.87 cm	> 0.79 cm	> 0.71 cm
Prosper, ND					
Extra-large achenes	1661 ^{d}[†]	26 ^{c}	67	72	84
Large achenes	2064 ^{c}	35 ^{a}	69	82	86
30% Polymer A	2146 $^{b\ c}$	32 $^{a\ b}$	75	86	93
35% Polymer A	2279 ^{b}	34 ^{a}	78	86	92
30% Polymer B IH[‡]	2371 $^{a\ b}$	35 ^{a}	73	91	96
35% Polymer B IH	2579 ^{a}	34 ^{a}	71	90	95
30% Zeolite	2127 ^{b}	34 ^{a}	75	88	59
35% Zeolite	2299 ^{b}	32 ^{a}	77	85	92
30% Lime	2353 $^{a\ b}$	34 ^{a}	73	85	92
35% Lime	2498 ^{a}	34 ^{a}	76	89	96
Minot, ND					
Extra-large achenes	1594 ^{d}	27 $^{a\ b}$	10	18	88
Large achenes	1875 $^{b\ c}$	31 ^{a}	8	13	93
30% Polymer A	1926 ^{b}	32 ^{a}	5	12	95
35% Polymer A	2069 ^{b}	30 ^{a}	3	7	93
30% Polymer B IH[‡]	-	-	-	-	-
35% Polymer B IH	-	-	-	-	-
30% Zeolite	-	-	-	-	-
35% Zeolite	-	-	-	-	-
30% Lime	-	-	-	-	-
35% Lime	2245 ^{a}	29 $^{a\ b}$	4	10	90

[†] Means followed by the same letter within the same column are not statistically significant at $P \leq 0.05$

[‡] In-house coating type

contrary to the test weight of coated kernel treatments supports the need of hulling the XL achenes for improved field performance.

Surprisingly, all the coated kernel treatments both at Minot and Prosper showed statistically similar test weights despite having statistically different achene sizes. Despite the shorter grain filling period at Minot due to moisture stress, which influenced both grain yield and achene size, the plants produced kernels with acceptable test weights.

Conclusions

Coating the hulled sunflower kernels improved both singulation and emergence as compared to XL achenes. Improved emergence and plant spacing resulted in more uniform stand establishment and grain yield for the coated kernel treatments compared with the XL achene treatment. Coated kernel treatments showed little or no emergence stress as compared to both XL and large planted achenes. Overall, coated kernel treatments were shown to be superior to XL and large achenes. Evaluation of coated kernel treatments should be scaled up to larger field testing. All steps involved in hulling, separation, handling, and coating must be as gentle as possible to the kernel.

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PAPER 3: PLANT STAND UNIFORMITY CHARACTERIZATION WITH UNMANNED AERIAL VEHICLE (UAV) IMAGING OF A CONFECTIONARY SUNFLOWER FIELD

Abstract

Both seed emergence and seed singulation contribute to the plant stand uniformity, and quantifying plant stand uniformity is an important aspect to evaluate the ability of precision planting the seeds. Plant stand uniformity is traditionally chacterized manually for seedling emergence and other phenotypic traits. Both UAV technology and image processing methods provide an efficient alternative to the traditional time consuming and labor intensive approach. The objective of this study was to automate the plant stand uniformity characterization of the confectionary sunflower field when seedlings are between V2 and V4 growth stages using freeware ImageJ. Data collected manually from the small scale 2017 precision-planted sunflower field trials at Prosper, ND were used as a reference. An aerial image of the field was collected on the same day using a UAV equipped with an RGB camera. The image processing methods included conversion of the images to grayscale followed by background noise reduction, morphological, and segmentation operations. Seventeen thresholding algorithms were compared for the purpose of background noise reduction. Two morphological operations, “Open” and “Close”, were able to join the separated leaves of the same seedling. Clusters of multiple seedlings were then separated into segments representing discrete seedlings with a segmentation operation, “Watershed.” Minimum thresholding algorithm gave the best agreement with manual counts with p values of 0.0035 and 0.018, respectively, for the emergence counts and number of multiples. Seedling vigor and seed-to-seed spacing were also quantified using the Minimum algorithm. Seed-to-seed spacing was further used to quantify the plant stand uniformity in the field. This study demonstrated the semi-autonomous prediction of emergence counts, number of

multiples, seedling vigor, and seed-to-seed spacing of the sunflower field using an image processing method. This method can be adapted to bigger scale sunflower fields by calibrating the method with a small area from the field, by calculating a correction factor for adjusting the predictions from the rest of the field.

Introduction

A key component of the performance of any crop is the complex combination of different traits such as seed singulation, seedling emergence, and seedling vigor. Successful seed placement with the desired seed-to-seed spacing is a crucial step for a successful crop production. Poor seed singulation during planting results in inconsistent seed-to-seed spacing caused by multiples and skips. Multiples result due to the placement of more than one seed at the intended point in the field, whereas in the case of skips, no seed is placed at the intended point. As a result, multiple seedlings planted together negatively impact the grain yield as those seedlings have to share the vital resources essential for their development; whereas skips result in the unutilized field space. Both seedling emergence and seed singulation determines the plant stand uniformity.

Traditionally, the emergence counts are manually collected from the field by visual inspection. Multiples and skips due to poor seed singulation are also manually identified in the field. Multiples are identified once the seedlings emerge from the ground; whereas skips are estimated based on the missed seedling on the intended planting point. Skips and multiples can also be retrieved from the precision planter data loggers; however, not all the planted seeds emerge which makes it difficult to characterize the plant stand uniformity.

Several manual methods exist to test the seed vigor with the most common methods based on the growth of the seedlings (Filho, 2015). However, all of these methods are very laborious. Automated methods to collect the field data during the early growth stages can more rapidly quantify the emergence counts, number of multiples, seedling vigor, and seed-to-seed spacing. Having access to this type of data from the very early seed developmental stages can provide tremendous insight into the future of the field and to guide management decisions accordingly.

Image analysis has found applications in various industries such as manufacturing, military, medical, and photography. Application of image processing techniques to agriculture is still in the very young stage. Seedling vigor of corn in terms of seedling size was predicted using image analysis techniques by Matthews and Powell (2011). Image processing methods have also been used for the automated field stand counts for corn (Gnadinger and Schmidhalter, 2017; Varela et al., 2018); however, the issue of separating/segmenting multiple seedlings with overlapping leaves has not been addressed. It is challenging to count seedlings with overlapping leaves; clusters of seedlings may be counted as one seedling in image processing techniques. Ducournau et al. (2004) developed an automated image processing method for monitoring the emergence of sunflower seeds in laboratory conditions, however, the issue of identifying multiples was not addressed in that study.

Achieving evenly-spaced sunflower seeds is inherently challenging due to the shape and size of these seeds. Extra-large confectionary sunflower seeds perform poorly with precision planters, resulting in skips and multiples (Lilleboe, 2016). Sidhu et al. (2017) conducted a field study at Prosper, ND with the precision planting of eight coated kernel treatments to evaluate the effect of various coating materials and build-up levels on the emergence and seed placement of coated kernels. The authors collected the data manually by visual inspection in the field for emergence counts and number of multiples. Collecting such data manually from the field trials is very time consuming and labor intensive. Thus, the need to develop an image processing method to automate the collection of emergence counts and number of multiples from the sunflower field is high.

Software such as Matlab and Python have been commonly used in the literature for the image processing applications in the field of agriculture. However, users must have the programming skills to process the images using these software, which requires significant training. A freeware software called ImageJ has a suite of tools that can be adapted easily for various applications, and may be well suited for the plant stand uniformity characterization of a sunflower field

In this study, an image processing method was developed based on a freeware software called as ImageJ to automate the plant stand uniformity characterization of the field trials of confectionary sunflower seeds from the UAV- collected aerial imagery. Emergence counts, number of multiples, and seed-to-seed spacing data was predicted using the aerial imagery for the plant stand uniformity characterization. Seed-to-seed spacing data was further used to identify the possible skips in the field. Average seedling area was also predicted to monitor the health of the seedlings. A successful automated image processing method will find its application in comparing the different coated sunflower kernel treatments, and to adapt these methods for the similar data collection for the bigger field as well.

Materials and methods

Field study

A field study was conducted at Prosper, ND (46°58′ N, 97° 4′ W) in 2017, with ten seed treatments: two controls (large planting achenes, and extra-large achenes) and eight coated kernel treatments (Zeolite 30%, Zeolite 35%, Lime 30%, Lime 35%, Polymer A 30%, and

Polymer A 35%, Polymer B In-house 30%, and Polymer B In-house 35%). Planting at Prosper was done on June 1, 2017, using an Almaco SeedPro precision planter (Almaco, Ames, IA). The experimental design was a randomized complete block design with six replicates. Experimental units (individual plots) consisted of four planted rows spaced 0.76 m apart with 28 seeds at a 4.5 cm depth in each row with 27 cm seed-to-seed spacing. More details on this field study are included in the methods of Sidhu et al. (2018).

Seedling emergence counts and number of multiples from all four rows of each replicate was determined by visual inspection at ground level on 21 day after planting (DAP). Seedlings spaced less than 13 cm apart (in clusters) were considered to be multiples. The number of multiples in each row was determined by summing the number of seedlings present in all the clusters in a row. Emergence counts were taken by counting all the seedlings in a row, including the number of multiples present in that row. Emergence counts and number of multiples, as determined above, were used as reference values to determine which methods of image analysis were most accurate.

Unmanned aerial vehicle (UAV) imaging

Aerial images were acquired with a DJI Phantom 4 UAV (DJI Technology Company, Shenzhen, China) equipped with a 12-megapixel RGB camera with a focal length of 35 mm and a maximum aperture of F/2.8. Images were taken on 21 DAP with clear sky conditions at an azimuth of 132.62 and solar elevation of 59.85. Reference data from the field was also taken on this 21 DAP. Images were taken at flight height of 23 m when all the seedlings were between the V2 and V4 growth stages. A description of the growth stages of sunflower has been reported by Schneiter and Miller (1981). The resulting images from the UAV were stitched together using

Agisoft PhotoScan software (version 1.4.2., St. Petersburg, Russia). The size of each pixel in the collected images was 3.16 cm^2.

Image processing method development

Images from four out of six replicates of controls and coated kernel treatments were used to develop and compare image pre-processing methods, with the remaining two replicates used for validation of the most promising methods. For that purpose, the four replicates used for developing image processing methods are referred to as calibration dataset, and the two replicates used for validating the developed models are referred to as a validation dataset. The images were processed with ImageJ software (version, 1.41, NIH, Bethesda, MD). The schematic of image processing methods used in this study is shown in Fig. 4.1.

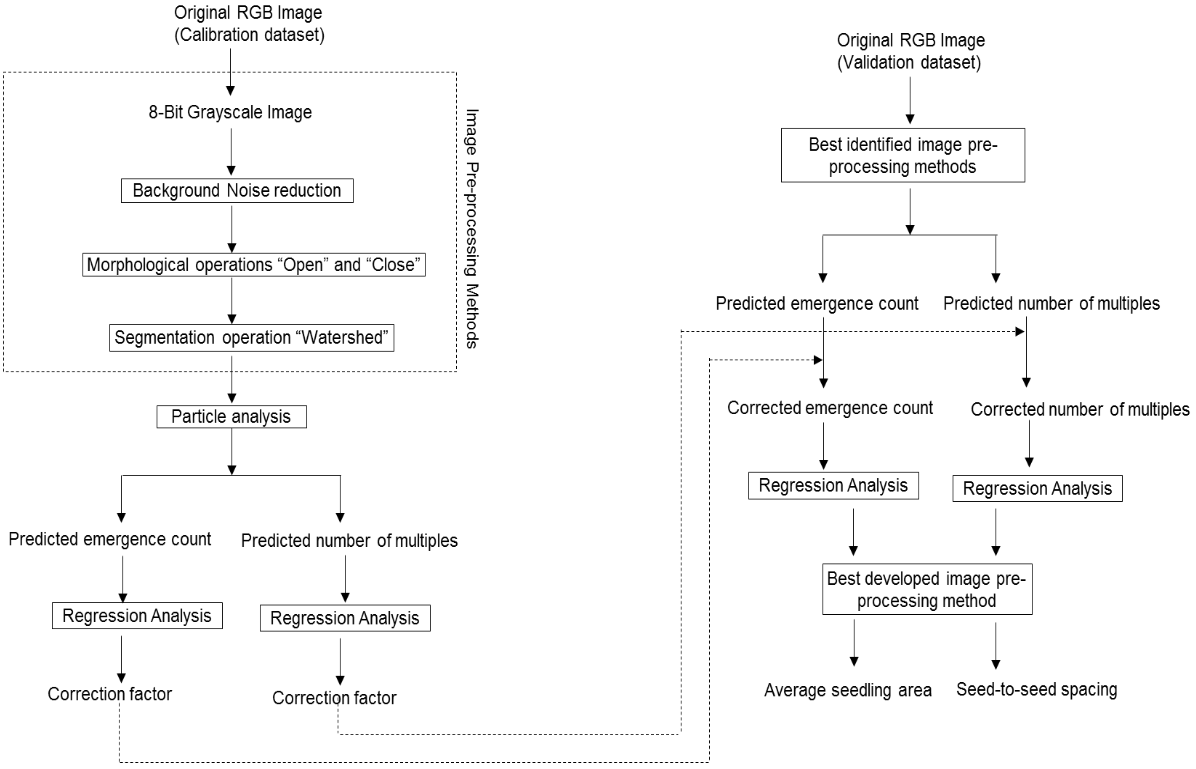

Fig. 4.1. Flowchart showing the image processing methodology.

The color images obtained by the camera were converted to 8-bit grayscale images as shown in Fig. 4.2 (a) and 4.2 (b). This conversion consolidates the three RGB color channels of each pixel into a single grayscale representation and assigns the numeric value (0-255) depending on the different shades of gray. Further, the 8-bit grayscale images were converted to binary images using “Make Binary” function as shown in Fig. 4.3 (c)

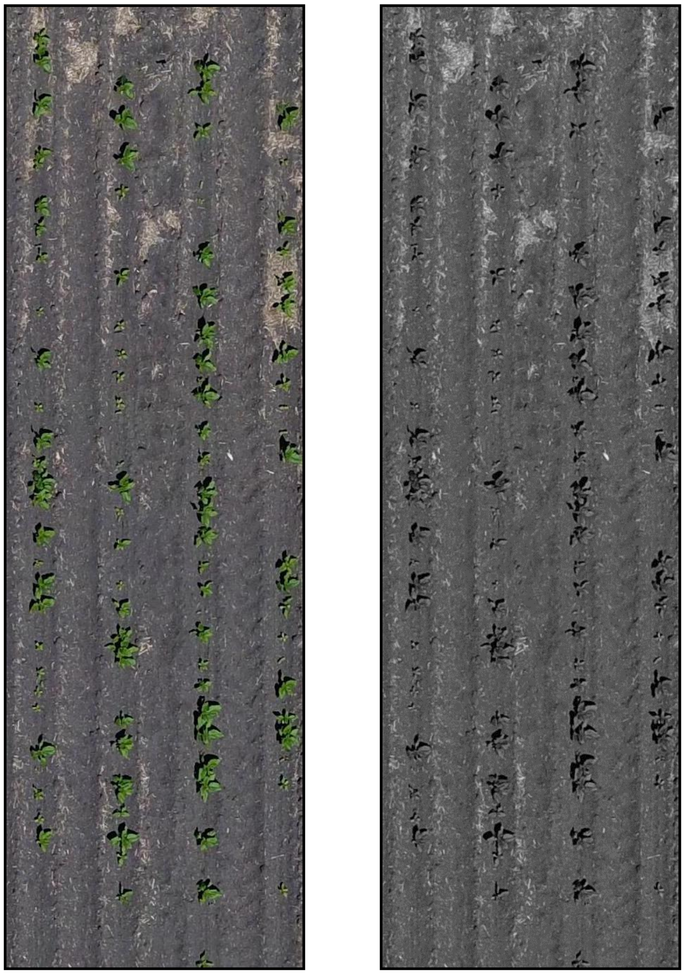

Fig. 4.2. Pre-processing step to convert the original RGB image into an 8-bit grayscale image: (a) Original image of a replicate of the 2017 confectionary sunflower field study (756 cm x 30.5 cm plot) (b) processed 8-bit grayscale image.

Noise in the images due to soil texture, straws, weeds, or any other ambiguity caused due to lighting conditions must be eliminated in order to identify the region of interest. To reduce the background noise from the grayscale images, two different approaches to thresholding, area-based, and color-based, were investigated and are explained in more detail below. Thresholding algorithms eliminate the unwanted pixels in the image and highlight the pixel clusters of interest, which should correspond only to the seedlings in this study. Further, it was observed that leaves of the same seedling appeared as separate segments as shown in Fig. 4.3a. To join these separate segments of the same seedling, the following two morphological operations were used: "Open" and "Close". Lomenie and Stamon (2008) describes the algorithms related to the "Open" and "Close" operations. An example of the segmented leaves of the same seedling being joined by "Open" and "Close" operations is shown in Fig. 4.3b. These operations assign the same pixel value as of the seedling segment pixel value to certain adjacent pixels, resulting in joining all the leaves of the same seedling.

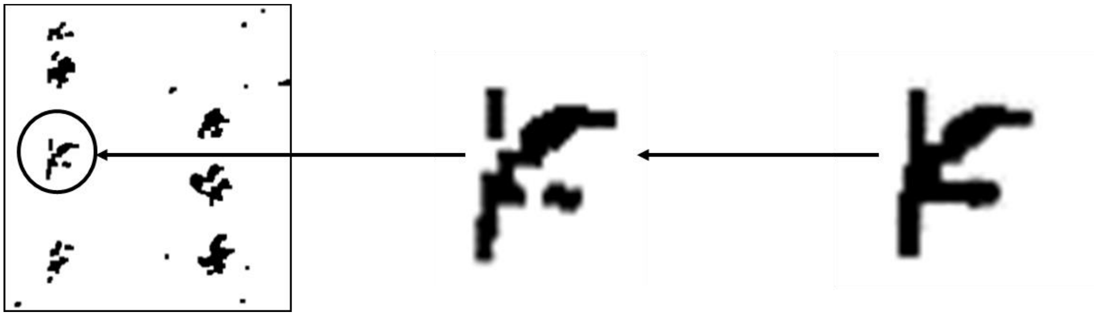

Fig. 4.3. Effect of "Open" and "Close" operations on the segmented leaves of the same seedling: (a) different leaves of the same seedling appearing as different segments, (b) different segments of the seedling joint as one.

It was observed that after using "Open" and "Close" operations, some of the neighboring seedlings were also joined to form clusters, particularly in the case of multiples. To segment the cluster of multiple seedlings, a segmentation operation "Watershed" was used. An example of the cluster being segmented into multiple seedlings using the "Watershed" operation is shown in Fig. 4.4. The "Watershed" operation segments the cluster of seedlings based on the proximity of the pixels of the selected objects in the image. The algorithm associated with the "Watershed" operation have been described by Roerdink and Meijster (2001).

Before and after segmentation, the images were subjected to the "Analyze Particle" operation, which labels each particle with a number in each row as shown in Fig 4.5. Each image consists of a series of discrete particles, with each particle representing one seedling. Each row was manually selected for the "Analyze Particle" operation by drawing a rectangle around it. The particle count gave the emergence count for each row. The number of multiples for each row was determined by taking the difference between the two counts. "Analyze Particle" operation also calculates the area of each particle; the average particle area of each row was reported as the average seedling area. The "Analyze Particle" operation can also be used to further reduce noise by excluding the particles of a specified size with the aid of the "Masks" option.

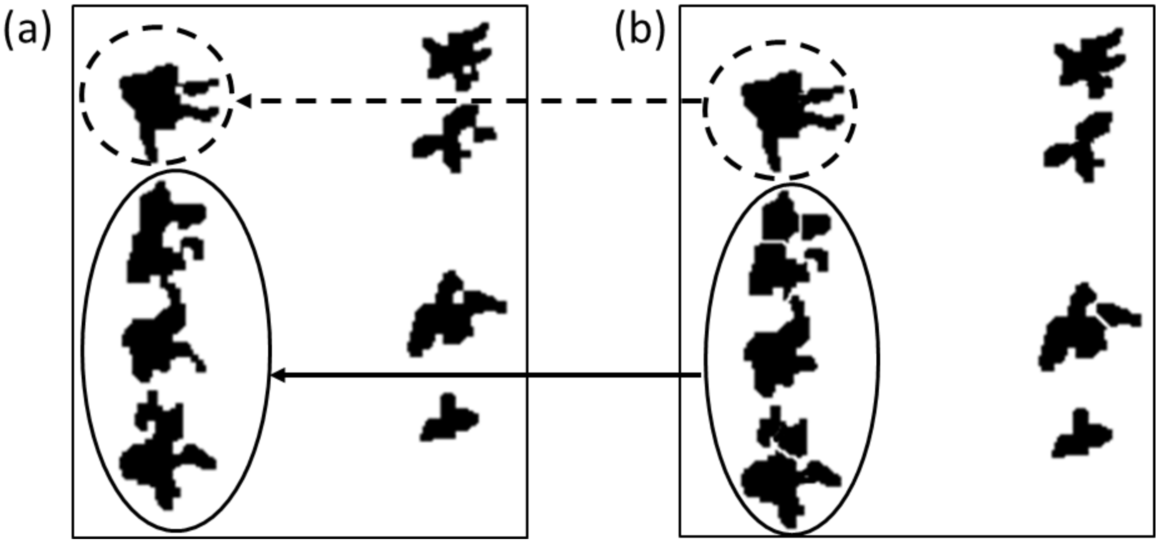

Fig. 4.4. Effect of "Watershed" operation on a cluster of seedlings: (a) cluster of seedlings appearing as one big entity, (b) segmentation of big cluster into smaller seedlings. Solid circle indicates the breaking of the big cluster, while a dashed circle indicates a smaller segment remaining untouched.

The seed-to-seed (STS) spacing of each row was also deduced from the "Analyze Particle" operation by including the centroid function. Centroid function was able to find the centroid of each particle, thus giving the centroid-to-centroid distance for all the particles. The targeted STS for the 2017 field study of confectionary sunflower seeds was 27 cm. In order to measure the deviation from the targeted STS, STS obtained with the image processing methods was divided into increments of 5 cm starting from 0 cm to 55 cm. STS between 26 to 30 cm was considered to be resulted from the seeds that were placed near the actual precision planter targeted space of 27 cm. Thus, the STS category of 26 - 30 cm was considered as the desired STS category. A number of seeds that fell in the category of STS of 0-5 cm and 6-10 cm were considered to be resulted from the multiples placed closely by the precision planter; whereas, a number of seeds that fell in the STS categories of 51-55 cm, and 55+ cm were the possible skips during planting. It is tricky to identify the skips as the non-present seedling point could be a result of the non-germinated seedling too.

The predicted values of calibration dataset for both emergence counts and a number of multiples were compared with their respective reference values (as described in the section "*Field study*") to check the accuracy of the predicted counts obtained with the ImageJ software. The four image processing methods that yielded the best correlations with reference values for the emergence counts and the number of multiples were chosen for the validation. The best image processing method identified during the validation step was used for estimating the average seedling area and seed-to-seed (STS) spacing. Seedling area is an important trait to determine the future plant health and grain yield. STS spacing data can give an insight into the plant stand uniformity by estimating the possible number of skips and multiples planted.

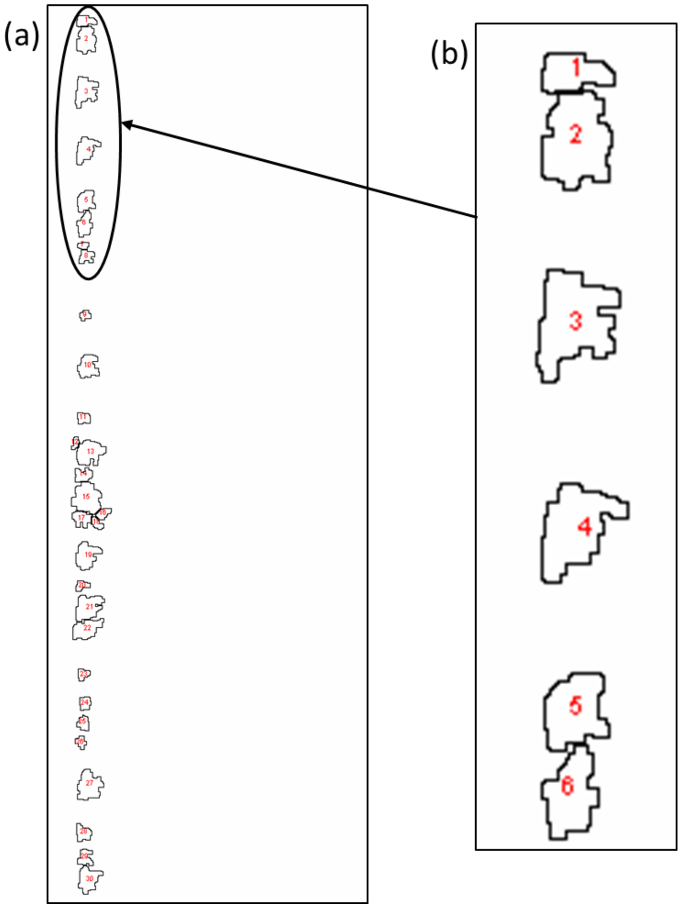

Fig. 4.5. Individual particles counted using the “Analyze Particle” operation: (a) counts for each row, (b) zoomed-in section of the counts.

Area-based thresholding algorithm

The 8-bit grayscale images were converted to binary images (black and white images) using an automated thresholding algorithm in ImageJ available in the “Make Binary” operation. This step renders the background as white and the seedlings as black. The noise was reduced from the binary image using the “Analyze Particles” operation by excluding the entities in the image which were less than 4 cm^2 in size. Images were then subjected to “Open” and “Close” operations followed by the “Watershed” operation. Using the “Watershed” operation also generates a few smaller segments resulting in false counts. To address this issue, these particles were excluded from the final count by specifying the size range in the “Particle Analyze” operation. This was an iteration based step, where different lower size limits ranging from 10 to 20 cm^2 were tested with infinite size as the upper limit. It was observed that the size range from 16 cm^2 to infinite resulted in the closest fit for emergence count when compared with the reference values. Using the “Analyze Particle” operation, emergence count, number of multiples, average seedling area, and seed-to-seed (STS) spacing in every row were obtained.

Color-based thresholding algorithm

The 8-bit grayscale images obtained were subjected to the sixteen different thresholding algorithms available in ImageJ: Image-J Default, Huang (Huang and Wang, 1995), Intermodes (Prewitt and Mendelsohn, 1966), Isodata (Ridler and Calvard, 1978), Li (Li and Tam, 1998), MaxEntropy (Kapur et al., 1985), Mean (Glasbey, 1993), MinError (I) (Kittler and Illingworth, 1986), Minimum (Prewitt and Mendelsohn, 1966), Moments (Tsai, 1985), Otsu (Otsu, 1979), Percentile (Doyle. 1962), RenyiEntropy (Kapur et al., 1985), Shanbag (Shanbag, 1994), Triangle (Zack et al., 1977), and Yen (Yen et al., 1995). These thresholding algorithms were developed for various image processing applications, and detailed information on these algorithms can be found in the ImageJ help files. Images processed with each of the sixteen threshold algorithms were further processed with “Open”, “Close”, and “Watershed” operations as described in section *“Area-based thresholding”*. Emergence count, number of multiples, average seedling area, and seed-to-seed (STS) spacing in each row was obtained using the “Particle Analyze” operation.

Validation of the developed image processing methods

The four best-performing image processing methods identified during the image processing method development were further validated by obtaining corrected emergence counts and the number of multiples of each row for the validation dataset. The corrected emergence counts and number of multiples were calculated by dividing the predicted values by the ratio of predictions.

Data analysis

Emergence count and number of multiples data were averaged for all the four rows over all the four replicates for image processing method development. The coefficient of determination and ratio of predictions were computed to compare the predicted data with the reference data. The ratio of predictions was computed by dividing the predicted values by the reference values.

For validation of the best performing image processing methods, the corrected emergence counts and number of multiples were estimated for the validation dataset. The coefficient of determination and root mean square error (RMSE) were computed for comparing the corrected and reference data. RMSE was calculated using the following equation:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{predicted} - y_{reference})^2}$$

where n is the number of samples, $y_{predcited}$ is the predicted data using image processing methods, and $y_{reference}$ is the reference data collected from the field.

Data was also analyzed using analysis of variance (SAS v9.3, SAS Institute Inc., Cary, N.C.), and an F-protected LSD ($p \leq 0.05$) was calculated for comparisons of the main effect means.

Results and discussion

Image processing method development for emergence count and number of multiples

Seventeen different image processing methods were applied to predict the emergence count and number of multiples from the UAV-collected RGB images using ImageJ image processing methods. Four replicates of the controls and coated sunflower kernel treatments were used as calibration dataset for these image processing methods. Seventeen thresholding algorithms mentioned in section *"Image processing method development*" were investigated to reduce the noise from the images. It was noted that using just the thresholding algorithms, predicted emergence counts were six times higher on average than the reference emergence counts, for all the thresholding algorithms. This was mainly because the leaves of the same seedling often appeared as separate segments as shown in Fig. 4.3a. ImageJ counted each separate segment (leaf) as one seedling leading to the over counting of the emergence counts. Conversely, clusters of multiple seedlings as shown in Fig. 4.4 led to undercounting of the emergence counts as a group of multiples was counted as one seedling. Thus, to achieve a more accurate count the above-mentioned thresholding algorithms were used in conjunction with "Open", "Close", and "Watershed" operations as mentioned in section *"Image processing method development*".

The coefficients of determination (R^2) from a comparison of the predicted and reference values for the calibration dataset for all the seventeen thresholding algorithms used in conjunction with "Open", "Close", and "Watershed" operations are presented in Table 4.1. Overall, the four best algorithms for thresholding for the emergence count were ImageJ-Default, Intermodes, MaxEntropy, and Minimum algorithm (with $R^2 \geq 0.86$ and ratio of predictions $\leq$ 1.70). All of these best algorithms for emergence counts were identified as the best algorithms for number of multiples too resulting in $R^2 \geq 0.72$ and ratio of predictions ≤ 1.46 for prediction of the number of multiples (Table 4.1).

Table 4.1. Coefficient of determination (R^2) and mean ratios between the predicted and reference values for emergence counts and number of multiples (n = 10).

Thresholding	Emergence counts[1]		Number of multiples[1]	
Algorithms	R^2	Ratio of predictions	R^2	Ratio of predictions
Binary	0.80	1.97	0.58	2.07
ImageJ-Default	0.89	1.70	0.79	1.38
Huang	0.72	2.98	0.66	1.6
Intermodes	0.86	1.26	0.73	1.41
Isodata	0.84	1.78	0.70	1.51
Li	0.85	1.86	0.69	1.55
MaxEntropy	0.88	1.47	0.72	1.46
Mean	0.85	1.68	0.73	1.42
MinError (I)	0.77	2.56	0.51	3.09
Minimum	0.91	1.06	0.80	1.33
Moments	0.65	3.25	0.51	2.34
Otsu	0.68	3.17	0.51	2.47
Percentile	0.79	3.04	0.58	2.19
RenyiEntropy	0.73	3.78	0.57	2.72
Shanbag	0.62	3.87	0.51	3.16
Triangle	0.68	3.41	0.55	2.97
Yen	0.72	3.24	0.56	2.89

[1] Data is averaged for the calibration dataset.

Validation of developed method for the emergence counts and number of multiples

The four best algorithms identified in conjunction “Open”, “Close”, and “Watershed” operations were used for predicting both the emergence counts and the number of multiples for the validation dataset.

It was noted during the image processing method development that the four best-identified thresholding algorithms always overestimated both the emergence counts and number of multiples. The ratio of the predictions with the ImageJ-Default, Intermodes, MaxEntropy, and Minimum thresholding algorithms to the reference data were 1.70, 1.26, 1.47, and 1.06, respectively (Table 4.1). The ratio of predictions for number of multiples were 1.38, 1.41, 1.46, and 1.33, receptively for the ImageJ-Default, Intermodes, MaxEntropy, and Minimum thresholding algorithms (Table 4.1).

Therefore, the predicted emergence counts and number of multiples for the validation dataset with each of the thresholding algorithm were divided by their respective ratio of predictions to get the corrected values. These corrected values were then compared with the corresponding reference data (Fig. 4.6 and 4.7, respectively). The reference values for both the emergence counts and number of multiples were in whole numbers; whereas the corrected values were obtained as decimal numbers.

The corrected emergence counts using the ImageJ-Default and Minimum thresholding algorithm have a very good fit (R^2 = .92 and .94, respectively) to the reference emergence counts (Fig. 4.6). ImageJ-Default and Minimum thresholding algorithms resulted in R^2 of 0.90 and 0.93, respectively for the corrected number of multiples (Fig. 4.7). The ImageJ-Default thresholding algorithm automatically derives a threshold from a gray level histogram by an iterative approach to differentiate the background from the object of interest. The average pixels for both the background and the object is computed at initial threshold followed by repeating of this process by incrementing threshold. This process is repeated until the threshold value reaches the average pixel value of the background and the object. Minimum thresholding algorithm determines the threshold value by iteratively smoothing the frequency distribution histogram of the pixels until only two distinct peaks referring to background and object are obtained. It was developed for distinguishing different types of human blood cells (Prewitt and Mendelsohn, 1966). Overall, the Minimum thresholding algorithm resulted in both the highest R^2 and lowest RMSEP for both the corrected emergence counts and number of multiples for each row (Fig. 4.6 and 4.7). Further, with the Minimum thresholding algorithm, the corrected emergence counts and corrected number of multiples for the validation dataset are significantly correlated with the respective reference data resulting in the p-values of 0.0035 and 0.018.

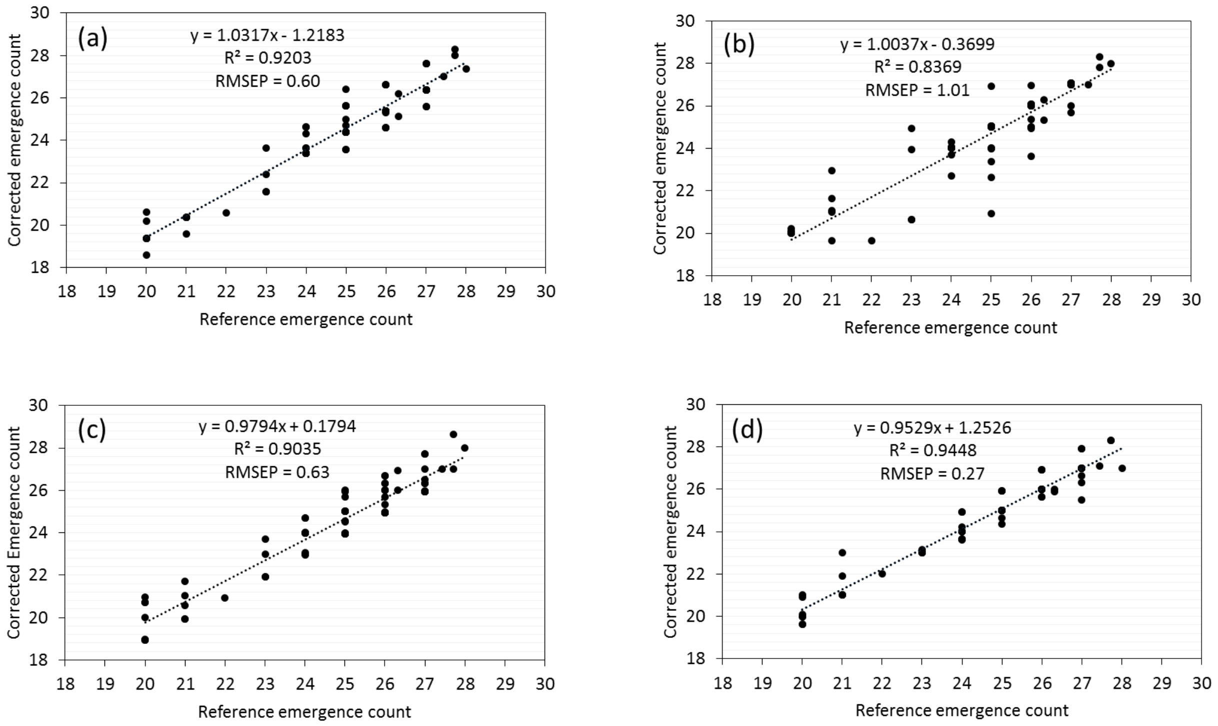

Fig. 4.6. Relationship between the corrected emergence counts and the reference emergence counts for the validation dataset with ImageJ thresholding algorithms: a) ImageJ-Default, (b) Intermodes, (c) MaxEntropy, and (d) Minimum (n = 80). Predicted values were divided by the corresponding ratio of predictions to get the corrected emergence counts.

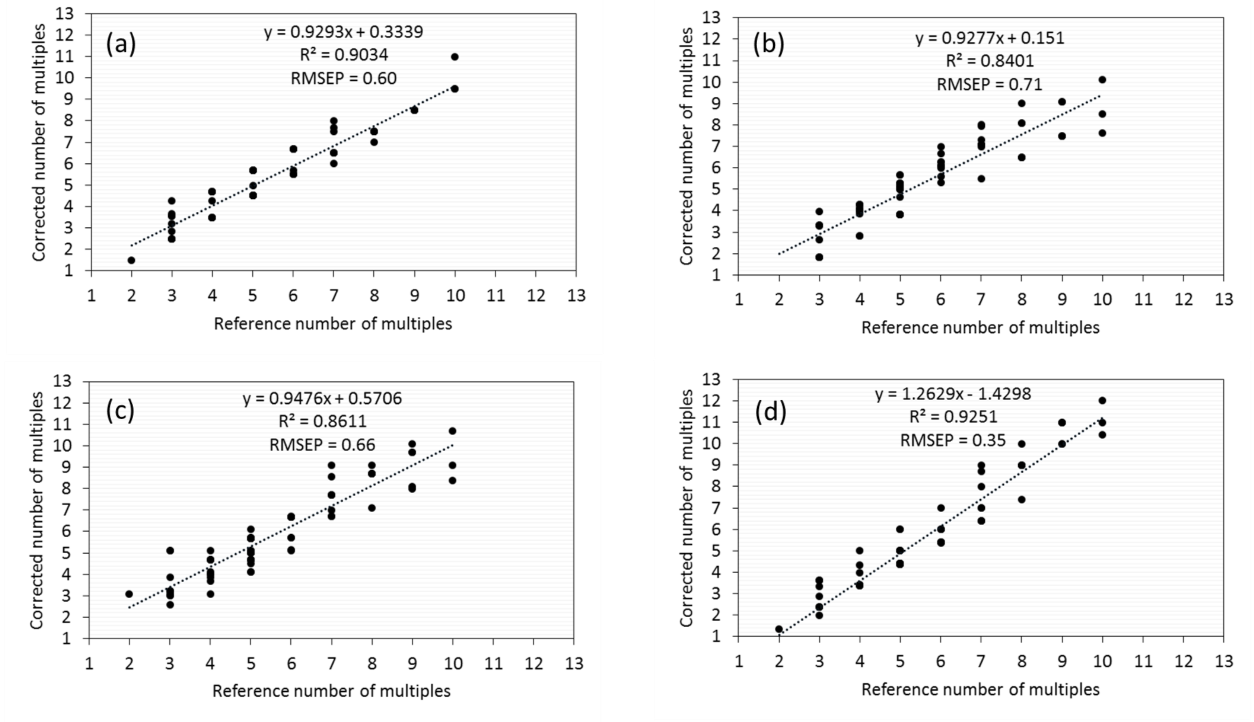

Fig. 4.7. Relationship between the corrected number of multiples with the reference number of multiples for the validation dataset with ImageJ thresholding algorithms: a) ImageJ-Default, (b) Intermodes, (c) Isodata, (d) Li, (e) MaxEntropy, (f) Mean, and (g) Minimum (n = 80). Predicted values were divided by their mean ratio of estimates to get the corrected number of multiples.

Average seedling area

Average seedling area of each treatment type can give the indication of the seedling vigor which can be used to predict the relative grain yield during harvest. The grain yield can be impacted by environmental factors, thus it is not possible to predict the grain yield accurately based on the average seedling area on 21 DAP. Thus, in this study, an effort to predict the relative grain yield based on the average seedling size on 21 d post-planting was investigated.

The average seedling area for each treatment type for the calibration dataset calculated by Minimum thresholding algorithm in conjunction with “Open”, “Close”, and “Watershed” operations is presented in Table 4.2. Minimum thresholding algorithm was identified as the best algorithm to estimate both emergence count and number of multiples; therefore, it was assumed that this algorithm, when used in conjunction with “Open”, “Close”, and “Watershed” operations, can be trusted with the estimation of average seedling area for sunflower field.

As expected, the average seedling area associated with extra-large seeds (XL) is significantly lower by18% as compared to the coated kernel treatments (Table 4.2). The average seedling area for each treatment type is significantly correlated with the grain yield (Sidhu et al., 2018; Table 5) at the Pearson’s correlation coefficient of 0.85 at the p-value of 0.002.

Table 4.2. Predicted seedling area in each row of each treatment type using Minimum thresholding algorithm.

Treatment type	Average Seedling Area[1] (cm^2)	Grain Yield [2] (kg/ha)
Large planting seed	54±5^{c}	2064^{c}
Extra-large seed	48±11^{d}	1661^{d}
Zeolite 30%	57±7^{b}	2127^{b}
Zeolite 35%	58±6^{a}	2299^{b}
Lime 30%	59±4^{b}	2353^{ab}
Lime 35%	60±5^{a}	2498^{a}
Polymer A 30%	57±6^{b}	2146^{bc}
Polymer A 35%	58±7^{a}	2279^{b}
Polymer B IH[3] – 30%	58±4^{a}	2371^{ab}
Polymer B IH – 35%	59 ±5^{a}	2579^{a}

Means followed by the same letter within the same column are not statistically significant at P≤0.05

[1] Seedling area averaged for the calibration dataset.

[2] Grain yield data taken from Sidhu et al. (2018)

[3] In-house coating type

Seed-to-seed spacing (STS)

STS for each treatment type for the calibration dataset calculated by Minimum thresholding algorithm is presented in Fig. 4.8. As expected, the XL seeds showed poor plant stand uniformity as only 49% of the seeds were placed in desired STS category of 26 – 30 cm as opposed to 74% of large planting seed in the desired STS category. XL seeds also resulted in the largest number of seeds in the STS categories related to multiples (STS categories of 51 cm +)

and possible skips (STS categories of 0-5 cm and 6-10 cm) as compared to the large seed and all of the coated kernel treatments. Both Zeolite 35% and Polymer B-IH coated kernel treatments resulted in 77% of the seeds in the desired STS category with < 7% seeds both in the multiple STS category and the possible skips category.

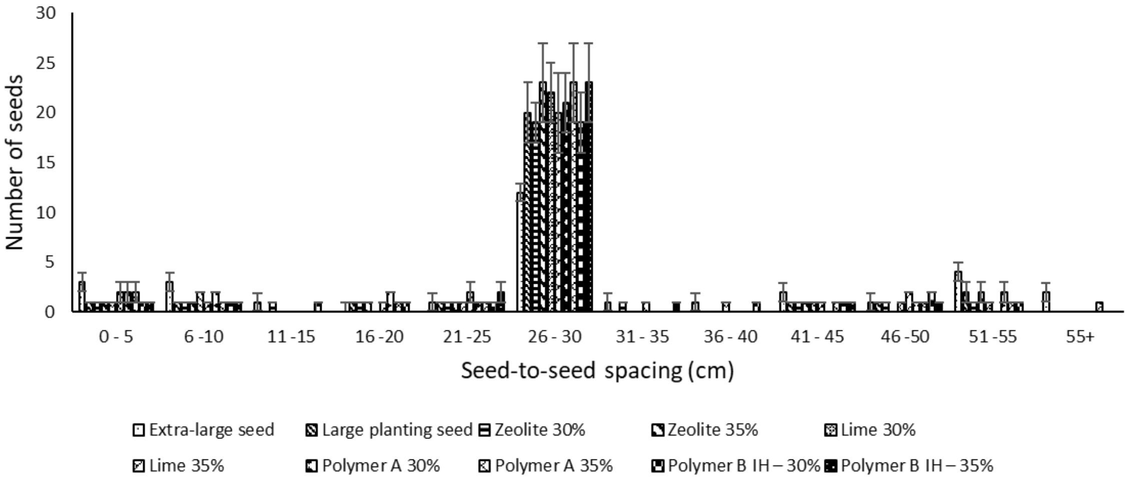

Fig. 4.8. Predicted seed-to-seed spacing in each row for the calibration dataset of each treatment type using Minimum thresholding algorithm.

Limitations of existing ImageJ methods

It was noted that the predicted values were always overestimated for both emergence counts and number of multiples. Using the existing image processing methods available in ImageJ with a correction factor, the best image processing method involving the use of Minimum thresholding algorithm resulted in R^2 of 0.94 and 0.92 for determining emergence counts and number of multiples, respectively. The Minimum thresholding algorithm was originally developed for the identification and counting of human blood cells. For some of the cases, using the “Watershed” operation broke the cluster of multiple seedlings into more than the actual number of seedlings present in the cluster. Developing an image processing method specifically for determining the emergence counts and identifying the number of multiples for the sunflower field can result in better accuracy of the predicted values. This could be achieved by developing an ImageJ plugin that can identify both the shape and color of the sunflower seedlings. Additionally, ImageJ is able to process the image size of 2GB at a given time, thus larger sized images must be broken down to appropriate size for processing.

Conclusions

This study showed that Minimum thresholding algorithm in conjunction with “Open”, “Close”, and “Watershed” operations was the most appropriate image processing method for estimating both automated emergence counts and automated number of multiples. This developed image processing method reduced both the labor and time to manually collect the emergence counts and number of multiples from the field. The time required to collect the before mentioned data reduced by 85% when the developed image processing method was used as opposed to the traditional method of visual data collection in the field for emergence counts and number of multiples count which took 6 h. This developed image processing method was also used to calculate the average seedling area which can be used as an indicator of future grain yield. Seed-to-seed spacing was deduced from this image processing method which gives an indication of number of multiples and possible skips in the field.

Though this image processing method is developed for a small scale field study, this method can easily be adapted for larger-scale fields too. Use of a correction factor, based on the difference between predicted and reference values from a small area of the field, to correct the predicted values for the rest of the field is recommended. We further recommend to validate the developed image processing method in this study in different field conditions too.

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GENERAL CONCLUSIONS

In recent years, the demand for extra-large (XL) confectionary sunflower seeds as snack food has increased and this trend will likely continue to grow. Yet, the acceptability of these seeds by growers remains a challenge because of the poor viability and plantability of these XL seeds. Thus, the option of coating the hulled sunflower kernels was investigated in this dissertation. It was hypothesized that coating the hulled kernels of XL seeds would retain the viability of hulled kernels while increasing the plantability of the hulled kernels. The performance of coated kernels was checked against not only XL seeds, but also with the large planting seeds that are currently being used by farmers for planting. Methods to evaluate the quality of coated kernels were also investigated in this dissertation.

It was noted that the coating on hulled kernels delayed the water uptake for most of the coated kernels, resulting in delayed emergence. As a result, the standard AOSA method to test sunflower seed germination was modified to take the germination count on the tenth day instead of the standard seventh day to account for late emerging seedlings. A quick screening test, tetrazolium (TZ), was found to be acceptable to check the viability of coated kernels in three days.

During the lab testing of coated kernels, the post-planting emergence of the coated kernels with the following materials was comparable to both large and XL seed: zeolite, lime, pumice, and three types of polymers. The kernels coated at a 34% build-up level had the highest seed singulation while preserving the kernel viability. Furthermore, it was shown that the use of the seed lubricants with the coated kernels inside the planting equipment increases the coated kernel plantability, both in terms of singulation and preserving the post-singulation germination.

Field testing of the coated kernels at two different locations (Prosper, ND, and Minot, ND) showed improvements for increasing both viability and plantability of coated sunflower kernels as compared to the XL seed. Minot location had moisture stressed growing conditions. At both locations, all the coated kernel treatments resulted in significantly higher live seed emergence and grain yield than XL seeds. Polymers, Zeolite, and lime at both 30% and 35% build-up levels were found to be suitable during these field trials. Polymer A and lime were identified as the two best coating materials. All of the coated kernel treatments (except 30% build-up level at one type of polymer) showed superior grain yields when compared to the large seeds.

An automated image processing method to characterize the plant stand uniformity from the RGB images collected using unmanned aerial vehicle (UAV) from the field trails at Prosper location. From all the 17 tested, algorithms, Minimum thresholding algorithm in conjunction with “Open”, “Close”, and “Watershed” functions were found to be suitable image processing methods. These automated methods reduced the manual data collection time by 90% for taking the emergence counts and number of multiples count.

RECOMMENDATIONS FOR FUTURE WORK

Hulling and coating

- Further refinement of controlling the seed orientation of XL seeds entering the shearing rolls should be investigated.
- Efficient ways to separate the broken and intact kernels generated during the hulling of XL seeds should be researched. Use of optical sorters with size and shape characterization abilities would be especially effective.
- Economic analysis of the seed hulling and coating process should also be performed on the best performing coating materials and build-up levels. This analysis will help to facilitate the decision of recommending best coating treatments to the sunflower industry.

Testing of coated kernels

- Additional smaller scale field studies should be conducted at several locations for two growing seasons with atleast the four best coating materials at build-up level of 35% along with controls (XL seeds and large seeds) with a precision planter to validate the performance of sunflower coated kernels.
- Talc should be added to the coated kernels during planting for protecting the coated kernels inside the planting equipment while improving the seed plantability.
- Incorporation of active ingredients (such as fertilizers, fungicides, pesticides, nutrients) in sunflower kernel coating should be investigated.
- Dust-off tests should also be conducted to measure the amount of dust created by coated sunflower kernels with active ingredients during planting.

Imaging methods

- The image processing method developed in this dissertation should be validated under different field conditions for the sunflower field.
- RGB images acquired for the conducted study for this dissertation can be converted to HSI (hue-saturation intensity) color space to process the images to characterize seedling vigor.
- Better accuracy of predictions could be achieved by the development of an ImageJ plugin that can identify the sunflower seedling shape and color.