

## Lesquerella seed and oil yield response to split-applied N fertilizer



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### ABSTRACT

Agronomic management information is critical for successful commercial production of new crops such as lesquerella [*Physaria fendleri* (A. Gray) O'Kane & Al-Shehbaz]. Response of lesquerella to six nitrogen (N) fertilizer rates under well-watered and water-stressed treatments were studied in irrigated desert conditions in the southwestern United States to establish guidelines for lesquerella N management. Seed and oil yield, seed and straw N concentrations, N harvest index, and total N uptake were recorded at harvest and destructive biomass samples were also collected during growing season for plant N status. Although soil NO<sub>3</sub>-N was low due to a cover crop to remove excessive N in the soil, pre-plant N fertilizer did not affect lesquerella growth at the early growth stage. For the treatments with high N fertilizer rates (224 and 336 kg ha<sup>-1</sup>), lesquerella plant N concentrations were relatively stable before the first bloom, decreased drastically until mid-bloom (one month after the first bloom), and then continued to decrease at a lower rate until harvest. As N fertilizer rates increased, seed and straw N concentration increased linearly under both well-watered and water-stressed conditions. Lesquerella seed and oil yield had a quadratic relationship with N fertilizer rate. Because seed oil concentration decreased as N fertilizer increased, N fertilizer rate for the optimum oil yield was lower than the N fertilizer rate for the optimum seed yield. According to seed and oil yield as well as plant N status under different N fertilizer rates, we provided a guideline for N fertilizer management for lesquerella.

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### 1. Introduction

Lesquerella, a member of the mustard family, is native to the southwestern United States and northern Mexico and adapted to the arid environments in the region (Rollins and Shaw, 1973). Lesquerella seeds contain a unique triglyceride seed-oil which is dominant by hydroxy fatty acids (Dierig et al., 2011). The oil can be used for a high performance biodiesel, an additive to diesel fuels to reduce engine damage and wear, or to produce a number of other bioproducts such as plastics, inks, and adhesives (Roetheli et al., 1992; Goodrum and Geller, 2004). Lesquerella oil is similar to castor oil, which is the current source of hydroxy fatty acids but contains ricin (a deadly poison) and other harmful allergens in its seed meal. Lesquerella contains none of these chemicals and can be handled both at the farm level and the oilseed processing level with

standard equipment and technology (Moser et al., 2008; Dierig et al., 2011).

Crop breeding and improved management practices have improved both seed yields and oil content significantly in recent years (Dierig et al., 1998, 2004, 2006). Agronomic studies over the past 20 years have also enabled lesquerella to be profitably grown in producers' fields. The information on planting date, plant population, water use, timing of harvest, and pest management has been studied in Arizona and other locations (Roseberg, 1993, 1996; Coates, 1996; Brahim et al., 1998; Hunsaker et al., 1998; Nelson et al., 1999; Adamsen et al., 2003; Puppala et al., 2005; Adam et al., 2007; Blackmer and Byers, 2009). The information is needed by growers to make decisions on crop adoption and management and by industry to make decisions on seed/oil costs and to understand how oil supplies can be stably available to meet future projected demand.

The effects of N fertilizer on lesquerella seed and oil yield have been studied in Arizona (Nelson et al., 1996, 1999; Adamsen et al., 2003). All three studies showed that lesquerella seed and oil yield

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**Table 1**  
Basin irrigation date and amount for well-watered and water-stressed lesquerella treatments in 2011–2012 and 2012–2013 growing seasons.

Irrigation	2011–2012 growing season			2012–2013 growing season		
	Date	Well-watered (mm)	Water-stressed (mm)	Date	Well-watered (mm)	Water-stressed (mm)
1	November 3, 2011	157	157	November 13, 2012	143	143
2	November 10, 2011	166	166	November 19, 2012	65	65
3	November 18, 2011	85	85	November 28, 2012	56	56
4	December 9, 2011	74	74	January 3, 2013	80	80
5	January 6, 2012	44	44	January 22, 2013	71	71
6	February 2, 2012	48		February 15, 2013	73	
7	February 9, 2012		104	March 12, 2013	170	170
8	February 24, 2012	98		March 26, 2013	90	
9	March 5, 2012	102		April 5, 2013	118	118
10	March 16, 2012	79	79	April 15, 2013	105	
11	March 30, 2012	74		April 26, 2013	123	123
12	April 13, 2012	54	66	May 8, 2013	131	
13	April 23, 2012	92		May 17, 2013	152	152
14	May 4, 2012	101	101			
15	May 15, 2012	114				
Total		1286	876		1376	978

were strongly affected by N fertilizer rates. In these studies, however, either the N fertilizer rates (0, 60, 120 kg ha<sup>-1</sup>) were limited to three (Nelson et al., 1996; Adamsen et al., 2003) or the treatments of different N fertilizer rates were only applied at peak bloom stage (Nelson et al., 1999; Adamsen et al., 2003), making it difficult to use the data to establish N application guidelines for farmers. Due to the complex N dynamics in the soil, information on plant N status and uptake is needed to establish N fertilizer application guidelines for lesquerella.

In the irrigated deserts of southwestern United States, nearly all crop water requirements are provided by irrigation. In addition to the two to three irrigations to establish the crop, lesquerella is irrigated about 10 times during the growing season, typically using flood irrigation. Irrigation enables farmers to split N fertilizer into multiple applications. Furthermore, since lesquerella could be grown on marginal cropland, optimal N fertilizer application rate could be different under well-watered and water-stressed conditions. Therefore, the aims of this study were to investigate the effect of N fertilizer on lesquerella seed and oil yield and plant N status during growing season and to establish N fertilizer application guidelines for farmers under well-watered and water-stressed conditions.

## 2. Materials and methods

### 2.1. Experimental site and design

The study was carried out in 2011–2012 and 2012–2013 winter growing seasons at the University of Arizona's Maricopa Agricultural Center at Maricopa, Arizona (33.067547° N, 111.97146° W). The soil texture at the site was Casa Grande sandy loam and sandy clay loam (fine-loamy, mixed, superactive, hyperthermic

Typic Natrargid). Average air temperature was 16.9 and 16.0 °C for the first and second growing seasons, respectively. Precipitation amounted to 41 mm and 80 mm in the 2011–2012 and 2012–2013 growing seasons, respectively.

Two irrigation treatments were established in this study: well-watered and water-stressed. Both treatments were irrigated from November to January with 526 mm and 415 mm of water for crop establishment and early growth in the 2011–2012 and 2012–2013 growing seasons, respectively (Table 1). From February 2 to May 17 in 2012, 762 mm and 351 mm of water was applied to well-watered and water-stressed treatment, respectively. In the 2012–2013 growing season, 962 mm and 563 mm of water was applied to well-watered and water-stressed treatment, respectively, from February 15 to May 17. Individual irrigations varied in amount from 44 to 170 mm. The well-watered and water-stressed treatment was irrigated based on periodic soil moisture measurements, previously reported lesquerella water requirements (Hunsaker et al., 1998), and the AZSched irrigation scheduling software (Martin, 2007). Irrigation treatments started after lesquerella establishment (Table 1). The irrigation treatments were not replicated, due to field size requirements for flood irrigation.

Within each irrigation treatment, a randomized complete block design with four replications and a plot size of 7 m wide and 13 m long was used to study the effects of six N fertilizer levels (0, 56, 112, 168, 224, and 336 kg ha<sup>-1</sup>) on lesquerella growth and yield. Nitrogen fertilizer was split-applied at pre-plant, 6–10 leaf stage, first bloom, mid-bloom (one month after first bloom), and peak bloom (two months after first bloom) in both growing seasons (Table 2). Nitrogen fertilizer in the form of urea with 46% of N was manually applied to each plot at different growth stages. The fertilizer was incorporated into the soil with flood irrigation immediately after application.

**Table 2**  
N fertilizer rates at different growth stages in the 2011–2012 and 2012–2013 growing seasons.

Growth stage	2011–2012	2012–2013	N fertilizer rate (kg ha <sup>-1</sup> )					
			N1	N2	N3	N4	N5	N6
Pre-planting	November 3, 2011	November 13, 2012	0	11.2	22.4	33.6	44.8	67.2
6–10 leaf	February 2–9, 2012 <sup>a</sup>	January 18, 2013	0	11.2	22.4	33.6	44.8	67.2
First bloom	March 16, 2012	March 8, 2013	0	11.2	22.4	33.6	44.8	67.2
Mid-bloom	April 13, 2012	April 5, 2013	0	11.2	22.4	33.6	44.8	67.2
Peak bloom	May 4, 2012	May 3, 2013	0	11.2	22.4	33.6	44.8	67.2
Total N (kg ha <sup>-1</sup> )	–	–	0	56	112	168	224	336

<sup>a</sup> N fertilizer was applied to the well-watered treatment on February 2 and to water-stressed treatment on February 9 due to different irrigation schedule.

## 2.2. Field management

Sudangrass [*Sorghum bicolor* (L.) Moench var. sudanense] was grown as a cover crop in the summer before lesquerella planting to remove excess N and reduce soil fertility variations in the soil. As a result, pre-plant soil samples showed that there was less than  $3 \text{ mg kg}^{-1}$   $\text{NO}_3\text{-N}$  available in the top 90 cm of the soil profile. The final cutting of sudangrass occurred in early September of each growing season, and fields were plowed, disked twice, laser-leveled, and pressed with a cultipacker to form a firm soil surface for lesquerella planting. Lesquerella cultivar 'Gail' was broadcast into dry soil on flat seed beds at a rate of  $12 \text{ kg ha}^{-1}$  on November 3 in 2011 and November 13 in 2012. The field was pressed again with a cultipacker to ensure good seed and soil contact. To ensure N fertilizer was the only limiting factor in the study,  $56 \text{ kg ha}^{-1}$  of phosphate in the form of triple super phosphate (45%  $\text{P}_2\text{O}_5$ ) was applied before diskings. Other nutrients were sufficient according to pre-plant soil analysis.

Bensulide (Prefar 4E, Gowan Company, Yuma, AZ, USA) was applied at  $6.7 \text{ kg a.i. ha}^{-1}$  at planting in the 2011–2012 growing season for weed control. In the 2012–2013 growing season, both Bensulide and Pendimethalin (Prowl 3.3 EC, BASF Corporation, Research Triangle Park, NC, USA) were applied at 6.7 and  $0.56 \text{ kg a.i. ha}^{-1}$ , respectively, at planting for weed control at early growing season. Clopyralid (Stinger, Dow Agrosciences, Indianapolis, IN, USA) was applied at the rate of  $0.32 \text{ kg a.i. ha}^{-1}$  on January 18, 2012 and March 20, 2013 for post-emergence weed control. One hand weeding was conducted on March 25, 2012 in the 2011–2012 growing season. No insecticide or fungicide was needed in both growing seasons.

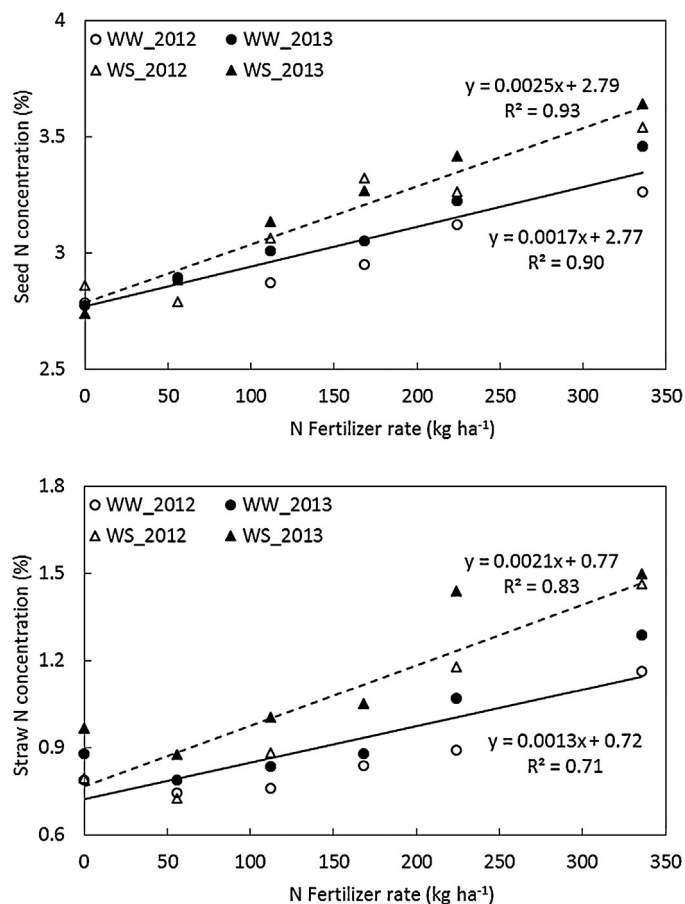
## 2.3. Data collection

Plant biomass samples were cut at ground level from  $900 \text{ cm}^2$  areas in each plot on January 19, March 1, March 15, May 1, May 18, June 20 in 2012 and January 17, February 12, March 7, March 25, April 4, April 18, May 2, May 22, June 11 in 2013. All samples were oven-dried at  $65^\circ\text{C}$  with ventilation and weighed. Plant samples from individual plots were then ground to 0.5 mm and analyzed for total N concentration on a Carlo Erba elemental analyzer (model NA1500 N/C, Carlo Erba Instruments, Milan, Italy).

One week before harvest, paraquat (Gramoxone SL, Syngenta Crop Protection, Greensboro, NC, USA) was sprayed to lesquerella at a rate of  $0.84 \text{ kg ha}^{-1}$  to desiccate the crop. An area of 1.8 m wide and 10 m long in each plot were harvested with a small-plot combine harvester on June 20, 2012 and June 11, 2013. Three  $1 \text{ m} \times 0.5 \text{ m}$  areas from each plot were also hand-harvested. These hand-harvested samples were brought back to laboratory to separate seed from other plant structures. All samples were oven-dried for total N analysis as above. N harvest index was calculated as seed N uptake divided by total N uptake. Oil yield was obtained by multiplying seed yield and seed–oil concentration for each plot.

## 2.4. Data analysis

Linear functions were fitted to lesquerella seed and straw N concentration and total N uptake under well-watered condition as well as seed–oil concentration as dependent variables and N fertilizer rates as independent variable. Power functions were fitted to total N uptake under water-stressed condition and N fertilizer rates. Quadratic functions were fitted to the relationship of lesquerella seed yield and oil yield with N fertilizer rates. The N fertilizer rates with the maximum seed and oil yield were determined by making the first derivatives of the quadratic functions equal to zero. Analyses were performed in SAS 9.3 (SAS Institute, Cary NC).



**Fig. 1.** Lesquerella seed and straw N concentration affected by N fertilizer rate under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in two growing seasons.

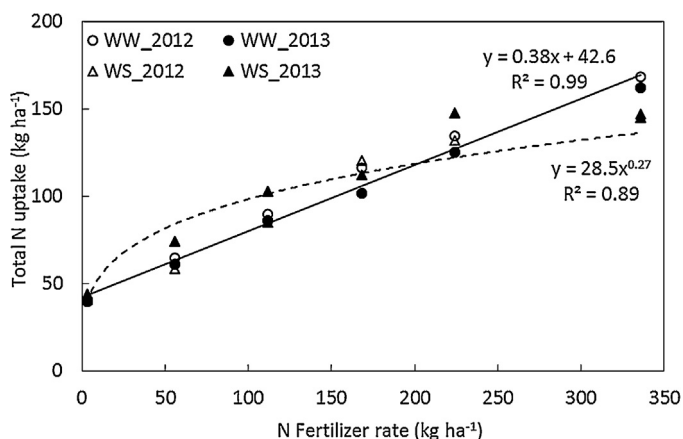
## 3. Results

### 3.1. Seed and straw N concentration and total N uptake affected by N fertilizer rate

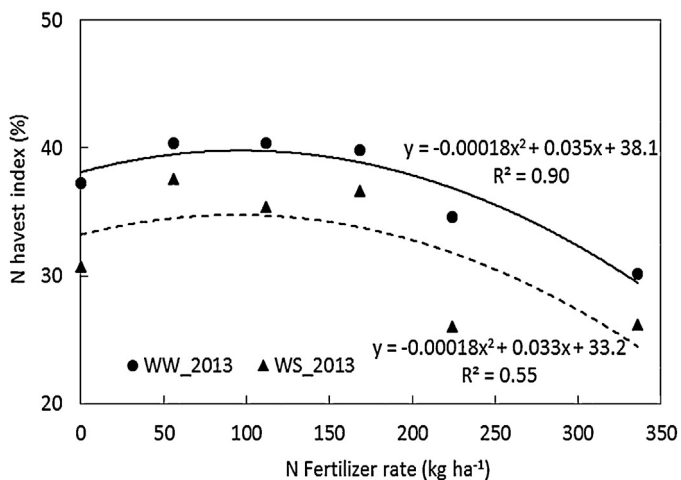
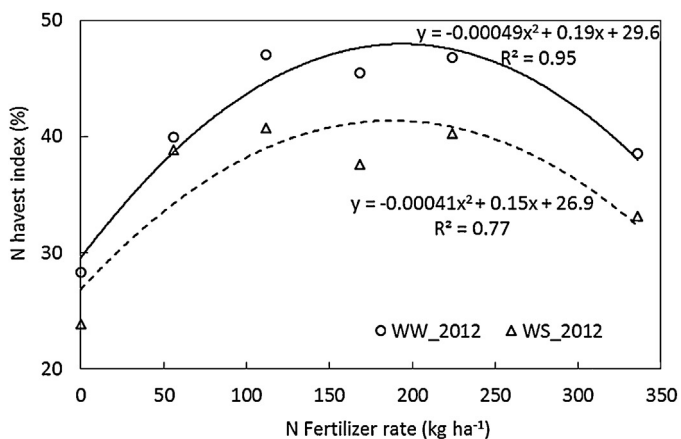
There were no significant interactions between year and seed N concentration, straw N concentration, or total N uptake. Therefore, the data from both years were combined for analysis. Seed and straw N concentration increased linearly with N fertilizer rates under both well-watered and water-stressed conditions (Fig. 1). Water stress increased seed and straw N concentrations and the rate of increase was higher with higher N fertilizer rates. The seed N concentration increased 0.17% and 0.25% for every  $100 \text{ kg ha}^{-1}$  N fertilizer increased under well-watered and water-stressed conditions, respectively. The straw N concentration ranged from 0.75 to 1.5% in all treatments and increased by 0.13 and 0.21% for every  $100 \text{ kg ha}^{-1}$  N fertilizer increased under well-watered and water-stressed conditions, respectively.

In the well-watered treatment, the total N uptake at harvest increased linearly as N fertilizer rate increased (Fig. 2). For every  $\text{kg ha}^{-1}$  of N fertilizer applied, the total N uptake increased  $0.38 \text{ kg ha}^{-1}$ . In the water-stressed treatment, the increase of total N uptake showed a power function relationship with N fertilizer rate, indicating that total N uptake plateaued at higher N fertilizer rate under water-stressed conditions.

The data on N harvest index from two growing seasons were separated for analysis due to significant year by treatment interactions. Lesquerella N harvest index ranged from 26 to 47% in all treatments (Fig. 3). The relationship of N harvest index and N

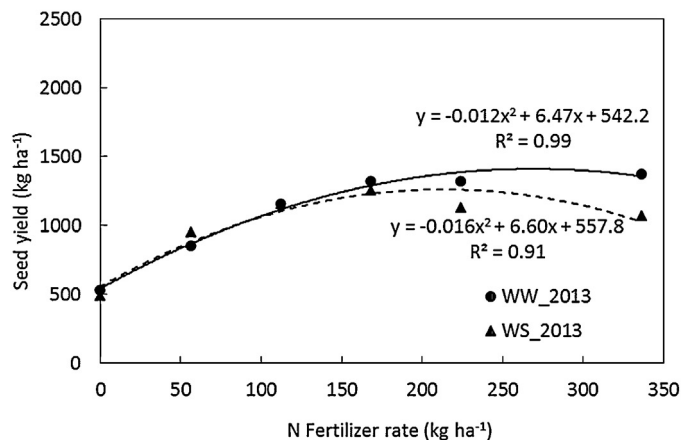
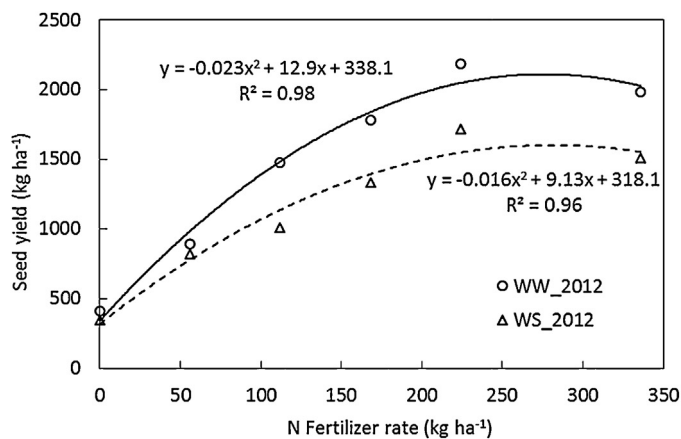


**Fig. 2.** Lesquerella total N uptake affected by N fertilizer rate under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in two growing seasons.



**Fig. 3.** Lesquerella N harvest index affected by N fertilizer rate under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in the 2011–2012 (top) and 2012–2013 (bottom) growing seasons.

fertilizer rate was quadratic under both well-watered and water-stressed conditions. The highest N harvest indices were obtained at 194 and 188 kg ha<sup>-1</sup> under well-watered and water-stressed conditions, respectively, in the 2011–2012 growing season. In the 2012–2013 growing season, the highest N harvest indices were obtained at 97 and 94 kg ha<sup>-1</sup> for well-watered and water-stressed treatments. The lower N fertilizer rate to obtain the highest N

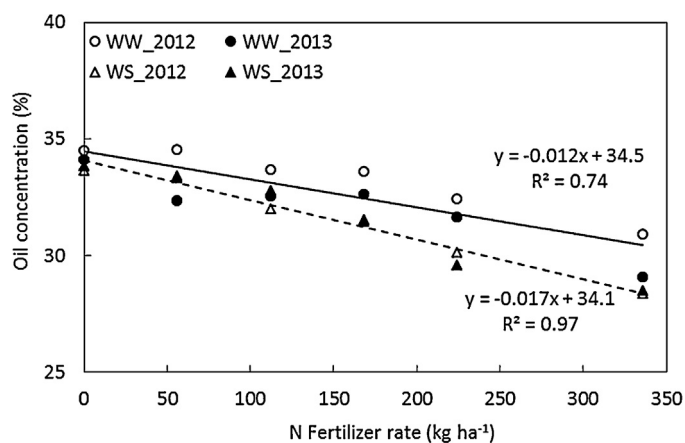


**Fig. 4.** Lesquerella seed yield affected by N fertilizer rate under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in the 2011–2012 (top) and 2012–2013 (bottom) growing seasons.

harvest indices in the 2012–2013 growing season were possibly due to lower plant population caused by Prowl herbicide injury (2.5 m plant ha<sup>-1</sup>) compared to the 2011–2012 growing season (9.3 million plant ha<sup>-1</sup>).

### 3.2. Lesquerella seed yield affected by N fertilizer rate

Due to significant differences in lesquerella yield among the two growing seasons, the data from two years were presented separately. Lesquerella yield was higher in the 2011–2012 growing season (Fig. 4). This was probably due to herbicide injury that slowed plant growth in the first month of 2012–2013 growing season. The higher plant population in the 2011–2012 growing season might contribute to the differences, although a previous study showed that lesquerella yield was not different from 0.75 to 1.5 million plant ha<sup>-1</sup> plant population (Brahim et al., 1998). Lesquerella yield had a quadratic relationship with N fertilizer rate. Seed yield increased with N fertilizer and decreased when N fertilizer rate exceeded a threshold. The maximum seed yields were obtained with 276 and 282 kg ha<sup>-1</sup> N fertilizer rates under well-watered and water-stressed conditions, respectively, in the 2011–2012 growing season. In the 2012–2013 growing season, the maximum seed yields were reached at 269 and 213 kg ha<sup>-1</sup> N fertilizer rates under well-watered and water-stressed conditions, indicating lower N fertilizer rates should be applied under water-stressed conditions.



**Fig. 5.** Lesquerella seed-oil concentration affected by N fertilizer rate well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in two growing seasons.

### 3.3. Seed-oil concentration and oil yield affected by N fertilizer rate

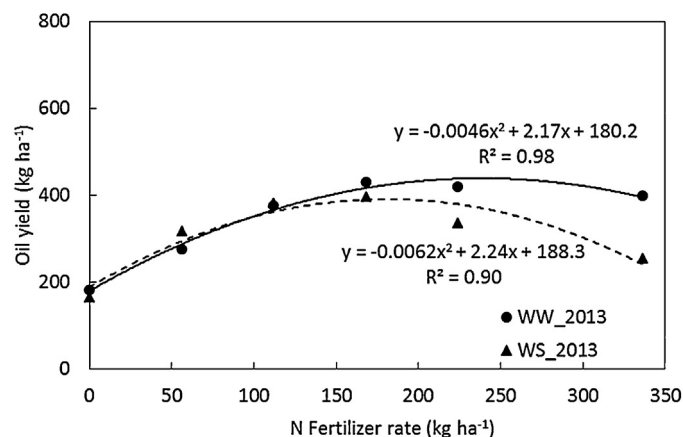
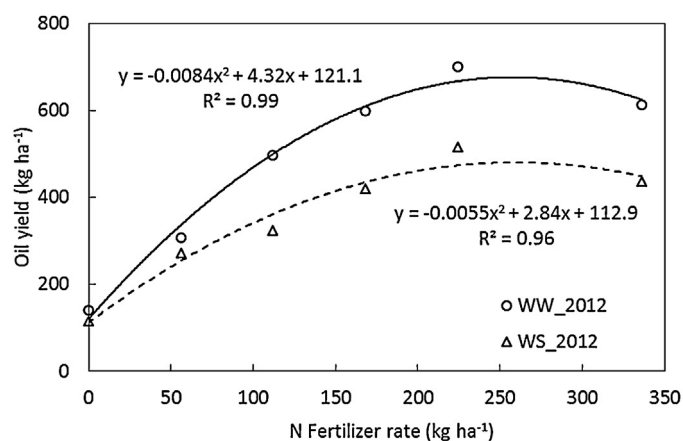
Lesquerella seed-oil concentration decreased with increased N fertilizer rate (Fig. 5). The oil concentration ranged from 34.6% to 28.5% with different N fertilizer rates. For every 100 kg ha<sup>-1</sup> increase in N fertilizer, seed-oil was reduced by 1.2% in the well-watered treatment and 1.7% in the water-stressed treatment.

Similar to seed yield, lesquerella oil yield had a quadratic relationship with N fertilizer rate (Fig. 6). However, the N fertilizer rate to obtain maximum oil yield were lower compared to the rate for maximum seed yield. The maximum oil yields were obtained at 257 and 258 kg ha<sup>-1</sup> N fertilizer rate in the well-watered and water-stressed treatments, respectively, in the 2011–2012 growing season. In the 2012–2013 growing season, the N fertilizer rates to obtain maximum oil yields were 236 and 181 kg ha<sup>-1</sup> for well-watered and water-stressed treatments, respectively. This is due to the fact that the N fertilizer rate to reach the highest oil yield was affected by both seed yield and seed-oil concentration.

## 4. Plant N status over growing season

Pre-plant N fertilizer application did not affect lesquerella biomass (data not shown) and plant N concentrations at 77 days after planting in the 2011–2012 growing season and 65 days after planting in the 2012–2013 growing season (Fig. 7). For the treatments with high N fertilizer rates (224 and 336 kg ha<sup>-1</sup>), lesquerella plant N concentrations were relatively stable before the first bloom, and then decreased drastically in one month after the first bloom. After that, plant N concentrations continue to decrease at a lower rate until crop harvest. For lower N fertilizer rates, plant N concentrations decreased at earlier stages and faster.

From plant N status measurements and crop yield data, we provided N fertilizer recommendation guidelines based on plant N concentrations for lesquerella N management (Fig. 8). Pre-plant N application is not recommended due to the fact that lesquerella grows very slowly in the first two months. Multiple irrigations are also needed to establish the crop and could leach N fertilizer applied at pre-plant. The first post-plant N application is recommended at 6–10 leaf stages if plant N concentration is below 3%. During growing season, plant N concentrations should be kept in the optimum range. Plant N concentrations are recommended to be kept from 1.5 to 1.8% at peak bloom stage (about two months after the first bloom), at which the last split N application is recommended to be applied. While crop yield could be affected when plant N concentrations fall below the optimum range, applying excessive N fertilizer



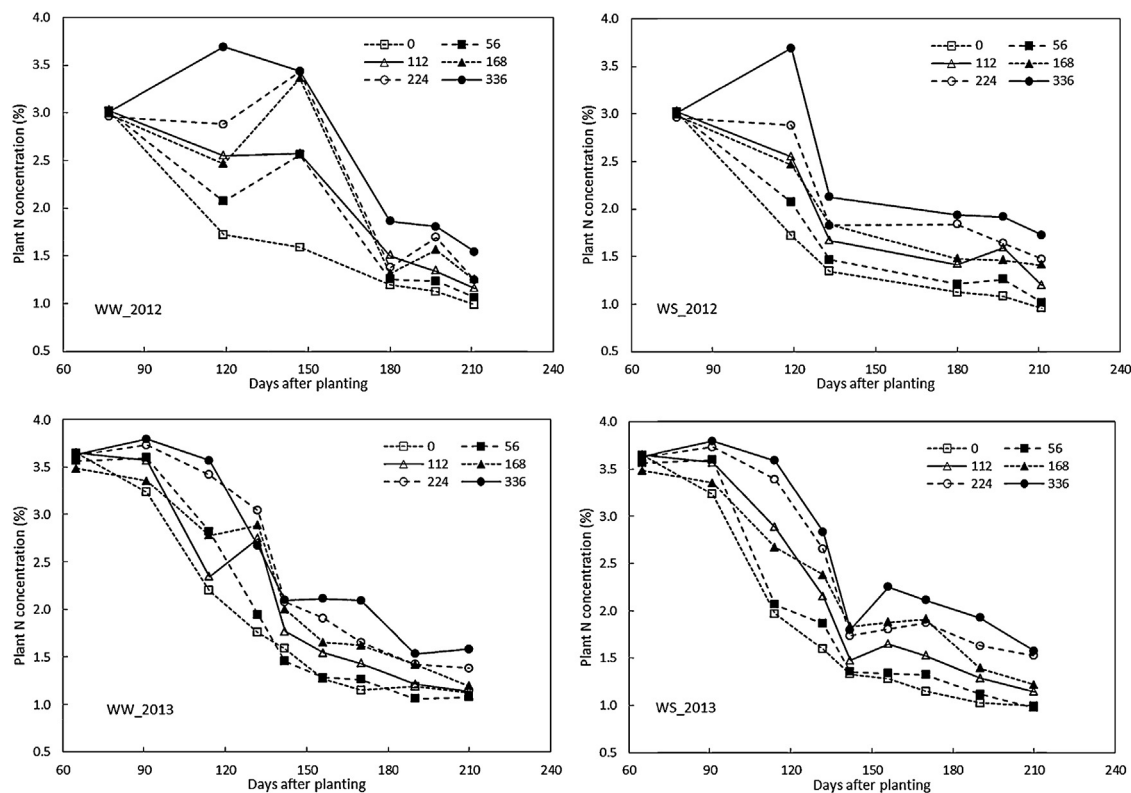
**Fig. 6.** Lesquerella oil yield affected by N fertilizer rate under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in the 2011–2012 (top) and 2012–2013 (bottom) growing seasons.

could also reduce lesquerella seed and oil yield and reduce growers' profitability.

## 5. Discussion

Consistent with previous reports, our study showed that lesquerella seed yield was positively affected by N fertilizer rate and that oil concentration decreased as N fertilizer rate increased (Nelson et al., 1996, 1999). As a result, the N fertilizer rates for the highest oil yield were lower than the rates for the highest seed yield. The N fertilizer rates for the highest oil yields ranged from 257 to 258 kg ha<sup>-1</sup> in the 2011–2012 growing season and 181–236 kg ha<sup>-1</sup> in the 2012–2013 growing season, which were 91–93% and 85–88% of the N fertilizer rates for the highest seed yields. In the production field, it is important for farmers that produce the seed and the industry that purchases the seed to understand the N rates for highest seed yield and oil yield are different. It is also necessary to point out that a sudangrass cover crop was planted before our experiments to remove excessive N in the soil profile. N fertilizer application rates could be reduced if more N is available in the soil.

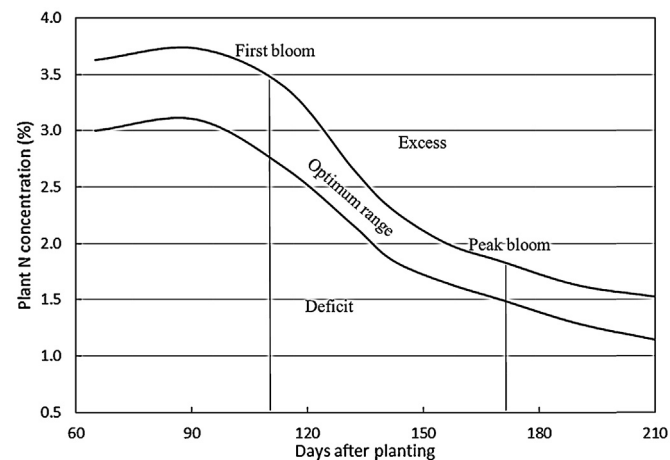
Rainfall in the deserts of the southwestern United States is very low during the lesquerella growing season. The crop is almost totally dependent on irrigation, which provides the possibility for multiple N fertilizer applications with irrigation water. In the second season of this study, 70 mm of rain fell in January, which resulted in the small yield difference between water stressed and well-watered plots. Split N applications in lesquerella have been shown to increase crop yield significantly (Nelson et al., 1999). Split



**Fig. 7.** Plant N concentrations affected by N fertilizer rate during growing season under well-watered (WW, solid line) and water-stressed (WS, dotted line) conditions in two growing seasons.

N applications probably also reduced N loss due to leaching and denitrification and increased N use efficiency.

At harvest, lesquerella straw N concentration ranged from 0.75 to 1.5%, which is significantly higher than other cultivated species in the same family, such as *Brassica napus* (Chamorro et al., 2002; Kessel et al., 2012). Low N harvest index was caused by the high straw N concentration and low harvest index of 16.5% in lesquerella compared to that of 0.40 in *B. napus* (Kessel et al., 2012; Dierig et al., 2013). Breeding new cultivars with improved harvest index and increased N remobilization from straw to seed could improve lesquerella yield and N use efficiency.



**Fig. 8.** Lesquerella N management guidelines based on plant N status. The first N fertilizer should be applied at 6–10 leaf stage (about 75 days after planting) to keep plant N concentration above 3%. Plant total N concentration is recommended to be kept in the optimum range during the growing season.

Although similar trends in lesquerella yield to N fertilizer rate were observed in two years, crop yield was significantly lower in the second growing season. This was probably due to the lower plant population in the second growing season. However, the plant population in this study was significantly higher than the optimum 0.75–1.5 million plant  $\text{ha}^{-1}$  plant populations in previous studies (Brahim et al., 1998). Crop yield for long-cultivated field crops such as durum wheat (*Triticum durum* Desf.) and cotton (*Gossypium hirsutum* L.) can be stable with proper crop management in the irrigated desert due to less variation in climates (Silvertooth et al., 2001; Ottman, 2011). However, yields for new crops can vary significantly, making viable commercial production difficult (Cline, 2012). Improvement in management practices and crop breeding could reduce yield variations and increase success rate for commercial production.

## 6. Conclusions

Both lesquerella seed and oil yield were positively affected by N fertilizer. High straw N concentration and low harvest index resulted in low N harvest index in lesquerella. The maximum oil yields were obtained at 257 and 258  $\text{kg ha}^{-1}$  N fertilizer rate in the well-watered and water-stressed treatments, respectively, in the 2011–2012 growing season. In the 2012–2013 growing season, the N fertilizer rates to obtain maximum oil yields were 236 and 181  $\text{kg ha}^{-1}$  for well-watered and water-stressed treatments, respectively. The N fertilizer rate were 91–93% and 85–88% of the N fertilizer rates for the highest seed yields in the 2011–2012 and 2012–2013 growing season, respectively, due to the fact seed–oil concentration was negatively related to N fertilizer rate. In the production field, it is important for farmers that produce the seed and the industry that purchases the seed to understand the N rates for highest seed yield and oil yield are different. Pre-plant N fertilizer

application did not affect lesquerella biomass and plant N concentrations in the first two months. For the treatments with high N fertilizer rates, lesquerella plant N concentrations were relatively stable before the first bloom, decreased drastically in one month after the first bloom, and continue to decline until harvest. This study agrees with previous research that yield stability over different growing seasons is one of the major limiting factors for new crops such as lesquerella and more agronomic research and crop breeding work is needed for successful commercial production.

## Acknowledgements

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