EXPLORING THE POTENTIAL COMMERCIAL SIGNIFICANCE OF *RATIBIDA COLUMNIFERA* (NUTT.) WOOTEN & STANDL. FOR THE GREEN INDUSTRY

A Dissertation

by

KAITLIN ANN HOPKINS

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Co-Chair of Committee,	Michael A. Arnold
Co-Chair of Committee,	Charles Hall
Committee Members,	Brent Pemberton
	Marco A. Palma
Head of Department,	Patricia Klein

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ABSTRACT

Ratibida columnifera (Nutt.) Wooton & Standl. is a perennial that exhibits large variations in morphological characteristics. This dissertation sought to characterize requirements for *R. columnifera* vegetative and seed propagation, as well as elicit consumer preferences of selected traits using conjoint analysis. Stem development stage, application of auxin, genotypic variation, and effects of bottom heat applications were assessed to determine impacts on adventitious root regeneration. Younger apical stem sections readily produced better quality root systems than more lignified basal sections. Optimal rooting percentages and quality occurred with 0.10% to 0.30% IBA (indole butyric acid) quick dips, with 0.30% optimal for most genotypes. Application of 26°C bottom heat improved rooting in both seasons compared to ambient and bottom heat of 32°C. Heat of 32°C improved rooting measures during the cool season, but not the warm season. Overnight hydration, storage condition variations, stratification, scarification, and seed maturation effects were assessed to determine impacts on viability and percent germination. Overnight hydration did not impact germination. Germplasm had a significant effect on germination for all remaining experiments. Seed maintained viability through 18 months. Cold storage(3°C) had no effect on viability or germination. All three germplasms exhibited increased percent germination with some stratification, and declined with all acid scarifications. Most germplasm benefit from between 30 to 60 days of cold moist stratification. There was an interaction effect among germplasms, location on the inflorescences, and maturity stages for *R. columnifera*. Data suggests that seed should be harvested as close as possible to when natural dispersement would occur for optimum germination. Conjoint analysis was used to gain insight on consumer preferences for attributes and levels attributed to *R. columnifera*. A ratings-based conjoint analysis of petal colors, petal shapes, petal numbers, and prices was conducted to elucidate willingness to pay using data from 1000 subjects recruited using Amazon Mechanical Turk (MTurk). Petal color was the most important attribute, followed by price, petal shape, and petal number. Utility values revealed a preference for bicolor petals. Preferences for price went from least expensive to most expensive. Circular petals were favored over other shapes. Subjects also preferred inflorescences to have 10 petals or more. Cluster analysis yielded three consumer segments which differed in their utility values.

DEDICATION

This dissertation is lovingly dedicated to my devoted husband, Marc Klekar. Thank you for the unwavering support you provided in this endeavor

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Contributors

This work was supervised by a dissertation committee consisting of Professor Michael A. Arnold (co-advisor), Professor Charles Hall (co-advisor) and Professor Brent Pemberton of the Department of Horticultural Sciences and Professor Marco Palma of the Department of Agricultural Economics.

All other work conducted for the thesis dissertation was completed by the student under the advisement of Professor Michael A. Arnold and Professor Charles Hall of the Department of Horticultural Sciences, with field assistance from student workers, and statistical analysis assistance from Dr. Melinda Knuth.

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CHAPTER I

INTRODUCTION

Background and Rationale

Ratibida columnifera (Nutt.) Wooton & Standl. [syn. *Lepachys columnifera* (Nutt.) J.F. Macbr., *Ratibida columnaris* (Pursh) D. Don, *Rudbeckia columnaris* Pursh, *Rudbeckia columnifera* Nutt.], is most commonly known as prairie coneflower or Mexican hat, but is also referred to by a number of regional common names including columnar prairie coneflower, longhead-coneflower, redspike Mexican-hat, thimble flower, or upright prairie coneflower (Tropicos, 2018; USDA, 2012). *Ratibida columnifera* is an herbaceous perennial in the *Asteraceae* Bercht. & J. Presl (nom. alt. et cons.: *Compositae*) family found in a large natural range in the United States (Tropicos, 2018). This wildflower exhibits a large variation in both floral and vegetative characteristics (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). These variants have potential to produce a variation that could be a candidate for a nursery crop. *Ratibida columnifera* is readily available as seed, but not common as a nursery crop (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). This could be due to the lack of information on the cultural requirements to produce a quality crop. Collection of wild germplasm and characterization of the cultural requirements of this plant could eventually lead to a commercialized variety not yet seen on the market.

Literature Review

Growth, Flowering, and Distribution

Ratibida columnifera is a perennial wildflower commonly encountered in much of the United States. Distribution of this wildflower extends from the United States into Mexico (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). Native distribution in the United States includes from Montana and North Dakota to New Mexico and Texas, and then from New Mexico to the East coast in the Southern United States. It can be found in many other states, but this is believed to have been introduced and not native (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). The growing season of *R. columnifera* ranges from March to November, partially due to its wide distribution (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). It will typically flower in the first year of growth. This plant exhibits physiological stress tolerance and can also be considered drought resistant (Hind, 2006). *Ratibida columnifera* has also been examined for salt tolerance (Niu et al., 2012). This study exposed several wildflower species to a range saline solution concentration (Niu et al., 2012). The solution was made up of sodium chloride (NaCl), magnesium sulfate ($MgSO_4$:$7H_20$), and calcium chloride ($CaCL_2$) at 87:8:5 weight ratio. This combination of salts reflects the typical salt combination of saline municipal water. Plants were subjected to electrical conductivity (EC) concentrations of 1.5, 2.8, 4.1, 5.1, and 7.3 $dS \cdot m^{-1}$ (Niu et al., 2012). Out of the selected species for that study, *R. columnifera* was the most tolerant of salt application (Niu et al., 2012). *Ratibida columnifera* had a 90-100 percent survival rate for all treatments (Niu et al., 2012). It also had high visual ratings regardless of salinity treatment (Niu et al., 2012). *Ratibida columnifera* prefers a full sun environment but will perform in partial shade (Hind, 2006).

Floral characteristics vary within the species for *R. columnifera*. Petal color can range from solid yellow to a dark maroon, with many combinations between (Hind, 2006). Some flowers will have defined color blocks of red and yellow, while others have a gradual color change from red to yellow. Other unique variations include different petal shape and double petals (Hind, 2006).

This herbaceous perennial ranges in height from 20-100 cm (Hind, 2006). Usually there are a few stems with each node producing a flowering shoot (Hind, 2006). The Leaves are simple at the base and obovate. The rest of the leaves are ovate to obovate in outline, and usually once pinnate with the terminal leaflet (Hind, 2006). The inflorescence is a solitary capitula with a peduncle length of 18-40 cm. The chromosome count for *R. columnifera is* 2n=28 (Löve, 1982). The disc florets are perfect flowers (Hind, 2006). Ray florets are neuter, or lacking reproductive parts (Hind, 2006). *Ratibida columnifera* is a self-sterile plant, meaning it must outcross with another individual to produce viable seed (Jahns, 1976). *Ratibida columnifera* is also entomophilous, or insect pollinated (Lonard and Judd, 1989). The fruit is an achene that is 2.3-3 mm long by 2-2.2 mm wide (Hind, 2006). In order to breed *R. columnifera*, prior to the anthesis of the first disc flower the heads are bagged to prevent pollen contamination using commercial paper laboratory tissue (Kimwipes) (Jahns, 1976). Crosses are made by removing the bags of the two flowers to be crossed, then rubbing the heads together, and then rebagging the heads twice daily until at least one of the heads terminates flowering

(Jahns, 1976). The reason for pollinating twice a day is that the disc flowers mature from the base of the column to the top, so one pollination event would not pollinate the entire seed head. The heads remain bagged until the achene's reach full maturity around five to seven weeks later (Jahns, 1976).

Propagation and Production

Ratibida columnifera is readily available as seed and is a common component of many wildflower seed mixes (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). It is more uncommon to see it sold as a container nursery crop. Available literature stresses the need for stratification of *R. columnifera* seeds for successful germination rates (Niu et al., 2012; Romo and Eddleman, 1995). *Ratibida columnifera* seed do not require light to germinate (Romo and Eddleman, 1995). Other factors that can affect germination and therefore future use of collected seed are storage conditions and duration. Germplasm within a species can vary drastically, especially when there are geographical differences. Genetic variation among populations along a geographical gradient can suggest the presence of clinal variation, as was the case in *Solidago* (Weber and Schmid, 1998). Literature lacks detailed information about pretreatments and seed harvesting considerations for *R. columnifera.* This study aims to establish protocols necessary for the storage and pretreatments necessary to maintain *R. columnifera* seed.

Commercial production fields should be planted in rows at 82 pure live seed (PLS) per linear meter row (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). Row spacing ranges from 60 to 90 cm depending on cultivation equipment. For example, with 60 cm spacing the recommended seeding rate is 1.1 $kg{\cdot}ha^{-1}$ PLS

(United States Dept. of Agric. Natural Res. Conservation Serv., 2006). Seed harvest is effective using several methods including swathing and combining or direct-combining. Direct-combining is done when the seed has begun to shatter from the top of the ripened seed head (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). Processing of the seed works well over a 2-3 screen fanning mill with final cleaning over an indent cylinder or gravity table (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). Seed production of 336 to 560 $kg \cdot ha^{-1}$ is expected under irrigated conditions, with stands being productive for three years or two seed crops (United States Dept. of Agric. Natural Res. Conservation Serv., 2006).

Documenting the extent of plant trait variation in native populations is important when determining ornamental nursery crop potential (Carver et al., 2014). Not only are aesthetics of the traits important, but also the ability of the plant to tolerate commercial container nursery / greenhouse production techniques is crucial to industry acceptance. Identifying the adaptability of the plant both to landscape use and current production technology will serve as the foundation for future improvement of selections (Carver et al., 2014). Use of exotic non-native plants in the landscape causes several issues (Wilde et al., 2015). These issues include replacing native vegetation which is a problem for organisms that depend on native plants, decreasing diversity of insects and herbivores, and decreasing the energy available to the food web due to lack of native plants (Wilde et al., 2015). A challenge to increasing the use of native species is in providing ornamental plants that are both ecologically functional and economically viable (Wilde et al., 2015).

Marketing and Consumer Preferences

The U.S. green industry includes nursery and greenhouse producers, landscape service providers as well as wholesale and retail distributors (Hall et al., 2006). The wholesale value of plant production by nurseries in the top 17 U.S. states was estimated at $4.65 billion in 2006 and $4.2 billion in 2008 for commercial greenhouses in the top 15 states (Dennis et al., 2010). Consumer and market interest in non-horticultural business practices has encouraged discussions about sustainable production and certification in industry (Dennis et al., 2010). The goal of sustainable production is to reduce environmental degradation, maintain agricultural productivity, promote economic viability, conserve resources and energy, and maintain stable communities and quality of life (Dennis et al., 2010). Use of native plants can increase biodiversity, and decrease some inputs to production like water use due to the plants being more adapted to the location (Wilde et al., 2015). The transformation on resident native plant communities to other kinds of landscapes will result from subdivision developments, resort planning, and urbanization (Zadegan et al., 2008). This transformation, along with an interest in non-native species in developed landscapes, is becoming a major concern for environmentalists, conservationists, and restoration ecologists (Zadegan et al., 2008). One potential response to these concerns is to install more native plant species in either traditional or more naturalistic landscape designs providing the community with an ecology-based solution to help maintain or restore biological diversity (Zadegan et al., 2008).

As growers consider adoption of sustainable practices, if they find positive customer value tradeoffs exist for sustainable practices, they may be more willing to adopt sustainable practices (Hall et al., 2009). Thus, positive value for sustainable practices may affect grower's willingness to adopt sustainable practices (Hall et al., 2009). Previous studies show that consumer demand for product-stewardship or environmentally conscious products and business practices is rapidly rising (Yue et al., 2012). Markets consist of different groups of consumers who have different preferences and attitudes toward environment-related product attributes. Consumers with environmental concerns are willing to purchase and pay a premium for plants labeled as environmentally friendly products (Yue et al., 2012). This was also reflected when surveyed individuals identified cost as one of the least important factors limiting their purchase decision of native plants (Wilde et al., 2015). From this we know that consumers will choose native plants as an environmentally friendly option, but one issue is that consumers are not always informed of the native status of the plants (Yue et al., 2012). In one survey, only 4% of participants were "always" informed, 18% were informed "most times", 38% were "sometimes" informed, 30% were "seldom" informed, and 10% were 'never' informed (Yue et al., 2012). By labelling plants as native and sustainable, one might be able to increase this awareness in consumers and increase sales of native plants (Yue et al., 2012). In an experimental auction, about 16% of the participants always increased their bids for a plant that was labeled as "native" and "non-invasive" regardless of the plants' other characteristics (Yue et al., 2012). This auction also allowed for the estimate of the price premium or discount the different groups of consumers might place on plants if native or invasive plants are labeled (Yue et al., 2012). The group of consumers in this auction that always increased willingness to pay when a plant was labeled native, “the nativists”, were willing to pay $0.83 more for native plants (Yue et al., 2012). Consumers may also think and act differently in response to new products and services, and products adapted to be more environmentally friendly (Behe et al., 2010). Nursery crop production of wildflower seed sales were valued at $8,784,400.00 in 2014 (National Agricultural Statistics Service, 2014). There were a total of 73 total operations spanning over 1244 hectares. Increasing the number of variations available in both seed and potted *R. columnifera* could show an increase in value to these growing sectors. Further development of native ornamentals with flowering, architecture, and drought-tolerance traits may be a strategy to increase native plant use among a large segment of the consumer market (Wilde et al., 2015).

The objectives of this dissertation were to characterize variation of traits of ornamental interest in *R. columnifera* across its native range, explore methods for optimizing seed and vegetative propagation of *R. columnifera*, determine acceptable substrates and fertility requirements during container production of *R. columnifera*, and estimate consumer preferences for key market-related traits of *R. columnifera*.

Collection Strategies

In this study, both vegetative cuttings and seed collection were gathered to develop into stock plant materials. Selections of potential collections were first based on the flower color, petal shape, number, and location. These traits will more likely persist through vegetative cuttings. Traits like flower, petal and foliage size, flower number, and growth habit will likely differ once put into nursery growing conditions. For both types of collections, certain information was recorded. This includes assigning a collection code to the plant. For example, a code of “TX-RC-1” is the first *R. columnifera* collection from Texas. The date of collection and the latitude/longitude of collection site were recorded. Additionally, a physical description of the collection site was gathered. Finally, any notes about the plant’s significant characteristics were recorded. Locations for collections generally include easily accessible roadsides on public property, or on private property with permission from the owner. In order to gather collections from more distant areas in the native range, an assistance request letter was sent to colleagues, native plant societies, and contacts through the ASHS member database detailing collection protocol and where to mail the collections.

The vegetative propagation method used was a stem cutting with at least three nodes and floral parts removed. These cuttings were treated with Hormodin 2 rooting hormone (0.3% IBA, OHP, INC., Mainland, PA) and inserted into moistened perlite (Sunshine® Perlite, premium grade, Sungro, Agawan, MA). They were then placed under intermittent mist (10 s every 15 min from dawn to dusk) until roots formed. Light conditions in the greenhouse were an average of 319.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Once roots were formed, the plants were transplanted into 1.14 L deep square pots (4.5 inch square deep vacuum pot press fit, Dillen products, The HC Companies INC, Middlefield OH) containing nursery crop substrate (Sungro Metro Mix®902, Agawam, MA) and top dressed with 15N-3.933P-9.96K slow release fertilizer (Osmocote plus, 15-

9-12, Patterned release fertilizer, 8-9 month, Everris ICL, Dublin, OH). These plants were then grown outdoors under 50% light exclusion screen using collected rainwater for irrigation. Photosynthetically active radiation (PAR) under the shade cloth were an average of 655.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL).

For seed collection, significant characteristics needed to be identified before the petals fell off. This means that plants were tagged with construction tape and seed was collected when mature at a later date. Once seed heads are mature, they are cut from the plant and placed into a paper envelope and fully sealed. The seed heads remained in the envelopes until dry, and were then placed into sealed plastic bags and put into storage at 4°C.

Vegetative and seed propagation of wild gathered *R. columnifera* plants were used to establish an *R. columnifera* stock plant collection. Light conditions in the greenhouse where the mist benches were located were an average of 319.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Once roots were formed on vegetative cuttings, the plants were transplanted into 1.14 L deep square pots (4.5 inch square deep vacuum pot press fit, Dillen products, The HC Companies INC, Middlefield OH) containing nursery crop substrate (Sungro Metro Mix®902, Agawam, MA) and top dressed with 15N-3.933P-9.96K slow release fertilizer (Osmocote plus, 15-9-12, Patterned release fertilizer, 8-9 month, Everris ICL, Dublin, OH). These plants were then grown outdoors under 50% light exclusion screen using collected rainwater for irrigation.

Photosynthetically active radiation (PAR) under the shade cloth were an average of 655.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Once hardened off, plants were transplanted into 2.37 L pots (Trade size #1(C300S), blow molded-classic line, Nursery Supplies Inc., Chambersburg, PA), and then into 5.68 L and 14.5 L pots respectively (Trade size #2, Trade size #5, blow-molded-classic line, Nursery Supplies Inc., Chambersburg, PA). The *R. columnifera* were transplanted using nursery crop substrate (Sungro Metro Mix®902, Agawam, MA) and top dressed with 15N-3.933P-9.96K slow-release fertilizer (Osmocote plus, 15-9-12, Patterned release fertilizer, 8-9 month, Everris ICL, Dublin, OH). During warmer months of the year (April-October) the stock plants were in full sun nursery conditions, watered on an as needed basis, and fertigated weekly with 20-20-20 water soluble fertilizer (Peters® Professional 20-20-20 General Purpose, Everris, ICL, Dublin, OH). Photosynthetically active radiation (PAR) in the full sun location were an average of 1638 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). During cooler months of the year (November-March) stock plants were moved into an overwintering house (Polyethylene sheeting, no climate control) in order to reduce damage due to cold temperatures. The overwintering house averaged 833.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Depending on the time of year, both seed and vegetative cuttings were collected from the stock plants in these various locations.

CHAPTER II

VEGETATIVE PROPAGATION OF *RATIBIDA COLUMNIFERA* (NUTT.) WOOTEN & STANDL.

Vegetative propagation is vital to preservation of unique natural variants and allows capture of both additive and nonadditive variance in breeding programs (Wassner and Ravetta, 2000). This allows for efficient clonal reproduction of desired germplasm. Propagation techniques vary depending on species; therefore, it would be effective and efficient to know a propagation protocol for the species in question. *Ratibida columnifera* (Nutt.) Wooton & Standl. [syn. *Lepachys columnifera* (Nutt.) J.F. Macbr., *Ratibida columnaris* (Pursh) D. Don, *Rudbeckia columnaris* Pursh, *Rudbeckia columnifera* Nutt.], is most commonly known as prairie coneflower, but is also referred to by a number of regional common names including columnar prairie coneflower, longhead-coneflower, redspike Mexican-hat, thimble flower, or upright prairie coneflower (Tropicos, 2018; USDA, 2012). This species is readily available via seed and seedlings but does not currently have a vegetative propagation protocol utilizing stem cuttings. In tissue culture, *R. columnifera* will respond to cytokinin application by growing shoots, and auxin by growing callus and roots (Holden et al., 1976). This is promising and suggests that auxin may also initiate callus and rooting in stem cuttings for *R. columnifera*. In a study of *Grindelia*, another member of Asteraceae Bercht. & J. Presl, cuttings were exposed to Indole-3-butyric acid (IBA) in various concentrations, which resulted in differential effectiveness of adventitious rooting (Wassner and Ravetta, 2000). It is important to identify the optimum concentration of IBA to use, because too low or too high levels can adversely impact rooting quality. In general, when using stem cuttings, young basal shoots are typically more suitable than mature stems for most wildflower species (Trinklein, 2014). In the study of *Grindelia*, cutting position was the most important variable influencing rooting success (Wassner and Ravetta, 2000). In that study they used apical (upper 6-8 nodes) and basal (lower 6-8 nodes) in order to evaluate the effect of cutting position on rooting. Other studies have also shown that more heavily lignified cuttings are more difficult to root than younger stems (Hartmann et al., 2010). For this reason, the developmental stage of the cutting was examined for *R. columnifera*. The study on *Grindelia* also showed that rooting success differed among different geographical accessions (Wassner and Ravetta, 2000), which leads us to believe that might be the case for *R. columnifera* accessions as well. Environmental conditions such as differences in temperature can also have a significant effect on rooting (Castañeda-Saucedo et al., 2020), and therefore needs to be explored further in *R. columnifera*. A seasonal rooting capacity is reported in other perennial species between summer and autumn rooted cuttings (Sharma and Aier, 1989). This suggests a need for replication of studies in both cooler and warmer seasons.

This study is designed to answer several cultural components for effective adventitious rooting of stem cuttings of *R. columnifera* including the effects of developmental stage of cuttings on rooting percentage and root quality, optimum hormone concentrations for maximum rooting responses, effects of bottom heat during rooting, and variation in adventitious rooting from stem cuttings among genotypes.

Materials and Methods

There were two vegetative propagation experiments (effects of hormone concentrations and developmental stages on rooting; effects of bottom heat application and germplasm on rooting) with the bottom heat experiment replicated once in warm temperature conditions and once in cool temperature conditions. The experiment involving factorial combinations of hormone concentrations and developmental stage of cuttings examined rooting effects of developmental stages (young versus mature) and four concentrations of IBA (0, 0.1%, 0.3%, or 0.8% IBA, OHP INC., Mainland, PA) on 30 cuttings of each treatment combination for a total of 240 cuttings.

Rooting Hormone and Developmental Stage

Rooting hormone and development stage experiments involved one germplasm, accession TX RC 8 (College Station, TX, 30.61167 N 30° 36'42.02903", -96.35342 W 96° 21'12.31423"). This experiment took place in June 2019. TX RC 8 stock plants were located in a full-sun section of the nursery. Stock plants were planted in nursery crop substrate (Sungro Metro Mix®902, Agawam, MA) and top dressed with 15N-3.933P-9.96K slow-release fertilizer (Osmocote® plus, 15-9-12, Patterned release fertilizer, 8-9 month, Everris ICL, Dublin, OH). Photosynthetically active radiation (PAR) in the full sun location were an average of 1638 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day, whereas in the overwintering house averaged 833.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Plants were grown in 14.5 L pots (Trade size #5, blow-molded-classic line, Nursery Supplies Inc., Chambersburg, PA). During warmer months of the year (April-October) the stock plants were in full sun nursery conditions, watered on an as needed basis, and fertigated weekly with 20-20-20 water soluble fertilizer (Peters® Professional 20-20-20 General Purpose, Everris, ICL, Dublin, OH). Greenhouse temperatures where the misting benches were located were 32°C maximum and 21°C minimum. Light conditions in this greenhouse were an average of 319.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Each treatment combination was comprised of 30 stem cuttings each for a total of 240 cuttings. The vegetative propagation method used was a stem cutting with at least three nodes. Shoots of *R. columnifera* have inflorescences on the apical portion of mature stems. Cuttings were taken from developmentally uniform shoots with inflorescences, which were removed prior to planting. The young developmental stage was comprised of apical three node long stem tip cuttings. The mature developmental stage cuttings were three node long cuttings taken from the more basipetal portion of the stem that had become fibrous. Aqueous solutions of IBA at four concentrations (0, 0.1%, 0.3%, or 0.8% IBA OHP, INC., Mainland, PA) were applied to the basal 4 cm of cuttings via a 5 s soak prior to planting in moistened perlite (Sunshine® Perlite, premium grade, Sungro, Agawan, MA) in 10 × 36 × 51 cm (4 × 14 × 20 in) black plastic nursery flats (Dyna-flat™, Kadon Corp., Dayton, OH). They were then placed under intermittent mist (10 s every 15 min from dawn to dusk) for the duration of 30 days with natural photoperiods. Greenhouse temperatures were 32°C maximum and 21°C minimum. At 30 days after planting, cuttings were harvested by gently removing them from the perlite and rinsing in a beaker of water to clean off the media. Qualitative measurements such as callus/root formation and a visual rating were gathered. The visual rating was on a scale of 1 (poor) to 5 (excellent) with a rating of 3 being the minimally acceptable quality rooting for commercial production (Fig. 1). Quality was judged by how vigorous rooting was, for example if there were many dense fibrous roots as opposed to a few long sparse roots. A rating of 1 (poor) was given to cuttings that failed to generate callus or roots. A rating of 2 was given to cuttings that generated some callus and a few non-branching roots. A rating of 3 was given to cuttings that produced many roots with some branching. A rating of 4 was given to cuttings with even more roots with branching. A rating of 5 (excellent) was given to cuttings that produced numerous branching roots.

Figure 1. Rating scale 1 (poor) to 5 (excellent) with a rating of 3 being the minimally acceptable quality rooting for commercial production.

Quantitative measurements included the measurement of the five longest roots (cm), root number, dry mass (g), and proportion of cuttings generating one or more roots (rooted cuttings) and those exhibiting rooting or callus (callused cuttings).

A randomized complete block design was used for this experiment. Factorial combinations of two developmental stages and four IBA combinations were arranged in three blocks, each containing ten replicates of each treatment combination. Analysis of variance, generalized linear model (GLM), and Tukey's HSD were used for the interactions among rooting hormone concentrations and developmental stages with $P \leq 0.05$ for significance using JMP Pro 15 (SAS Institute Inc., Cary, NC, 1989-2021) for continuous and categorical variables was conducted.

Seasonal Effects of Bottom Heat and Genotype

This experiment examined rooting effects of bottom heat application [no additional heat (ambient with greenhouse temperature of approximately 23°C), 26°C, or 32°C] and three genotypes (accession TX RC 8, College Station, TX, 30.61167 N 30° 36'42.02903", -96.35342 W 96° 21'12.31423", accessions TX RC 29 and TX RC 30, Somerville, Texas, 30.522288 N, -96.429397 W) at 30 cuttings (3 replications of 10 cuttings) of each combination for a total of 270 cuttings. This experiment was replicated once during the warm season (September 2019), and once during cool season (January 2020). Results from earlier experiments with IBA and developmental stages were utilized to determine the use of 0.3% IBA and young developmental stage for all cuttings in the bottom heat and genotype experiments. Stock plants during September were located in a full-sun section of the nursery, whereas stock plants in January were located in filtered light in the overwintering house (polyethylene sheeting, not climate controlled). Photosynthetically active radiation (PAR) in the full sun location were an average of 1638 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day, whereas the overwintering house averaged 833.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Stock plants were planted in nursery crop substrate (Sungro Metro Mix® 902, Agawam, MA) and top dressed with 15N-3.933P-9.96K slow-release fertilizer (Osmocote plus, 15-9-12, Patterned release fertilizer, 8-9 month, Everris ICL, Dublin, OH). Plants were grown in 14.5 L pots (Trade size #5, blow-molded-classic line, Nursery Supplies Inc., Chambersburg, PA). Stock plants were watered on an as needed basis, and fertigated weekly with 20-20-20 water soluble fertilizer (Peters® Professional 20-20-20 General Purpose, Everris, ICL, Dublin, OH). Aqueous solutions of 0.3% IBA were applied to cuttings via a 5 s soak prior to planting in moistened perlite. Germination mats (Model PM-9A, Pro-Grow Supply Corp., Brookfield, WI; Redi-Heat™ Model RHD2105, Phytotronics Inc., Earth City, MO) with temperature soil probes were utilized to consistently heat the media to the appropriate temperature for the duration of the experiment (Redi-Heat™ Thermostat, Model RHT4, Phytotronics, Inc., Earth City, MO). During this experiment, greenhouse temperatures were 32°C maximum and 21°C minimum, and light conditions were an average of 319.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). Cuttings were placed under intermittent mist (10 s every 15 min from dawn to dusk). At 30 days after planting, cuttings were harvested by gently removing them from the perlite and rinsing in a beaker of water to clean off the media. Qualitative measurements such as callus/root formation and a visual rating (as described above) were gathered. Quantitative measurements included the measurement of the five longest roots (cm), root number, root dry mass (g), and proportion of cuttings generating one or more roots. A randomized complete block design was used for this experiment. Factorial combinations of three bottom heat temperatures and three genotypes were arranged in three blocks, each containing ten replicates of each treatment combination. The entire experiment was then replicated in time. When appropriate, analysis of variance, generalized linear model (GLM), and Tukey's HSD were used for the interactions among bottom heating and genotypes with $P \leq 0.05$ for significance using JMP Pro 15 for continuous and categorical variables was conducted.

Results and Conclusions

Rooting Hormone and Developmental Stage

Table 1. Effect of IBA concentration and developmental age of cutting on rooting success of germplasm TX RC 8.

Developmental stage	Hormone treatment	Root length (cm)	Root number	Dry Mass (g)
Young	0.00% IBA	3.48 b	9.13 d	0.20 c[z]
	0.10% IBA	6.54 a	26.9 c	0.27 abc
	0.30% IBA	4.58 b	39.47 a	0.22 bc
	0.80% IBA	4.28 b	30.8 bc	0.20 c
Mature	0.00% IBA	5.02 ab	14.6 d	0.29 ab
	0.10% IBA	4.81 b	24.9 c	0.26 bc
	0.30% IBA	5.18 ab	34.9 ab	0.30 ab
	0.80% IBA	3.71 b	38.4 a	0.34 a
Treatment effects	Whole model	<0.0001*	<0.0001*	<0.0001*
	Hormone conc.	0.0001*	<0.0001*	0.618
	Dev. Age	0.8875	0.1838	<.0001*
	Conc. x age	0.0003*	0.0010*	0.0001*

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Table 2. Effect of IBA concentration and developmental age of cutting on rooting quality rating of germplasm TX RC 8.

Main effect	Treatment	Rooting quality rating
IBA concentration	0.00% IBA	3.57 b[z]
	0.10% IBA	4.10 a
	0.30% IBA	4.15 a
	0.80% IBA	3.33 b
Developmental age	Young	3.95 a
	Mature	3.63 b
Treatment effects	Whole model	<0.0001*
	Hormone conc.	<0.0001*
	Dev. Age	0.0253*
	Conc. x age	0.0749

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*$P \leq 0.05$*). Effects that are statistically significant are labeled *.

Hormone concentration and developmental age of cuttings significantly impacted many aspects of root regeneration (Table 1). Models for all four root regeneration measures were significant. Interactions among IBA concentrations and stem developmental stage were significant for root length, number, and root dry mass (Table 1). Young cuttings with 0.10% IBA, outperformed other young cuttings with a root length average of 6.5 cm. Mature cuttings with 0.00% IBA and 0.30% IBA were not significantly different than the young cuttings with 0.10% IBA. Young cuttings had longer roots with 0.10%, and had less rooting success when no IBA was applied. Mature cuttings root lengths were not significantly different from one another. Root number was significantly affected by the main effects of hormone concentration and age

(*P*<.0001) and had a significant interaction (Table 1). Young cuttings produced the most roots at 0.30% IBA, followed by 0.80% IBA, then 0.10% IBA, with the least number of roots resulting from 0.00% IBA. Mature cuttings produced the most roots at 0.80% IBA, followed by 0.30% IBA, then 0.10% IBA, with the least number of roots being produced with 0.00% IBA. Hormone concentration and developmental age had significant main effects on rooting quality, but their interaction was not significant (Table 2). In terms of rooting quality ratings, young cuttings and 0.10% IBA and 0.30% IBA improved rooting quality (Table 2). Both young and mature cuttings produced the best quality roots in the mid-range hormone concentrations (0.10% IBA and 0.30% IBA, and the worst quality roots in the hormone concentration extremes (0.00% IBA and 0.80% IBA). There was a significant interaction for dry mass among IBA concentrations and developmental stages (Table 1). Overall, the mature cuttings had a larger mass than the young cuttings. Hormone concentration and developmental stage of cutting did not have a significant effect on the proportion of callused/rooted cuttings (*P*=0.7788, data not presented).

This experiment involved many significant interaction effects between cutting developmental stage and hormone concentration. Across the board, a low to mid-range hormone concentration of 0.10% or 0.30% improved rooting quality on both developmental stages in comparison to the control. This reinforces the suggestion that application of auxin will initiate root formation (Holden et al., 1976), specifically in members of the Asteraceae (Wassner and Ravetta, 2000). Higher concentrations had more numerous roots that were slightly shorter in length. Absence of hormone generated fewer roots that were slightly longer in length. A desirable ratio of root number to root length could be achieved by application of a mid-range hormone concentration. This is reflected in the root quality scale, where 0.10% and 0.30% on young tissue yielded the highest quality roots. This is consistent with the claim that young tissue is more suitable for vegetative propagation than mature tissue (Trinklein, 2014). This may be due to the heavier lignification that is often found in mature tissues (Hartmann et al., 2010). For this reason, it is recommended that for the optimum overall root quality in *R. columnifera*, one should apply 0.30% IBA to young developmental stage cuttings.

Seasonal Effects of Bottom Heat and Genotype

Warm Season

Table 3. Significant interaction effects of bottom heat application on root number and rooting quality of germplasm TX RC 8, TX RC 29, and TX RC 30 during warm seasons.

Germplasm	Bottom heat temperature (°C)	Root number	Rooting quality rating scale
TX RC 8	Ambient (23°C)	18.8 b	1.60 bcd[z]
	Bottom heat (26°C)	37.9 a	3.00 a
	Bottom heat (32°C)	23.0 b	1.82 bc
TX RC 29	Ambient (23°C)	3.97 c	1.17 d
	Bottom heat (26°C)	6.20 c	1.07 d
	Bottom heat (32°C)	4.23 c	1.13 d
TX RC 30	Ambient (23°C)	8.07 c	1.30 cd
	Bottom heat (26°C)	18.3 b	1.97 b
	Bottom heat (32°C)	5.47 c	1.13 d
Treatment effects	Whole model	<0.0001*	<0.0001*
	Bottom heat	<0.0001*	<0.0001*
	Germplasm	<0.0001*	<0.0001*
	Bottom heat x germplasm	0.0021*	<0.0001*

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Table 4. Effect of bottom heat application on rooting success of germplasm TX RC 8, TX RC 29, and TX RC 30 during warm season.

Main effect	Treatment	Root length (cm)	Dry mass (g)	Proportion rooting (%)
Bottom heat (°C)	Ambient (23°C)	1.31 b	0.12 a	54 b[z]
	Bottom heat (26°C)	2.21 a	0.13 a	80 a
	Bottom heat (32°C)	1.34 b	0.12 a	64 b
Germplasm	TX RC 8	2.12 a	0.14 a	84 a
	TX RC 29	0.93 b	0.10 b	48 c
	TX RC 30	1.79 a	0.11 b	67 b
Treatment effects	Whole model	<0.0001*	0.0003*	<0.0001*
	Bottom heat	<0.0001*	0.107	0.0006*
	Germplasm	<0.0001*	<0.0001*	<0.0001*
	Bottom heat x germplasm	0.1113	0.3624	0.9312

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Germplasm and bottom heat application to cuttings during warm ambient temperatures significantly impacted many aspects of adventitious rooting (Table 3, Table 4). Interactions among bottom heats and germplasm were present for root number and root quality ratings, but were not significant for root length, root dry mass, or proportion of cuttings rooting (Table 3, Table 4). Main effects of germplasm were significant for all root regeneration measures and the main effects of bottom heats were significant for all but root dry mass (Table 3, Table 4). The ambient and 32°C treatments were not significantly different from each other except in regards to dry mass and proportion rooting (Table 4). TX RC 8 and TX RC 30 had significantly longer roots than TX RC 29 regardless of which bottom heat treatment they received (Table 4). For TX RC 8, 26°C bottom heat application yielded the most roots, with ambient temperature and 32°C having no significant difference from each other. TX RC 29 had no significant differences in number of roots regenerated between the three bottom heat applications. TX RC 30 had the most roots with 26°C, with ambient and 32°C having no significant difference from each other (Table 3). TX RC 8 had the best root quality with 26°C bottom heat application, with ambient temperatures and 32°C treatments having no significant difference. TX RC 29 had no significant differences between the three temperatures on root quality. TX RC 30 had the best quality roots at 26°C, with ambient and 32°C having no significant differences (Table 3). TX RC 8 had significantly more mass than TX RC 29 or TX RC 30. The bottom heat application 26°C treatment had a significantly higher proportion of cuttings root than the other two heat applications (Table 4). TX RC 8 had the highest proportion of rooting out of the three germplasms, followed by TX RC 30 and then TX RC 29 (Table 4).

Recommendations for bottom heat use when vegetatively propagating *R. columnifera* during the warm season are as follows. Regarding the bottom heat treatments, 26°C bottom heat temperatures significantly improved the following aspects: root length, root number, root quality, and proportion rooting in a majority of the germplasms. Application of 26° heat to the rooting substrate proved to be beneficial to rooting success, aligning with the claim for other species that environmental conditions such as differences in temperature can also have a significant effect on rooting of another member of Asteraceae, *Stevia rebaudiana* (Bertoni) Bertoni (Castañeda-Saucedo et al., 2020). In almost every treatment combination, the germplasm TX RC 8 had improved rooting characteristics. One interesting result was that TX RC 29 rooting characteristics were unaffected by bottom heat application in all but proportion of cuttings rooted. This shows that different germplasm react differently to bottom heat treatments. This coincides with the suggestion that rooting success differed among different geographical accessions of *Grindelia* (Wassner and Ravetta, 2000).

Cool Season

Table 5. Effect of bottom heat application on rooting success of Germplasm TX RC 8, TX RC 29, and TX RC 30 during cool seasons.

Main effect	Treatment	Root length (cm)	Root number	Rooting quality rating scale	Proportion rooting (%)
Bottom heat (°C)	Ambient (23°C)	11.25 b	14.60 a	1.32 a	59 b[z]
	Bottom heat (26°C)	22.30 a	20.79 a	1.58 a	78 a
	Bottom heat (32°C)	21.00 a	17.49 a	1.54 a	79 a
Germplasm	TX RC 8	16.77 b	18.31 b	1.43 b	70 b
	TX RC 29	10.56 c	6.03 c	1.12 c	56 b
	TX RC 30	27.24 a	28.53 a	1.89 a	90 a
Treatment effects	Whole model	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Bottom heat	<0.0001*	0.1034	0.0677	0.0017*
	Germplasm	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	Bottom heat x germplasm	0.1856	0.0974	0.4045	0.0667

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Germplasm and bottom heat application to cuttings during cool ambient temperatures significantly impacted several aspects of adventitious root formation, but did not result in any significant interactions among germplasms and bottom heats (Table 5). Main effects of germplasm were significant for all rooting measures during the cool season and main effects of bottom heat were significant for root length and rooting proportions (Table 5). The 26°C and 32°C bottom heat applications stimulated growth of longer roots, when compared to ambient temperatures (Table 5). TX RC 30 had the longest roots, followed by TX RC 8, with TX RC 29 having the shortest roots. TX RC 30 produced the most roots, followed by TX RC 8 and TX RC 29. TX RC 30 produced the highest quality roots, followed by TX RC 8 and then TX RC 29. Dry mass had no significant differences among the treatment combinations ($P \leq 0.0776$, data not presented). Proportion of rooting was significantly impacted by bottom heat application and germplasm ($P \leq .0001$), with no interaction effects ($P \leq 0.0667$). The 32°C and 26°C bottom heat applications had a greater proportion of total cuttings generate roots when compared to the ambient temperature treatment for all genotypes during the cool season. TX RC 30 had the greatest proportion of cuttings root, followed by TX RC 8 and then TX RC 29 (Table 5).

The cool season replication of the bottom heat application experiment had differing results from the warm season. In aspects where there were significant differences, 26°C and 32°C were favorable over ambient temperatures. There was no significant difference between the 26°C and 32°C temperature treatments during the cool season, whereas 32°C did not improve rooting in the warm season. The germplasm performance was very consistent from treatment to treatment with TX RC 30 always outperforming TX RC 8, and TX RC 8 always outperforming TX RC 29. This shows that rooting success differs among different geographical accessions for *R. columnifera* (and appears to be consistent within, but not necessarily between seasons), similar to results reported on *Grindelia* (Wassner and Ravetta, 2000). Reports on *S. rebaudiana*, states that temperatures, both of the environment and substrate, have significant effects on rooting (Castañeda-Saucedo et al., 2020) held true for *R. columnifera*. Differing environmental temperatures between the cool and warm season did affect the seasonal rooting capacity for *R. columnifera*, as has been reported with woody perennial species between summer and autumn rooted cuttings (Sharma and Aier, 1989). It is recommended that when attempting to root *R. columnifera* cuttings in the cool season, one should apply between 26°C and 32°C bottom heat to achieve optimum rooting. The application of 26°C bottom heat improved rooting in both the cool and warm seasons.

Unless specific data is available for a given genotype, results of our experiments suggest using a 26°C bottom heat, young recently matured shoot tips, and 0.10% IBA to 0.30% IBA quick dips to maximize adventitious root development. Results of these studies also suggest that to maximize commercial production, candidate genotypes for introduction should be screened for general rooting potential prior to release.

CHAPTER III

SEED PROPAGATION METHODS FOR *RATIBIDA COLUMNIFERA* (NUTT.) WOOTEN & STANDL.

Ratibida columnifera is readily available as seed and is a common component of many wildflower seed mixes (United States Dept. of Agric. Natural Res. Conservation Serv., 2006). In order to develop novel germplasm from native sources into a marketable product, seed and vegetative collections must be obtained for breeding. It is not enough to just collect seed; one must be able to store and germinate it. Some species have simple needs for germination, while others require treatments to break physiological dormancy and/or quiescence. Available literature stresses the need for cold moist stratification of *R. columnifera* seeds for successful germination (Niu et al., 2012; Romo and Eddleman, 1995). Most studies stratify from three to eight weeks, at varying temperatures. An optimum yield of 60% total germination was obtained by stratifying at 15°C for 28 days (Romo and Eddleman, 1995). Studies have examined optimum temperatures for stratification of *R. columnifera* seeds, but there is a lack of literature defining the number of days that allow maximum percent germination. In one study, *R. columnifera* seeds were cold moist stratified for eight weeks in trays of sterile germination mix (Middleton et al., 2015). Another study did not produce sufficient germination until seed was stratified at 5°C for 3 to 6 weeks (Niu et al., 2012). *Ratibida columnifera* seed do not require light to germinate (Romo and Eddleman, 1995). Other factors that can affect germination and therefore future use of collected seed are storage conditions and duration. Some species can store for years at room temperature, while others begin to have lowered germination rates over time and struggle to maintain viability without cold storage (Hong and Ellis, 1996). Germplasm within a species can vary drastically, especially when there are geographical differences. Genetic variation among populations along a geographical gradient can suggest the presence of clinal variation (Weber and Schmid, 1998). In an experiment on *Solidago altissma* L. and *Solidago gigantea* Aiton there were major differences in phenology, indicating that the populations were distinct in their physiological requirements for floral initiation (Weber and Schmid, 1998). Climate differences along latitudinal, longitudinal, and altitudinal transects are one of the most important abiotic factors regarding species distribution, and can lead to adaptation under natural selective pressures (Monty and Mahy, 2009). Selection of genotypes adapted to the region in which they intend to be sold may be necessary to achieve a high-performance valued consumer product. Influences occurring in the maternal environment that can affect seed quality in plants are conditions that are present after formation of the embryo, but before seed dispersal takes place (Valencia-Diaz and Montaña, 2005). This phenomenon is known as the maternal effect (Valencia-Diaz and Montaña, 2005). Characteristics of the seed that can be affected by maternal plant stress include seed fill and seed size. These characteristics correlate heavily with viability and germination rates (Valencia-Diaz and Montaña, 2005). Due to the acropetal floral development in *R. columnifera*, there is concern that seed maturation and viability varies along the conical inflorescence and among inflorescence maturities as described for *Erigeron* (Harris et al., 1991). For these reasons, several germplasms were grown in a common environment. Seed was harvested from trial beds in a nursery crop setting, for which many environmental stresses that a plant in nature would experience were drastically reduced.

The following experiments seek to establish protocols for optimum germination and storage conditions necessary for maintenance of *R. columnifera* seeds. Objectives that were examined included: 1) if soaking seed influences germination, 2) if fresh seed will germinate, or 3) if treatments such as stratification or scarification needed to be applied, 4) seed storage duration and conditions were examined for effects on germination, and 5) to determine the impacts of stage of seed maturity on germination.

Materials and Methods

General Conditions

Seeds which were collected from five open pollinated genotypes (TX RC 8, TX RC 12, TX RC 30, TX RC 44, TX RC 48) grown in trial plots (Somerville, Texas, 30.522288 N, -96.429397 W) were utilized for germination studies. The trial plots are in a full sun location in the nursery in native soil. Photosynthetically active radiation (PAR) was an average of 1638 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD during mid-day (Fieldscout® Quantum Foot-Candle Meter, Spectrum® Technologies, Inc., Aurora, IL). These plants are irrigated on an as needed basis, and fertigated weekly with 20-20-20 water soluble fertilizer (Peters® Professional 20-20-20 General Purpose, Everris, ICL, Dublin, OH). Due to the non-uniform maturation of inflorescences on *R. columnifera*, seed were harvested from fully dried seed heads when mature. Seed were harvested in the late summer, and were placed into a paper envelope and fully sealed. The seed heads remained in the envelopes until dry, and were then placed into sealed plastic bags and put into storage at 3°C. Seed for germination experiments were used within 6 months of their harvest date (excluding storage condition and seed maturity experimentation).

Germination studies were performed under controlled laboratory conditions (23.3°C). In order to estimate if germination requirements varied among a population of seedlings, seed from open pollinated parent plants with varied phenotypic morphology were tested. These were utilized for the following germination experiments.

Prior to each germination experiment, a percent viability test using a triphenyltetrazolium chloride (TTC, Sigma-Aldrich, St. Louis, MO) staining method (Riebkes et al., 2015) was conducted on the seed from each maternal genotype. Soaking in 1% TTC solution for 48 hr caused the embryo within the seed to stain red if viable (Riebkes et al, 2015). This seed viability test was performed on a subsample of 100 seeds from each treatment combination at the beginning of each germination trial. Germination data were normalized to the proportion of seed that are staining viable for each maternal genotype.

Effect of Overnight Hydration

The first germination experiment examined effects of soaking seed in water prior to germination (December 2018). Nine hundred seeds consisting of 300 seeds per each of three genotypes (TX RC 30, TX RC 44, TX RC 48) were soaked overnight in 50 ml of double distilled water and then surface dried. Nine hundred additional seeds remained dry until placement into petri dishes (polystyrene disposable sterile, 100 x 15mm Cat# 25384-302, VWR International). One hundred seeds were placed into each sterile petri dish on moist filter paper (9cm, Qual. 415 Cat No. 28320-041, VWR International), for a total of eighteen petri dishes. There were three replicates of each treatment. There were 9 dishes with 100 soaked seeds in each, and 9 dishes with 100 unsoaked seeds in each for a total of 1800 seeds tested. Seeds were placed under grow lights, with 16 hours on/8 hours off photoperiod increments (60W, PhilipsF96T12/CW/EW, Alto collection, USA) in laboratory conditions (23.3°C). Photosynthetically active radiation (PAR) under the grow lights under laboratory conditions averaged 39.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. Seed were checked for germination every 48 hr for four weeks after planting. Germination was defined as the emergence of the radicle (>1 mm).

A completely random design was used for this experiment. Factorial combinations of three genotypes (TX RC 30, TX RC 44, TX RC 48) and two hydration treatments (control, overnight hydration). Analysis of variance and Tukey's HSD were used for the interactions among treatments and genotypes with $P \leq 0.05$ for significance using JMP Pro 15 for continuous variables.

Effects of Storage Conditions on Viability

To examine optimum storage temperature for maintaining viability, a cold storage versus room temperature storage experiment was conducted. This experiment was replicated every six months to express the maintenance or degradation of viability, with continuation until depletion of available seeds after 18 months (began December 2018). Seed were harvested in the late summer 2018 (August), and were placed into a paper envelope and fully sealed. The seed heads remained in the envelopes until dry, and were then placed into sealed plastic bags and put into storage at either 23°C or 3°C. Three genotypes were used (TX RC 30, TX RC 44, TX RC 48), at two different storage temperatures, and replicated three times for a total of 1800 seeds. There were three hundred seeds in each treatment combination at storage temperatures of 23°C and 3°C conditions. Seeds were stored away from light and kept dry in sealed semipermeable plastic bags. At the predetermined storage length, seeds were placed into sterile petri dishes (Polystyrene disposable sterile, 100 x 15mm Cat# 25384-302, VWR International) on filter paper (9cm, Qual. 415 Cat No. 28320-041, VWR International) moistened with double distilled water. Germination conditions and data collection were as described for the hydration experiment described above. Three replicates of each genotype were placed in culture conditions in a completely random design. Analysis of variance, generalized linear model (GLM), and Tukey's HSD were used for the interactions among treatments and genotypes with $P \leq 0.05$ for significance using JMP Pro 15 for continuous variables.

Effects of Scarification and Cold Stratification Pretreatments

Cold stratification and scarification were examined in the same experiment (May 2019). Treatments included control (0 stratification and no scarification), three moist stratification durations (30, 60, and 90 days at 3°C stratification), and three sulfuric acid scarification (51%, Macron Fine Chemicals™, Center Valley, PA) durations (5, 10, 15 min. acid scarification). Seed were harvested in August 2018 and placed in semipermeable bags at 3°C. Sufficient seeds from storage were placed into stratification at 30, 60, and 90 days prior to germination tests to allow scarification and stratification germination effects to be tested simultaneously with the control and scarification treatments. There were 300 seeds per treatment. Seeds were placed into sterile petri dishes on moist filter paper. Seeds were checked for germination every 48 hr for four weeks after planting. Three replicates of 100 seeds per petri dish for each stratification and scarification treatment were arranged in a completely randomized design. Analysis of variance and Tukey's HSD were used for the interactions among treatments and genotypes with $P \leq 0.05$ for significance using JMP Pro 15 for continuous variables.

Effects of Seed Maturity on Germination

Requirements for seed maturity were examined by harvesting the columnar inflorescences at three stages of development: immature in appearance (green with ray flowers still attached), full size (ray flowers senesced, just beginning to change from green to grey-brown), and fully mature (ray flowers senesced, inflorescence brown to black-brown) but not shattered. Each seed head was divided into thirds: basal, middle, and apical. Seeds were aggregated for inflorescences of a given genotype and maturity stage. This was done with three different genotypes. Three hundred seed subsamples from each maturity stage x inflorescence segment x genotype were stained with TTC to estimate viability. This experiment took place in June 2020. Seed were harvested in June 2020 over the course of several weeks due to the non-uniform maturation of seed heads. Harvested seed was allowed to air dry in paper envelopes, and then transferred into semipermeable plastic bags for dry storage at 3°C. Three petri dishes, each containing 100 seeds, were placed on moistened filter paper for 24 hr and placed under grow lights in laboratory conditions as described above. Seeds were checked for germination every 48 hr for four weeks after planting. Germination was defined as the emergence of the radicle (>1 mm). Factorial combinations of treatments were used in this completely randomized design. Analysis of variance and Tukey's HSD were used for the interactions among treatments and genotypes with $P \leq 0.05$ for significance using JMP Pro 15 for continuous variables was conducted.

Results and Discussion

Effect of Overnight Hydration

Application of overnight hydration did not have a significant effect ($P \leq 0.05$) on percent germination for the interaction among germplasm x soaking, nor for either of the main effects of soaking or germplasm (data not presented). This suggests that growers should not waste time and resources by soaking *R. columnifera* seed prior to planting.

Effects of Storage Conditions on Viability

Table 6. Effect of seed storage temperature and duration on percent germination of *Ratibida columnifera* germplasms TX RC 30, TX RC 44, and TX RC 48.

Main effect	Treatment	Germination (%)
Germplasm	TX RC 30	82.5 a[z]
	TX RC 44	74.5 b
	TX RC 48	68.2 c
Duration	0 months	77.4 a
	6 months	76.5 a
	12 months	75.1 a
	18 months	71.2 b
Treatment effects	Whole model	0.0001*
	Temperature	0.2170
	Germplasm	0.0001*
	Temperature x germplasm	0.9365
	Duration	0.0226*
	Temperature x duration	0.5445
	Germplasm x duration	0.2820
	Temperature x germplasm x duration	0.3196

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*$P \leq 0.05$*). Effects that are statistically significant are labeled *.

No significant interactions among germplasm, duration of storage, or storage temperature were found. The main effect of storage temperature was also not significant ($P \leq 0.05$). However, germplasm had a significant main effect on percent germination (Table 6). TX RC 30 had the highest percent germination at 82.5%, followed by TX RC 44 at 74.5%, with the lowest percent germination being TX RC 48 with 68.2%. All three germplasm differed statistically from each other ($P \leq 0.05$). Storage duration also had a significant main effect on germination. The data shows no significant difference in percent germination up to 12 months in storage (Table 6). By 18 months of storage there was a slight but statistically significant reduction in percent germination resulting in about a 10% loss in viability (Table 6). This minimal reduction in viability after 18 months suggests that seed can potentially be stored for longer periods if needed.

There was a statistically significant difference for this study when looking at the germplasm and duration main effects (Table 6). TX RC 30 had the highest percent germination of the three selections, followed by TX RC 44 and then TX RC 48. This indicates that the ability to retain viability over time can depend on germplasm selection. Seed longevity for *R. columnifera* has shown in this study is in concurrence with the observation that seed longevity can vary among accessions within other species because of differences in genotype and provenance (Hong and Ellis, 1996). In addition to genetic influences, differences in germination among accessions can be due to the cumulative effect of the environment during seed maturation, harvesting, drying, the time of seed harvest, duration of drying, and the period before the seed is placed in storage (Hong and Ellis, 1996). Each of the germplasms in the present experiment had maximum germination percentages well over the 60% observed in the Romo and Eddleman (1995) study. The *R. columnifera* in the Romo and Eddleman study was collected from the University of Saskatchewan's Matador Research Station, a native environment. Our seed was collected from well fertilized and irrigated stock in a nursery setting. The differences in cultural protocols of the stock plant could be why our seed had higher germination percentages overall. This may also be why our seeds were able to maintain a percent germination of 71.2% after 18 months of storage regardless of the temperature of storage. Storage duration also had a significant effect on germination (Table 6). There was no significant difference in germination for the first year of storage. It was not until 18 months that we began to observe statistically significant reductions in germination. This implies that seed may not need to have cold storage to retain viability in short term storage. Future studies should be performed to determine if temperature has significant effects on retaining viability in long term, multi-year storage durations and what impact freezing seeds may have on viability.

Effects of Scarification and Cold Stratification Pretreatments

Table 7. Effect of 3°C stratification on percent germination of *Ratibida columnifera* germplasms TX RC 30, TX RC 44, and TX RC 48.

Germplasm	Stratification	Germination (%)
TX RC 30	Control	89.7 a[z]
	30 day stratification	85.7 abc
	60 day stratification	82.3 abc
	90 day stratification	64.7 bc
TX RC 44	Control	88.7 ab
	30 day stratification	78.7 abc
	60 day stratification	76.0 abc
	90 day stratification	68.7 abc
TX RC 48	Control	75.0 abc
	30 day stratification	62.7 c
	60 day stratification	65.0 abc
	90 day stratification	76.7 abc
Treatment effects	Whole model	0.0033*
	Stratification	0.0116*
	Germplasm	0.0121*
	Stratification x germplasm	0.0354*

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Application of stratification significantly affected the percent germination of the three *R. columnifera* genotypes (Table 7). There was a significant interaction effect among the three genotypes and the pretreatments (Table 7). Thus, the effect that the seed pretreatment had on percent germination depended on which genotype was examined. Stratification proved to be an important factor in germination success, as the literature suggests (Romo and Eddleman, 1995). TX RC 30 had no significant difference in germination from the control at 30 and 60 day stratification, and experienced a significant decrease at 90 days stratification. TX RC 44 and TX RC 48 had no statistical evidence of enhanced germination with any of the stratification treatments. Increased stratification to 90 days either reduced percent germination or had no benefit (Table 7). Sulfuric acid treatments of 5 to 15 min had substantial deleterious effects on germination of all three genotypes (Data not presented).

None of the three genotypes exhibited a statistically significant increase in percent germination with stratification (Table 7). All three genotypes exhibited statistically significant negative effects from sulfuric acid scarification in this experiment. Our results indicate that 30 and 60 days of cold stratification does not negatively impact percent germination of regional genotypes of *R. columnifera* and that sulfuric acid scarification as tested herein was not beneficial (data not presented, no significant effects). Though this is true for these regional selections, stratification may be necessary for selections from other geographical locations. For this reason, we suggest a 30-60 day cold moist stratification screening for *R. columnifera* selections. Future studies could examine additional stratifications times between 0 and 60 days in order to further refine recommendations for individual maternal genotypes. Of perhaps greater need is investigation of potential variation in chilling requirements for germination across the large geographic range of *R. columnifera*. Although not promising from the results of this study, sulfuric acid scarification using a more dilute solution or shorter duration could be tested.

Effects of Seed Maturity on Germination

Table 8. Effect of inflorescence developmental stage and location on the inflorescence on percent germination of *Ratibida columnifera* germplasms TX RC 8, TX RC 12, and TX RC 30.

Main effect	Treatment	Germplasm	Germination (%)
Developmental stage	Immature	TX RC 8	9.6 cd
		TX RC 12	3.1 d
		TX RC 30	18.8 bc
	Mid-Age	TX RC 8	20.8 b
		TX RC 12	6.2 d
		TX RC 30	50.9 a
	Mature	TX RC 8	19.9 b
		TX RC 12	46.6 a
		TX RC 30	53.4 a
Region	Basal	TX RC 8	25.2 cd
		TX RC 12	20.0 de
		TX RC 30	49.2 a
	Middle	TX RC 8	13.1 e
		TX RC 12	20.0 de
		TX RC 30	41.1 ab
	Apical	TX RC 8	11.9 e
		TX RC 12	15.9 e
		TX RC 30	32.8 bc
Treatment effects		Whole model	<0.0001*
		Developmental stage	<0.0001*
		Region	<0.0001*
		Germplasm	<0.0001*
		Germplasm x developmental stage	<0.0001*
		Germplasm x region	0.0093*
		Developmental stage x region	0.0678
		Germplasm x developmental stage x region	0.0676

[z]Tukey's HSD mean comparison levels not followed by same letter within a column are significantly different (*P≤0.05)*. Effects that are statistically significant are labeled *.

Developmental stage of the inflorescence and a seed’s location on the inflorescence had a significant effect on percent germination (Table 8). There was a statistically significant two-way interaction for germination percentages among germplasms and developmental stage, and germplasm and region on inflorescence (Table 8). Mid-age and mature developmental stages had increased percent germination for all three germplasms. TX RC 12 had the lowest percent germination out of the three germplasms, except in reference to the mature inflorescences. Basal and middle portions of the inflorescence produced greater germination percentages than the apical portions, except for TX RC 30. In TX RC 12 the immature and mid-age developmental stages were not statistically different from one another. The mature developmental stage of TX RC 12 germinated more readily than the less mature stages and was comparable in germination percentages to the greater levels observed for TX RC 30 on mid-age and mature sections. There was no statistical evidence of differences in percent germination when looking at location on inflorescences of TX RC 12. TX RC 30 had increased percent germination in the mid-age and mature developmental stages, with the immature stage being significantly lower but greater than similar maturity stages on TX RC 12 or TX RC 8 (Table 8). The basal and mid-inflorescence seed locations outperformed the apical portions of the inflorescences for most stages of development. This was most pronounced on immature inflorescences which is consistent with the apical portions containing largely immature seeds whereas those in basal portions of the inflorescence would be more mature based on earlier chronological flowering as the inflorescence matured. Thus, for commercial seed production harvest should be from fully mature inflorescences to encourage maximum and uniform germination. If collection cannot wait for full inflorescence maturity, then only the basal portions of the inflorescence should be retained.

Developmental stage of the inflorescence and a seed's location on the inflorescence had a significant effect on percent germination (Table 8). There was a statistically significant interaction effect between the germplasms and developmental stage, and germplasms and region on the inflorescence. In all germplasm, mid-age to mature inflorescences had an increase in percent germination when compared to the immature inflorescences. Due to this, it is recommended to plan seed harvests for when a majority of the inflorescences have dropped their ray petals and turned brown. Two out of the three germplasm had significantly higher percent germination when looking at the basal and middle portions of the seed head, with the third germplasm having no differences among locations. This makes sense, due to the fact that *R. columnifera* disc florets open first on the basal portion of the inflorescence, and finish opening at the apical portion. This would mean that seed at the basal portion of the inflorescence has had longer time to mature than apical seed. This phenomena happens in other Asteraceae species as well, such as *Artemisia annua* L., where among genotypes there were differences ranging from those having capitula with open ray and disc florets, to those with capitula that are tightly enclosed within involucral bracts (Wetzstein et al., 2014). For this reason, knowledge of the timing of flower development in key genotypes, as well as the relative developmental timing of ray and disk florets is of critical importance for successful crossing (Wetzstein et al., 2014). All of this data implies that *R.*

columnifera is consistent with the more generalized recommendation for wildflowers that seed should be harvested near a stage when they would disperse naturally for optimum germination yields (Hong and Ellis, 1996). The impact of genotype on final germination rates was evident in this experiment (Table 8) as well as those discussed earlier (Tables 6 and 7). In this experiment TX RC 30 had the greatest germination rates on middle and fully mature seeds just as it had in earlier studies, whereas TX RC 12 had comparable rates only on basal portions of fully mature inflorescences and TX RC 8 had much reduced germination even on basal portions of fully mature inflorescences.

Summary

This study provides an insight into pretreatments, storage conditions, and harvest protocols that have proven valuable to optimizing the percent germination of *R. columnifera* seeds**.** Much of the data gathered from these experiments yielded higher percentages of germination than previous literature stated (Romo and Eddleman, 1995). This may be because of conditions that the stock plants from which seed were collected were grown. Added stresses in the development of the seed can influence the longevity of the mature seed (Harrington, 1972). These stresses may lead to maturity of a seed without its being fully developed or while it still lacks essentials (Harrington, 1975). Stresses to the mother plant that can reduce seed longevity may also include water stress, temperature stress, high salinity, disease, insect infestation, and frost damage (Harrington, 1972). The stock plants from which seed was gathered for our experiments were well fertilized, irrigated, and protected from many of the stresses that a *R. columnifera* plant may face in nature. This may be why our germination percentages outperformed that of the previous literature. The data also reflected the general sentiment that in many species there can be differences in germination responses in different accessions due to germplasm and provenance differences (Hong and Ellis, 1996). Seed pretreatments and storage conditions also reflected the ideas brought forth in the literature (Hong and Ellis, 1996). Improvement in percent germination for *R. columnifera* seeds can be made by using 30-60 day cold moist stratification pretreatments and harvesting seed in the correct developmental stages which can vary by genotype. Sulfuric acid scarification pretreatment and extended stratification caused negative effects on percent germination, and therefore are not recommended. Hydration of seed prior to planting had no significant impact on percent germination. Percent germination is not significantly changed by storage temperatures in the short term, but may be in longer terms. Germplasm effects were found to be significant throughout the series of experiments. The germplasms accession location could prove to be an important factor in how the plants respond to physiological stresses (Weber and Schmid, 1998). Future studies are needed to fine tune propagation protocols, such as investigating frozen seed storage, optimizing germination temperatures, optimizing germination substrates, determining provenance impacts on germination requirements and seed storage protocols, and determining if any residual impacts of germination treatments correlate to vigor of seedling growth beyond germination.

CHAPTER IV

CONSUMER PREFERENCES OF *RATIBIDA COLUMNIFERA* (NUTT.) WOOTEN & STANDL. FLORAL CHARACTERISTICS

Ratibida columnifera is a wildflower that exhibits a large variation in both floral and vegetative characteristics (USDA NRCS, 2018). Consumers can currently purchase this plant via seeds, and occasionally as a potted plant, however there are few cultivars or novel variations developed. Only a single cultivar, 'Red Midget' which is a shorter cultivar with an upright habit to 60 cm and red-brown disk flowers rimmed in orange and yellow, was found in the trade (Roots and Rhizomes, 2020). However, in order to ensure a successful commercialization of identified *R. columnifera* variants being developed in this research effort, these new product introductions must resonate with potential consumers.

To that end, we tested the overall preferences of *R. columnifera* flowers by performing a conjoint analysis using selected key product traits. From the extensive literature using conjoint methods, we know that consumers typically base their purchasing decisions on simultaneous evaluation of several product characteristics, as opposed to just one characteristic (Behe et al., 1999). Conjoint analysis allows for isolation of a number of factors and can be used to determine importance of each factor (Behe et al., 1999). Conjoint analysis involves evaluative rankings or multi-attribute alternatives by individuals (Baidu-Forson et al., 1997). This allows for measurement of consumer preferences among items with multiple attributes (Baidu-Forson et al., 1997). Results of this test will reveal which floral traits are important to consumers, as well as their willingness to pay for certain combinations of traits. Consumer preference, willingness to pay for the selected traits, and prices could aid in future selections of germplasm candidates for commercialization. Considering that *R. columnifera* is a native plant to a large portion of North America, it would also be useful to be able to gauge the participant's knowledge of native plants in the questionnaire.

According to the 2017 Census of Agriculture, bedding/garden plants accounted for a value of sales of $3.8 billion. This category includes annuals, herbaceous perennials, vegetable plants, and hanging baskets. The USDA NASS Floriculture crops report stated that the wholesale value of all bedding and garden plants for 2019 amounted to $2.01 billion (USDA NASS, 2019). This plant category was the largest contributor to total value sales of 2019 for floriculture crops. Potted herbaceous perennials totaled $600 million in 2019 and accounted for 30% of the total bedding and garden category (USDA NASS, 2019). *Ratibida columnifera* is a wildflower in the family *Asteraceae* Bercht. & J. Presl (*Compositae*), just like *Rudbeckia* L. (coneflowers). *Rudbeckia* was sold as a potted herbaceous perennial plant and accumulated total sales of $9.5 million in the 2014 USDA Census of Agriculture. *Rudbeckia* in nature is a wildflower, and now has developed cultivars that are commercialized and are likely the cause of these large sales. This is a similar path that we strive to take with development of *R. columnifera.* Increasing the number of variations available in both seed and potted *R. columnifera* could increase value as a commercial product. Both *R. columnifera* and *Rudbeckia* are native plants, which potentially gives them unique value to consumers.

Native plants make up approximately 9.1% of total sales of the nursery industry in the United States (Khachatryan et al., 2020). Through surveys conducted of nursery retailers, landscape architects, and Master Gardeners three general factors were considered important (Wilde et al., 2015). These factors were availability of native plants, consumer preferences regarding ornamental qualities in comparison to exotic species, and knowledge about native plants (Wilde et al., 2015). Increasing availability would be best accomplished through existing supply chains that are currently primarily supplying exotics (Wilde et al., 2015). Another study showed that the main reason surveyed nurseries supplied native plants was client request (21.7%) followed by low maintenance (17.8%), ecological reasons (16.3%), and that native plants are best adapted to difficult planting sites (15.5%) (Brzuszek et al., 2009). From the same study, nurseries listed their main reason for not selling more native plants was that there was not enough customer interest (36.4%) and unfamiliarity with natives (20.2%) (Brzuszek et al., 2009).

Native plants need to meet consumer preferences regarding ornamental qualities and need to compete with exotic alternatives in the marketplace (Wilde et. al, 2015). Though native plants can be more expensive than exotic plants due to small scale of production, price was identified as one of the least important factors limiting consumer acceptance (Wilde et al., 2015). It was determined in a conjoint choice survey that consumers would pay more for well-designed landscapes that included native plants rather than lawns. Another study revealed that consumers were willing to pay more for plants labeled as non-invasive and native (Wilde et al., 2015). Based on this finding, environmental traits of native ornamentals could be considered value-added traits (Wilde et. al, 2015). In the same study, half of consumers purchased ornamentals based on plant traits, not origin or invasive potential (Wilde et. al, 2015). Consumers are also willing to pay more for alternative designs that incorporate any form of prairie garden compared to a conventional lawn (Helfand et al., 2006). It has been noted, that due to the hypothetical bias of survey-based methods; willingness to pay elicited from hypothetical decision tasks almost always exceed willingness to pay elicited from non-hypothetical decision tasks (Chang et al., 2009). In a survey of Florida native plant producers, they indicated that lack of desired species was the biggest limitation to the Florida native wildflower market (Kauth and Pérez, 2011). Other limiting factors identified in this survey included education of customers and industry, accuracy of labeling, reliability of seed sources, need for new market development, and the availability of desired species (Kauth and Pérez, 2011). In a study focusing on landscape architects' use of native plants, they reported that residential projects ranked highest in use (30%), followed by commercial (25.1%), municipal (16.1%), and finally federal projects (8.5%) (Brzuszek et al., 2007). When these same landscape architects were asked why native plants were selected, they indicated that natives were best adapted to the site conditions (31.2%) (Brzuszek et al., 2007). Less than 20% of these respondents claimed to plant native plants at the request of clients, which could indicate that landscape architects are selecting native plants independently from client demand (Brzuszek et al., 2007). Aesthetic qualities of native plants can vary widely (Zadegan et al., 2008). Many native plant species have a tendency to grow in groups or colonies rather than single species stands and often produce small flowers with short bloom periods (Zadegan et al., 2008).

A better understanding of consumers' preferences for native plants, their attitudes towards bringing a more naturalistic design into the built environment, and purchasing behavior is needed to better characterize the market for producers of native plants and other horticultural professionals (Zadegan et al., 2008). Nevada survey respondents rated the importance of four major benefits of native species, the most important being drought resistance with 75.8% of respondents (Curtis and Cowee, 2007). The next most important trait of a native plant to Nevada respondents was the natural appearance of the plant (60.2% rating) (Curtis and Cowee, 2007). The third most important characteristic was protection from invasive plant species post wildfire (40.8%), followed by erosion control (22.7%) (Curtis and Cowee, 2007). Traits that are popular with consumers include extended flowering, novel floral morphology, compactness, and disease resistance (Wilde et al., 2015).

Native plants attract native insects and birds to a greater percentage than areas without native plants (Burghardt et al., 2009). There is also evidence that the presence of non-native plants can reduce native lepidopteran (order *Lepidoptera* L.) insect species (Burghardt et al., 2010). In addition to lepidopteran visitations, native plants are also preferred by 23 bee species as opposed to non-native plants that were visited by only 1 bee species (Morandin and Kremen, 2013). They also found more native bee species on native plants (Morandin and Kremen, 2013). In mature hedgerow sites where native floral cover was similar to exotic floral cover, they found that honey bees preferred native plants (Morandin and Kremen, 2013). They suggested that creation of native plant hedgerows in intense agricultural areas may benefit honeybee colony health (Morandin and Kremen, 2013). Further development of native ornamentals with improved flowering, architecture, and drought-tolerance traits may be a strategy to increase native plant use among a large segment of the consumer market (Wilde et al., 2015).

Conjoint analysis has been used to identify consumer segments based on their preferences for green industry products (Behe et al., 2014). These analyses allow us to understand the effects of product attributes as well as the influence of demographics on choice decisions (Behe et al., 2014). Conjoint analysis on landscape plant material revealed that landscapes with annual and perennial color increased the perceived value of homes (Behe et al., 2005). Color differences have also shown to be very important factors in purchasing decision making (Behe et al., 1999). Plant type was also seen as important in a separate study which investigated local and sustainable plant production characteristics (Behe et al., 2013). Price can be a major contributing factor for consumers in making decisions to purchase a product (Mason et al., 2008). By performing conjoint analysis on attributes of *R. columnifera*, we may be able to develop it into a value-added product that will better resonate with potential customers.

Materials and Methods

The online survey consisted of four parts: 1) a section eliciting recent plant purchasing behaviors, 2) a section determining existing native plant knowledge and preferences, 3) a section related to the *R. columnifera* conjoint analysis, and 4) a final section garnering demographic characteristics of the respondents. The conjoint design used for this study included a combination of categorical plant and product attributes and levels that represent four flower colors, three petal shapes, two petal numbers, and three price levels per container (Table 9) that align with common price points for similar products for a 4 x 3 x 3 x 2 factorial design (Knuth et al., 2018). Attributes and levels pertaining to *R. columnifera* floral characteristics were based off of variation found in nature. The four flower colors were red, yellow, bicolor, and marble. Petal shapes included oval, lobed, notched, and circular. The two petal number levels were less than ten and greater than or equal to 10. The three price levels were $10.00, $15.00, and $20.00. These prices were chosen based on current market sales price data for trade 1-gallon (approximately 3 L) *R. columnifera* containers. This resulted in 54 hypothetical combinations, or profiles, which was too numerous to combat subject fatigue. A conjoint analyzer software was used to select 16 of these combinations to be utilized in the consumer questionnaire, which is few enough combinations to reduce subject fatigue and time investment of the respondent (Knuth et al., 2018).

Table 9. Attributes and levels within the conjoint analysis of container-grown *Ratibida columnifera*.

Attributes	Levels
Flower color	Bicolor
	Marble
	Red
	Yellow
Petal shape	Circular
	Notched
	Lobed
	Oval
Petal number	Less than 10
	Greater than or equal to 10
Price	$10.00
	$15.00
	$20.00

The conjoint analysis will aid in assessing consumer's valuation on each product attribute, meaning we can determine levels of part-worth utility associated with each attribute, and the product as a whole (Knuth et al., 2018). The survey used digital images consisting of a photo of the plant in a container photographed against a neutral background with the accompanying price and characteristics listed above the image (Knuth et al., 2018). The analysis included use of a balanced Likert scale to allow respondents to rate the likelihood of purchase for each profile (Wollaeger et al., 2015). An example of a likelihood to purchase question would show the profile and then ask the consumer "How likely would you be to purchase this plant?". The respondent would then have the ability to respond with a whole unit on the Likert scale of 0 (very unlikely) to 5 (very likely) (Wollaeger et al., 2015).

Conjoint images consisted of a description of attributes above the image of the plant, with the Likert scale below the image (Fig. 2). This conjoint design will provide knowledge of consumer's willingness to pay, which is valuable information when it comes to marketing strategy, pricing decisions, and new product development (Breidert et al., 2006). In conjoint experiments, participants rank different products in order to estimate a preference from which willingness to pay can be derived (Breidert et al., 2006).

Figure 2. An example screenshot of one conjoint image shown to 1000 subjects in an online survey pertaining to *Ratibida columnifera* plant attributes and native plants.

The survey also solicited information regarding native plant use in landscapes, as well as gauging respondent knowledge on other native plant topics. Lastly, demographic questions such as number of adults in household, children in household, age, gender, ethnicity, education level, area of residence/developed environment, and income were asked (Wollaeger et al., 2015). The survey was developed and administered in Qualtrics

(Provo, UT) (Wollaeger et al., 2015) and was active until survey completion (number of needed responses achieved).

Potential survey respondents were contacted from a pool maintained by MTurk and invited to participate in the survey (Wollaeger et al., 2015). A recent study looking at the demographics and dynamics of Amazon Mechanical Turk participants (referred to as workers), and there were some differences found (Difallah et al., 2018). In their study, the workforce gender was balanced, though the age tended to be younger than the overall population. It was also found that MTurk workers have household incomes that were slightly below the average US population (Difallah et al., 2018). By asking demographics questions within our survey, we will be better able to claim whether choices made in our study might reflect that of the population as a whole. Both the survey development and methodology of data collection were approved by the university committee involving research with human subjects (Texas A&M University IRB# 2018-1655M Exempt AC 3YR).

Conjoint analysis identified importance values for main attributes and part-worth values for each level of the attributes that were then used to segregate the sample into clusters using SAS software (version 25, Cary, NC, USA) PROC CLUSTER. Principle component analysis describes the strength and direction of correlated variables in terms of their potential to quantify unobservable constructs (Knuth et al., 2020). Principal component analysis was performed using SAS and retained 10 items out of 19 possible items due to their factor loadings being ≥ 0.500 (Knuth et al., 2020). These 10 items were also chosen based on leveling off of eigenvalues in the scree plot. The loading value is used in principle component analysis to indicate the mean value for each item (question), being highest among all of the mean values for that item when testing for linear combinations (Knuth et al., 2020). Statistical analysis utilized in this study was as follows: principal components analysis (PCA) on native plant questionnaire using IBM SPSS Statistics 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.), conjoint analysis using TRANSREG and FASTCLUS in SAS (version 25, Cary, NC, USA), and distribution analysis of demographic data in JMP Pro 15 (SAS Institute Inc., Cary, NC, 1989-2021).

Results and Discussion

The survey was administered to 1384 potential participants beginning July 13, 2020. Subjects who agreed with the consent form and passed the survey check (where subjects were directed to answer in a specific way to ensure subjects were reading every question carefully) totaled 1000.

The mean age of respondents was 38.2 years (±10.4 years) and respondents were predominantly male (60%, Table 8). Respondents were primarily white (56%), followed by Asian (34%). African American (5%), Hispanic (3%), Native American (1%), Other (1%), Prefer not to respond (1%), and Pacific Islander (0%). Over half of respondents had a four-year degree (54%), followed by some college (14%) and master's degrees (14%). Respondents mainly resided in suburban regions (40%), followed by metropolitan regions (32%), with the remaining living in rural areas (28%). Mean yearly income for respondents was $52, 370 (± $37,157).

Table 10. Demographic variables of overall samples and by cluster[1].

Demographic Variables		Total Sample	Cluster 1	Cluster 2	Cluster 3	Statistic, p-Value
		Mean (S.D.) or %				
(Categorical)		**N = 1000**	N = 181	N = 418	N = 353	
Gender (M=1; F=2)	Male	60%	60%	61%	60%	X^2 = 0.018, 0.9910
	Female	40%	40%	39%	40%	
Ethnicity	African American	5%	6%	5%	3%	*F* = 13.494, <.0001
	Asian	34%	42%	25%	44%	
	Hispanic	3%	1%	4%	3%	
	Native American	1%	1%	1%	1%	
	Pacific Islander	0%	0%	0%	0%	
	White/Caucasian	56%	50%	62%	47%	
	Other	1%	1%	2%	1%	
	Prefer not to respond	1%	1%	1%	0%	
Education	Less than high school	0%	0%	0%	1%	*F* = 5.499, 0.0042
	High school/GED	9%	8%	10%	8%	
	Some College	14%	13%	16%	11%	
	2-year college degree	8%	6%	11%	6%	
	4-year college degree	54%	58%	52%	56%	
	Master's degree	14%	14%	11%	17%	
	Professional degree (JD, MD)	1%	1%	1%	1%	
	Doctoral degree	0%	0%	0%	1%	
Area of residence	Metropolitan region	32%	29%	33%	33%	*F* = 5.183, 0.0058
	Rural region	28%	25%	21%	39%	
	Suburban region	40%	45%	46%	29%	
Age (years old)		38.2 (±10.4)	36.7 (±9.6)	39.4 (±10.9)	36.8 (±9.5)	
Yearly income (USD $, 000)		52.3 (±37.2)	56.2 (±39.1)	52.5 (±35.6)	51.1 (±38.4)	*F* = 1.155, 0.3155

[1]Data analyses were generated using chi-square and F-test in JMP Pro 15 software (SAS Institute Inc., Cary, NC, 1989-2021).

The overall mean age of the subjects was 38.2 years, with cluster 1 having the youngest group (36.7) and cluster 2 having the oldest (39.4). Yearly income overall was $52, 370 (S.D. = $37, 157). Cluster 1 had the highest income $56,243.09 (S.D. =

$39,132.63), followed by cluster 2 $52, 488.04(S.D. = $35, 624.35) and cluster 3 $51, 076.49 (S.D. = $38, 375.79).

Recent plant purchasing behavior was quantified using a five-question purchasing pattern survey (Table 11). In the total sample (N = 1000), 41% of participants stated that they spent $1-$100 on plants and gardening supplies in the past six months, with the next highest expenditure being $101-$200 at 29%. All clusters had the most participants indicating expenditure of $1-$100 in the past six months. When asked what percent of yearly plant purchases were locally produced, 23% of participants spent 26%-50% on locally produced products. Cluster 1 indicated expenditure of 26%-50% on locally produced products. Cluster 2 had 21% indicate only spending 1%-25% on locally produced goods, and also had the highest percentage of participants that did not know if the source was local or not (21%). Participants in cluster 3 purchased locally sourced plants the most with 53% of participants spending 26%-75% on locally sourced plants. Independent, free-standing garden centers and home improvement stores were mainly frequented by our participants, with 49% of participants purchases over the past six months. Participants purchased 28% from supermarkets and grocery stores. Participants also utilized internet sales (28%), which outcompeted mass merchandisers and print catalogs. Participants purchased most of their plant and gardening supplies from independent free-standing garden centers (39%) and home improvement stores (32%). Cluster 1 frequented garden centers and home improvement stores equally (48%, 48%), and closely aligned with the total sample in regard to distribution of locales. Cluster 2 had more purchases from home improvement stores (58%), and garden centers

(56%), and had the least amount of internet sales out of all clusters (16%). Cluster 3 purchased more plants from grocery stores (33%), the internet (25%), and mass merchandisers (16%) than other clusters. Impacts of covid-19 restrictions on purchasing sources for this study are unknown, but could conceivably have enhanced online purchases relative to brick-and-mortar outlets.

Vegetable plants (53%), annual flowering plants (40%), and herbs (36%) were the main types of plants that were purchased overall by participants, followed closely by flowering perennials (32%) and indoor flowering potted plants (30%). Cluster 1 and cluster 3 purchased plants similarly to the total sample. Cluster 2 purchased more herbs (40%), perennials (35%), and indoor flowering potted plants (39%) than the total sample

Table 11. Purchasing pattern survey for overall sample and by cluster[1].

Purchasing patterns survey		Total sample	Cluster 1	Cluster 2	Cluster 3	Statistic, p-Value
		Mean (S.D.) or %				
Categorical		**N = 1000**	**N = 181**	**N = 418**	**N = 353**	
Thinking back over the plants and gardening supplies you purchased over the past six months, approximately how much did you spend (in total) on gardening supplies and plants (excluding mechanical equipment like lawn mower and tillers)?	$0	8%	6%	7%	6%	X^2 = 21.84, 0.0393
	$1-$100	41%	44%	45%	35%	
	$101-$200	29%	29%	27%	33%	
	$201-$300	12%	12%	11%	13%	
	$301-$400	6%	2%	6%	9%	
	$401-$500	3%	4%	3%	3%	
	$500 or more	2%	2%	1%	2%	
Approximately what percent of your yearly plant purchases for landscape or garden supplies are LOCALLY produced?	0%	7%	3%	7%	6%	X^2 = 32.16, 0.0014
	1%-25%	20%	24%	21%	20%	
	26%-50%	23%	25%	19%	29%	
	51%-75%	19%	16%	18%	24%	
	76%-99%	10%	12%	10%	7%	
	100%	4%	3%	5%	3%	
	Do not know	18%	17%	21%	12%	
From which type(s) of stores did you purchase plants and gardening supplies over the past six months?	Independent, free-standing garden center	49%	48%	56%	44%	X^2 = 11.03, 0.0040
	Home improvement or hardware store	49%	48%	58%	40%	X^2 = 27.28, <.0001
	Supermarket or grocery store	28%	31%	24%	33%	X^2 = 8.52, 0.0141
	Mass merchandiser	14%	12%	15%	16%	X^2 = 1.66, 0.4350
	Internet	20%	22%	16%	25%	X^2 = 10.91, 0.0043
	Print catalog	2%	1%	2%	3%	X^2 = 1.60, 0.4483
	None of the above	7%	6%	6%	5%	X^2 = 0.35, 0.8389
	Prefer not to respond	0%	1%	0%	0%	X^2 = 2.20, 0.3333
From which one type of stores did you purchase MOST of your plants and gardening supplies over the past six months?	Independent, free-standing garden center	39%	36%	42%	37%	X^2 = 63.75, <.0001
	Home improvement or hardware store	32%	30%	39%	24%	
	Supermarket or grocery store	10%	15%	5%	14%	
	Mass merchandiser	4%	3%	3%	6%	
	Internet	7%	7%	4%	11%	
	Print catalog	1%	1%	0%	2%	
	None of the above	7%	6%	6%	6%	
	Prefer not to respond	0%	1%	0%	0%	

Table 11 Continued

Purchasing patterns survey		Mean (S.D.) or %				
		Total sample	Cluster 1	Cluster 2	Cluster 3	Statistic, p-Value
Categorical		**N = 1000**	**N = 181**	**N = 418**	**N = 353**	
In thinking about your plant purchases over the past six months, please check the box beside all the types of plants that you purchased in the past six months.	Annual flowering plants (e.g., petunia, marigold, impatiens)	40%	38%	45%	37%	X^2 = 6.78, 0.0336
	Vegetable plants (e.g., tomato, pepper)	53%	56%	56%	50%	X^2 = 3.37, 0.1857
	Herbs (e.g., basil, parsley, sage)	36%	36%	40%	33%	X^2 = 4.16, 0.1248
	Flowering perennials (e.g., Hosta, chrysanthemum, day lily, cone flower)	32%	33%	35%	30%	X^2 = 2.58, 0.2752
	Flowering shrubs (e.g., hydrangea, lilac, etc.)	21%	20%	19%	26%	X^2 = 6.99, 0.0304
	Non-flowering shrubs (e.g., boxwood, taxus, etc.)	5%	2%	6%	6%	X^2 = 5.10, 0.0780
	Fruit producing trees (e.g., apple, pear, etc.)	15%	13%	14%	20%	X^2 = 7.75, 0.0207
	Evergreen trees or shrubs (e.g., pines, conifers, junipers)	7%	9%	7%	7%	X^2 = 0.93, 0.6267
	Shade trees (e.g., maple, oak, etc.)	6%	7%	7%	6%	X^2 = 0.32, 0.8542
	Indoor flowering potted plants (e.g., orchid, African violet, etc.)	30%	29%	39%	22%	X^2 = 23.88, <.0001
	None of the above	9%	7%	9%	7%	X^2 = 2.15, 0.3408

[1]Data analyses were generated using chi-square and F-test in JMP Pro 15 software (SAS Institute Inc., Cary, NC, 1989-2021).

Conjoint findings

Overall, petal color was the most important attribute out of the four that were tested. This was followed by price, petal shape, and petal number (Table 12). Within petal color, bicolor was preferred over red, yellow, and marble, respectively. Levels for the price attribute went from least expensive to most expensive in order of preference. The most preferred petals shape overall was circular, followed by oval, notched, then lobed. Greater to or equal to 10 petals were preferred over less than 10 petals for the petal number attribute.

There were some differences among the clusters in regards to preference. All clusters ranked petal color as most important. However, cluster 2 was more price conscious than the other two clusters. Cluster 1 was least conscious of price among the clusters. Cluster 3 had a slightly higher preference for color shape than other clusters.

Utility scores had differences among clusters. Cluster 1 is unique in the preference of red flower color, where the other clusters mainly preferred bicolor. Cluster 3 least preferred the red colored petals, whereas cluster 1 and cluster 2 least preferred the marble coloration. Cluster 3 would pay more ($15.00) than the other two clusters.

Table 12. Conjoint analysis showing mean relative importance scores and standard errors (S.E.) for each attribute overall and by cluster[1].

Attribute	Mean (S.E.) Relative Importance				
	All	By cluster			
	Subjects	Cluster1	Cluster2	Cluster3	(DF) *F*, p-Value
	N = 952	N = 181	N = 418	N = 353	
Petal color	41.93 (19.32)	44.28 (18.13)	44.98 (20.02)	37.11 (18.13)	(2) 18.18, <.0001
Petal shape	21.99 (11.74)	23.93 (12.08)	16.99 (8.26)	26.91 (12.69)	(2) 83.81, <.0001
Petal number	12.36 (10.50)	12.18 (11.01)	11.00 (10.06)	14.06 (10.52)	(2) 8.27, 0.0003
Price	23.72 (16.29)	19.60 (12.33)	27.02 (19.04)	21.93 (13.59)	(2) 17.08, <.0001

Attribute	Level	Mean (S.E.) Utility Score				
		All	By cluster			
		Subjects	Cluster1	Cluster2	Cluster3	(DF) F, p
Petal color	Bicolor	0.31 (0.60)	-0.33 (0.37)	0.67 (0.55)	0.22 (0.41)	(2) 300.85, <.0001
	Marble	-0.29 (0.53)	-0.37 (0.47)	-0.50 (0.55)	-0.00 (0.38)	(2) 106.85, <.0001
	Red	0.19 (0.60)	0.41 (0.53)	0.41 (0.59)	-0.20 (0.42)	(2) 150.98, <.0001
	Yellow	-0.20 (0.58)	0.30 (0.51)	-0.58 (0.52)	-0.02 (0.36)	(2) 265.68, <.0001
Petal shape	Circular	0.06 (0.31)	0.11 (0.30)	0.09 (0.31)	0.01 (0.29)	(2) 9.40, <.0001
	Lobed	-0.07 (0.30)	-0.06 (0.27)	-0.12 (0.33)	-0.01 (0.26)	(2) 14.14, <.0001
	Notched	-0.02 (0.29)	-0.06 (0.32)	0.01 (0.29)	-0.02 (0.27)	(2) 3.84, 0.0225
	Oval	0.02 (0.28)	0.01 (0.27)	0.03 (0.29)	0.02 (0.28)	(2) 0.14, 0.8669
Petal number	< 10	-0.12 (0.26)	-0.10 (0.25)	-0.18 (0.29)	-0.06 (0.22)	(2) 20.84, <.0001
	≥ 10	0.12 (0.26)	0.10 (0.25)	0.18 (0.29)	0.06 (0.22)	(2) 20.84, <.0001
Price	$10.00	0.21 (0.47)	0.11 (0.32)	0.46 (0.52)	-0.04 (0.28)	(2) 145.22, <.0001
	$15.00	0.00 (0.24)	-0.01 (0.24)	0.01 (0.25)	0.00 (0.22)	(2) 0.48, 0.6201
	$20.00	-0.21 (0.47)	-0.10 (0.29)	-0.47 (0.52)	0.04 (0.28)	(2) 154.06, <.0001

[1]Data analysis was generated using TRANSREG and FASTCLUS in SAS (version 25, Cary, NC, USA)

Principal Component Analyses

The principal component analyses of 10 questions on native plant knowledge (Table 13) yielded one component. It was labeled Native Knowledge (Table 14). When comparing mean scores for the component across clusters, cluster 3 had the higher native plant knowledge mean, followed by cluster 1 and then cluster 2.

Table 13. Principle component analysis component matrix for native knowledge questionnaire used to determine native knowledge value for clusters.

Questions	Component 1
I consider myself knowledgeable about native plants	0.841
I keep current on the most recent developments in native perennial plants.	0.781
I can recognize plants native to my area	0.781
I can recall many plants native to my area from memory.	0.775
Dense and compact landscape plants are important to me.	0.653
I know where to purchase plants native to my area.	0.632
I am interested in perennial native plants.	0.563
Work should be done to develop new native plant selections.	0.529
Longer blooming seasons are important to me.	0.509
Ratibida columnifera (Mexican Hat Wildflower) would be attractive in a cut flower arrangement.	0.507

Table 14. Mean comparisons of the principal components by cluster[1].

Component	Means Comparison						
	Means (S.D.)						
	Cluster1		Cluster2		Cluster3		*F*, p-Value
Native plant knowledge	0.04 (1.01)	ab	-0.09 (0.96)	b	0.20 (0.94)	a	8.37, 0.0003

[1]Different letters within a row indicate significant differences of means at $P<0.05$.

Conjoint clusters

Three clusters formed from the cluster analysis, which were then compared using analysis of variance of the demographic characteristics including age, gender, ethnicity, education, area of residence, income, native plant knowledge, number of purchase locations, and number of plant types purchased within the past 6 months (Table 15)

Table 15. Cluster proportions including principal component analysis and part-worth utility values[1].

Variable	Single color oval petal lovers N = 181		Price conscious bicolor petal lovers N = 418		Knowledgeable marbled petal lovers N = 353		p-Value
	Mean (S.D.)						
Age	36.73 (9.57)	b	39.41 (10.88)	a	36.76 (36.76)	b	0.0003*
Gender	1.39 (0.49)	a	1.39 (0.49)	a	1.40 (0.49)	a	0.9910
Ethnicity (% non-white)	0.50 (0.50)	b	0.62 (0.48)	a	0.47 (0.50)	b	<.0001*
Education	4.59 (1.15)	ab	4.39 (1.19)	b	4.67 (1.22)	a	0.0042*
4 year college degree or more (% with)	0.73 (0.45)	a	0.63 (0.48)	b	0.75 (0.44)	a	0.0015*
Area of residence	2.16 (0.85)	a	2.13 (0.88)	a	1.96 (0.78)	b	0.0058*
Income (USD $, 000)	56.2 (39.10)	a	52.5 (35.60)	a	51.1 (38.40)	a	0.3155
Native knowledge	0.04 (1.00)	ab	-0.09 (0.96)	b	0.20 (0.94)	a	0.0003*
Number of purchase locations	1.69 (0.93)	a	1.77 (0.93)	a	1.65 (0.95)	a	0.2354
Number of plant types purchased	2.42 (1.46)	ab	2.68 (1.78)	a	2.37 (1.66)	b	0.0298*
Petal number relative importance							
< 10	-0.10 (0.25)	a	-0.18 (0.29)	b	-0.06 (0.22)	a	<.0001*
≥ 10	0.10 (0.25)	b	0.18 (0.29)	a	0.06 (0.06)	b	<.0001*
Petal color relative importance							
Bicolor	-0.33 (0.37)	c	0.67 (0.54)	a	0.22 (0.41)	b	<.0001*
Marble	-0.37 (0.47)	b	-0.50 (0.55)	c	-0.00 (0.38)	a	<.0001*
Red	0.41 (0.53)	a	0.41 (0.59)	a	-0.20 (0.42)	b	<.0001*
Yellow	0.30 (0.51)	a	-0.58 (0.52)	c	-0.02 (0.36)	b	<.0001*

Table 15 Continued

Variable	Mean (S.D.) Single color oval petal lovers N = 181		Price conscious bicolor petal lovers N = 418		Knowledgeable marbled petal lovers N = 353		p-Value
Petal shape relative importance							
circular	0.11 (0.30)	a	0.09 (0.31)	a	0.01 (0.29)	b	<.0001*
lobed	-0.06 (0.27)	ab	-0.12 (0.33)	b	-0.01 (0.26)	a	<.0001*
notched	-0.06 (0.32)	b	0.01 (0.29)	a	-0.02 (0.27)	ab	0.0225*
oval	0.013 (0.27)	a	0.03 (0.29)	a	0.02 (0.28)	a	0.8669
Price relative importance							
$10.00	0.11 (0.32)	b	0.46 (0.52)	a	-0.04 (0.28)	c	<.0001*
$15.00	-0.01 (0.24)	a	0.01 (0.25)	a	0.00 (0.21)	a	0.6201
$20.00	-0.10 (0.29)	b	-0.47 (0.52)	c	0.04 (0.28)	a	<.0001*

[1]Different letters within rows indicate significant differences of means at $P < 0.05$. Effects that are statistically significant are labeled ‘*’.

Among the three clusters that emerged, gender, income, number of purchase locations, preference for oval petal shape, and price of $15 did not vary. Cluster 1 had young participants and intermediate education, non-white participants, native plant knowledge, and number of plant types purchased. Cluster 1 participants mainly reside in suburban regions. They had intermediate petal number preference in comparison to the other clusters. They preferred red or yellow flowers more so than the other clusters and had intermediate preference for marble coloration. They least preferred bicolor flower color the most out of all clusters. Oval and circular petal shapes were preferred the most by this cluster, with intermediate feelings towards notched and lobed shapes. This cluster was willing to pay $15.00 and ranked intermediate in preference for other price levels. Given these characteristics, cluster 1 was labeled as "Single color oval petal lovers" (Table 15).

Cluster 2 had the highest mean age average at 39.4 years. This cluster also had the highest percentage of non-white participants. This cluster had the least educated participants out of the three clusters. This cluster resided mainly in suburban areas. Cluster 2 also had the lowest native plant knowledge out of the three clusters. This cluster did however purchase a larger variety of plant types than the other clusters. They had strong feelings of preference for petal number, having the least preference for less than 10 petals, and a greater preference for greater than or equal to 10 petals when compared to other clusters. They stood out among clusters in their strong preference for bicolor petal color and aligned with cluster 1 in their preference for red petal color. They disliked marble and yellow petal colors the most when compared to other clusters. Petal shape preferences for this cluster aligned with other clusters except for their strong dislike for the lobed petal shape. This cluster preferred the lowest price possible ($10.00) and least preferred the highest price ($20.00). Due to this, we labeled this cluster as "Price conscious bicolor petal lovers" (Table 15).

Cluster 3 was intermediate in age and non-white participants. This cluster had high education when compared to the "Price conscious" cluster. Cluster 3 had more rural participants, as well as the most plant knowledge out of the three clusters. They purchased the fewest types of plants. Cluster 3 differed from the others in their preference for less than 10 petals, and least preferred 10 petals or more. Cluster 3 preferred marble coloration the most out of the three clusters. Cluster 3 was intermediate in preference for the other petal color levels. Cluster 3, which preferred oval and lobed petal shapes, was intermediate in preference for notched petal shape, and least preferred circular petal shape. Cluster 3 least preferred the lowest price ($10.00) and had the most interest in paying higher prices ($15.00, $20,00), so we labeled this cluster as "Knowledgeable marbled petal lovers." (Table 15).

Demographics Characteristics

This study was 60% male, which is much higher than the 49.2% male population of the United States in 2019 reported by the U.S. Census (U.S. Census, 2020). The median age of subjects in this study (38.2 years) was very close to the median age of the U.S. (38.5 years) (U.S. Census, 2020). Our subject sample had a greater proportion of people of color when compared to the U.S. census which had 72% White, 12.8% African

American, 0.9% American Indian and Alaska native, 5.7% Asian, 0.2% native Hawaiian and other Pacific Islander, 5% other, and 3.4% with two or more races. Individuals of Hispanic backgrounds make up 18.4% of the population. In particular, our study had a high number of Asian participants (34%) (U.S. Census, 2020). The 2019 U.S. Census had 88.6% high school graduate or higher, whereas 100% of our subject sample had education of high school or higher (U.S. Census, 2020). The U.S. Census had 33.1% with education of a 4-year degree or higher, and our study had 69%, showing that our subject sample was more highly educated (U.S. Census, 2020). In 2019 the median earnings were $43, 215, and our study had an overall median income of $52, 370 (U.S. Census, 2020).

Conjoint Findings and Previous Literature

The conjoint study findings were consistent with prior research in regard to the importance of flower color and price (Behe et al., 1999; Mason et al., 2008). Overall petal color was the most important attribute, followed by price, petal shape, and petal number. Petal color and price being the most important factors is a reoccurring theme in conjoint analysis results (Behe et al., 2001). Petal number is commonly the least important attribute in conjoint analyses where it is included (Grygorczyk et al., 2016). In roses, subjects preferred full petaled roses, as opposed to single-semi double petals and double petals (Grygorczyk et al., 2016). Due to the results of the conjoint analysis, improvements of *R. columnifera* in regard to petal color would prove to be the most valuable from a consumer purchasing standpoint. Utility values give more insight into which colors are more desirable.

Part-Worth Conjoint Utility Values

Bicolor petals were preferred over other petal color levels overall according to the part-worth utility values. A preference for two-toned petals was also observed with a conjoint survey performed on roses (*Rosa* L.) (Grygorczyk et al., 2016), and impatiens (*Impatiens hawkeri* W. Bull) (Berghage and Wolnick, 2000). After bicolor, the colors in order from most preferred to least preferred in the overall sample were red, yellow, and finally marble. Red is a popular preference for many floral products like roses (Grygorczyk et al., 2016), geraniums (*Pelargonium* × *hortorum* L.H. Bail.) (Behe et al., 1999), impatiens (Berghage and Wolnick, 2000), and poinsettias (*Euphorbia pulcherrima* Willd. *ex* Klotzsch) (Behe et al., 1997). Since the bicolor *R. columnifera* is the "wild type" coloration that is readily available, it could be profitable to develop a solid red *R. columnifera* seeing as that is the color that is preferred across many species. Price preference followed what would logically be expected, the lowest price, $10.00, was most preferred, followed by $15.00 and $20.00 respectively. When it comes to floral products, it is a common theme to have lower prices preferred to higher prices (Behe et al., 1999). Overall, a circular petal shape was preferred over all other shapes (oval, notched, lobed). There is little to be found in the literature regarding consumer preference of petal shapes. Subjects preferred inflorescences with ten petals or more, as opposed to one with less than 10 petals. This is the opposite reaction from that of the rose study from Grygorczyk (Grygorczyk et al., 2016). In that study, double petaled roses were least preferred.

When looking at the data for the entire sample, preferences of our subjects reflect the findings of conjoint analyses in the previous literature. Consumers preferred *R. columnifera* with partial (bicolor) or complete red coloration over other options, lower prices, more petals, and entire circular or oval petals.

Conjoint Clusters

The price conscious bicolor petal lovers fall into the age range of the Gen X generation (individuals born between 1965 and 1980), as opposed to the single color oval petal lovers and the knowledgeable marbled petal lovers who are both in the Gen Y population (individuals born between 1981 and 1995) (Knuth et al., 2020). This group is the most price conscious, having the greatest preference for lower prices and greatest aversion to higher prices. This cluster had the least formal education, and the least knowledge of native plants out of the three clusters. Gen X consists of 44 million people, and tend to value money, possessions, and the shopping experience more than older generations (Behe et al., 2016). These Gen X trends were reflected in our data for the price conscious bicolor petal lovers, both in their price and store type preferences. This group purchased mainly from brick and mortar storefronts, and utilized print catalogs and the internet the least.

The single color oval petal lovers and knowledgeable marbled petal lovers both are heavily weighted towards the Gen Y population. This generation is said to be the most ethnically and culturally diverse age cohort in America today (Behe et al., 2016). The data from our survey did not reflect that sentiment. Both of these groups were very similar in distribution of ethnicity. These groups achieved higher degree educations than their Gen X counterparts. The single color oval petal lovers resided in more suburban regions, whereas the knowledgeable marbled petal lovers reside in more rural locations. Gen Y are often referred to as digital natives, meaning they have always had access to the internet (Behe et al., 2016). This may explain the more frequent internet sales seen in the two Gen Y groups.

Variance and Risk

In probability theory and statistics, variance is a measure of how far a set of numbers is spread out. It is one of several descriptors of a probability distribution, describing how far the numbers lie from the mean (expected value). To illustrate the concept of variance, it is useful to consider an example from the field of finance. Understanding the concept of variance along with three typical asset classes — money market, bonds, stocks — can aid in building a financial portfolio for any. Money market investments are very safe, they almost never go in the red, but they also do not pay high returns. Stocks are on the opposite end of the spectrum, going back and forth between red and black from year to year frequently, but over longer periods of time they usually pay higher premiums. Bonds are somewhere in the middle. They are safer than a stock, but riskier than a money market and their average returns reflect that risk.

Variance within samples in research studies such as this can also be correlated to risk as indicated above. Sometimes there may not be statistically different means between treatment combinations, but the variance can be larger or smaller than other treatment combinations. You can determine the variance by observing the standard deviation alongside means. Variance can be both a positive or negative aspect in that a larger variance generally means larger risk, higher reward potential, and more variability in the results. For instance, a researcher or plant breeder attempting to obtain the best performing genetics for their cultivation program might choose the treatment that includes lower and higher extremes in certain plant attributes in hopes of achieving that higher level of performance. On the other hand, a lower variance may infer less risk, a more dependable reward, or less variability in results. A production greenhouse grower might choose this option in aims of producing a uniform crop and reducing the risk of having unsaleable outliers in their crop. This idea applies to decisions in marketing *R. columnifera* in the sense that we can make more or less risky decisions depending on what market segment we are considering.

Hypothetical bias

The treatment combinations of *R. columnifera* in our survey do not exist currently. Therefore, our survey was *ex ante* in design, meaning it is a forecast used to guide a decision about what to do in the future, because the particular good does not exist yet (Loomis, 2014). This is opposed to *ex post,* where choices are based on actual revealed preferences rather than forecasts. Thus, while it is appropriate for researchers to elucidate preference on yet-to-be-developed goods, hypothetical bias can pose issues when ascertaining if the participant's real world purchasing decisions will indeed reflect the survey findings. Hypothetical bias is when there is a disconnect between actual cash willingness to pay, and stated willingness to pay, quite often it is an overstatement of willingness to pay (Loomis, 2014). As it relates to our results, that means that it is a likely possibility that consumers will actually purchase 1-gallon containers of *R.*

columnifera for less than the $10 stated willingness to pay. Hypothetical bias can be minimized with *ex ante* survey design approaches such as consequentiality, insisting on honesty, explaining hypothetical bias to respondents, and reducing social desirability (Loomis, 2014). It can also be minimized with *ex post* approaches such as data screening, market calibration, and uncertainty recoding. There is no universal agreement on the best method to correct for hypothetical bias, and that maybe incorporating several approaches may better capture the true willingness to pay (Loomis, 2014).

Conclusions

This survey has provided valuable results in regard to decisions on what direction to take in making decisions for *R. columnifera* breeding selections. This research objective is important because a great deal of time, energy, and resources are put into cultivar development programs. It would be logical to consider developing cultivars with attributes that follow consumers preferred trends. This would ensure that a product is developed that resonates with consumers.

Results from this survey provide insights regarding consumer preferences for *R. columnifera* in the following ways. We examined a combination of petal color, petal number, petal shape, and price of willingness to pay for *R. columnifera*. Consistent with previous literature we found that petal color was the most important attribute (Behe et al., 1999; Mason et al., 2008). Price was the next most important attribute, followed by petal shape and petal number. This order of importance is seen in previous literature as well (Behe et al., 2001; Grygorczyk et al., 2016). Consumers preferred *R. columnifera* with partial (bicolor) or complete red coloration over other options, lower prices, more petals, and entire circular or oval petals. If these results were to be implemented into a breeding program, it is suggested that petal color be a main focus, followed by petal shape and petal number in regard to floral characteristics. Future research could include additional variation in tints, tones, or shades of red and bicolor patterns. There is a distinct lack of literature on consumer preferences for petal shape, therefore additional research on petal shape could be useful. Floral characteristics alone are not the only important driving factors in decision making in regard to ornamental plants. Consumers have shown preference and aversion to differing plant sizes and growth habits (Baidu-Forson et al., 1997; Behe et al., 2005). In *R. columnifera*, conjoint analysis could be performed on different foliage characteristics, plant size and plant habit as well. Implementing improvements into *R. columnifera* selection that follow the trends of this survey would aid in creation of a product with improved market potential over the species type.

CHAPTER V

CONCLUSIONS

One study established key information for development of improved *R. columnifera* (Nutt.) Wooten & Standl. cultivars with characteristics that will resonate with consumers. Additional experiments provide insights into responses of *R. columnifera* to seed storage, seed treatment, and vegetative propagation practices, and revealed additional research questions that could be the topics of future studies. In addition to optimization of cultural practices, consumer preferences and willingness to pay values can be used to direct breeding selection and efforts towards a consumer driven marketable product.

Ratibida columnifera (Nutt.) Wooten & Standl. propagation may be achieved both by seed and by stem cuttings assuming the correct prerequisites are met. Vegetative propagation experiments involved many interaction effects between cutting developmental stage and hormone concentration. Overall, a low to mid-range hormone concentration of 0.10% or 0.30% IBA improved rooting quality on both young terminal cuttings and those that were more highly lignified (Table 1). Absence of hormone generated fewer roots that were longer in length, whereas higher concentrations produced root systems with more numerous short roots. A mid-range hormone concentration would produce a more ideal ratio of root number to root length. Younger tissues yielded higher quality root systems than mature cuttings. It is recommended that and application of 0.30% IBA is used on young apical stem cuttings for best results. Germplasm was found to be a significant effect when testing the use of bottom heat applications. In cool seasons it is recommended that one should apply 26°C or 32°C bottom heat to achieve optimum rooting (Table 5). Application of 26°C bottom heat improved rooting during both cool and warm seasons (Table 3, Table 4, and Table 5). Overall, results of our experiments suggest using 26°C bottom heat, young recently matured shoot tips, and 0.10% to 0.30% IBA quick dips to maximize adventitious root development. These results also indicate that to maximize commercial production, candidate genotypes for introduction should be screened for rooting potential prior to release. Germplasm was a significant effect in all experiments except for the overnight seed hydration pre-treatment test. This reflects responses found in many species where there are differences in germination responses in different accessions due to geographical and provenance effects (Hong and Ellis, 1996). Seed stored at 3°C or 23°C in a dry state can maintain viability for at least 12 months (Table 4). Some seed pretreatments had a positive influence on germination percentage and viability, while some had negative impacts. Overnight hydration did not hinder germination, but it also did not result in increased germination percentage or uniformity. Acid scarification had a negative impact on percent germination, and is not suggested as a pretreatment for *R. columnifera.* Stratification (moist, 3°C) between 30 and 60 days does not negatively impact germination percentages in most germplasm, however 90 day stratification had a negative impact (Table 7). Developmental stage of the inflorescence and a seed's location on the inflorescence had a significant effect on percent germination (Table 8). There was an interaction effect between germplasm and developmental stage, and germplasm and region on the inflorescence. In all germplasm, mid-age to mature inflorescences had an increased percent germination when compared to immature inflorescences. Therefore, it is recommended to plan seed harvests for when a majority of the inflorescences have dropped their ray petals and turned brown. There were varied impacts from a seed's location on the inflorescence. Two of the germplasm had higher germination percentage on the basal and middle portions of the conical inflorescence, while another genotype had no differences among locations. This likely reflects the acropetal flowering of the inflorescence which would lead to acropetal seed maturation. This is consistent with observations that knowledge of the timing of flower development and relative timing of ray and disc florets is critical for successful crossing in *Asteraceae* (Wetzstein et al., 2014). Much of the data from these experiments yielded higher percent germination than previous literature stated (Romo and Eddleman, 1995). This may be because our seed was derived from well managed stock plants, and therefore lacking much of the stress that a mother plant in nature would experience. These stresses to the mother plant can reduce seed longevity and viability (Harrington, 1975). Future studies are needed to optimize propagation protocols further. Such studies could include testing frozen seed storage, optimizing germination temperatures, optimizing germination substrates, determining provenance impacts, and determining if any residual impacts of germination treatments correlate to vigor of seedling growth beyond germination. Establishment of these protocols are crucial to successfully propagating *R. columnifera* via seed, which will be important for germinating seedlings from controlled crosses.

The consumer preferences survey provided valuable information regarding which floral characteristics and prices would result in the most marketable cultivars. Knowledge of consumer preferences regarding these characteristics allows breeders to make more educated decisions when making selections for crosses. Attributes tested included, in order of importance, were petal color, price, petal shape and petal number (Table 12). In considering the utility values of these attributes, consumers preferred *R. columnifera* with partial (bicolor) or complete red petal coloration over other options, lower prices, more petals, and circular or entire petals (Table 12). Three conjoint clusters emerged, which differed in utility values, native plant knowledge, and demographics (Table 15). This is indicative of a heterogenous market, where consumers have different attitudes and preferences, and their behavior differs in regards to the purchase and use of products (Behe et al., 2013). Future research could include additional variation in tints, tones, or shades of red and bicolor patterns. Future studies could also examine other important aspects of *R. columnifera* including foliage variation, plant size, and plant growth habit.

This study has established key protocols for the perpetuation of *R. columnifera* genetics, and provided awareness of the public's opinion on the potential of *R. columnifera* as a value-added nursery crop. A foundation of optimized protocol, and a better understanding of consumer's decisions, has been assembled for future researchers to build upon and produce a novel, commercialized, sustainable nursery product.

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