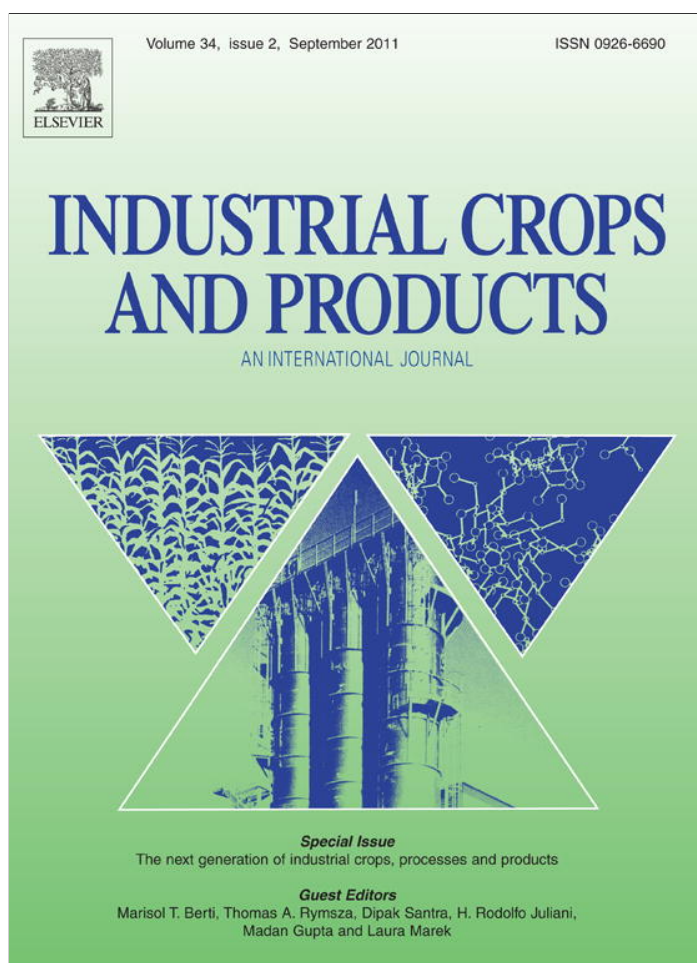


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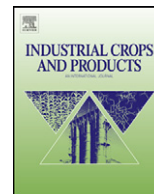
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## Industrial Crops and Products

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## Lesquerella: New crop development and commercialization in the U.S.

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### ABSTRACT

While *Lesquerella fendleri* Gray (Wats.) is not yet a commercial crop, its history serves as a model for new crop development. The most important characteristic is the absence of any significant biological barriers to commercialization. Other potential crops may have valuable, high-demand products but possess traits difficult to overcome such as seed shattering or poor yield capacity. *Lesquerella* has a distinctive plant architecture that is conducive to seed productivity under a variety of conditions, and the trait could be further exploited. The plant also has high amounts of within-species and interspecific genetic diversity allowing breeding improvements in traits including oil quantity and quality. The unique seed oil is predominately composed of a hydroxy fatty acid, lesquerolic acid (C20:1OH), that is similar to ricinoleic acid (C18:1OH) found in castor oil. Improvements in agronomics will help increase seed yields, water use efficiency, while reducing crop production costs. New tools offered by remote sensing will help plant breeders and growers assess crop development. Defining herbicides and obtaining registrations for use in *lesquerella* appears to be the biggest obstacle for commercialization of this crop. The improvements in agronomics, breeding, genetics, and the expansion of new markets started in the 1980's, and has made *lesquerella* a viable potential crop that could utilize thousands of hectares in arid climates of the world provided research continues.

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### 1. Introduction

Traditional crops used in agriculture today have undergone thousands of years of selection. Most were domesticated for food purposes and have been selected and bred for traits adapted to agriculture as management of farms evolved. Traits such as grain or seed shattering in many crops were major barriers to domestication. Selection for plants that could hold on to ripe seed or grains to allow effective field harvest was a key step. This has been referred to as the hallmark of domestication in rice (Li et al., 2006; Zhang et al., 2009). Indeterminate growth has also been suggested as a major hurdle for domestication along with photoperiod sensitivity, low growth habit, and small seed size (Garcia et al., 1997).

New industrial crops fit a new model for crop domestication. These crops, often considered wild or undomesticated, have products for markets that in many cases have only recently developed. The only consideration has been the uniqueness of the products with little concern for barriers to growth, yield, and harvest. In some cases, high value products and/or co-products could be a tradeoff for reduced yields due to shattering or indeterminate growth. These

specialty crops usually have limited markets and might command higher prices so that low yields could be acceptable. An example may be cuphea (*Cuphea* spp.), an oilseed crop in the process of development for use as a replacement for tropical palm oil. It is very rare that oilseeds originating from temperate regions produce short chain fatty acids (C8:0, C10:0, C12:0 and C14:0). Very limited trial production has occurred and yields are depressed from the potential of the crop because of seed shattering. It is possible that the price for specialty biofuel markets is high enough that growers could profit from yields of 600 kg/ha (Geller et al., 2003; Berti et al., 2008).

*Lesquerella fendleri* may be a different model to consider for new industrial crops. It was discovered for its unique industrial seed oil in 1960 (Jones and Wolf, 1960; Smith et al., 1961). The hydroxy fatty acids (HFAs) dominate the seed oil profile and in some species, 90% of the oil is HFA. It is a New World genus native to the U.S., Mexico, and some species are found throughout the Americas (Rollins and Shaw, 1973) and belongs to the mustard family. Key domestication traits in *lesquerella* include seed retention, photoperiod neutrality, and oil seed quality. Optimizing pest management and agronomic practices (e.g., date of planting, fertilizer management, and irrigation regimes) for *lesquerella* will also facilitate commercial production (Roseberg, 1993).

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Lesquerella can be severely affected by weed competition due to its slow growth during establishment and its short stature. In Arizona, lesquerella is broadcast seeded in flood irrigated level-basins, similar to alfalfa or small grains. In this type of production system, mechanical cultivation for weed management is difficult. Thus, identifying herbicides for chemical weed management in lesquerella is crucial for commercial production. Markets for hydroxylated oils are currently filled by castor oil, grown in countries other than the U.S. Some of these unique markets include feedstocks for lithium greases, polymers in paints and coatings, base stocks for lubricants, nylon-11, hydraulic fluids, and applications in the personal care industry (Roetheli et al., 1992). The hydroxyl group of these oils makes them prime candidates as additives to diesel fuel to improve lubricity (Naughton, 1992). Goodrum and Geller (2005) demonstrated that lesquerella oil has superior performance compared to castor, soybean, and rapeseed methyl esters at concentrations as low as 0.25% in reducing wear and damage to diesel engines, primarily with fuel injection systems. Castor oil also contains high amounts of HFAs, but the main HFA, ricinoleic acid, is two carbons shorter than lesquerolic acid, which imparts slightly different physicochemical properties to the oil. Lesquerella could be established as a reliable, domestic oilseed supply of HFAs for a variety of industrial applications (Roetheli et al., 1992). Lesquerella will not replace current commodity crops but instead will be placed in a rotation with these crops (e.g., a 2-year, 3-crop rotation of lesquerella, grain sorghum, and cotton). Lesquerella still is not a commercial crop despite all of its excellent qualities. There have been advances in breeding, agronomics, tools such as remote sensing for agro-management decisions and crop evaluations, and utilization research. There is a need to continue this research and focus on key areas to move lesquerella into commercialization. The objective of the manuscript is to outline the current potential for lesquerella production by providing a review of the most current and relevant research.

## 2. Planting and production

Although lesquerella is a short-lived perennial, it can be produced as a winter annual crop in the southwestern U.S. The plant has tolerance to freezing temperatures ( $-5^{\circ}\text{C}$ ; Dierig, unpublished data) and is planted in fall and harvested in June throughout the southwestern U.S. The most important environmental factors affecting stand establishment include soil temperature, moisture, and depth of planting. The temperature range for germination has been reported to be between 5 and  $35^{\circ}\text{C}$ , with  $20^{\circ}\text{C}$  optimal for *L. fendleri* (Sharir and Gelmond, 1971). Adam et al. (2007) examined a number of *L. fendleri* lines and found optimal temperature for germination varied by germplasm used. Improved lines had higher optimal temperatures ( $28^{\circ}\text{C}$  in one case) than unimproved accessions. Having a similar planting and harvesting period as wheat and other small grain crops, lesquerella could fit into southwestern growers' cropping systems as an alternative crop. Many farmers already have equipment for cultivating lesquerella. For example, lesquerella can be planted with Valmar granule applicators and Brillion planters (commonly used to plant alfalfa) and harvested with conventional grain combines with a slight modification of the sieves (the same as for alfalfa).

Planting dates significantly affect lesquerella growth and yield. Late planted lesquerella does not require as much time as early plantings to form a closed canopy. However, our research showed that late planting reduced plant biomass significantly due to shorter growing period before flowering. As a result, the number and mass of buds, flowers, and siliques were all reduced at the late planting (January and February) compared to the October planting in Arizona (Dierig and Wang, unpublished data). Crop yield at late

plantings was also lower by 25% or more compared to early planting. Similar results were also reported in previous studies in Arizona (Nelson et al., 1996) and Oregon (Roseberg, 1993). For these reasons, the recommended planting dates are October in Arizona and late September in Texas and New Mexico. Earlier planting in Arizona also results in earlier harvest before a monsoon rain season, which could avoid rainfall induced shattering and further reductions in crop yield. The planting method for establishing lesquerella is crucial because of the small seed size requiring broadcast planting with very little soil covering of the seed. Sandy loam soils lend themselves to this type of planting because they can cover the seed without burying it too deep. Optimal planting rate is between 8 and 12 kg/ha (Brahim et al., 1996). This is equivalent of 13–20 million seeds per ha. Brahim et al. (1996) suggested a population of a million to be optimum for seed yield. Lower populations can be used without lowering seed yield if seed distribution is uniform.

Another critical factor for stand establishment is soil preparation (Wang, unpublished data). Leveling the field for flood irrigation and then driving over it with a barbed roller (cultipacker) causes small indentations in the soil that are desirable for germination of small seeded crops. Higher plant populations have resulted with a Valmar 2055 granule applicator (used for fertilizer, herbicides, and any other pelleted or dry material) to broadcast the seed compared to the use of a Brillion planter, commonly used to plant alfalfa. The field is then rolled again. The Valmar equipment appears to keep the seed closer to the surface. The critical step is to make sure the soil surface is firm so seed is uniformly sprayed and does not drop too deep to germinate, which includes a level or slight slope for even irrigation distribution. Many stand failures can be attributed to a lack of soil moisture for seed germination and emergence. Therefore, fields should be kept moist to avoid soil crusting until emergence is complete (about 10 days to 2 weeks). Water requirements during the winter months are general low. Multiple establishment irrigations are required to keep the soil surface moist. A minimum of 3 irrigations are required on sandy loam soils depending on air temperatures and wind conditions at the time of planting. Lesquerella requires 635–762 mm of water during the growing season (Hunsaker et al., 1998). The greatest water requirements occur between late February and May when lesquerella produces 90% of its growth.

Lesquerella responds well to nitrogen fertilizer (Nelson et al., 1996, 1999; Adamsen et al., 2003). Nitrogen should be applied in split applications: 60 lb/A at planting and 60 lb/A at the onset of flowering in February. High nitrogen application reduced seed oil content in lesquerella (Nelson et al., 1996, 1999). However, total oil yield can still be improved with nitrogen application even though oil content may be reduced. Currently, no information is available on the response of lesquerella to phosphorus or other nutrients.

As canopy flowering declines and seed mature in May and June due to increasing temperatures, plants are desiccated for harvest. Plants are dried by stopping irrigation and when plants begin to turn brown, a desiccant (e.g. Paraquat) is applied to complete the process. This allows the crop to be harvested using a conventional combine about 3–4 weeks after the last irrigation, usually in June. Heavy rains during that period can cause seed shattering since plants are more vulnerable when drying compared to when they are green.

## 3. Breeding

### 3.1. Oil content and profile

Lesquerella lines with higher total oil and lesquerolic acid contents are available through public releases and plant variety

protection (Dierig et al., 1998, 2006a). Recurrent selection has produced individual plants with seed oil contents of 45% (Dierig et al., 2006a) but not yet varieties. This may be the upper limit for lesquerella or it could be higher since the value represents an average from a half gram of seeds from a single plant, which is necessary for nondestructive oil content analysis. The half gram sample is composed of approximately 900–1000 seeds, all derived from the same maternal source but potentially a different pollen source (half sibs). A single seed weighs approximately 0.0006 g (1000 seed weight = 0.6 g) where some seeds would be higher and some lower to produce this average. A technique has been developed by Isbell et al. (2008) to analyze half seeds and preserve the genotype by growing the other half but the technique has not been used to screen populations for genetic diversity of seed oil content.

The half seed technique has been used to screen seed oil profiles of lesquerella populations (Dierig et al., 2006b). It has allowed the discovery of mutants in the fatty acid biosynthesis, such as, a high oleic fatty acid/0 hydroxy fatty acid mutants (Dierig, unpublished date). Oleic acid is the precursor to lesquerolic acid (Broun et al., 1998). Disruption of key enzymes, such as desaturases, affects hydroxylation of oleic to ricinoleic acid, which is elongated to lesquerolic acid (Dauk et al., 2007). Other enzymes affect the functionality of the triglyceride and have important consequences for breeding improvement.

The bifunctionality of the triglyceride of *L. fendleri* restricts the lesquerolic acid content to about 67% as an upper limit. This can be beneficial since some market applications prefer this level, such as nylon-11. Other applications will require higher levels of HFAs to be more economically competitive. Other *Lesquerella* species with trifunctionality are being utilized to introgress the trait (three filled positions of the triglyceride) into *L. fendleri* (Dierig et al., 2004). An improved line with this trait would fit most of the same markets as imported castor and would lower the unsaturated fatty acids linoleic and linolenic acids (Broun et al., 1998). There will be a need for different varieties to be developed with different seed oil profiles for the appropriate end-use.

### 3.2. Seed yield

The current seed yields are approximately 1800 kg/ha, but the crop has the potential of yielding 2500–3000 kg/ha. This increase will come through a combination of improved agronomic practices and breeding. Some agronomic issues include more precise plant spacing, better irrigation management, and more efficient harvest. Finding more productive varieties through plant breeding includes selection based on harvest index and for specific environments. *L. fendleri* is the most productive of all species so far tested because of its extensive branching leading to high seed yields. Selecting for branching at warmer or cooler temperatures will identify plants more adapted for growth in different climates. Plants that reach maturity in a shorter time period will save production costs. This will require selection based on seed germination at cooler temperatures, or plants that will branch at lower temperatures.

### 3.3. Autofertility

A few lesquerella species are autofertile including *L. pallida* and *L. mcvaughiana*. Both have the same chromosome number as *L. fendleri* and are a potential source to introgress autofertility since *L. fendleri* is an outcrossing species. The breeding program has developed interspecific hybrids with *L. fendleri* and both *L. pallida* and *L. linheimeri* to improve the oil profile. There are some promising hybrid lines with autofertility that have resulted and are currently being tested (Dierig and Ray, 2009).

There have also been some single plant selections made within *L. fendleri* that could be further developed to incorporate autofertility.

The advantage of autofertility is that pollinators are not needed for seed yield. Pollinators can pose a problem when weather prohibits them from visiting flowers and the resulting lack of pollination results in yield reduction. There are also years when feral bee populations are not as abundant resulting in lower yields, unless managed bees are used. The cost of bees for pollination obviously results in higher production costs and can be significant due to problems beekeepers are experiencing such as colony collapse disorder. Self pollination might prove to be detrimental due to inbreeding depression, but the trait still warrants investigation.

### 3.4. Other traits

There are many other traits that could be beneficial to lesquerella production. Herbicide tolerance could open more options for additional herbicides to be used. Some preliminary work to screen plants for Dicamba resistance has begun (Foster, unpublished data) which the discovery of plant germplasm with herbicide resistance could have large effects on weed management strategies and reduce production costs.

Other areas of research may include development of varieties with higher amounts of co-products such as seed coat gums, glucosinolates in the seed meal, or seed oil estolides. These markets will need to be more established to know the direction necessary for breeding. Salinity and heat stress are also important areas for breeding of new crops in the southwestern U.S., especially as climate change becomes more of a reality.

## 4. Tools for crop improvement

It is important to have the capabilities to monitor the increase and decline in lesquerella flowering so decisions can be made for irrigation management, desiccant applications, and harvesting. This is also helpful for plant breeding to distinguish and quantify genotypes flowering earlier since counting flowers on indeterminate plants is extremely time consuming. Lesquerella canopies are known for their vibrant yellow flowers that are quite distinct from green vegetative plant parts and soil background. Two remote sensing approaches for monitoring the flowering status of lesquerella canopies were examined; one based on spectral processing of canopy reflectance data from a hand-held spectroradiometer and the other based on image processing of digital photos from a standard digital camera (Thorp et al., in press; Thorp and Dierig, in press).

A model developed by Thorp et al. (in press) from canopy reflectance data and measured flower counts was able to estimate flower counts. A moderate trend existed in the data, but the method may not be suitable if very precise flower count estimates are needed. Regression coefficients highlighted strong contrasts at 483, 565, 721, and 817 nm, which correspond to light in the blue, far green, red edge and near-infrared portions of the spectrum, respectively. A time series plot demonstrated the ability of the model to track the progression of flowering throughout the growing season and identify peak flowering in mid-April in Arizona.

A linear regression model developed with the percent flower cover data from digital images and measured flower counts was also able to estimate flower counts (Thorp and Dierig, in press). A key feature of the image processing approach included an image transform to the hue, saturation, and intensity color space for delineation of yellow lesquerella flower pixels. Time series plots also demonstrated the ability of the digital image-based model to track flowering throughout the growing season. In comparing the two methods, flower cover data from digital photos was generally better at estimating flower counts than the canopy spectral reflectance data. Digital imaging offers an inexpensive and quite

practical means for remote monitoring of flowering patterns in lesquerella canopies.

## 5. Weed management

Slow growth during establishment and the small stature of lesquerella results in significant open space (i.e., greater than 50% bare soil surface) at 2 months after fall plantings providing ample opportunity for weed establishment, growth, and competition. Because lesquerella is broadcast planted, it is not practical to cultivate for weed management. This increases the importance of finding herbicides that lesquerella can tolerate without severe injury and developing chemical weed management strategies. Roseberg (1993, 1996) reported that lesquerella had tolerance to the pre-emergence herbicides trifluralin (Trade name: Treflan), benefin (Balan), (ethalfluralin (Sonalan)), pendimethalin (Prowl) (all members of the dinitroaniline class of herbicides) and pronamide (Kerb) but did not report seedling density or harvest data. Preemergence herbicides can be applied prior to planting and mechanically incorporated (PPI) or they can be applied after planting and incorporated into the soil with irrigation or rainfall (PRE).

The timing of preemergence herbicide incorporation with water relative to germination is critical in order to avoid inhibiting lesquerella seedling emergence. Several preemergence herbicides screened in Arizona severely inhibited lesquerella emergence when they were applied just after planting and were incorporated with the same irrigation that germinated the lesquerella seed. A notable exception to this general trend was bensulide (4.5 and 6.7 kg/ha) applied 2 DAP (McCloskey and Dierig, unpublished data). Bensulide is sold under the trade name of Prefar and is registered for use in the Brassica (cole) leafy vegetable group so it was not unexpected that lesquerella would exhibit tolerance to this herbicide. Other general trends noted when screening preemergence herbicides were that there was much less effect on lesquerella seedling density when preemergence herbicides were applied after two irrigation at 21 DAP and the postemergence graminicides, fluzifop-*p*-butyl (Fusilade), sethoxydim (Poast) and clethodim (Select) had no effect on lesquerella seedling density or yield (McCloskey, unpublished data).

Several strategies for developing lesquerella weed management programs are suggested by Roseberg (1993, 1996) and McCloskey and Dierig (2009). Bensulide is an obvious choice for PPI and PRE applications with the first irrigation but other options that warrant further investigation are reduced rate PPI applications of benefin (Balan) or pendimethalin (Prowl) (and possibly other dinitroaniline herbicides) at rates between 0.56 and 0.84 kg/ha. These reduced rates would still provide suppression of grasses and some small seeded broadleaves particularly if used in conjunction with bensulide (Prefar). After three or four establishment irrigations, pendimethalin alone or tank mixed in combination with flumioxazin (Chateau) would provide long-term control of most grass and broadleaf weeds. Early in the crop cycle, grass specific herbicides such as fluzifop-*p*-butyl (Fusilade), clethodim (Select) or sethoxydim (Poast) could be used to control grass weeds that were already emerged at the time of PRE herbicide applications or that escaped control.

The most challenging weed management issue in lesquerella is postemergence broadleaf weed control. Clopyralid (Stinger) causes little lesquerella injury while oxyfluorfen and 2,4-DB cause slight injury and reduced seedling densities but do not affect yield (McCloskey and Dierig, 2009; Roseberg, 1996). Dicamba (Clarity) and flumioxazin (Chateau) with a nonionic surfactant can cause moderate injury and potentially reduce yield when applied post-emergence to lesquerella (McCloskey and Dierig, 2009; Roseberg, 1996) but may still be useful in protecting lesquerella from weed

competition. Other herbicides that caused severe injury to lesquerella plants included prometryn and a tank mixture of diuron and linuron (McCloskey and Dierig, 2009). Additional research is needed to evaluate postemergence herbicides for use in lesquerella.

## 6. Utilization

*L. fendleri* is rich in lesquerolic acid, a hydroxy fatty acid homologue of ricinoleic acid found in castor oil. *L. fendleri* contains approximately 60% hydroxy fatty acids compared to castor which has nearly 90% ricinoleic acid. The high linolenic acid (C18:3) content (12%) of lesquerella oil imparts reduced oxidative stability when compared to many other vegetable oils but antioxidant packages can be added to bring the oil performance to an acceptable level.

A number of lesquerella derivatives and products have been synthesized utilizing the hydroxy moiety of the oil with a particular focus on the development of estolides. Lawate (1995) synthesized triglyceride estolides from lesquerella oil by capping the hydroxy moiety with heptanoic, isostearic, adipic and fumaric acids. These fully capped triglyceride estolides were then evaluated for their thickening properties in a high oleic (C18:1) vegetable oil. The fumaric acid capped estolides had the largest impact on viscosity where a 5% mixture of estolides in the high oleic vegetable oil increased the solution viscosity by 23%. mono and fully capped triglyceride estolides were synthesized from lesquerella oil by condensation with a range of (C2–C18) fatty acid capping groups and their physical properties (Isbell and Cermak, 2002; Isbell et al., 2006). Lesquerella oil has a pour point of  $-21^{\circ}\text{C}$  with a  $40^{\circ}\text{C}$  viscosity of 127.7 cSt. The high viscosity of the oil precludes the pure oil from a number of lubricant applications. The pour points of the estolides were significantly reduced in comparison to the parent oil where a C6:0 fully capped estolide had a pour point of  $-36^{\circ}\text{C}$  and a viscosity of 87.9 cSt. These materials may find suitable applications as gear lubricants or as thickening agents in other base oils.

Further improvements in the physical properties of the oil can be made by synthesizing estolides (Cermak et al., 2006) directly from lesquerella fatty acids. Oleic and 2-ethylhexanoic acid capping groups gave excellent pour points of  $-48^{\circ}\text{C}$  and  $-54^{\circ}\text{C}$ , respectively as their 2-ethylhexyl esters. The viscosities of these estolides are sufficiently low enough to meet many lubricating applications that the triglyceride estolides and the parent oil could not meet. In addition, the simplest oil derivative, methyl ester, also has unique lubricating properties. Methyl lesquerolate has better performance in providing lubricity to low sulfur diesel fuel blends than the corresponding castor and soybean methyl ester derivatives (Moser et al., 2008; Goodrum and Geller, 2005). Methyl lesquerolate at 0.2 wt% in ultra low sulfur diesel provided sufficient lubricity to pass the high frequency reciprocating rig (HFRR) test ISO limit of 0.45 mm wear scar where both soybean and castor methyl esters failed. Castor methyl ester blends passed at 0.5 wt% and soybean required 3% for the blend to meet the ISO specification.

## 7. Challenges with commercialization

Lesquerella has evolved from a wild plant to a crop suitable to grow in a commercial setting. It faces the same issues that many new crops have or will face when going to the next level of commercialization, which is how to scale-up production from small number of acres to thousands of acres. An important challenge is finding suitable herbicide options and obtaining registrations. There are still a number of agronomic questions to be answered. Little is known about potential pests or diseases that may affect production on a large scale. Soil types and irrigation management

will need further research. The utility of the meal and gum also need to be better defined.

## 8. Conclusions

Lesquerella offers a unique oil product along with several coproducts that makes commercialization attractive. Plant breeding is needed to continue to improve oil content and quality to make lesquerella more competitive with castor. Agronomics will play an important role in improving seed yield and water use efficiency while reducing production costs. New tools offered by remote sensing will help plant breeders and growers assess crop development. Defining herbicides and obtaining registrations for use in lesquerella appears to be the biggest obstacle for commercialization of this crop. The hydroxy oil would reduce dependence on imported castor oil and provide a new crop for diversification of U.S. agriculture. The cost of crop production is competitive but there are still risks associated that a grower alone would not want to bear. We need to be able to quantify the risks of development needed and decide what is sustainable. Scale-up in production is the critical issue for commercialization of lesquerella.

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