Five-year field trial of the biomass productivity and water input response of cactus pear (Opuntia spp.) as a bioenergy feedstock for arid lands

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Abstract
Climate-resilient and highly water-use efficient crops are needed to meet the future food, feed, fiber, and fuel demands of a growing human population. Cactus pear (Opuntia spp.) are highly productive yet have crop water demands that are approximately 20% that of traditional crops due to their ability to perform crassulacean acid metabolism (CAM). In the first long-term field trial of Opuntia for bioenergy in the United States, the aboveground biomass (cladode and fruit) productivity and response to different water inputs of O. cochenillifera, O. ficus-indica, and O. streptacantha were evaluated at a planting density of 1418 plants ha−1 under three irrigation levels over a 5-year period to investigate their response to different water inputs. Mean cladode fresh weight, cladode dry weight, cladode count, fruit fresh weight, and fruit dry weight increased linearly by 1.26-, 1.88-, 1.53-, 1.89-, and 2.13-fold, respectively, with increasing irrigation from 300 to 716 mm year−1. Significant differences in irrigation response appeared in years 4 and 5 as the cactus trees grew larger. However, no significant differences were observed among the three Opuntia spp. for all parameters measured. Biomass productivity increased over 5 years by 140.8- and 132.5-fold for cladode fresh and dry weight, respectively. Mean annual total aboveground biomass at 300, 407, and 716 mm year−1 water input resulted in 8.25, 11.16, and 15.52 Mg dry mass ha−1 year−1, respectively, at the sparse planting density used here to separate irrigation blocks, which could have been easily quadrupled. O. ficus-indica performed better than O. cochenillifera and O. streptacantha in terms of cladode biomass and fruit count and quality. Biomass productivity was consistent with previous studies for Opuntia spp. demonstrating that cactus pear displays very high biomass and food/forage potential using substantially lower rates of supplemental irrigation than conventional crops grown in semi-arid and arid regions.

Keywords
biofuel feedstock, biomass productivity, cactus pear, crassulacean acid metabolism (CAM), marginal lands, Opuntia spp., semi-arid and arid
1 | INTRODUCTION

Modeling of global warming trends due to greenhouse gas (GHG) emissions predicts more intense and longer lasting drought events over 30% of the global land mass and a five-fold increase in water demand deficits during the 21st century (Naumann et al., 2018). Modeling also indicates that increased surface soil drying trends and rising vapor pressure deficits will lead to reduced terrestrial net primary productivity and carbon sequestration (Grossiord et al., 2020), altered geographic redistributions of plants (Crous, 2019), and an expansion of drylands (Dai et al., 2018; Huang et al., 2015; Koutoulis, 2019; Wei et al., 2019; Xu et al., 2019). Anthropogenic climate change will also lead to increased groundwater depletion (Cuthbert et al., 2019) and reduced seasonal water storage (Bormann et al., 2018), curtailing availability for agricultural use, which accounts for 70–80% of global water demands (FAO, 2017; Velasco-Muńoz et al., 2018). Traditional bioenergy crops used for the production of bioethanol, such as maize and sugar cane, have large water demands (Gerbens-Leenes et al., 2012; Gerbens-Leenes et al., 2009). Therefore, a clear and present need exists to adopt and expand the use of more climate-resilient and water-use efficient (WUE) bioenergy crops (Nabhan et al., 2020).

Highly productive and WUE crassulacean acid metabolism (CAM) crops, such as Agave and Opuntia spp., are potentially useful alternatives for the production of biomass and bioenergy in water-limited, marginal, abandoned, semi-arid, and arid agricultural areas (Borland et al., 2009; Cushman et al., 2015; Davis et al., 2014, 2015, 2019; Nobel, 1991; Yang et al., 2015). CAM species shift CO{2} uptake and fixation to the night to reduce water loss through transpiration, resulting in crop water demands of 16% and 28% compared with C_{3} and C_{4} photosynthesis crops, respectively, while maintaining comparable aboveground biomass (dry matter) productivities (Borland et al., 2009; Nobel, 1991). CAM species can also share a set of other biochemical and morphological co-adaptive traits that attenuate or limit water loss, further improving their ability to thrive under conditions of limited water availability (Niechayev et al., 2019). Opuntia ficus-indica offers greater latitudinal and temperature range adaptability and greater resilience to climate change than Agave spp. (Owen et al., 2016; Owen & Griffiths, 2014). O. ficus-indica is able to withstand a broad range of temperatures from −6°C to 55°C and up to 60°C when heat acclimated (Chetti & Nobel, 1988; Nobel, 1988; Nobel & de la Barrera, 2003; Nobel et al., 1986). O. ficus-indica can maintain growth under dry conditions, yet growth is highly responsive to rewatering following periods of water deficit (Scalisi et al., 2016) due to lateral or “rain” root proliferation in response to rainfall (Dubrovsky et al., 1998; North & Nobel, 1996) and rectifier-like root hydraulic conductivity that limit water loss during soil drying (Cui et al., 1993; Lopez & Nobel, 1991; North & Nobel, 1992, 1997).

Cactus pear possesses great economic value for arid and semi-arid regions of the world (Davis et al., 2019). For millennia, Opuntia spp. have been used as a source of human food, food products, nutraceuticals, cosmetics, and medicines (Bensadón et al., 2010; Brinker, 2009; del Socorro et al., 2017; El-Mostafa et al., 2014; Feugang et al., 2006; Sáenz et al., 1998; Sáenz-Hernández et al., 2002; Shedalkar et al., 2010; Stintzing & Carle, 2005; Zimmerman, 2017). Opuntia spp. have also been used as a source of animal forage and fodder in semi-arid and arid regions of the world (Lima et al., 2016; Mohamed-Yasseen et al., 1996; Nefzaoui & Ben Salem, 2001; Nobel, 1988; Russell & Felker, 1987; Zimmerman, 2017). Given their ability to grow in dry regions, Opuntia spp. are also useful for landscape, soil conservation, erosion control, carbon sequestration, ecosystem services, GHG emissions mitigation, and desert reclamation efforts (Le Houérou, 1996; Nefzaoui et al., 2014; Zimmerman, 2017). Interest in Opuntia spp. for bioenergy production in semi-arid and arid regions around the world has increased in recent years (Cushman et al., 2015; do Nascimento Santos et al., 2016; Krümpel et al., 2020; Sánchez et al., 2012; Zimmerman, 2017).

Global production of Opuntia spp. is estimated to occupy approximately 2.6 M ha with a majority of production occurring in Brazil, Tunisia, Mexico, Morocco, Algeria, Italy, South Africa, Ethiopia, and other countries in North and South America (Kumar et al., 2018). Under rain-fed conditions, average annual biomass productivity of Opuntia spp. ranges from 3 to 15 Mg DM ha\(^{-1}\) year\(^{-1}\) depending upon geographic location and ambient precipitation (do Nascimento Santos et al., 2016; Mason et al., 2015; Sánchez et al., 2012). However, under well-irrigated conditions, average annual productivities can range from 40 to 50 Mg DM ha\(^{-1}\) year\(^{-1}\) (Dubeux et al., 2006; Flores-Hernández et al., 2004; García de Córtazar & Nobel, 1991; Lima et al., 2016; Mason et al., 2015; Nobel, 1991, 1996; Nobel & García de Córtazar, 1991; Nobel et al., 1992; Reis et al., 2016). These biomass productivities of Opuntia spp. are comparable to or exceed those of other bioenergy feedstocks, such as maize, switchgrass, poplar, and sugarcane, but with far lower water inputs (Borland et al., 2009; Cushman et al., 2015; Davis et al., 2011; Somerville et al., 2010). Opuntia spp. are considered a useful second-generation feedstock for both bioethanol and biogas (methane) production due to their high productivity, ease of propagation, suitability for coppicing, and low lignin content (Cushman et al., 2015; do Nascimento Santos et al., 2016; Lueangwattanapong et al., 2020; Mason et al., 2015; Yang et al., 2015; Zimmerman, 2017).

An important consideration for growing Opuntia spp. on abandoned or marginal semi-arid and arid regions of the world is their ability to conserve valuable groundwater
resources. Absolute minimum water inputs are estimated to range from 150 to 200 mm year\(^{-1}\) (Le Houérou, 1996). Optimal ambient precipitation amounts for \textit{O. ficus-indica} production range from 300 to 600 mm year\(^{-1}\) (Kumar et al., 2018; Mason et al., 2015). Many \textit{O. ficus-indica} production areas have Mediterranean climates, where most precipitation occurs in the winter and supplemental irrigation is required during the summer. Irrigation can lead to increased cladode and fruit production (Garcia de Cortázár & Nobel, 1991, 1992) and improved fruit yield (Mulas & D'Hallewin, 1997). Prolonged drought can reduce photosynthetic activity, water content, parenchymal thickness and chlorophyll content (Pimiento-Barrios et al., 2007), and turgor (Goldstein et al., 1991), and accelerate fruit ripening while reducing fruit size (Nerd & Nobel, 2000). However, no long-term studies have reported on how different water inputs impact biomass productivity for multiple \textit{Opuntia} spp. within the United States.

The goal of the current study was to perform the first long-term field trial of \textit{Opuntia} spp. to determine biomass yields using different irrigation inputs under arid conditions in the southwestern USA. The 5-year field planting used a 3 \(	imes\) 3 factorial design with three different \textit{Opuntia} spp. (\textit{O. cochenillifera}, \textit{O. ficus-indica}, and \textit{O. streptacantha}) and three different irrigation treatments in the desert agricultural region within the Moapa Valley of southern Nevada where annual precipitation averages 136 mm\(^{-1}\) year\(^{-1}\). The goal of the study was to determine whether differences in cladode and fruit biomass and WUE occurred in response to different water inputs and if these differences were species-dependent.

The overall results showed that each \textit{Opuntia} spp. was suitable for agricultural production in the southwestern USA with significant differences in the response to different water inputs emerging in years 4 and 5 of the study. After 3 years, annual dry matter biomass production rates at comparable planting densities exceeded those observed for \textit{Agave americana}, a high-yielding commercial CAM crop (Davis et al., 2017; Jones et al., 2020).

\section{MATERIALS AND METHODS}

\subsection{Site description and weather conditions}

A 5-year field experiment was conducted at the University of Nevada Cooperative Extension field plot located at Logandale, NV (36°32'36"N, 114°26'49"W, and altitude of 420 m). The predominant soil at the experimental field site is classified as Overton silty clay (Taxonomic classification: fine, smectitic, calcareous, thermic Aeric Endoaquepts; Survey Staff, 2019). The 5-year field trial weather data average (2015–2019) and 28-year weather data average (1992–2019) were obtained from the Desert Research Institute, Western Regional Climate Center weather station located approximately 1000 m away from the research plots. Average monthly air temperature and accumulated monthly precipitation were collected for the growing seasons of both cladodes and fruits (Figure S1a–c; Tables S1 and S2). Average monthly insolation rates (direct normal irradiance, 1998–2016) were reported for Las Vegas, NV, which is located 90 km west of the field site in Logandale, NV (Figure S1d; Sengupta et al., 2018).

\subsection{Experimental design and irrigation treatments}

Treatments included three different \textit{Opuntia} spp. (\textit{O. cochenillifera}, \textit{O. ficus-indica}, and \textit{O. streptacantha}). Three different supplemental irrigation levels (Figure S2; Table S3) were used to obtain a combined total of ambient precipitation and irrigation of 290, 398, and 707 mm\(^{-1}\) year\(^{-1}\) (Figure S2; Tables S4 and S5). Treatments were arranged in a 3 \(	imes\) 3 factorial using a randomized complete block design (RCBD) with four replications of each treatment combination. The three \textit{Opuntia} spp. were selected for field trials based upon their biomass production potential during a previously performed productivity survey of 12 different \textit{Opuntia} spp. (i.e., \textit{O. littoralis}, \textit{O. streptacantha} [two accessions], \textit{O. santa-rita}, \textit{O. robusta} [two accessions], \textit{O. basilaris} [two accessions], \textit{O. ficus-indica} [four accessions], \textit{O. megacantha}, and \textit{O. cochenillifera}) obtained from the Riverside Botanical Gardens and the University of California, Riverside agricultural operations collections (established by José Luis Andrade and Park Nobel). These species were grown in triplicate under greenhouse conditions for 2 years (2009–2011). Mature pads were propagated by planting in 7.6-liter pots containing a 3:1 ratio of Sunshine MVP soil mix (Sun Gro Horticulture) and decomposed granite. One-year-old cladodes were placed approximately 5 cm into the soil. Standard greenhouse conditions at the Nevada Agricultural Experiment Station (Reno, NV) included natural light in the range of 1100–1500 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) and temperature in the range of 28–32°C day/17–18°C night. Pots were watered once (October–March) or twice (April–September) a week and fertilized monthly with Miracle Gro\textsuperscript{®} fertilizer (Scott’s MiracleGro, Inc.) according to the manufacturer’s instructions. Insect pests were kept in check by application of systemic insecticide treatment (Marathon\textsuperscript{®} 1% Granular, OHP) every 3 months. Aboveground biomass was determined gravimetrically in triplicate for all species and accessions.

\subsection{Plot establishment and management}

Prior to planting the three \textit{Opuntia} spp., the field site was well prepared to eliminate the presence of perennial weeds,
shrubs, or residues from previously grown crops. The field site was also tilled twice to facilitate broad-bed formation and leveled to improve water distribution ensuring uniform plant growth and establishment.

All three *Opuntia* spp. were planted on March 18, 2014 as single cladodes with a plant spacing of 2.32 m apart within a row and 3.04 m apart between rows (i.e., planting density = 1418 plants ha⁻¹) to facilitate mechanical tilling for weed management and minimize the interference between replicated blocks. Single cladodes were planted with the lower third buried into the ground in a north-south orientation to increase the amount of intercepted east-west solar radiation, which facilitates higher net assimilation rates (NAR) of cladodes, thereby increasing plant productively (Bakali et al., 2016; Garcia de Cortázar et al., 1986). Cladode establishment was 95% following the initial planting. Any cladodes that failed to thrive were replaced from nursery stock that was planted at the same time. Plants were cultivated for 5 years with average minimum and maximum air temperatures of 7.6°C and 34.4°C, respectively. The 28-year average (1992–2019) minimum and maximum air temperatures for the field site were 8.0°C and 33.4°C, respectively (Figure S1; Table S1).

The mean annual ambient precipitation in Logandale, NV was 151.1 mm year⁻¹ for cladodes and 171.2 mm year⁻¹ for fruit during the growing seasons of 2014–2019 (Figure S1b,c; Table S2). The mean historical ambient precipitation averaged in Logandale, NV from 1992 to 2019 was 136.3 mm year⁻¹ (Table S2). Cladodes were harvested in the winter and fruits were harvested in the summer; therefore, the growing seasons and corresponding supplemental irrigation totals differed. Ambient precipitation was supplemented with drip irrigation using pressure compensating emitters. Irrigation was withheld during the wet winter months on the following dates: December 1, 2015 to February 23, 2016; December 23, 2016 to February 7, 2017; December 5, 2017 to January 28, 2018; and December 31, 2018 to March 19, 2019 (Figure S2; Tables S3–S5). Fertilization was uniformly applied through the drip irrigation system on a monthly basis using All Purpose LiquaFeed® fertilizer N:P:K 12:4:8 (Scott’s MiracleGro, Inc.). The field plot was protected by perimeter fencing to prevent herbivory by local megafauna (e.g., pocket gophers, cotton tail rabbits, and ground squirrels). Individual plants were not pruned or otherwise managed in any way to influence cladode or fruit production or size.

### 2.4 Data collection and calculations

Data collection included the number of cladodes (cladode count, CC), number of fruits (fruit count, FC), cladode fresh weight (CFW), cladode dry weight (CDW), fruit fresh weight (FFW), and fruit dry weight (FDW) per plant from each plot annually. To determine the aboveground cladode biomass, one plant was destructively harvested from each block annually on March 18–20, 2015; January 14–15, 2016; January 19–20, 2017; January 18–19, 2018; and January 16–18, 2019. Mature fruits were harvested and counted from all plants on August 18, 2015, July 21, 2016; July 7, 2017; July 12–13, 2018; July 16–17, 2019. Both fresh cladode and fruit biomass were weighed at the field site upon collection using an Explorer® Precision EX24001 electronic balance (Ohaus, Inc.). To evaluate dry mass, cladodes and fruits were dried separately using a forced-air drying oven (Yamato Scientific America, Inc., models DKN810 or DKN812, Santa Clara, CA or Grieve Corp., model SC-550) at 70°C for 2 weeks or until no further change in weight was observed. Due to abundance epicuticular wax, both cladodes and fruit were diced into small pieces after harvest and prior to drying to promote complete dehydration.

The dry weight fruit-to-biomass ratio (FBR) was computed as the ratio of FDW divided by the total aboveground dry weight of biomass (cladode plus fruit) for each plot.

Water-use efficiency (WUE, kg biomass plant⁻¹ mm⁻¹ water used) of CFW, CDW, FFW, and FDW of the cactus was computed for each plot based on annual water use (WU) using the soil–water balance equation as described below (Gesch & Johnson, 2015):

\[
\text{WU} = \pm \Delta SW + P + I - RO - DP, \quad (1)
\]

where stored soil water content (±ΔSW), precipitation (P), water input (I), surface runoff (RO), and deep percolation (DP) denote weekly soil water storage variation (mm); ambient precipitation (mm); supplemental irrigation (mm); surface runoff (mm); and vertical water flow in the soil (mm) over a year, respectively. Due to the fine textured and poorly drained nature of soil at the site, ±ΔSW, RO, and DP were considered minimal. Thus, the Equation (1) was modified as:

\[
\text{WU} = P + I. \quad (2)
\]

WUE was computed based on the ratio between biomass yield (Yld = yield of fresh or dry matter, kg plant⁻¹) under each irrigation level and annual WU as described below (Lima et al., 2018):

\[
\text{WUE} = \frac{\text{Yld}}{\text{WU}}. \quad (3)
\]

WUE of all plots was computed prior to the statistical analysis.

### 2.5 Statistical analysis

Assumptions of normality using the Shapiro–Wilk's test and homogeneity were performed using Levene’s test for the treatment effects on the parameter data within the
generalized linear mixed models (GLMM) using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., 2019). Irrigation, species, year, and their interactions were treated as fixed effects, whereas replication was considered as the random effect. The Gaussian distribution, the identity as a link function, the estimation technique of restricted maximum likelihood (REML), the optimization technique of dual quasi-newton, and the containment as the degree of freedom method were used during the analyses. $P$ values were reported based on three-way analysis of variance (ANOVA) tests of fixed effects. The mean separation was determined using Fisher's protected least significant difference (LSD) test using the PDIFF statement in the LSMEANS option. The lines option in the LSMEANS statement was used to add letter groups for multiple comparisons. Responses in the study were considered significant at $p < 0.05$ unless otherwise specified. Orthogonal polynomial contrasts (OPC) were performed to identify the trends of measured parameters in response to irrigation. Pearson's correlation coefficients among the CFW, CDW, FFW, FDW, CC, FC, limb damage (LD), fruit to biomass ratio (FBR), and WUE were created using the PROC CORR procedure of SAS 9.4 (SAS Institute Inc., 2019). Regression analyses were also performed for the measured traits that correlated to quantify the relationship. Data were visualized using GraphPad Prism version 9 (GraphPad Software, www.graphpad.com).

### 3 RESULTS

#### 3.1 Temperature and ambient precipitation data

Weather data during the 5 years of the experiment and the 28-year average (1992–2019) revealed that there were relatively consistent patterns in mean monthly air temperature ($^\circ$C) from 2014 through 2019 and in the 28-year average monthly values except for a decreasing trend in the months of February and May of 2019 (Figure S1a; Table S1). Despite the lowest mean monthly temperature of $5.7^\circ$C (December 2015) and a mean monthly low temperature of $8^\circ$C (December), no significant cold damage was observed for any of the three Opuntia spp. tested. The annual average ambient precipitation of the site was 151.1 mm for cladodes and 171.2 mm for fruit for the 5-year interval (2014–2019) of the study and 136.3 mm for the 28-year average (1992–2019; Figure S1b,c; Table S2). The ambient precipitation amounts were 20 mm lower for cladodes than fruit due to the differences in the annual growing season for cladodes (typically January to January) and fruits (typically July to July; Figure S1b,c; Table S2). The ambient mean precipitation amounts (and supplemental irrigation) inputs were also lower for cladodes and higher for fruit due to the differences in the annual growing season (Figure S2; Tables S3–S5). Accumulated monthly precipitation during the experimental period deviated for each year compared to the 28-year average, with many of the atypical precipitation events occurring in the months of August and October in 2015, December and January in 2016, January and July in 2017 and 2018, January and February in 2019, and December through March for all years (Figure S1b,c; Table S2). Final measurements were completed in July of 2019 after 5 years of growth. Average monthly insolation rates (direct normal irradiance, 1998–2016) were reported for Las Vegas, NV (Figure S1d; Sengupta et al., 2018).

#### 3.2 Supplemental irrigation data

Ambient precipitation was supplemented with drip irrigation at three different rates. The three field irrigation levels were selected based upon previously published yield responses of *O. ficus-indica* to different irrigation inputs (Dubeux et al., 2006; Neftaoui & Ben Salem, 2001; Nobel, 1988). Ambient precipitation was supplemented with drip irrigation to deliver a mean of 321.7 mm year$^{-1}$ for cladodes and 333.5 mm year$^{-1}$ for fruit (Figure S2a,b; Table S3). The total mean irrigation amounts were slightly lower for cladodes and slightly higher for fruit due to the differences in ambient precipitation during the annual growing season (Tables S4 and S5). The combined mean ambient and supplemental irrigated water inputs resulted in a mean cumulative total water inputs of 290, 398, and 707 mm year$^{-1}$ for cladodes and 310, 417, and 726 mm year$^{-1}$ for fruit, respectively (Figure S2c,d; Table S6). The combined mean ambient and supplemental irrigation rates were 300, 407, and 716 mm year$^{-1}$ for total aboveground biomass (Tables S5 and S6).

#### 3.3 Overall cladode biomass and fruit productivity and water-use efficiency

The 5-year mean cladode dry matter (DM) biomass of all three species increased significantly ($p < 0.05$) with a linear trend from 7.8 to 14.6 Mg ha$^{-1}$ as water inputs increased from 290 to 707 mm (Figure 1a; Table S6). Mean fruit DM biomass of all three species also increased linearly from 0.42 to 0.9 Mg ha$^{-1}$ as water inputs increased from 310 to 726 mm (Figure 1c; Table S6). Total aboveground biomass of all three species increased linearly from 8.25 to 15.53 Mg ha$^{-1}$ as water inputs increased from 300 to 716 mm (Figure 1e; Table S6). In contrast, WUE, followed a quadratic trend ($p < 0.05$) with the lowest WUE occurring at the lowest irrigation rate (300 mm year$^{-1}$) for cladodes, fruit, and total aboveground biomass (Figure 1b,d,f; Table S6). WUE was highest for cladodes, fruit, and total biomass at the middle water input rate (407 mm year$^{-1}$; Figure 1b,d,f; Table S6).
The WUE at the middle water input rate increased significantly \((p < 0.05)\) and was 1.85-fold, 2.8-fold, and 1.9-fold greater than the lowest water input rate for cladodes, fruit, and total biomass, respectively. Lastly, at the highest water input rate \((716 \text{ mm year}^{-1})\), WUE declined slightly for cladodes, fruit, and total biomass, but these differences were not significant (Figure 1b,d,f; Table S6).

### 3.4 Year-over-year productivity gains in cladode biomass and fruit production

The mean CFW of all three *Opuntia* spp. combined increased significantly over years 2015–2019 (Table 1; Figure 2a; Table S7); however, the effects of water input, species, and their interactions were not significant (Table 1). The mean CFW for the three *Opuntia* spp. increased across 5 years according to a power curve \((1.148 x^{1.415})\) for each additional increase in year (where \(x = \text{number of years}\) from 2015 through 2019 (Figure 2a; Table S7). These increases were 7.5-, 30-, 58.1-, and 140.8-fold in 2016, 2017, 2018, and 2019, respectively (Figure 2a; Table S7). The mean CDW of the three *Opuntia* spp. was significantly impacted by water input, year, and the interaction between water input and species and water input and year (Table 1). CDW increased according to a power curve \((0.004 x^{5.464})\) for each additional increase in year with increases of 6-, 17.5-, 32.5-, and 110.9-fold in 2016, 2017, 2018, and 2019, respectively (Figure 2b; Table S7).

FFW was impacted by the effects of water input, year, and the interaction of water input and species (Table 1). FFW increased according to a power curve \((0.8015 x^{1.438})\) for each additional increase in year with the greatest fruit production by weight occurring in 2018 (Figure 2c; Table S7). FFW increased from 0.144 kg plant\(^{-1}\) in 2015 to 6.89 kg plant\(^{-1}\) in 2019. This increase was 9.5-, 24.1-, 57.4-, and 48.2-fold in 2016, 2017, 2018, and 2019, respectively. FFW production decreased by 1.2-fold in 2019 compared with that of 2018, but this change was not significant (Figure 2c; Table S7).

FDW was affected by water input, species, and year, and the interaction of water input and year, and species and year (Table 1). FFW increased according to a power curve
TABLE 1  Three-way analysis of variance (ANOVA) of fixed effects for cladode fresh weight, cladode dry weight, fruit fresh weight, fruit dry weight, cladode count, fruit count, limb damage, fruit to biomass ratio, and water-use efficiency (cladode fresh weight, cladode dry weight, fruit fresh weight and fruit dry weight) of three *Opuntia* spp. (*O. cochenillifera*, *O. ficus-indica*, and *O. streptacantha*) as affected by water input, species and year, and their interactions, grown in Logandale, NV from 2014 to 2019

| Parameter                  | df   | Cladode fresh weight | Cladode dry weight | Fruit fresh weight | Fruit dry weight | Cladode count | Fruit count | Limb damage | Fruit-to-biomass ratio | Water-use efficiency |
|----------------------------|------|----------------------|--------------------|--------------------|------------------|---------------|-------------|-------------|-----------------------|----------------------|
| Water input                | 2    | 0.229                | 0.001              | 0.004              | 0.003            | 0.005         | 0.438       | 0.0309      | 0.095                 | 0.0106               |
| Species                    | 2    | 0.936                | 0.391              | 0.335              | 0.002            | 0.311         | 0.572       | <0.0001     | 0.102                 | 0.607                |
| Water input × Species      | 4    | 0.347                | 0.025              | 0.028              | 0.713            | 0.037         | 0.545       | 0.4878      | 0.548                 | 0.181                |
| Year                       | 4    | <0.0001              | <0.0001            | <0.0001            | <0.0001          | <0.0001       | <0.0001     | <0.0001     | 0.045                 | <0.0001              |
| Water input × Year         | 8    | 0.493                | <0.0001            | 0.212              | 0.017            | 0.0036        | 0.624       | 0.159       | 0.572                 | 0.036                |
| Species × Year             | 8    | 0.999                | 0.934              | 0.883              | 0.001            | 0.829         | 0.551       | 0.253       | 0.999                 | 0.937                |
| Water input × Species × Year| 16   | 0.951                | 0.161              | 0.599              | 0.776            | 0.676         | 0.514       | 0.935       | 0.935                 | 0.424                |

Note: Bold font indicates significant effects.
Abbreviation: df, degrees of freedom.
(0.1179 $x^{1.267}$) with the greatest fruit production by weight occurring in 2018 (Figure 2d; Table S7). FDW increased from 0.07 to 1.04 kg DM plant$^{-1}$ from 2015 to 2019. This increase was 1.3-, 5-, 13.9-, and 9.1-fold in 2016, 2017, 2018, and 2019, respectively (Figure 2d; Table S7). The FDW values in 2018 and 2019 were consistently higher than the values observed in 2015, 2016, and 2017. In contrast to FFW, the FDW was 1.4-fold higher in 2018 compared to 2019.

Like CFW, the number of cladodes (CC) per plant was influenced significantly by water input and year, and the interaction effects of water input and species, and water input and year (Table 1). From 2015 through 2019, CC increased according to a power curve ($2.549 x^{2.697}$) in response to year (Figure 2e; Table S7). The mean CC per plant increased by 4.3-, 2.7-, 2.3-, and 1.8-fold in 2016, 2917, 2018, and 2019, respectively. The number of fruits per plant (FC) was influenced significantly only by the year (Table 1). Like CC, the FC displayed a power curve ($0.9163 x^{3.723}$) in response to year (Figure 2f; Table S7). FC increased from a mean of only 2.39 fruits per plant in 2015 to 370.3 fruits per plant in 2019. The year-over-year FC increase was 8.3-, 27-, 60.2-, and 153.3-fold in 2016, 2017, 2018, and 2019, respectively, with a very large variation in FC occurring in 2019 (Figure 2f; Table S7).

### 3.5 Fruit-to-biomass ratio

The FBR was mostly affected by year; however, water input levels also showed a trend among the three *Opuntia* spp. cultivated over the 5-year period (Table 1). The FBR changed significantly over the years (Table S7). In year 2015, the FBR was highest due to the high relative proportion of fruit to cladode biomass. During years 2016–2018, the FBR declined, but did not change significantly as the plants grew larger with a proportionate increase in fruit production. However, during 2019, the FBR declined dramatically as the cladode biomass of the plants increased significantly exceeding fruit production (Table S7). With respect to the effects of the three water input levels, FBR was highest at 417 mm of irrigation, but was lower at the 310 and 726 mm levels of water input displaying a quadratic trend ($p = 0.031$; Table S8). With respect to species effect, *O. cochenillifera* showed the highest FBR followed by *O. streptacantha* and *O. ficus-indica*, respectively.
(Table S9). However, overall these differences were not significant.

### 3.6 Water input effects on cladode biomass and fruit production

We next examined the effects of water input across all three *Opuntia* spp. Water inputs at the two lower levels (290 and 398 mm) did not result in a significant difference in CFW. However, the highest level (707 mm) resulted in an increase in CFW of all three *Opuntia* spp., but this increase was not significant ($p = 0.11$, Figure 3a; Table S8). Increased water input resulted in a linear increase in mean CDW for all three *Opuntia* spp. from 290 to 707 mm with a significant increase at 707 mm ($p = 0.0002$, Figure 3b; Table S8). Like CDW, increasing water input resulted in a linear increase in mean CDW for all three *Opuntia* spp. from 290 to 707 mm, with a significant increase in CC at 716 mm ($p = 0.0001$), but not at the two lower rates of irrigation (Figure 3c; Table S8). Similar to CDW, there was an increase in FFW with increasing water input with a significant increase at 726 mm ($p = 0.001$, Figure 3d; Table S8). Similarly, FDW increased linearly with increasing water inputs with significant increases at both 417 and 726 mm ($p = 0.001$, Figure 3e; Table S8). As with CFW, there were no significant differences in FC across the three water input levels, primarily due to the large variation in FC at 417 mm of water input, which also produced the largest number of fruit (Figure 3f; Table S8).

### 3.7 Species effects on cladode biomass and fruit production

A comparison of the production differences among the three *Opuntia* spp. did not reveal significant differences across the different parameters measured with the exception of FDW ($p = 0.002$, Figure 4a–f; Table 1). Among fruit production parameters, FFW did not differ significantly among the three different species (Figure 4d). In contrast, FDW was significantly different with *O. cochenillifera* producing a significantly higher FDW than the other
two species ($p = 0.003$, Figure 4e; Table S9). Notably, *O. ficus-indica* produced a 2-fold higher FC than either *O. cochenillifera* or *O. streptacantha*. However, due to the large variations in the FC observed for *O. ficus-indica* over the course of the experiment, these results were not significant ($p > 0.05$) (Figure 4f; Table S9).

**Figure 4** Species effect on cladode and fruit biomass production. Effect of three *Opuntia* spp. (*O. cochenillifera* [Oc], *O. ficus-indica* [Ofi], and *O. streptacantha* [Os]) on (a) cladode fresh weight, (b) cladode dry weight, (c) cladode count, (d) fruit fresh weight, (e) fruit dry weight, and (f) fruit count, grown in Logandale, NV from 2014 to 2019. Values represent means ± SEM ($n = 60$). Values indicated by the same lowercase letters were not significantly different by species; ns, not significant ($p > 0.05$), three-way analysis of variance (ANOVA) with Fisher’s least significant difference (LSD) test (protected t-test) for multiple comparisons.

**Figure 5** Effects of water input and species on limb breakage due to wind. Effects of (a) water input and (b) species on limb breakage of three *Opuntia* spp. (*O. cochenillifera* [Oc], *O. ficus-indica* [Ofi], and *O. streptacantha* [Os]) grown in Logandale, NV during the winter of 2018–2019. Values represent means ± SEM ($n = 36$). Values indicated by the same lowercase letters were not significantly different by water input or species ($p > 0.05$), three-way analysis of variance (ANOVA) with Fisher’s least significant difference (LSD) test (protected t-test) for multiple comparisons.
3.8 Water input and species effects on limb breakage

Wind damage can have a significant impact on the extent of limb breakage and increase the need for increased orchard management in wind-prone areas. Therefore, the number of limb breakage events per plant was documented during the fifth year after a series of severe winter storms. Limb breakage was mostly affected by the species and water input rate (Table 1; Figure 5; Tables S8 and S9). Increasing water input from 290 mm to 398 or 706 mm resulted in a significant increase in limb breakage (Figure 5a; Table S8). Interestingly, *O. cochenillifera* was highly resistant to limb damage by wind (*p* = 0.0001), whereas *O. streptacantha* was highly susceptible to such damage, with *O. ficus-indica* showing an intermediate, but significant amount of damage (Figure 5b; Table S9).

3.9 Effects of water input and species on cladode biomass and fruit production

In addition to the effects on limb breakage by wind, the interaction of water input and species showed significant effects on CDW (*p* = 0.025), CC (*p* = 0.037), and FFW (*p* = 0.028; Table 1). CDW values were significantly different at the 398 and 707 mm water input levels. At the 398 mm level, *O. cochenillifera* produced a greater CDW than *O. ficus-indica* or *O. streptacantha* (Figure 6a; Table S10). However, at the 707 mm level, *O. ficus-indica* produced the highest CDW compared with the other two species (Figure 6a; Table S10). For *O. cochenillifera*, CDW was highest at 398 mm. In contrast, *O. ficus-indica* and *O. streptacantha* showed the highest CDW at 707 mm (Figure 6a; Table S10). Trends for CC were also similar to those for CDW. CC was highest at 398 mm for *O. cochenillifera*, whereas for both *O. ficus-indica* and *O. streptacantha*, FFW was highest at 707 mm (Figure 6b; Table S10). As with CDW, *O. ficus-indica* showed a significant stimulation in cladode production at the highest water input level, whereas *O. streptacantha* showed higher cladode production at both 398 and 707 mm levels of water input. Trends in FFW production matched those of CDW and CC. FFW was highest at 726 mm for *O. cochenillifera*, whereas for both *O. ficus-indica* and *O. streptacantha*, FFW was highest at 726 mm (Figure 6c; Table S10). Notably, *O. ficus-indica* showed a significant stimulation in fruit production at the highest water input level.

3.10 Effects of water input and year on cladode biomass and fruit production

Next, we examined the interaction between water input and year, which revealed significant differences as the cactus trees grew increasingly larger in years 4 and 5 of the study.

CDW across all three species increased significantly with increasing water inputs only in 2019 when a linear growth response was apparent as water input was increased from 290 to 707 mm (*p* < 0.0001, Figure 7a; Table S11). Although
CDW did increase with increasing water input during years 1–3; these increases were not significant. However, by year 4 (2018), increased CDW at the highest two water inputs became significant compared with years 1 and 2 (2015–2016). With respect to the two-way interaction effect between water input and year, CC trends resembled those observed for CDW with significant increases occurring in years 2018 and 2019 along with significant increases in CC in response to increasing water input levels in both years ($p = 0.004$, Figure 7b; Table S11). Within each water input level, the number of cladodes exhibited a power trend increase across the different years of the study (trendline not shown). The number of cladodes increased by 32.5-, 52.5-, and 64.3-fold in response to 290, 398, and 707 mm levels, respectively, from 2016 to 2019 (Figure 7b; Table S11). With respect to the two-way interaction effect of water input and year, FDW differed at the 398 and 707 mm water inputs across different years ($p = 0.017$, Figure 7c; Table S11). At the 417 mm water input, FDW was significantly higher in 2018 and 2019 compared with the earlier three growing years (2015–2017). Interestingly, FDW was significantly higher at the 726 mm water input in 2018 and 2019 compared with the two lower water input levels. In both 2018 and 2019, FDW was similar or higher at both the 417 and 726 mm water input levels than at the 310 mm water input level, thus illustrating the impact that increased water input also had on fruit production (Figure 7c; Table S11).

The interaction between species and year on FDW was also significant across all years and species (Table S12). Regarding the two-way interaction of species and year, among different years (within each species), FDW was significantly different only for $O. \text{cochenillifera}$ and $O. \text{streptacantha}$ (Table S12). For $O. \text{cochenillifera}$, FDW was the highest in 2018 and significantly greater than the other 4 years. However, $O. \text{streptacantha}$ produced the highest FDW in 2018 followed by 2019 compared with 2015, 2016, and 2017. $O. \text{ficus-indica}$ produced the highest FDW in both 2018 and 2019 (Table S12).

### 3.11 Water-use efficiency

The WUE associated with CFW, CDW, FFW, and FDW was influenced significantly by water input and year (Table 1). Correlations among these parameters and their associated WUEs are presented in Table S13. Increasing water inputs resulted in a quadratic trend ($p = 0.0115$) response in the WUE of CFW wherein WUE increased from 290 mm, plateaued at 398 mm, and decreased at 707 mm (Table S14). The WUE of CDW showed both a linear ($p = 0.0325$) and quadratic ($p = 0.0286$) improvement with increasing water inputs from 290 mm to 707 mm (Table S14). The WUE of CDW showed a significant increase in 2019 with the higher levels of water input (398 and 707 mm) showing a significant increase over the 290 mm water input level (Figure 7d; Table S14). With respect to the water input response of fruit, WUE of FFW showed both linear and quadratic trend improvements with
increasing water input from 310 to 726 mm (Table S14; p value for linear =0.071 and quadratic =0.075). In contrast, the WUE of FDW was significantly impacted by water input, species, year, and the interaction between species and year (Table 1). The response of water input on WUE of FDW showed both linear (p = 0.02) and quadratic (p = 0.0006) trends as water input increased from 310 to 726 mm (Table S14). The effect of species on WUE was not significant for CFW, CDW, and FFW. However, the WUE of FDW production was significantly higher (p = 0.012) for O. cochenillifera compared with the other two species (Table S15).

In addition to water input, the age of the plants had a significant effect on WUE for CFW, CDW, FFW, and FDW (Table 1; Table S16). Over a 5-year period (2015–2019), the WUE of CFW increased 4 to 490 g of CFW plant$^{-1}$ mm$^{-1}$ of water used (Table S16). The fold year-over-year increase in CFW was 6.75-, 3.39-, 0.54-, 1.34-fold in years 2016, 2017, 2018, and 2019, respectively (Table S16). FFW increased from 0.3 to 13.1 g FFW plant$^{-1}$ mm$^{-1}$ of water used in 2015 through 2019, respectively, with significant increases occurring in 2017 and 2018–2019 (Table S16). Over the 5-year period, WUE of FDW increased from 0.14 to 1.82 and to 1.25 g plant$^{-1}$ mm$^{-1}$ from 2015 through 2018 and 2019, respectively (Table S16).

With respect to the two-way interaction of water input and year, the WUE of CDW increased significantly year-over-year at all three water input levels tested (p = 0.036, Figure 7d; Table S17). The highest WUE occurred in the last year (2019) as the biomass of the plant increased dramatically (Figure 7d). With increasing water input from 290 to 707 mm, the WUE nearly doubled (Figure 7d; Table S17). At the highest water input level, WUE increased from 0.5 to 59.4 g CDW plant$^{-1}$ mm$^{-1}$ of water consumed and a year-over-year 5.3-, 3.0-, 1.6- and 4.6-fold increase in 2016, 2017, 2018, and 2019, respectively (Table S17).

With respect to the two-way interaction of water input and species, the WUE of CDW was significantly higher for O. ficus-indica than for O. cochenillifera at the 707 mm water input level. At the 398 mm water input level, the WUE of CDW for O. cochenillifera was significantly higher than at

![Figure 8](image-url)

**Figure 8** Correlation analysis of biomass parameters. The relationships among parameters of *Opuntia* spp. grown under three irrigation levels over a 5-year period: (a) cladode fresh weight (CFW) versus cladode dry weight (CDW), (b) cladode fresh weight (CFW) versus fruit fresh weight (FFW), (c) CFW versus cladode count (CC), (d) CC versus CDW, (e) CC versus FFW, and (f) CC versus fruit count (FC). $R^2$ values indicate the relative degree of correlation between parameters.
the lowest and the highest water input levels. Overall, the WUE of CDW across the three species was not significantly different ($p > 0.078$) (Table S18). However, for *O. ficus-indica*, the WUE of CDW was higher at 398 and 707 mm of water input compared with the 290 mm water input level (Table S18). The trends for WUE for FFW were similar to those observed for CDW. For *O. cochenillifera*, WUE was significantly higher at 417 mm of water input than at the lowest and the highest water input levels. However, for *O. ficus-indica*, the WUE for FFW was significantly higher at the 726 mm of water input than at the lower levels of water input. Overall, the WUE of FFW across the three species was not significantly different ($p > 0.066$; Table S18).

### 3.12 Correlation among parameters for cladode biomass and fruit production

Lastly, we explored the relationships between the various parameters tested to identify which ones were correlated with one another. As one might expect, CDW and CFW and CC and CFW showed the highest correlation values among the different parameters tested, with greater variation observed as the plant became larger (Figure 8a,c). CDW and CC were less well correlated with one another (Figure 8d). FFW was weakly correlated with either CFW or CC (Figure 8b,e). Lastly, FC was very weakly correlated with CC due to large variations in these two data sets (Figure 8f).

### 4 DISCUSSION

This study provides the first long-term field evaluation of the vegetative biomass and fruit production of three *Opuntia* spp. in the United States under three different levels of water input. Modeling studies have shown that *O. ficus-indica* exhibits higher CO$_2$ uptake potential across a wider range of maximal daytime temperatures (Owen & Griffiths, 2014) and greater resilience to climate change than *Agave tequilana* and related *Agave* spp., which are used for beverage production and show great potential as emerging bioenergy and biomaterials crops (Corbin et al., 2015; Escamilla-Treviño, 2012; Holtum et al., 2011; Jones et al., 2020; Li et al., 2014; Owen et al., 2016; Yan et al., 2011). Modeling projections for *O. ficus-indica* production in Australia (Owen & Griffiths, 2014), North America (Yang et al., 2015), and globally, estimated a maximal productivity of up to 40 Mg DM ha$^{-1}$ (Owen et al., 2016). A productivity range of 40–50 Mg DM ha$^{-1}$ year$^{-1}$ was in agreement with earlier studies of biomass productivity that used supplemental irrigation (Garcia de Cortázar & Nobel, 1991, 1992; Nobel, 1991, 1996; Nobel & García de Cortázar, 1991; Nobel et al., 1992).

In the current study, mean annual aboveground biomass production of 11.6 Mg DM ha$^{-1}$ was obtained for the three different levels of water inputs tested and was roughly similar across all three species tested. This level of biomass production was less than the above modeling estimates due to the relative low planting densities of 1418 plants ha$^{-1}$ used to isolate the different irrigation-level blocks in the current study. These aboveground biomass estimates did not include the specific contribution of fruit, which can contribute an additional 6% to annual DM accumulation according to the results reported here (Table S6). However, the planting density could easily be increased by 4-fold to 5672 plants ha$^{-1}$, thereby achieving productivity values of 46.4 Mg DM ha$^{-1}$. Higher aboveground biomass production of 15.5 Mg DM ha$^{-1}$ year$^{-1}$ was obtained at the highest water input level used (716 mm), suggesting that greater water inputs could boost production further if adequate water resources were available. Notably, this higher planting density is still on the low end of some high-density studies with the goal of forage production, which employed planting densities in the range of 20,000–50,000 plants ha$^{-1}$ year$^{-1}$ (de Queiroz et al., 2015; Dubeux et al., 2006; Lima et al., 2016; Table 2). Comparable cladode production of *O. ficus-indica* was observed in Portugal where production ranged from 2.98 to 11.95 Mg DM ha$^{-1}$ (Reis et al., 2016). Other field trials of *Opuntia* spp. across several semi-arid or arid regions of the world produced mean yields that ranged from 3.5 to 5.1 Mg DM ha$^{-1}$ (Grünwaldt et al., 2015; Guevara et al., 2011; Krümpel et al., 2020; Liguori et al., 2014), whereas other field trials in Ethiopia and India produced lower mean biomass yields in the range of 0.08–1.48 Mg DM ha$^{-1}$ (Mangalassery et al., 2017; Soni et al., 2015; Tarekgen et al., 2017). Overall, our current results indicate that productivity of >40 Mg DM ha$^{-1}$ year$^{-1}$ could be accomplished without difficulty employing standard drip irrigation practices with low annual water inputs in the range of 407–716 mm. Furthermore, these annual biomass production rates were comparable with those of other bioenergy feedstocks such as maize, sugarcane, switch grass, and popular (Borland et al., 2009; Davis et al., 2011; Somerville et al., 2010).

### 4.1 Effect of year on aboveground biomass productivity

As cactus pear is a perennial crop, evaluation of its biomass yield over multiple years is an important metric used to determine the time it takes to reach maximum productivity. The year-over-year biomass productivity increase followed a power curve (Figure 2). After 3 years, annual dry matter biomass production rates for the three *Opuntia* spp. exceeded those observed for *A. americana* at comparable planting densities of 2500 plants ha$^{-1}$ (Davis et al., 2017).
### Table 2: Comparative study of cactus pear (Opuntia spp.) cladode biomass across geographic locations of the world

| Geographic regions         | Climate         | No. of trials | Year(s) of cultivation | Rainfall (mm) | Plant population (count ha$^{-1}$) | Design | Replications | Biomass range (Mg DM ha$^{-1}$ year$^{-1}$) | Mean biomass (Mg DM ha$^{-1}$ year$^{-1}$) | Citations                      |
|----------------------------|-----------------|---------------|------------------------|---------------|-------------------------------------|--------|--------------|--------------------------------------------|--------------------------------------------|-------------------------------------|
| Northeast Brazil           | Semi-arid       | 80            | 4 years                | 750           | 5000 (low), 40,000 (high)           | RCBD   | 4            | 6–17 (low), 17.8–33.7 (high)               | 11.53 (low), 23.8 (high)                  | Dubeux et al. (2006)                  |
| Northeast Brazil           | Semi-arid       | 12            | 2 years                | 473           | 50,000                              | RCBD   | 12           | 8.62–34.71                               | 19.18                                      | Lima et al. (2016)                    |
| Southern Nevada, USA       | Semi-arid       | 45            | 4 years                | 142.2         | 1418                                | RCBD   | 4            | 7.8–14.7                                  | 10.98                                      | This study                          |
| Northeast Brazil           | Semi-arid       | 10            | 3 years                | 618           | 20,834                              | RCBD   | 4            | 6.93–9.15                                 | 8.18                                       | de Queiroz et al. (2015)              |
| Mendoza plain, Argentina   | Arid            | 18            | 3 years                | 293           | 3334                                | —      | —            | 2.53–6.83                                 | 5.16                                       | Grünwaldt et al. (2015)$^a$           |
| Castelo Branco, Portugal   | Semi-arid       | 3             | 2 years                | 670           | 2667                                | RCBD   | 3            | 2.98–11.95                                | —                                         | Reis et al. (2016); Reis et al. (2018)$^b$ |
| Laikipia, Kenya            | Semi-arid       | 2             | 2 years                | 600           | 3333 (low), 8000–20,000 (high)      | RCBD   | 3            | 2.5–8.2                                   | 4.56 (low), 5.4 (high)                  | Krümpel et al. (2020)$^c$             |
| Mendoza plain, Argentina   | Arid            | 3             | 5 years                | 293           | 667                                 | RCBD   | 3            | 0.97–6.71                                 | 3.52                                       | Guevara et al. (2011)                 |
| Western Sicily, Italy      | Semi-arid       | —             | 4 years                | 600           | 333                                 | —      | —            | —                                         | 3.6                                       | Liguori et al. (2014)$^d$             |
| North Gondar, Ethiopia     | Humid, subtropical | 1          | 1.5 years              | 1052          | 6667                                | RCBD   | 3            | 0.98–2.75                                 | 1.48                                      | Tarekegn et al. (2017)               |
| Northwestern India         | Arid            | 60            | 2 years                | 346           | 2500                                | RCBD   | —            | 0.11–1.35                                 | 0.4                                       | Mangalassery et al. (2017)$^e$         |
| Bikaner, India             | Arid            | 14            | 2 years                | 275           | 2500                                | RCBD   | 5            | 0.025–0.166                               | 0.08                                      | Soni et al. (2015)                    |

$^a$In Mendoza plain, Argentina study, the experimental design and number of replications were not reported.

$^b$Mean value for cladode biomass production of the 18 O. ficus-indica ecotypes surveyed was not reported.

$^c$Plants were harvested 50 cm above ground level and biomass was collected three times per year.

$^d$The western Sicily, Italy study was conducted from May to November 2010. Weight of cladodes was measured in 1-year, 2-year, 3-year, and 4-year-old plants. The measurement was done for only six trees from the orchard. No. of trials, experimental design, and number of replications were not presented in the study. Biomass range was also not mentioned.

$^e$In the northwestern India study, the number of replications was not clearly mentioned.
A. americana produced a mean of 9.3 Mg DM ha−1 year−1 at 530 mm year−1 irrigation, whereas the Opuntia spp. produced a mean aboveground of 10.3 Mg DM ha−1 year−1 with a mean water input of 407 mm year−1 and a comparable planting density of 2500 plants ha−1 (Table S6). The current results showed that the three Opuntia spp. produced greater aboveground biomass with slightly lower water inputs than A. americana when identical years of growth and planting densities were considered.

Even greater aboveground biomass production occurred during years 4 and 5, when mean biomass increased to 19.35 and 68.5 Mg DM ha−1 year−1, respectively (Figure 2; Tables S6 and S7). As plants grew larger, they increased the amount of intercepted photosynthetically active radiation (PAR), which, in turn, resulted in higher biomass production (Dubieux et al., 2006). Larger cactus trees also produced the highest fruit numbers and biomass (Figure 2; Table S7), which was consistent with previous reports documenting commercial fruit numbers and biomass (Figure 2; Table S7). As plants grew larger, they increased the amount of intercepted photosynthetically active radiation (PAR), which, in turn, resulted in higher biomass production (Dubieux et al., 2006). Larger cactus trees also produced the highest fruit numbers and biomass (Figure 2; Table S7), which was consistent with previous reports documenting commercial-level fruit yields after 4–5 years (Inglese et al., 2002; Kumar et al., 2018). Overall, annual yield results indicated that maximal cladode production measured as fresh or dry weight or cladode counts occurred in year 5 (Figure 2; Table S7). In terms of fruit fresh and dry weight production and fruit count, maximal fruit production plateaued by year 4 with significant fruit production occurring in year 3. The observed trend in fruit production was similar to other fruit crops, such as wine grape, which requires about 3 years before an economically harvestable crop is obtained (Inglese et al., 2002).

4.2 | Opuntia shows few production barriers

During the course of this long-term study, the Opuntia spp. tested showed several distinct advantages over other water-wise bioenergy feedstocks. The three Opuntia spp. evaluated in the current study showed no significant cold damage at the field location where the mean lowest temperature was 7.6°C (December; Figure S1a; Table S1). O. ficus-indica exhibits freezing tolerance down to about −7°C with cold acclimation (Nobel & de la Barrera, 2003). This level of cold tolerance is very similar to the cold sensitivity documented for A. americana (Nobel & McDaniel, 1988). Among three Agave species tested, A. americana showed higher cold tolerance than either A. fourcroydes or A. tequilana in field trials in Maricopa, AZ subjected to extreme cold events (Davis et al., 2017, 2019). Although Opuntia spp. are known to host insect pests, including cochineal scale insect (i.e., Dactylopius opuntiae, D. coccus) and the cactus moth (i.e., Cactoblastis cactorum; Zimmerman, 2017), these pests were not observed in the current field study, likely due to the geographic isolation of the site. In contrast, A. americana can suffer production losses due to the Agave snout weevil, particularly in larger plants grown with higher irrigation levels (Davis et al., 2017). Opuntia spp. can also suffer significant production losses from Opuntia stunting disease likely caused by a bacterial or viral pathogen (Felker et al., 2019; Perales-Segovia et al., 2018). However, symptoms of this disease were not observed.

4.3 | Effect of water input on aboveground biomass productivity

Water input rate had a profound effect on biomass productivity in Opuntia spp. In the current study, biomass productivity increased linearly in response to increasing mean water inputs for cladodes and fruit, regardless of the species, with significant differences among treatments becoming apparent in years 4 and 5 as the cactus trees increased in size. The increase from 290 to 707 mm of total water input increased mean CDW and FDW by 1.8- and 2.1-fold, respectively (Figure 1a,c,e, Figure 3b,e, Table S6). The highest mean water input rate for total aboveground biomass (716 mm) used in this study produced the highest biomass. This result is similar to the conclusion that 740 mm of total irrigation resulted in maximal cladode production in Coahuila, Mexico (Flores-Hernández et al., 2004).

Under semi-arid (rain-fed) conditions with annual precipitation rates of 200–400 mm, average aboveground productivity for Opuntia species varied from 3 to 15 Mg DM ha−1 year−1 (do Nascimento Santos et al., 2016; Le Houérou, 1996; Mason et al., 2015; Nobel, 1991). O. ficus-indica biomass productivity based on Geographical Information Systems (GIS) modeling with estimated annual precipitation amounts of 250 or 500 mm, the average annual productivities of 4.2 or 9.4 Mg (dry matter) ha−1 year−1, respectively, were reported in the Almería province, Spain (Sánchez et al., 2012). However, when supplied with sufficient irrigation (and fertilizer), average annual biomass productivities were 40–50 Mg ha−1 year−1 in studies conducted in Brazil, Chile, and Mexico (Garcia de Cortázar & Nobel, 1991, 1992; Nobel, 1991, 1996; Nobel et al., 1992). Notably, O. ficus-indica showed the greatest response to increasing water inputs compared to the other two species (Figure 6; Table S10), indicating that this species would be desirable for maximizing cladode biomass and fruit production if greater irrigation resources were made available. In contrast, decreasing water inputs negatively impacted CDW and CC (Figure 3; Tables S8 and S11), likely due to reductions in the extent of vegetative meristem initiation, particularly as plants grew larger after year 3 and beyond and required more water. Insufficient irrigation also negatively impacted FFW, FDW, and FC (Figure 3; Tables S8 and S11), likely due to reductions in the extent and timing of floral meristem initiation and poor fruit maturation, particularly as plants increased in overall size in years 4 and 5 (Inglese et al., 1995; Nerd & Nobel, 2000).
4.4 Effect of species on aboveground biomass productivity

Comparison of the three species tested showed no significant differences in overall cladode biomass productivity and FFW (Figure 4a–d; Table S9). However, among the three species tested *O. cochenillifera* showed the highest FFW production (Figure 4e). Overall, *O. ficus-indica* performed the best in terms of CFW, CDW, and FC, but these differences were not significant. Notably, *O. ficus-indica* produced the highest FC and the most desirable fruit quality (Figure 4f), confirming its utility in commercial production settings (Felker et al., 2005; Mondragon-Jacobo & Bordelon, 1996). Related studies using drip irrigation delivering 740 mm year\(^{-1}\) in Durango, Mexico reported that *O. ficus-indica* produced more fruit biomass than three other species identified as either *O. amyclaea* or *O. megalantha* (Flores-Hernández et al., 2004). Similarly, *O. ficus-indica* showed higher CFW compared with *O. robusta* (Snyman, 2013). *O. ficus-indica* generally outperformed in studies conducted across five locations in NE Brazil with higher CFW (89.7 Mg ha\(^{-1}\) year\(^{-1}\)), but slightly lower CDW (7.9 Mg ha\(^{-1}\) year\(^{-1}\)) than *O. (Nopalea) cochenillifera* (do Nascimento Santos et al., 2016). These values were lower than the mean biomass productivity observed in the current study for CFW (136.7 Mg ha\(^{-1}\) year\(^{-1}\)) and CDW (10.9 Mg ha\(^{-1}\) year\(^{-1}\)). Fruit production and quality for *O. ficus-indica* can vary widely depending on the specific accession or cultivar under investigation (Felker et al., 2005; Parish & Felker, 1997). FFW values in the current study varied by species: *O. cochenillifera* (4.7 kg plant\(^{-1}\)), *O. ficus-indica* (3.7 kg plant\(^{-1}\)), and *O. streptacantha* (3.8 kg plant\(^{-1}\)). Notably, these FFW values were lower than values reported for Italian ecotypes of *O. ficus-indica*, such as “Gialla” (5.17 kg plant\(^{-1}\)) and “Bianca” (5.10 kg plant\(^{-1}\)), in a large survey of 18 *O. ficus-indica* populations in Portugal (Reis et al., 2018). However, fruit production values for the other *O. ficus-indica* ecotypes surveyed were well within the range of FFW values of 0.9–3.8 kg plant\(^{-1}\) reported in this study. The mean FC in the current study ranged from 88.5 to 185 fruit plant\(^{-1}\) (Table S10). In year 3, the FC ranged from 43.3 to 87.7 fruit plant\(^{-1}\) depending on the level of water input (Table S12). This range of values was higher than the range of 11–68 fruits produced after 3 years from 18 *O. ficus-indica* ecotypes grown in Portugal (Reis et al., 2018). The lower FC observed in this study might be attributed to the evaluation of commercial varieties and ecotypes that were selected to produce fewer, larger fruit.

4.5 Fruit-to-biomass ratio (FBR)

FBR is a measure of vegetative growth relative to reproductive growth and is mainly affected by the age of the plant among other factors such as climatic conditions, cultivars or species, as well as the management practices, such as planting density and irrigation rates (Kumar et al., 2018). The age of the plants had a major effect on the FBR, whereas other factors such as species or irrigation rates did not show significant effects (Table S1). Pearson correlation analysis showed that FBR was negatively correlated with aboveground cladode biomass production (Table S13), suggesting an inverse relationship between vegetative and reproductive growth of plants. The FBR changed significantly over time with the ratio changing from high to low as the plants increased cladode production dramatically, but with fruit production not increasing at the same rate when measured on a dry mass basis (Table S7). The mean FBR across all years was 0.12 kg kg\(^{-1}\), which was lower than the 0.35 and 0.45 kg kg\(^{-1}\) for the first and second flush, respectively, reported for commercial plantations in India (Kumar et al., 2018). Although *Opuntia* spp. can produce fruit as early as the first year following planting, fruit production is associated with the optimal balance between cladode DM and fruit production, which is estimated to be at least 33 g of cladode DM (Garcia de Cortázár & Nobel, 1992). The lowest fruit yields in 2019 were likely attributed to the shading of upper cladodes in older trees, which might not have accumulated the greater than 33 g DM cladode\(^{-1}\) required for fruit production (Garcia de Cortázár & Nobel, 1992). FBR also increased in response to increasing total water inputs (Table S8) and differed among the three species tested (Table S9). Many factors, such as soil water status, temperature, and intercepted PAR, are thought to be critical for maintaining the balance between cladode DM and fruit production (Garcia de Cortázár & Nobel, 1991, 1992; Nobel, 1988).

4.6 Effects of wind damage on *Opuntia* production

Wind damage is an important factor in *Opuntia* spp. orchard management particularly in wind-prone areas. During the winter of 2018–2019, several severe storms with high winds allowed for the assessment of differences in wind damage as measured by limb breakage among the different irrigation levels and species. The lowest water input level resulted in significantly lower rates of limb breakage (Figure 5a; Table S8). The higher rates of limb breakage at the two higher water input rates could be attributed to greater turgidity of the cladodes, which presumably caused them to be more easily broken by wind shear forces. *O. cochenillifera* showed significantly lower rates of limb breakage than *O. ficus-indica*, whereas *O. ficus-indica* showed significantly lower rates of limb breakage than *O. streptacantha* (Figure 5b; Table S9). These differences were likely due to anatomical and biochemical differences among the three species and deserve further investigation. Notably, these field observations clearly indicate
that *O. cochenillifera* is well suited for minimizing orchard maintenance particularly in wind-prone regions and for use as wind or fire breaks when grown as hedge rows.

### 4.7 Highest water-use efficiency at moderate irrigation levels

WUE is defined as the unit of CO$_2$ fixed per unit of water lost to transpiration and is an important parameter for assessing the water input requirements of highly drought-tolerant biofuel crops such as *Opuntia* spp. Determining the optimal WUE for crops grown in semi-arid and arid regions is of paramount importance in regions where water supply is limited. The greatest WUE efficiency for both cladode and fruit biomass occurred at the intermediate water input rate (398–417 mm year$^{-1}$) and declined slightly as water input levels increased to 707–726 mm year$^{-1}$ (Figure 1b,d,f; Tables S6, S14 and S17). The lowest water input rate (290–310 mm year$^{-1}$) resulted in the lowest WUE. These results indicated that while increasing water inputs might improve overall biomass and fruit production, these increasing water inputs come at the expense of lower WUE. Similar WUE trends were observed for *A. americana* for which the highest WUE values were observed when intermediate levels of irrigation inputs were used in the range of 460–530 mm year$^{-1}$ (Davis et al., 2017). Determining the species with the highest WUE is also an important factor for water-limited areas. The three species used in this study showed fairly consistent WUE values among the three *Opuntia* spp. *O. ficus-indica* and *O. cochenillifera* displayed very similar WUE values in terms of cladode production with *O. ficus-indica* showing higher WUE for CFW (Table S15). However, *O. cochenillifera* showed slightly higher WUE values for fruit production than *O. ficus-indica* (Tables S15 and S18). In contrast, *O. streptacantha* showed the lowest WUE values for both cladode and fruit production, suggesting that this species would be less preferred in regions where water resources are limited. WUE also changed significantly for all production parameters with improved WUE as the plants grew larger over time. The greatest WUE was attained in year 5 for cladode production (Tables S16 and S17). In contrast, the highest WUE for fruit production occurred in year 4. These results point toward the potential water-saving benefits of perennial crop species for sustainable bioenergy production through soil moisture conservation (Wang et al., 2017).

### 4.8 Correlations among biomass parameters

A number of significant positive correlations were observed among the different biomass parameters tested. As might be expected for cladode measures, the highest correlations occurred between CFW versus CC ($r = 0.83$) and CDW versus CC ($r = 0.87$; Table S13; Figure 8). A higher numbers of cladodes resulted in a greater cladode area index, which, in turn, resulted in the capture of greater PAR, presumably as a result of greater CO$_2$ assimilation and biomass production (Dubeux et al., 2006; García de Cortázar & Nobel, 1991, 1992; Pinheiro et al., 2015). The correlation between CFW versus CDW was slightly lower ($r = 0.74$), likely due to differences in cladode anatomy and water-storage capacity differences among the three *Opuntia* spp. evaluated. The regression analysis also indicated that approximately 54.5% and 55.3% variation in CDW and CC, respectively, could be attributed to CFW (Figure 8a,c). Less variation (39.5%) in CC could be attributed to CDW (Figure 8d). As might also be expected for fruit measures, the highest correlations occurred between FFW versus FDW ($r = 0.77$) and FFW versus FC ($r = 0.64$), with FFW accounting for 27.4% of variation in CC (Table S13; Figure 8e). The correlation between FC versus CC ($r = 0.61$) was weaker, with FC accounting for only 4.4% of variation (Table S13; Figure 8f). Lastly, the correlation between FFW versus CFW ($r = 0.50$) was weaker, but FFW accounted for a greater percentage (25.7%) of variation in CFW (Table S13; Figure 8b).

### 5 Conclusions and future directions

This study provided the first long-term investigation into the performance of three different cactus pear species under three different water input levels. Overall, water input was the main factor influencing biomass productivity, with little difference in the biomass productivity observed among the three *Opuntia* spp. evaluated. Importantly, the highest water input level (716 mm year$^{-1}$) resulted in significantly higher CDW, CC, FFW, and FDW production than the two lower irrigation levels (300 or 407 mm year$^{-1}$). However, CFW and FC were not significantly different among the three irrigation levels due primarily to large variations in these parameters. Biomass trends over the 5-year period were *O. ficus-indica* > *O. cochenillifera* > *O. streptacantha* indicated that *O. ficus-indica* was the preferred choice for commercial production, although the three *Opuntia* spp. were not significantly different. Furthermore, *O. ficus-indica* was more responsive to higher water input inputs and produced superior fruit quantities and quality, reinforcing the desirability of this species for commercial production. This study produced a reliable estimate of aboveground biomass production of 11.6 Mg DM ha$^{-1}$ year$^{-1}$, which could easily be quadrupled to achieve 46.6 Mg DM ha$^{-1}$ year$^{-1}$. This value is consistent with legacy and more contemporary studies. With reliable biomass productivity estimates now in hand, detailed
life cycle assessment and life cycle costing analysis are currently underway for both bio ethanol and biogas production and will be the subject of future reports. To embrace the full biomass production and carbon sequestration potential of cactus pear species, future studies are needed to better understand various factors that impact growth and productivity including optimal nutrient requirements (e.g., NPK and micro-nutrient requirements), soil characteristics (e.g., pH, cation exchange, and water retention capacity), planting density, and geographic location using multi-location field trials. Lastly, detailed molecular, anatomical, biochemical, and physiological analyses of *O. ficus-indica* and other *Opuntia* spp. will improve our understanding of the outstanding WUE of this important water-wise CAM crop.

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DATA AVAILABILITY STATEMENT

Data available in article supplementary material. The data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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