

Contents lists available at ScienceDirect

Agricultural Water Management



journal homepage: www.elsevier.com/locate/agwat

# Guayule growth and yield responses to deficit irrigation strategies in the U. S. desert



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# ARTICLE INFO

Handling Editor - Dr. Z. Xiying

Keywords: Rubber Soil water Water productivity Evapotranspiration Crop coefficient

# ABSTRACT

Deficit irrigation can maximize the water productivity (WP) of guayule and increase the percent rubber (%R) in shrubs compared to irrigation meeting full crop evapotranspiration (ET<sub>c</sub>). In this study, we hypothesize that certain deficit irrigation strategies that impose soil water deficits during specific periods of growth or throughout the growing season might produce higher %R and equivalent rubber yield (RY), thereby, increasing WP compared to full irrigation. Herein, growth and yield responses of direct-seeded guayule to different water deficit schemes were evaluated in an experiment on a silty clay loam soil, in a field in central Arizona using furrow irrigation. Two guayule cultivars (AZ2 and AZ6) were grown for 22.5 months (Apr. 2020-Mar. 2022) in a splitplot design, with six irrigation treatments in whole plots and cultivars in split-plots. After homogeneous irrigation for two months, irrigation treatments were begun. A control treatment was irrigated to meet full ETc. The other five treatments were irrigated with less water using various deficit irrigation strategies imposed during the two-year growing period. Measurements included plant height (h), cover fraction ( $f_c$ ), soil water contents, harvest of dry biomass (DB), RY, resin yield (ReY), %R, and percent resin (%Re). Total water applied (TWA) by irrigation and precipitation to treatments varied from 2780 to 1084 mm and DB varied from 20.5 to 9.1 Mg ha-The h and f<sub>c</sub> were significantly greater at higher irrigation levels, while they were also significantly greater in AZ6 than AZ2. The DB, RY, and ReY generally increased linearly with TWA. However, it was found that a treatment applying every other irrigation of the control resulted in statistically equivalent yields to the control, with 36% less irrigation. The %R generally decreased with TWA, while %Re did not change. However, DB, %R, and %Re were significantly greater for AZ2 than AZ6, as were RY, ReY, and WP. Among the deficit treatments evaluated, every other irrigation offers the best strategy to significantly increase guayule WP without causing a yield penalty.

# 1. Introduction

Guayule (*Parthenium argentatum*, A. Gray) is a perennial shrub native to the desert of northcentral Mexico and southwestern Texas that produces high quality natural rubber (NR) appropriate for usage in commercial-grade tires (Rasutis et al., 2015; Eranki et al., 2018). Besides NR, guayule produces valuable co-products such as resin and biofuels (Nakayama, 2005; Rasutis et al., 2015). Guayule commercialization efforts to achieve a domestic supply of NR have a longstanding history in the U.S. desert Southwest (Ray et al., 2010), where efforts have accelerated in recent years due to increasing NR demands and disruptions in imported NR supplies from overseas (Eranki et al., 2018). A crucial breakthrough to advancing commercialization is that guayule crops can now be successfully established in fields by direct-seeding in soil rather than by transplanting, a method which has proven to be cost-prohibitive for growers (Elshikha et al., 2021; Wang et al., 2020). Optimal seed

https://doi.org/10.1016/j.agwat.2022.108093

Received 23 August 2022; Received in revised form 9 November 2022; Accepted 4 December 2022

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germination rates for the most available cultivar, AZ-2, occur when average daytime air temperatures are about 22 °C (Wang et al., 2020). The optimal planting windows for germination in the region are either late Apr. to mid-May, or mid-Oct. The Apr.-May planting is desirable because of the subsequent weather conditions needed for achieving active growth.

A significant challenge to expanding guayule production is that drastic water reductions for agriculture are occurring due to ongoing drought conditions in U.S. desert regions. Direct-seeded guayule can require a significant amount of irrigation for germination and crop establishment. The shallow-planted seed needs to be wetted on a neardaily basis to keep the surface soil wet until emergence is complete, which may take two-three weeks (Bucks et al., 1986). Following emergence, irrigation is needed until the crop is fully established, two-three months after planting (Bucks et al., 1986). The amount of irrigation needed to meet crop evapotranspiration (ET<sub>c</sub>) of guayule has been determined to be on the order of 1300-1500 mm per year (Bucks et al., 1985b; Nakayama, 1991; Hunsaker and Elshikha, 2017). During guayule's active growing period in the U.S. desert (early-spring through mid-fall), biomass, measured on a dry weight basis (DB), increases with irrigation, typically linearly for two- to four-year old shrubs (Bucks et al., 1985c; Nakayama et al., 1991; Hunsaker et al., 2019). While the rubber-bearing tissues are produced in the stems, branches, and roots during active growth, it is widely believed that the percent rubber (%R) content of the shrub is increased during periods of limited growth due to soil water deficit and other stresses, such as cold temperatures (Benedict et al., 1947; Kelly, 1975; Wang et al., 2022). As a perennial desert shrub, once guayule is established after planting, it is extremely drought tolerant and can survive extended periods without water application (Bucks et al., 1985a; Foster and Coffelt, 2005). However, the relative gain in %R due to high soil water stress and limited growth may or may not be enough to achieve rubber yield (RY) similar to or greater than that for fully-irrigated guayule. Disparate results on the relationship between RY and soil water depletion exist in the literature. Many studies on guayule grown in lighter soil types in U.S. desert areas have shown that the RY was significantly greater when the root zone soil water depletion during active growth was maintained at less than  $\approx 55-65\%$ versus higher depletion levels (Hunter and Kelley, 1946; Miyamoto et al., 1984; Bucks et al., 1985a, 1985b; Hunsaker and Elshikha, 2017; Elshikha et al., 2021). However, exceptions to those results are found in the literature. For example, Veihmeyer and Hendrickson (1961), on a sandy loam soil in the state of California, showed no difference in RY after two years between a well-irrigated treatment (maximum  $\approx 40\%$ depletion), a treatment irrigated about every other time of the former, and a treatment not irrigated during the entire second year. The literature on guayule yield responses to irrigation for studies conducted on heavier soil types that have higher soil water retention is not extensive, though the body indicates that less rather than more irrigation increases RY. The Hunter and Kelley (1946) results on a silty clay loam soil in California show significantly less RY was attained when soil water depletion was maintained at  $\approx$  25% versus much higher depletion levels. They provide evidence that maintaining low soil water depletion during the first year of growth and then terminating irrigation during the second summer of guayule does not curtail DB but does increase %R compared to the well-watered treatments. Further, Hunter and Kelley (1946) show significantly greater %R and RY for treatments with minimal irrigation after crop establishment, where soil water depletion reached  $\approx$  100% for an appreciable amount of time. In more recent studies in Arizona using direct-seeding on a clay to silty clay loam soil, Elshikha et al. (2021) and Wang et al. (2022) reported greater RY for the treatment receiving 50% less irrigation (maintained at about 75% soil water depletion) than that applied to the 100% ET<sub>c</sub> replacement treatment (55% maximum soil water depletion). Also, significantly greater water productivity (WP; rubber yield per unit of total water applied, TWA) [Pereira et al. (2012)] was achieved with 50% less irrigation in that study. Benedict et al. (1947) and Veihmeyer and Hendrickson

(1961) suggest that a significant increase in %R accumulation can be forced in guayule by alternating periods of low and high soil water deficits compared to maintaining low soil water deficits over the growing period, as attained with fully-irrigated guayule. They indicate these alternating periods of soil water deficit should be maintained throughout the active growing season, rather than in the non-active winter growth period.

The natural plant resin in guayule is found in all parts of the shrub including the leaves. Compared to RY, there has been less attention focused on studying irrigation strategies to enhance the resin yield (ReY) of guayule. The percent resin (% Re) content of the whole plant generally varies from about 7–10% Ray et al. (2010) and has been shown to either increase or decrease with irrigation amount in field studies (Bucks et al., 1985b; Hunsaker and Elshikha, 2017; Hunsaker et al., 2019; Elshikha et al., 2021). For guayule grown in the above studies for  $\approx$ two years, the ReY increased (often significantly) at higher irrigation amounts on lighter soils but showed less variability among irrigation levels on the heavier soil (Elshikha et al., 2021).

Guavule growers in the U.S. Southwest will likely not have an irrigation delivery close to that needed to meet 100% of  $ET_c$  (1300–1500 mm per year). For guayule grown on lighter soils, some literature indicates an irrigation deficit of 20-25% for full ET<sub>c</sub> would not reduce RY significantly (Bucks et al., 1985a, 1985b; Hunsaker and Elshikha, 2017; Elshikha et al., 2021). However, on heavier soils there appears to be a greater opportunity to reduce irrigation water use even much more than 25%. In this study, we hypothesize that certain deficit irrigation strategies that impose high water stress during a specific period of the guayule's ontogeny or throughout the entire growing season might result in equivalent or even higher rubber yields, due to increased rubber content, compared to fully-irrigated guayule. In particular, reduction of irrigation during summer when ET<sub>c</sub> demands and growth are highest may provide a significant increase in the RY-WP. To date, guayule irrigation research on growth and yield responses to specifically planned soil water deficits is limited and almost totally absent for direct-seeded guayule. Therefore, we conducted a field experiment on a heavier soil type in central Arizona, that exposed direct-seeded guayule to various pre-determined soil water deficit periods over a 22.5 month-long growing season. The objectives of the study were 1) to evaluate the effects of planned deficit irrigation on guayule growth parameters, DB, %R, %Re, RY, ReY, and WP and 2) to determine optimum deficit irrigation strategies for two cultivars that are being considered for guavule commercialization.

# 2. Materials and methods

### 2.1. Experimental design, planting, and crop establishment

A direct-seeded guayule irrigation experiment began in Apr. 2020 at a 1.60-ha field located at the Bridgestone Americas, Inc., Guayule Research Farm in Eloy, Arizona, USA (32.67 °N lat.; 111.63 °W long.; 482 m a.s.l.). The field consisted of a total of 36, 100-m long plots that were each 4.06 m wide. The experimental design was a randomized split-plot design, in which the field was divided into three blocks, having six irrigation treatments (denoted as I<sub>1</sub> to I<sub>6</sub>) in 18 whole plots and two guayule cultivars (denoted as AZ2 and AZ6) the split-plot treatment within whole plots. The six irrigation treatments were randomly assigned within each block and then AZ2 and AZ6 were randomly assigned within each irrigation treatment of the block. The field layout, irrigation methods, and irrigation treatments are described in more detail below in this section and in Section 2.4, while the two cultivars used in the study are described below.

On Apr. 20–21, 2020, seed of cultivar lines AZ2 and AZ6 were planted in the plot rows (1.016 m apart) using a four-row planter (Miniseeder,<sup>1</sup> a precision vacuum planter by Monosem Inc., Edwardsville, KS, USA), pulled behind a power train tractor. Seeding was in dry raised beds, 0.25-m wide and 0.15–0.20 m high. The AZ2 line is an interspecific hybrid with good seedling vigor and high biomass production (Ilut et al., 2017). In earlier trials, AZ6 achieved less biomass than AZ2 but produced higher %R than AZ2 (Ray et al., 1999). Of the six registered AZ cultivars, the AZ2 and AZ6 were shown to have the highest RY after two years of growth in the trial plots (Ray et al., 1999).

Following planting, a solid-set sprinkler system was installed in the field to irrigate near-daily in all plots for three weeks after planting (Apr. 22-May 11, 2020) for seedling germination, as recommended by Dissanayake et al. (2008). After sprinklers were removed, guayule seedlings in all plots were kept well-watered by irrigating several times during the next seven weeks (May 13-Jun. 21, 2020) using level furrow irrigation (Martin and Gilley, 1993), a common surface irrigation method used in the area. Irrigation treatments were initiated in mid-July 2020 using the level furrow irrigation method. The guayule crop was grown for approximately 22.5 months and the experiment was terminated following plot harvests on Mar. 01, 2022 (described in Section 2.5).

### 2.2. Climatic parameters

The weather data used in the study were obtained from the Arizona Meteorological Network station in Coolidge, Arizona (AZMET; https:// cals.arizona.edu/AZMET/05.htm), located  $\approx$  33 km directly north of the Eloy field site. Plots of averaged daily historical weather parameters at the station (Jan. 2003 through Dec. 2021) show the long-term patterns (Fig. 1a). Yearly maximum daily air temperatures (T<sub>a max</sub>) typically increase from around 20 °C in Jan.-Feb. to above 40 °C from early-Jun. through mid-Jul. The historical T<sub>a max</sub> values decline slightly below 40  $^\circ\text{C}$  during the mid-Jul. to mid-Sep. summer monsoon period. From start of fall through the end of Dec.,  $T_{a\ max}$  typically declines from about 36–16 °C, respectively. Long-term minimum daily air temperatures (Ta  $_{min}$ ) follow similar yearly patterns as T<sub>a max</sub> (Fig. 1a). The T<sub>a min</sub> can often be less than 0 °C during Dec. and Jan. months. The historical minimum relative humidity (RH<sub>min</sub>) and precipitation are lowest in May and Jun and highest during winter months and during summer monsoon months (Fig. 1a). Historical data indicate a mean yearly precipitation of 158 mm at the station, where the two primary rainy seasons occur from Dec. to Feb. and from Jul. to Sep (Fig. 1a). The historical daily standardized Penman-Monteith grass-reference evapotranspiration (ET<sub>o</sub>; Allen et al., 1998) varied from 1.6 mm in late-Dec. to 8.9 mm in mid-Jun (Fig. 1a). The mean yearly ET<sub>o</sub> for the 19 years was 1790 mm. At the station, seasonal differences in daily wind speeds at 2.0-m height  $(u_2)$  were not extreme, though they were highest in the months of Apr. to Jun. (averaging  $2.1 \text{ m s}^{-1}$ ) and lowest in the months of Sep. and Oct. (averaging 1.4 m s<sup>-1</sup>).

The daily weather parameters for the study period from late Apr. 2020 through the end of Feb. 2022 are shown in Fig. 1b. After planting, high temperatures and low humidity conditions were dominant during the two-month guayule germination/establishment period (Apr. 23 to Jun. 21, 2020), where daily  $T_{a\max}$  averaged 38.7 °C ( $\approx$ 3.0 °C higher than historical) and daily RH<sub>min</sub> averaged 5.4% ( $\approx$ 3.5% lower than historical). During this period, the daily ET<sub>o</sub> averaged 8.2 mm compared to 7.6 mm for historical. The climate during the summer growth period in 2020 continued to be hotter and dryer than historical norms, where daily  $T_{a\max}$  averaged 41.0 °C and RH<sub>min</sub> averaged 13.1% from Jun. 22 to

Sep. 20. Historically,  $T_{a\ max}$  and  $RH_{min}$  average 39.0  $^{\circ}C$  and 19.8% during the summer period, respectively. Precipitation during summer 2020 was also particularly scant (26 mm) compared to historical (57 mm). Similarly, fall 2020 and winter months in 2020-2021 (Dec. 2020-Mar. 2021) were dryer and warmer than normal. In Fall 2020, the daily RH<sub>min</sub> and T<sub>a max</sub> 11.3% and 30.0 °C, respectively, about 8.4% lower and 2.7 °C higher than historical, respectively. For winter months of 2020-2021, the differences in average RH<sub>min</sub> and T<sub>a max</sub> compared to historical were similar to those in fall 2020. Cumulative daily ET<sub>o</sub> from planting in Apr. thru Dec. 31, 2020 was 1517 mm. This was about 10% greater than the mean historical ET<sub>o</sub> during the same timeframe (1386 mm). Weather parameters during May and Jun. 2021 in the second year growing period were not especially different than those in 2020 (Fig. 1b). However, a big difference between the 2020 and 2021 guayule growing years occurred during summer months, where in 2021, the RH<sub>min</sub> and precipitation were well above historical for Jul.-Sep. For calendar year 2021, cumulative ETo totaled 1820 mm, which was only 2% higher than mean historical ET<sub>o</sub> (1790 mm). Overall, the spring to fall growing period in 2021 was more moderate than that in 2020 in terms of temperature and aridity. On the other hand, the daily u<sub>2</sub> were higher during the months of Apr. to Jun in 2021 (averaging  $\approx 2.5 \text{ m s}^{-1}$ ) than in 2020 (averaging  $\approx 2.0 \text{ m s}^{-1}$ ). Weather data for the final two months of the season in Jan. and Feb. 2022 were generally similar to those in Jan. and Feb. of 2021.

# 2.3. Soil characteristics

The field-site is mapped as a Gadsden series [Fine-loamy, montmorillontic (calcareous), hyperthermic Vertic Torrifluvents] (USDA-SCS, 1991). These soils are predominantly clay or silty clay loam, having > 35% clay content. The soil has relatively high water holding capacity but low water intake, which can impede water penetration to deeper soil layers in the profile.

Soil textures at the field were determined for soil samples collected in May 2020 during the installation of neutron meter access tubes (described in Section 2.4). The samples were obtained within each of the 36 treatment plots at soil-depths from 0 to 2.1 m in 0.3 m increments. The soil textures were analyzed using the Bouyoucos hydrometer method (Gee and Bauder, 1986). Average soil type varied in the profile from silty clay soil in the top 0-0.3-m to a loamy sand soil at the deepest depth (Table 1). The field capacity (FC), permanent wilting point (PWP), and available water (FC-PWP) water content characteristics were estimated for each 0–0.30-m soil depth using the Soil Water Characteristics routine of SPAW (Soil-Plant-Air-Water), a USDA hydrologic simulation model (Saxton and Willey, 2005), by entering the measured soil texture fraction values. Soil organic matter contents were adjusted in the USDA routine to reflect typical organic matter contents at the soil depths as reported by USDA-SCS (1991) for the Eloy soil type. The estimated available water for depths varied from 0.156 to 0.176  $m^3 m^{-3}$  for the heavier soil types to  $0.102 \text{ m}^3 \text{ m}^{-3}$  for the loamy sand.

# 2.4. Field layout, irrigation system, soil water measurement, and irrigation treatments

Fig. 2 illustrates the guayule field experiment layout showing the three blocks (RI-RIII), the 18 irrigation treatment whole plots within the blocks (I<sub>1</sub>-I<sub>6</sub>), and the two cultivars within each irrigation treatment plot (AZ2 and AZ6). The 36 separate experimental plots are enumerated as P1-P36 in Fig. 2. Irrigation water was supplied by a wet-well pumping station from an irrigation pond reservoir at the farm. Level furrow irrigation water delivery occurred via an underground 18-zone distribution manifold (101.6 mm-diam., schedule 40, PVC), controlled with 18 separate solenoid valves (V1-V18 in Fig. 2) located at the head end of each irrigation plot. The system delivered water at a rate of  $15.8 \text{ L s}^{-1}$ , as measured by a calibrated in-line propeller-type water meter. After sprinklers were removed, each of the 18 irrigation treatment plots were

<sup>&</sup>lt;sup>1</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.



Fig. 1. Historical [2003–2021 years] (a) and study-period [2020–2022 years] (b) daily weather parameters obtained from the AZMET station in Coolidge, AZ, including maximum and minimum air temperatures (Ta max and Ta min), minimum relative humidity (RHmin), precipitation, windspeed at 2.0 m height (u2), and grass-reference evapotranspiration (ETo).

Table 1

Percent sand, clay, and silt, soil texture, field capacity (FC), permanent wilting point (PWP) water contents, and available water (FC-PWP) for the field site at Eloy, Arizona. Data were averaged by depth over all treatment plots.

| Depth<br>(m) | Sand<br>(%) | Clay<br>(%) | Silt<br>(%) | Soil<br>Texture    | FC<br>(m <sup>3</sup> /<br>m <sup>3</sup> ) | PWP<br>(m <sup>3</sup> /<br>m <sup>3</sup> ) | FC-<br>PWP<br>(m <sup>3</sup> /<br>m <sup>3</sup> ) |
|--------------|-------------|-------------|-------------|--------------------|---|--|---|
| 0-0.30       | 14.2        | 42.3        | 43.5        | Silty clay         | 0.398                                       | 0.239  | 0.159   |
| 0.30-0.60    | 17.5        | 23.7        | 58.8        | Silty loam         | 0.308                                       | 0.136  | 0.172   |
| 0.60-0.90    | 22.3        | 13.7        | 64.0        | Silty loam         | 0.281                                       | 0.105  | 0.176   |
| 0.90-1.20    | 11.8        | 32.5        | 55.7        | Silty clay<br>loam | 0.353                                       | 0.179  | 0.174   |
| 1.20–1.50    | 9.3         | 35.5        | 55.2        | Silty clay<br>loam | 0.370                                       | 0.197  | 0.173   |
| 1.50 - 1.80  | 6.3         | 51.0        | 42.7        | Silty clay         | 0.455                                       | 0.299  | 0.156   |
| 1.80-2.10    | 71.8        | 4.0         | 24.2        | Loamy<br>sand      | 0.165                                       | 0.063  | 0.102   |

irrigated separately using the level furrow method. Irrigation plots were blocked on the far end of the field with berms so that all water applied remained in the plot area. Because the two guayule cultivars were embedded within each irrigation treatment, both cultivars in the treatment were irrigated simultaneously.

In May 2020, 2.25-m long, 51-mm diameter galvanized steel access tubes were installed vertically in the soil in the 3rd row of each 4-row plot. During installation of access tubes for the neutron probe, using a tractor mounted soil sampler (model 25-TS, Giddings Machine Comp., Windsor, CO, USA), soil samples were collected, as described before in Section 2.3. A field-calibrated, neutron moisture meter [NMM] (model 503, Campbell Pacific Nuclear, Martinez, CA, USA) was used to measure volumetric soil water contents ( $\theta_v$ , m<sup>3</sup> m<sup>-3</sup>) from 0.15 to 1.95 m below

the surface in 0.30 m increments. Measurements began on May 21, 2020 and were made at all 36 plot locations every 7–9 days (May-Nov. 2020 and Apr.-Nov. 2021) and about every two weeks during winter months.

Differential treatment irrigation amounts were initiated on July 16, 2020, following the two months of establishment irrigations (Apr.-Jun. 2020). One treatment ( $I_1$ ) served as the baseline control treatment whose irrigation scheduling was based on a soil water balance (SWB) of the rooting depth ( $Z_r$ ), Eq. 1:

$$D_{r,i} = D_{r,i-1} - P_i - I_i - CR_i + RO_i + ET_{c,i} + DP_i$$
(1)

where  $D_{r,i-1}$  and  $D_{r,i-1}$  are the root zone depletion (mm) at the end of day i and day i-1, respectively, and  $_{Pi}$ ,  $I_i$ ,  $CR_i$ , ROi,  $ET_{c,i}$ , and  $DP_i$  are amounts of precipitation, net irrigation depth, capillary rise, runoff from the soil surface, crop ET, and deep percolation, respectively, on day i, all in units of mm.  $CR_i$  and  $RO_i$  were considered zero due to the low groundwater table and the use of blocked furrows, respectively. The inputs for  $I_i$  were the average measured irrigation depths given to the three  $I_1$  treatment replicates. Inputs of  $_{Pi}$  were provided by the AZMET weather station for the site. When  $_{Pi}$  was less 1.0 mm, it was assumed negligible and not applied in Eq. 1. Daily values of total available water (TAW<sub>i</sub>) of the daily rooting depth ( $Z_{r,i}$ ) were calculated as:

$$TAW_i = 1000 Z_{r,i}(FC - WP)$$
<sup>(2)</sup>

where TAW<sub>i</sub> is in mm,  $Z_{r,i}$  in m, and field capacity (FC) and wilting point (WP) are in m<sup>3</sup> m<sup>-3</sup>. The FC and WP used for TAW<sub>i</sub> were the average field values provided in Table 2 over the estimated  $Z_{r,i}$ . The limits for  $D_{r,i}$  in Eq. 1 are zero (at FC) and TAW (at PWP). Since  $D_{r,i}$  cannot be less than zero on a given day i following irrigation and/or precipitation, an amount for DP<sub>i</sub> was computed, when necessary, to balance Eq. 1, if  $D_{r,i}$  was less than zero on day i. Daily percent soil water depletion (%SWD<sub>i</sub>)

| P36 | AZ2 | 18 | 16 | Ν |
|-----|-----|----|----|---|
| P35 | AZ6 | >  |    | Î |
| P34 | AZ2 | 17 | 12 | U |
| P33 | AZ6 | >  |    |   |
| P32 | AZ6 | 16 | 11 |   |
| P31 | AZ2 | >  |    | ≣ |
| P30 | AZ2 | 15 | 13 | " |
| P29 | AZ6 | >  |    |   |
| P28 | AZ6 | 14 | 14 |   |
| P27 | AZ2 | >  | •• |   |
| P26 | AZ2 | 13 | 15 |   |
| P25 | AZ6 | >  |    |   |
| P24 | AZ2 | 12 | 11 |   |
| P23 | AZ6 | >  |    |   |
| P22 | AZ6 | 11 | 12 |   |
| P21 | AZ2 | >  |    |   |
| P20 | AZ2 | 10 | 13 |   |
| P19 | AZ6 | >  |    | ≣ |
| P18 | AZ2 | 6/ | 14 | " |
| P17 | AZ6 | -  | •• |   |
| P16 | AZ2 | 8/ | 15 |   |
| P15 | AZ6 | ^  |    |   |
| P14 | AZ2 | 11 | 16 |   |
| P13 | AZ6 | _  |    |   |
| P12 | AZ2 | 9/ | 11 |   |
| P11 | AZ6 | _  |    |   |
| P10 | AZ2 | /5 | 15 |   |
| P9  | AZ6 | _  |    |   |
| P8  | AZ2 | 4  | 13 |   |
| P7  | AZ6 | ^  | 2  | ~ |
| P6  | AZ6 | /3 | 16 | - |
| P5  | AZ2 | _  |    |   |
| P4  | AZ6 | 12 | 14 |   |
| P3  | AZ2 | -  |    |   |
| P2  | AZ6 | 1  | 12 |   |
| P1  | AZ2 | ^  | .2 |   |

**Fig. 2.** Layout of the guayule field experiment in Eloy, Arizona, showing the three blocks (R I-III), each having six irrigation treatments (denoted as I1 to I6) and the two guayule cultivars (AZ2 and AZ6) randomly assigned within each irrigation treatment. P1-P36 indicate the 36 plots in the experiment. The 18 separate irrigation control valves are indicated as V1-V18. Description of irrigation treatments are summarized in Table 2.

# Table 2

Summary of irrigation regime and primary soil deficit periods for guayule irrigation treatments during 2020 (first year) and 2021 (second year) at Eloy, Arizona.

| Treatment      | Irrigation regime   | Period of soil water deficit <sup>a</sup>   |
|----------------|---|---|
| I <sub>1</sub> | Sufficiently irrigated in 2020 and 2021   | None  |
| I <sub>2</sub> | Same as $I_1$ through Jul. 2021, then<br>no more irrigation   | Final seven months before<br>harvest  |
| $I_3$          | Same as $I_1$ through Mar. 2021, then<br>two more irrigations (mid-May and<br>late Sep.)            | Summer months Jun. to Sep. in 2021  |
| I <sub>4</sub> | Same as $I_1$ through late Jun. 2020,<br>then every other irrigation of $I_1$<br>starting Jul. 2020 | Alternating high and low stress<br>periods during active growth<br>in 2020 and 2021 |
| I <sub>5</sub> | Four irrigations after establishment,<br>two each during the first and second<br>years of growth    | JulAug. in 2020 and Jun<br>Aug. in 2021   |
| I <sub>6</sub> | Two irrigations after establishment,<br>one each during the first and second<br>years of growth     | JulAug. in 2020 and May in 2021 through harvest                                     |

<sup>a</sup> Indicates most significant water deficit periods after guayule germination/ establishment irrigation ended June 21, 2020. was calculated as:

$$\text{\%SWD}_{i} = 100 \times \left[1 - \left(\text{TAW}_{i} - \text{D}_{r,i}\right)/\text{TAW}_{i}\right]$$
(3)

where  $SWD_i$  is in percent and  $TAW_i$  and  $D_{r,i}$  are as previously defined.

The daily  $Z_r$  for the SWB of Eq. 1 began with an initial depth of 0.60 m for the first 60 days after planting (DAP), was increased to a depth of 1.20 m for 61-210 DAP, and then was increased to a maximum Zr of 2.0 m from 211 DAP (mid-Nov. 2020) through the end of the growing season. The modeled changes in Zr were developed from soil water depletion patterns observed during the first seven months of spring-planted, direct-seeded guayule, as reported by Elshikha et al. (2021). The I<sub>1</sub> irrigation scheduling was intended to maintain %SWD<sub>i</sub> between 20% and 65%. To minimize DP<sub>i</sub>, irrigation application amounts for I1 were planned to restore 80% of calculated Dr.i (in mm). Limiting the maximum %SWD to 65% for I1 provided a depletion level that has been reported as sufficient for guayule to minimize the effects of reduced ET<sub>c</sub> due to water stress (Hunsaker and Elshikha, 2017; Bucks et al., 1985a, 1985b). Initiation of Eq. 1 was on the day of seeding (i.e., day i-1) where it was assumed that the  $D_{r,i-1}$  in Eq. 1 for the 0.60-m depth was the TAW (calculated as 99 mm).

During the first  $\approx$  30 DAP, prior to NNM measurements of  $\theta_v$ , Eq.1 used the dual crop coefficient procedures of FAO-56 (Allen et al., 1998) to estimate ET<sub>c,i</sub>:

$$\mathrm{ET}_{\mathrm{c},\mathrm{i}} = \left(\mathrm{K}_{\mathrm{cb},\mathrm{i}} + \mathrm{K}_{\mathrm{c},\mathrm{i}}\right) \times \mathrm{ET}_{\mathrm{o},\mathrm{i}} \tag{4}$$

where  $\text{ET}_{c,i}$  is estimated (mm),  $K_{cb,i}$  is the basal crop coefficient (unitless),  $K_{e,i}$  is the soil evaporation coefficient (unitless), and  $\text{ET}_{o,i}$  is the Penman-Monteith grass-reference evapotranspiration (mm), provided by the AZMET weather station for the site. Prior to  $\theta_v$  measurements, the  $K_{cb,i}$  was estimated as 0.20 based on  $K_{cb}$  data derived for early-season, well-watered, direct-seeded guayule (Elshikha et al., 2021). The  $K_{e,i}$  values were calculated following  $I_i$  and  $_{Pi}$  events using FAO56 methods, which require a separate SWB of the soil evaporation layer ( $Z_e$ ), assumed as 0.10 m:

$$D_{e,i} = D_{e,i-1} - P_i - I_i / f_{w,i} + E_{s,i} / f_{ew,i} + DP_{e,i}$$
(5)

where D<sub>e,i</sub> and D<sub>e,i-1</sub> are the cumulative depth of evaporation following wetting of the exposed and wetted fraction of the surface soil at the end of day i and end of day i-1, respectively,  $_{Pi}$  and  $I_i$  are as previously defined, E<sub>s,i</sub> is soil evaporation, and DP<sub>e,i</sub> is deep percolation loss if soil water content exceeds FC on day i, where all these variables are in units of mm. The  $f_{w,i}$  and  $f_{ew,i}$  are the fraction of the soil surface wetted by  $P_{Pi}$ and Ii and the exposed and wetted fraction, respectively, on day i (dimensionless). For the Ze SWB calculations, value of the readily evaporable water (REW) was 10 mm for the silty clay surface soil and total evaporable water (TEW) was calculated using the FC and WP values for the 0-30 m depth (Table 2) using the TEW equation in Allen et al. (1998). The TEW was calculated as 27.9 mm for the 0.10-m soil evaporation layer and TEW was the D<sub>e.i-1</sub> value used in the model at seeding. Following  $I_i$  and  $P_i$  events > 1.0 mm, depletion of  $Z_e$  was calculated on a daily basis by Eq. 5. Stage 1 drying (energy limiting) occurred until cumulative  $D_{e,i}$  was greater than REW. Stage 2 drying (falling rate) was determined by Eq. 6:

$$\mathbf{K}_{\mathbf{r},\mathbf{i}} = \left(\mathrm{TEW} - \mathbf{D}_{\mathbf{e},\mathbf{i}-1}\right) / (\mathrm{TEW} - \mathrm{REW}) \tag{6}$$

where  $K_{r,i}$  is the daily evaporation reduction coefficient calculated for  $D_{e,i\cdot1}>$  REW, whereas  $K_{r,i}=1.0$  during stage 1 drying. The  $K_{e,i}$  were computed as:

$$\mathbf{K}_{e,i} = \min \left[ \mathbf{K}_{r,i} \left( \mathbf{K}_{cmax,i} - \mathbf{K}_{cb,i} \right), \mathbf{f}_{ew,i} \times \mathbf{K}_{cmax,i} \right]$$
(7)

where  $K_{r,i} K_{cb,i}$ , and  $f_{ew,i}$  in Eq. 7 are as previously defined; and  $K_{cmax,i}$  is the maximum daily value of the combined  $K_{cb,}$  and  $K_{e,i}$  coefficients that can occur following  $I_i$  or  $_{Pi}$  and is a function of mean daily  $u_2$ , daily

 $\rm RH_{min},$  and estimated daily crop height (h). The limit for  $\rm K_{cmax,i}$  was constrained to  $\rm K_{cb,i}$  + 0.05 (Allen et al., 1998). Other parameters required in the  $\rm K_{e,i}$  calculations include fraction of canopy cover ( $\rm f_c$ ) and the wetting fractions ( $\rm f_w$ ) of the irrigation systems, which were 1.0 for both sprinkler and level furrow irrigation (i.e., 100% surface water coverage). For the h and  $\rm f_c$ , we used the average I<sub>1</sub> treatment height and cover that were measured intermittently (Section 2.5) and interpolated for daily estimates.

Starting on May 21, 2020, measured  $\theta_v$  was used to quantify the actual ET<sub>c</sub> (ET<sub>c act</sub>) for the I<sub>1</sub> treatment SWB model (Eq. 1). Actual ET<sub>c</sub> was calculated as the residual of the SWB for periods bounded by two adjacent dates of  $\theta_v$  measurements (Eq. 8):

$$ET_{cact} = (D_{r,2} - D_{r,1}) + I + P - DP$$
(8)

where  $\text{ET}_{c \text{ act}}$  is the total actual ET (mm) that occurred in the period from the first (1) to second (2) measurement date,  $D_{r,1}$  and  $D_{r,2}$  are the average measured depletion (mm) for the six I<sub>1</sub> plots (both cultivars) on the first and second date, respectively. The I, P, and DP, respectively, are total depth of average treatment measured irrigation (mm), total measured precipitation, and total deep percolation below the root zone (mm) that occurred during the period. The total ET<sub>c act</sub> for each period were used to model actual ET (ET<sub>c act,i</sub>) on a daily basis, where the sum of ET<sub>c act,i</sub> was required to be equal to the total ET<sub>c act</sub> in the period but whose individual values varied according to daily ET<sub>o</sub> and daily soil evaporation. The ET<sub>c act,i</sub> values in each period were found by iteration of the FAO56 dual K<sub>c</sub> calculations until daily values of (K<sub>cb</sub> + K<sub>e,i</sub>) ET<sub>o,i</sub> summed to total ET<sub>c act</sub> in the period (Eq. 9):

$$ET_{cact} = \sum_{i=1}^{j} 1^{1} (K_{cb} + K_{e,i}) = \sum_{i=1}^{j} 1^{1} ET_{cact,i}$$
(9)

where  $\text{ET}_{c \text{ act}}$  is the total (mm) for all days from 1 to j in a given period (determined in Eq. 9),  $K_{cb}$  is a uniform (single value) basal crop coefficient for each day i to j in a given period,  $K_{e,i}$  is the soil evaporation coefficient on day i, and  $\text{ET}_{o,i}$  is the PM  $\text{ET}_{o}$  (mm) on day i, and  $\text{ET}_{c \text{ act},i}$  is daily actual crop ET (mm) on day i. The  $K_{e,i}$  were determined with the FAO56 dual  $K_c$  procedures using the same  $K_e$  parameters described earlier.

After two months of guayule germination/establishment irrigations ending on Jun. 21, 2020, the I<sub>1</sub> treatment was irrigated another six times through mid-Nov. 2020. Irrigation for the I<sub>1</sub> treatment during the year 2020 was terminated after mid-Nov. 2020 irrigation. From March 2021 (late winter) onward, the I<sub>1</sub> treatment received a total of 10 irrigations in calendar year 2021, where its final irrigation before harvest was in late-Oct. 2021. The five other treatments, I<sub>2</sub>-I<sub>6</sub>, each received the same irrigation as I<sub>1</sub> through Jun. 21, 2020. The principles regarding the irrigations scheduling for the I<sub>2</sub>-I<sub>6</sub> treatments after the establishment irrigations are as follows:

Considering the suppositions of Hunter and Kelly (1946) on a silty clay loam soil, the I2 and I3 treatments were fully-irrigated for the first 15 and 11 months after planting, i.e., through early Jul. 2021, and early Mar. 2021, respectively (Table 2). Through those dates, the I<sub>2</sub> and I<sub>3</sub> were irrigated on the same days with essentially the same amounts as I<sub>1</sub>. The assumption was that  $ET_c$  and DB accumulation for the  $I_2$  and  $I_3$ treatments up to those points in the growing season would be comparable to that in the fully-irrigated I1 treatment. In previous direct-seeded guayule experiments at Eloy, fully-irrigated guayule achieved 100% cover at 10 months after planting in April, whereas maximum guayule biomass was achieved by 15 months (Elshikha et al., 2021; Wang et al., 2022). Thus, the premise behind the  $I_2$  treatment strategy was that after 15 months of growth, a significant amount of irrigation water could be saved by terminating irrigation altogether while achieving about the same DB expected for the full irrigation schedule of I<sub>1</sub> treatment. Further, the soil water stress that would be imposed upon I<sub>2</sub> during the final seven months prior to harvest (Table 2) might result in higher %R versus I1 and thus higher RY. The hypothesis for the I3 treatment strategy

was similar to that for  $I_2$  except that  $I_3$  after 11 months of full irrigation was given a single spring and a single fall irrigation in 2021 (Table 2). We considered that these two irrigations would boost the treatment's biomass accumulation somewhat in 2021 but by not irrigating  $I_3$  during the peak  $ET_c$  and growth period of summer, total water use would be reduced and rubber production would be stimulated due to the stress imposed during the summer period. Although final biomass for  $I_3$  under this scenario was anticipated to be less than that for the fully-irrigated  $I_1$ , it was also expected that%R would be higher for  $I_3$  than  $I_1$ , due to the high soil water deficit imposed during the summer of 2021 (Table 2). The irrigation amounts applied in spring and fall 2021 to  $I_3$  were based on soil water content measurements, such that the soil water depletion measured just prior to the irrigations was fully replenished to FC.

The I<sub>4</sub>-I<sub>6</sub> treatments were designed to re-consider some of the successful results of reduced irrigation presented in early guayule research by Hunter and Kelley (1946), Benedict et al. (1947), and Veihmeyer and Hendrickson (1961). All refer strategies to "force" higher %R accumulation in shrubs by alternating periods of high and low water stress during the active growing season. The I<sub>4</sub> treatment was to receive  $\approx 50\%$ of the irrigation water needed for the fully-irrigated guavule I<sub>1</sub> treatment after crop establishment. Starting in mid-Jul. 2020, the I<sub>4</sub> irrigation timing followed the I<sub>1</sub> schedule but I<sub>4</sub> irrigations were only applied  $\approx$  every other time the I<sub>1</sub> treatment was irrigated (Table 2). Thus, the I<sub>4</sub> scheduling regime was similar to a Veihmeyer and Hendrickson (1961) alternating irrigation treatment but it was started in the first year of our experiment. The irrigation water applied to I4 were planned to be the same amounts as those given to I<sub>1</sub> on every other irrigation date. Thus, when I4 was not irrigated, the soil water stress in the treatment would increase, whereas when not irrigated, the water stress would be alleviated. The I<sub>5</sub> and I<sub>6</sub> treatments (Table 2) were included to evaluate the effects of much more prolonged periods of extreme soil water deficit followed by infrequent periods of low water stress through occasional irrigation. After the last establishment irrigation, the I5 treatment was to receive only two additional irrigations in the first year of growth, planned for late summer-2020 and late winter-2021, plus two irrigations during the second year of growth, planned for mid-spring-2021 and early fall-2021. For I<sub>6</sub>, one additional irrigation was given during the first year of growth, (late summer-2020), plus one during the second year of growth (early-spring-2021). As for I<sub>3</sub>, the supplemental irrigation amounts applied to I5 and I6 replaced the soil water depletion measured just prior to the irrigations to FC.

Fertilizer was applied to all treatments on Jul. 15, 2020, in the form of urea-ammonium-nitrate (32% N), which was injected into the water to all treatment plots at a rate of 65 kg N ha<sup>-1</sup>. The same rate was applied to treatments in Mar. 15, 2021. This rate, 65 kg N ha<sup>-1</sup> per year, was suggested by Bucks et al. (1985a) for adequate growth for guayule plants and was used in recent guayule studies by Hunsaker and Elshikha (2017) and Hunsaker et al. (2019). The injection was done using a single head hydraulic diaphragm chemigation injection pump (Baldor Motor VL3504, Santa Fe Springs, CA, USA). The pump was connected, through injection ports, to the mainline delivering the water to the furrow plots.

# 2.5. Plant growth measurements

Manual measurements of guayule h and canopy widths were made for nine plants per plot starting on June 12, 2020. Thereafter, h and width measurements were made about every 30 days, which continued until Mar. 2022. Canopy width data were used to calculate  $f_c$  as a percent by Eq. 10:

$$f_c = (W_{ew} \times W_{ns})/(1/P_d) \times 100$$
<sup>(10)</sup>

where  $W_{ew}$  is plant width in the east-west direction, m,  $W_{ns}$  is plant width in the north-south direction, m, and  $1/P_d$  is planting area (1/plant density  $[P_d]$ ), m<sup>-2</sup>.

Whole plant samples were harvested on Mar. 01, 2022, 22.5 months

after planting. Three,  $2 \cdot m^2$  sections from each plot were hand-harvested. All plant harvests were limited to the inner two rows of each plot to minimize any edge effects on plant growth. Plants were cut at the ground level (stover) and immediately weighed for fresh weight and then airdried outdoors on shaded wire shelves for 7 days and re-weighed for dry weight (Coffelt and Ray, 2010). Plants were shredded and ground before a subsample was taken and placed in the oven at 140 °C for 24 h to remove remaining moisture and then re-weighed. Samples were analyzed at Bridgestone Americas Inc., Eloy, for %R and %Re, determined using a Soxhlet-based near-infrared spectroscopy (NIR) method that has high correlation to other guayule rubber analysis methods (Placido et al., 2020).

### 2.6. Statistical analyses

Irrigation and cultivar effects were analyzed statistically using a randomized split-plot model with Proc Mixed (SAS Institute Inc, 2016) for the following parameters: h, f<sub>c</sub>, %R, %Re, DB, RY and ReY, and the WP of DB, RY, and ReY denoted as DB-WP, RY-WP, and ReY-WP, respectively. Irrigation treatment in whole plots were the main effect (6 levels); cultivar was the split-plot effect (2 levels); and both effects were considered as fixed effects, as was their interaction. Random effects were block and block by irrigation. Proc Mixed estimated the random components and the residual by the residual maximum likelihood REML. If the *F* tests for irrigation treatment and/or cultivar for a parameter were significant (p < 0.05), then least squares means for irrigation treatments and/or cultivars were compared using the *P*diff option in SAS (with significance at p < 0.05). Linear regression for certain parameters were performed using the Data Analysis Tools in Microsoft Excel.

### 3. Results

# 3.1. Irrigation, precipitation, soil water depletion, and crop evapotranspiration

The irrigation amounts and the number of irrigations applied to the six treatments are summarized in Table 3. Prior to differential irrigation, a total of 511 mm was applied (Apr.-Jun. 2020) to all treatment plots, initially, through a sprinkler system (293 mm), then, by level furrow irrigation (218 mm). In 2020, irrigation was terminated in mid-Nov. for the I<sub>1</sub>-I<sub>4</sub> treatments, whereas the final irrigation in 2020 for I<sub>5</sub> and I<sub>6</sub> was in late summer. Effective precipitation (events > 1.0 mm per day) totaled 25 mm in Jul. and Aug. 2020 and totaled 27 mm from mid-Nov. 2020 to the end of Feb. 2021. Irrigation was resumed in early Mar. 2021 for all treatments, except I<sub>6</sub> (early Apr. 2021). The final irrigations of the experiment were applied to I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>-I<sub>5</sub>, and I<sub>6</sub> treatments on Oct. 28, Jul. 05, Sep. 29, and Apr. 08, 2021, respectively.

Cumulative effective precipitation from Mar. 01, 2021, through harvest (Mar. 01, 2022) was 209 mm, about 50 mm above the normal yearly precipitation at the location.

Level furrow irrigation amounts for the  $I_1$  treatment ranged from 42 mm in May 2020–160 mm in May and Jun. 2021. The  $I_1$  irrigation frequency varied from 1 to 3 irrigations per month. Highest irrigation frequencies occurred in the spring and summer in both 2020 and 2021 (Table 3). Total irrigation applied during the 22.5 month growth period varied for treatments from 2519 mm for  $I_1$  to 822 mm for  $I_6$  (Table 3). Total effective precipitation during the period was 261 mm. The trends for the cumulative total water applied (TWA), i.e., cumulative irrigation plus cumulative precipitation, with time are shown for irrigation treatments in Fig. 3. Compared to the final TWA of the  $I_1$  treatment (Table 3), the reductions in TWA for the  $I_2$ - $I_6$  treatments were 17%, 33%, 33%, 51%, and 61%, respectively.

Average measured %SWD for irrigation treatments (including both cultivars) are shown from the beginning of NMM measurements in late May 2020 through late Feb. 2022 (Figs. 4a and b). During the first year

### Table 3

Irrigation amounts (A) and number of events (B) for irrigation treatments described in Table 2. The data were summed for each season (Spring [Mar. 21-Jun. 20], Summer [Jun. 21-Sep. 20], Fall [Sep. 21-Dec. 20] and Winter [Dec. 21-Mar. 20]). Total water applied (TWA) by irrigation and precipitation and total number of irrigation events are summed at the bottom of each treatment column.

| (A)          |                         | $I_1$           | $I_2$ | $I_3$ | I <sub>4</sub> | I <sub>5</sub> | $I_6$          |
|--------------|-------------------------|-----------------|-------|-------|----------------|----------------|----------------|
| 2020-21      | Spring-(S) <sup>a</sup> | 293             | 293   | 293   | 293            | 293            | 293            |
|              | Spring-(F) <sup>a</sup> | 137             | 137   | 137   | 137            | 137            | 137            |
|              | Summer                  | 456             | 374   | 374   | 201            | 224            | 216            |
|              | Fall                    | 332             | 332   | 332   | 281            | 0              | 0              |
|              | Winter                  | 90 <sup>b</sup> | 143   | 143   | 143            | 143            | 0              |
| 2021-22      | Spring                  | 549             | 605   | 172   | 310            | 172            | 176            |
|              | Summer                  | 386             | 152   | 0     | 100            | 0              | 0              |
|              | Fall                    | 275             | 0     | 137   | 137            | 137            | 0              |
|              | Winter                  | 0               | 0     | 0     | 0              | 0              | 0              |
|              | Total irrigation        | 2519            | 2035  | 1588  | 1602           | 1105           | 822            |
|              | Total irr. + prec.      | 2780            | 2296  | 1849  | 1863           | 1366           | 1084           |
| ( <b>B</b> ) |                         | $I_1$           | $I_2$ | $I_3$ | $I_4$          | I <sub>5</sub> | I <sub>6</sub> |
| 2020-21      | Spring                  | 17              | 17    | 17    | 17             | 17             | 17             |
|              | Summer                  | 4               | 4     | 4     | 2              | 2              | 2              |
|              | Fall                    | 3               | 3     | 3     | 2              | 0              | 0              |
|              | Winter                  | 1               | 1     | 1     | 1              | 1              | 0              |
| 2021-22      | Spring                  | 4               | 4     | 1     | 2              | 1              | 1              |
|              | Summer                  | 3               | 1     | 0     | 1              | 0              | 0              |
|              | Fall                    | 2               | 0     | 1     | 1              | 1              | 0              |
|              | Winter                  | 0               | 0     | 0     | 0              | 0              | 0              |
|              | Total                   | 34              | 30    | 27    | 26             | 22             | 20             |

<sup>a</sup> Spring-(S) is the water applied during spring first 3 weeks after planting by sprinkler irrigation. Spring-(F) is water applied during spring through level furrow irrigation. S and F in spring were germination and establish irrigations. Note: all treatments received a furrow irrigation on Jun. 21, 2020 (first day of summer).

<sup>b</sup> The I<sub>1</sub> treatment irrigation water requirement was 143 mm for this late winter irrigation but was inadvertently underirrigated by the farm hand.



Fig. 3. Cumulative total water applied [TWA] (irrigation and precipitation) with time for the six irrigation treatments (I1-I6) described in Table 2.

of growth (Fig. 4a), average %SWD for the I<sub>1</sub> treatment slightly exceeded the 65% target by about 5% on two dates (Aug. 11 and Sep. 29, 2020). During the second year of growth (Fig. 4b), measured %SWD for the I<sub>1</sub> was  $\approx$  65% in mid-Apr. and early May but was less than 65% for all dates thereafter. Notably, higher %SWD than that of I<sub>1</sub> was observed for the I<sub>4</sub>-I<sub>6</sub> treatments starting in the latter-half of Jul. 2020, after initiation of soil water deficit periods for those treatments. Thereafter, the %SWD for the I<sub>5</sub> and I<sub>6</sub> treatments stayed consistently higher than that for I<sub>1</sub>, where they often exceeded 80–90% during their water deficit periods (Table 2). The irrigation regime of every other irrigation (I<sub>4</sub>) resulted in alternating periods of elevated and diminished differences in the I<sub>4</sub>%SWD compared to that for I<sub>1</sub>. The elevated %SWD mainly occurred during mid-Jul.-mid-Aug. in 2020, late-Jun.-early Aug. 2021, and early Nov. 2021-late Feb.





**Fig. 4.** Measured percent soil water depletion [%SWD] of irrigation treatments (averaged for AZ2 and AZ6 cultivars) in the first (A) and second (B) years of growth. Irrigation treatments in legends are described in Table 2.

2022 (Figs. 4a and b). During other times, I<sub>4</sub> and I<sub>1</sub> had similar %SWD. The measured %SWD for I<sub>4</sub> was greater than 80% on two dates (early-Aug. 2020 and early Ray 2021). As expected, marked increases in %SWD for the I<sub>2</sub> and I<sub>3</sub> treatments, compared to I<sub>1</sub>, did not arise until early spring 2021 (I<sub>3</sub>) and late summer 2021 (I<sub>2</sub>), following the cutback and termination of irrigation for those treatments (Table 2). The %SWD for I<sub>3</sub> was especially high during summer 2021 (82–89%), whereas it was 51–80% for I<sub>2</sub> during the same summer period. For measurement dates in Fig. 4a, the average and range of %SWD were 48 (11–71%), 47 (8–74%), 50 (9–78%), 53 (11–89%), 67 (8–91%), and 59% (3–83%) for I<sub>1</sub>-I<sub>6</sub>, respectively. For measurement dates in Fig. 4b, %SWD averaged 54 (34–68%), 69 (38–82%), 79 (55–89%), 72 (56–81%), 80 (56–93%), and 81% (65–88%) for I<sub>1</sub>-I<sub>6</sub>, respectively.

The combined estimated and actual daily ET<sub>c</sub> rate (averaged by month) and daily cumulative ET<sub>c</sub> for the 22.5 months of guayule growth are shown for the I<sub>1</sub> treatment in Fig. 5. In 2020, the daily ET<sub>c</sub> was estimated through May 20, 2020. The SWB calculations indicated that 71 mm of water percolated below the root zone during late-Apr.-late-May when germination/establishment irrigations were applied. Thereafter, calculated DP was minimal for the I<sub>1</sub> treatment and likely minimal in all other treatments, as well. Relatively high average daily ET<sub>c</sub> rates occurred during Apr. and May 2020, reflecting primarily soil evaporation contributions following frequent germination/establishment irrigations, first, through the sprinkler system and then by level furrow irrigation starting in early May. Starting in late Jun. 2020, level furrow irrigations to I<sub>1</sub> were much less frequent, resulting in less soil evaporation and lower average ET<sub>c</sub> values than in Apr.-May. As the crop grew during summer of 2020, average daily ET<sub>c</sub> increased from about 4.0 mm  $d^{-1}$  in Jun. until reaching maximum values ( $\approx 6.0 \text{ mm } d^{-1}$ ) in Aug. and Sep. 2020. Average monthly Kc values calculated for Jun., Jul., Aug., and Sep. 2020 data were 0.47, 0.68, 0.85, 1.03, respectively. After Aug., the monthly average ET<sub>c</sub> rate steadily declined until it fell to a minimum



**Fig. 5.** Estimated and actual crop evapotranspiration (ETc) showing monthly averaged daily ETc rates (open square symbols) and cumulative daily ETc (single line) for the I1 treatment during the 22.5 months of guayule growth. Estimated ETc data were from Apr. 20 to May 20, 2020. Actual ETc data were from May 21, 2020, to Feb. 24, 2022.

value in Jan. 2021 of  $\approx 1.0~\text{mm}~\text{d}^{-1}$ . During the winter dormancy months of Feb.-Mar. 2021, the ET<sub>c</sub> rate increased slightly, and then increased rapidly starting in spring 2021. The maximum monthly ET<sub>c</sub> rate was 10.3 mm d<sup>-1</sup> in Jun. 2021. The corresponding monthly K<sub>c</sub> value in Jun. was about 1.16. The climate in Jul. and Aug. 2021 was much more humid than Jun. and precipitation was above historical normal. During those months, average ET<sub>c</sub> declined to  $\approx 8.0~\text{mm}~\text{d}^{-1}$ . However, compared to Jun., the monthly K<sub>c</sub> for Jul. and Aug. increased (1.26 and 1.33, respectively), perhaps due to higher soil evaporation from the precipitation. The ET<sub>c</sub> continued to decline until it reached a minimum value (0.5 mm d<sup>-1</sup>) in Dec. 2021 and had a corresponding K<sub>c</sub> of about 0.30. The ET<sub>c</sub> rate increased to a value of 1.0 mm d<sup>-1</sup> in Jan. and Feb. 2022, shortly before harvest.

As the monthly averaged  $\text{ET}_c$  data would suggest, cumulative daily  $\text{ET}_c$  for the I<sub>1</sub> treatment rose rapidly from Jun. to Sept. 2020 and then leveled off during the winter dormancy period, starting in Dec. (Fig. 5). The rate of increase in cumulative  $\text{ET}_c$  was higher during the second summer of 2021 than during the first year of growth. The trend in cumulative  $\text{ET}_c$  during winter dormancy periods was similar in both years. Cumulative  $\text{ET}_c$  was 1250 mm from late Apr. through Dec. 2020, 1860 mm from Jan. 01 to Dec. 31, 2021, and 55 mm Jan.01-Feb. 24, 2022. For the 22.5 months of growth, the total  $\text{ET}_c$  was 3165 mm.

### 3.2. Plant growth

Guayule h for irrigation treatments with time are shown separately for the AZ2 and AZ6 cultivars (Figs. 6a and b, respectively). Statistical analysis indicated that h was significantly greater for AZ2 than AZ6 plants starting from the first measurements made in early Jul. 2020, while there was no significant differences for h between irrigation treatments until mid-Oct. 2020, or 5.5 months after planting. Differences in h between the cultivars were significant throughout the entire growing period with AZ2 having taller plants than AZ6. From early Nov. 2020 through late Apr. 2021, the h for I<sub>1</sub> was significantly greater than h for I<sub>4</sub>-I<sub>6</sub> but not the h for the I<sub>2</sub> and I<sub>3</sub> treatments. The h differences from late May-early Nov. in 2021 were consistently greater for I<sub>1</sub> and I<sub>2</sub> than for I<sub>5</sub> and I<sub>6</sub> for AZ2 but not for AZ6. Final measurements, just before harvest in 2022, revealed that mean h for the I<sub>1</sub> and I<sub>2</sub> were both significantly greater than those for I<sub>3</sub>-I<sub>6</sub> for AZ2, whereas the h for I<sub>1</sub> and I<sub>2</sub> were only significantly greater than the I<sub>6</sub> treatment for AZ6.

Percent fractional canopy cover increased for irrigation treatments from early Jul. until Nov. 2020 for both AZ2 and AZ6 (Figs. 7a and b, respectively). Similar to h results,  $f_c$  was significantly greater for AZ2 than AZ6 in Jul. and Aug. 2020, when differences for  $f_c$  between



**Fig. 6.** Plant height (h) with time for irrigation treatments for AZ2 (A) and AZ6 (B) guayule cultivars. Irrigation treatments are described in Table 2.



**Fig. 7.** Fractional canopy cover (fc) with time for irrigation treatments for AZ2 (A) and AZ6 (B) guayule cultivars. Irrigation treatments are described in Table 2.

irrigation treatments were not significant. For measurements from Sep. through early Feb. 2021, mean f<sub>c</sub> was greater for AZ2 than AZ6, though the differences varied between being significant and not significant during that time period. By late Apr. 2021 onward, the fc differences between cultivars were not significant. While AZ2 initially exhibited more vigorous cover development than AZ6 during the first summer, by early spring 2021, the mean  $f_c$  for AZ6 was the same as that for AZ2. Significant irrigation treatment effects on f<sub>c</sub> did not occur until mid-Nov. 2020, or about a month later than significant irrigation effects on h. In mid-Nov., the fc for treatments I1-I4 were significantly greater than for I6 but not I<sub>5</sub>. By late May 2021, the  $f_c$  were close to 100% for the I<sub>1</sub>-I<sub>4</sub> treatments in both cultivars and were significantly greater than those for the I<sub>5</sub> and I<sub>6</sub> treatments (f<sub>c</sub> less than 90%). From late Jun. onward, differences in f<sub>c</sub> due to irrigation were not significant, as the I<sub>5</sub> and I<sub>6</sub> treatments attained  $f_c$  greater than 90% in Jun. and  $\approx 100\%$  by early Oct. 2021.

# 3.3. Yield and water productivity

The DB means for both cultivars increased linearly with TWA and regression of DB vs TWA for treatments had a coefficient of determination ( $r^2$ ) of 0.85 (Fig. 8a). Statistically, both the main effect of irrigation and the cultivar effect on DB were significant, while irrigation by cultivar interaction was not significant. The DB mean for irrigation treatment was highest for the I<sub>1</sub> treatment and lowest for the I<sub>6</sub> treatment (Table 4). While the DB means for the I<sub>1</sub>, I<sub>2</sub>, and I<sub>4</sub> treatments were not significantly different from each other, they were each significantly greater than those for the I<sub>3</sub>, I<sub>5</sub>, and I<sub>6</sub> (Table 4).

Mean DB by cultivar was 9% higher for AZ2 than AZ6 and the difference was significant. As seen in Fig. 8a, DB differences between cultivars were more pronounced within the I<sub>3</sub>, I<sub>4</sub>, and I<sub>6</sub> treatments compared to the I<sub>1</sub>, I<sub>2</sub>, and I<sub>5</sub> treatments. However, statistical comparisons of cultivars within each irrigation treatment indicated that the DB mean only differed significantly between cultivars within the I<sub>4</sub> treatment (Table 4). It is notable that the mean DB of the AZ2 cultivar for the I<sub>4</sub> treatment was 19.2 Mg ha<sup>-1</sup>, which was only 8% lower than that for AZ2 for the I<sub>1</sub> treatment. Consistent with the irrigation mean results for cultivars, the AZ2 cultivar within the I<sub>4</sub> treatment had significantly greater DB than that for I<sub>3</sub>, which received essentially the same TWA as I<sub>4</sub>, as well as those for I<sub>5</sub> and I<sub>6</sub> (Table 4).

Irrigation and the cultivar effects on DB-WP were significant. Mean DB-WP for irrigation treatments was highest for I<sub>4</sub> closely followed by I<sub>5</sub>, where both treatments were significantly greater than the DP-WP for I<sub>1</sub> and I<sub>3</sub> (Table 4). While mean DB-WP for the I<sub>2</sub> and I<sub>6</sub> treatments were higher than for I<sub>1</sub> and I<sub>3</sub>, the differences were not significant. The mean DB-WP was significantly greater for AZ2 than AZ6, though statistical comparison of cultivars within each irrigation treatment indicated that only AZ2 within I<sub>4</sub> and I<sub>6</sub> treatments had significantly greater DB-WP than AZ6. The AZ2 cultivar within the I<sub>4</sub>, I<sub>5</sub>, and I<sub>6</sub> treatments achieved significantly greater DB-WP than all other irrigation-cultivar combinations, except for AZ6 within the I<sub>5</sub> treatment (Table 4). Numerically, DB-WP was the highest (1.03 kg m<sup>-3</sup>) for the AZ2 cultivar within the I<sub>4</sub> irrigation treatment.

The %R in plants at harvest was significantly different for irrigation treatment and cultivar and there was no interaction. The mean %R generally increased with decreasing TWA and increasing %SWD. Although mean %R for the I<sub>1</sub>, I<sub>2</sub>, and I<sub>4</sub> treatments was less than those the I<sub>3</sub>, I<sub>5</sub>, and I<sub>6</sub> treatments, significant differences in mean %R due to irrigation occurred only between the I<sub>5</sub> versus the I<sub>1</sub>, I<sub>2</sub>, and I<sub>4</sub> treatments (Table 4). For cultivars, mean %R was 20% higher for AZ2 than AZ6. Within all irrigation treatments, %R for AZ2 was significantly greater than those for AZ6. Statistically, %R was significantly greater for the AZ2 cultivar within the I<sub>5</sub> and I<sub>6</sub> treatments than those for all other irrigation-cultivar combinations, except for AZ2 within the I<sub>3</sub> treatment (Table 4).

Unlike %R, %Re was not significantly different due to irrigation.



**Fig. 8.** Means for AZ2 and AZ6 guayule cultivars obtained with different total water applied (TWA) for (a) dry biomass (DB), (b) rubber yield (RY), and (c) resin yield (ReY). The regression lines for DB and RY as a function of TWA had r2 of 0.85 and 0.59, respectively. Irrigation treatments (I1-I6) are summarized in Table 2.

While the cultivar effect on %Re was significant, interaction between irrigation and cultivar was also significant. Comparison of cultivars within irrigation treatments revealed that%Re was significantly greater for AZ2 than AZ6 within the I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, and I<sub>5</sub> treatments but not within the I<sub>4</sub> and I<sub>6</sub> treatments. Highest %Re occurred for the AZ2 cultivar within the I<sub>1</sub> and I<sub>2</sub> irrigation treatments, which were significantly

greater than %Re for all other irrigation-cultivar combinations, except those for AZ2 within the  $I_3$  and  $I_5$  treatments (Table 4).

As it was found for DB, the RY means for cultivars increased linearly with TWA, where the  $r^2$  of the linear regression was 0.59 (Fig. 8b). For RY, irrigation and cultivar effects were significant. The irrigation means for RY were highest for the I<sub>1</sub> treatment followed by I<sub>2</sub> and I<sub>4</sub>, which were not significantly different than for  $I_1$  (Table 4). Due to higher %R for the I<sub>3</sub> and I<sub>5</sub> compared to I<sub>2</sub> and I<sub>4</sub>, the RY for I<sub>3</sub> was not significantly different than that for I2, while RY for I5 was not significantly different than that for  $I_4$  (Table 4). The mean RY for the  $I_6$  treatment was significantly lower than the RY for all other treatments. The combination of higher DB and %R for the AZ2 than AZ6 cultivar resulted in AZ2 having 27% higher RY. The differences in RY for cultivars are clearly observed in Fig. 8b. Because differences in RY between cultivars were marked, linear regressions of RY versus TWA were performed separately for the cultivars, which gave high r<sup>2</sup> values to 0.86 and 0.91 for AZ2 and AZ6, respectively (data not shown). The maximum RY of 864 kg  $ha^{-1}$ was attained for AZ2 within the I1 treatment. The second and third highest RY were attained for AZ2 within the I4 and I2 treatments, which were about 10-11% lower than the RY for I<sub>1</sub>.

The RY-WP was also significantly affected by irrigation and cultivar, while there was no interaction. For irrigation, mean RY-WP was significantly greater for the I<sub>4</sub>, I<sub>5</sub>, and I<sub>6</sub> treatments than for I<sub>1</sub>, differences similar to those found for DB-WP (Table 4). However, for RY-WP, the mean was highest for the I<sub>5</sub> treatment then for I<sub>4</sub> vis-a-viz the DB-WP ranking. The mean RY-WP was significantly lower for the I<sub>1</sub> than all but the I<sub>2</sub> treatment. The cultivar differences were similar to that for RY, where mean RY-WP was 26% higher for AZ2 than AZ6. The best RY-WP was attained in the AZ2 cultivar within I<sub>5</sub>, closely followed by AZ2 within the I<sub>4</sub> and I<sub>6</sub> treatments (Table 4).

The ReY had similar irrigation treatment and cultivar trends with TWA to those shown for RY in Fig. 8b. The  $r^2$  of the linear regression of ReY vs TWA (Fig. 8c) was 0.69, higher than the  $r^2$  for RY vs TWA. For ReY, the irrigation and cultivar effects were both significant. Mean ReY was highest for the I<sub>1</sub> treatment but was not significantly greater than for I<sub>2</sub> and I<sub>4</sub> (Table 4). Both I<sub>1</sub> and I<sub>2</sub> means were greater than means for I<sub>3</sub>, I<sub>5</sub>, and I<sub>6</sub>, while the mean for I<sub>4</sub> was significantly greater than means for I<sub>5</sub> and I<sub>6</sub> only. For cultivars, the mean ReY for AZ2 was 24% greater than the mean for AZ6, as both DB and %Re were greater for AZ2 than AZ6. Because differences between cultivars were pronounced, linear regressions of ReY versus TWA were performed separately for the two cultivars. Those regressions increased the  $r^2$  to 0.93 and 0.85 for AZ2 and AZ6, respectively (data not shown). As with RY, maximum ReY was also obtained in the AZ2 cultivar within the I<sub>1</sub> treatment.

The mean ReY-WP were highest among irrigation treatments for I<sub>4</sub> and I<sub>5</sub>, which were both significantly greater than the ReY-WP for I<sub>1</sub> (Table 4). For ReY-WP, a significant cultivar effect occurred and there was no interaction. Within a given irrigation treatment, ReY-WP was significantly greater for AZ2 than AZ6 for the I<sub>1</sub>-I<sub>4</sub> treatments but not for the I<sub>5</sub> and I<sub>6</sub> treatments. Maximum RY-WP occurred for AZ2 within the I<sub>4</sub> treatment followed by AZ2 within the I<sub>5</sub> treatment.

The percent reductions in total irrigation (Total I) and TWA applied to the I<sub>2</sub>-I<sub>6</sub> treatments from that to the fully-irrigated I<sub>1</sub> amounts varied from 17% to 19% for I<sub>2</sub> to 61–67% for I<sub>6</sub> (Table 5). For the I<sub>2</sub>-I<sub>6</sub> treatments, the percent reduction in the mean yield parameters were less than those for TWA, except in the case of I<sub>3</sub>, which were about the same as the TWA reduction (Table 5). Relative to the reduction in TWA for treatments, yield reductions were the least for the I<sub>4</sub> treatment, where yields declined only about 15%, or less than one half the TWA reduction of 33%. When considering just the AZ2 cultivar, the I<sub>4</sub> reductions for DB and RY were less than 10%, or less than one third of the percent TWA reduction. Among the other deficit treatments, the most notable was the I<sub>5</sub> treatment having a 52% reduction in TWA, losing about 40% in DB but only 31% in RY. The percent reductions of the yield parameters for the I<sub>2</sub> and I<sub>6</sub> treatments compared to their TWA reductions were less substantial, i.e., reductions on the order of 6–9% lower for yield than

#### Table 4

Means by irrigation treatment (I<sub>T</sub>), cultivar (AZ2 and AZ6), and cultivar within irrigation treatment for dry biomass (DB), rubber yield (RY), and resin yield (ReY) and their water productivities<sup>a</sup> (DB-WP, RY-WP, and ReY-WP), along with percent rubber (R) and resin (Re), after 22.5 months of guayule growth. The irrigation treatments are described in Table 3.

| Effect                 |                | $I_{T}$        | DB<br>Mg ha <sup>-1</sup> | DB-WP<br>kg m <sup>-3</sup> | RY<br>kg ha <sup>-1</sup> | RY-WP<br>kg m <sup>-3</sup> | R<br>% | ReY<br>kg ha <sup>-1</sup> | ReY-WP<br>kg m <sup>-3</sup> | Re<br>% |
|------------------------|----------------|----------------|---------------------------|-----------------------------|---------------------------|-----------------------------|--------|----------------------------|------------------------------|---------|
| Irrigation             |                | I1             | 20.5a                     | 0.738bc                     | 780a                      | 0.028d                      | 3.8b   | 1533a                      | 0.055c                       | 7.5a    |
| U U                    |                | $I_2$          | 18.3ab                    | 0.796ab                     | 692ab                     | 0.030cd                     | 3.8b   | 1407a                      | 0.061bc                      | 7.7a    |
|                        |                | $I_3$          | 13.5c                     | 0.729bc                     | 566bc                     | 0.031bc                     | 4.2ab  | 1033bc                     | 0.056c                       | 7.6a    |
|                        |                | $I_4$          | 17.3ab                    | 0.930a                      | 660abc                    | 0.036ab                     | 3.8b   | 1297ab                     | 0.070a                       | 7.4a    |
|                        |                | I <sub>5</sub> | 12.4cd                    | 0.909a                      | 541c                      | 0.039a                      | 4.4a   | 933cd                      | 0.069a                       | 7.5a    |
|                        |                | I <sub>6</sub> | 9.1d                      | 0.837ab                     | 375d                      | 0.035abc                    | 4.2ab  | 667d                       | 0.061bc                      | 7.4a    |
| Cultivar               |                | AZ2            | 15.9a                     | 0.866a                      | 695a                      | 0.038a                      | 4.5a   | 1299a                      | 0.070a                       | 8.1a    |
|                        |                | AZ6            | 14.5b                     | 0.780b                      | 510b                      | 0.028b                      | 3.6b   | 991b                       | 0.054b                       | 6.9b    |
| Cultivar in irrigation | $I_1$          | AZ2            | 20.8a                     | 0.749bc                     | 864a                      | 0.031cde                    | 4.2b   | 1787a                      | 0.064bcd                     | 8.6a    |
|                        |                | AZ6            | 20.2a                     | 0.727c                      | 696bcd                    | 0.025e                      | 3.4c   | 1278bc                     | 0.046f                       | 6.3e    |
|                        | $I_2$          | AZ2            | 18.4abc                   | 0.803bc                     | 770abcd                   | 0.033cd                     | 4.2b   | 1613a                      | 0.070ab                      | 8.7a    |
|                        |                | AZ6            | 18.1abc                   | 0.790bc                     | 614de                     | 0.027de                     | 3.4c   | 1201bcd                    | 0.052df                      | 6.6de   |
|                        | $I_3$          | AZ2            | 14.5cd                    | 0.783bc                     | 671bcd                    | 0.036bc                     | 4.6ab  | 1191bcd                    | 0.064bcd                     | 8.2ab   |
|                        |                | AZ6            | 12.5d                     | 0.674c                      | 460ef                     | 0.025e                      | 3.7c   | 875de                      | 0.048ef                      | 7.0cd   |
|                        | $I_4$          | AZ2            | 19.2a                     | 1.028a                      | 782abcd                   | 0.042ab                     | 4.1b   | 1464ab                     | 0.079a                       | 7.6bc   |
|                        |                | AZ6            | 15.5bcd                   | 0.833bc                     | 539ef                     | 0.029de                     | 3.5c   | 1129bcd                    | 0.060bcde                    | 7.3c    |
|                        | I <sub>5</sub> | AZ2            | 12.6d                     | 0.921a                      | 622de                     | 0.045a                      | 5.0a   | 1004cde                    | 0.074ab                      | 8.0ab   |
|                        |                | AZ6            | 12.3d                     | 0.897ab                     | 460ef                     | 0.033cd                     | 3.8bc  | 863de                      | 0.063bcd                     | 7.0cd   |
|                        | I <sub>6</sub> | AZ2            | 9.9de                     | 0.915a                      | 459ef                     | 0.042ab                     | 4.8a   | 734de                      | 0.068abc                     | 7.5bc   |
|                        |                | AZ6            | 8.2e                      | 0.759bc                     | 291f                      | 0.027de                     | 3.6c   | 601e                       | 0.055cdef                    | 7.3c    |
|                        |                |                |                           |                             |                           |                             |        |                            |                              |         |

Means followed by different letters in rows within the effects sections for irrigation, cultivar, and cultivar within irrigation for each column parameter indicate significant differences (p < 0.05).

<sup>a</sup> Water productivity (WP) is based on total water applied (irrigation plus precipitation) from planting to harvest.

### Table 5

Mean increase (positive change) and mean decrease (negative change) for irrigation treatments compared to the I<sub>1</sub> treatment for irrigation, yield, and water productivity parameters.<sup>a</sup>

| $I_{T}$ | Total I | TWA  | Total I  | TWA   | DB    | DB-WP | RY    | RY-WP | ReY   | ReY-WP                       |
|---------|---------|------|----------|-------|-------|-------|-------|-------|-------|------------------------------|
|         |         | mm   |          |       |       |       |       |       | %     | 6 change from I <sub>1</sub> |
|         |         |      | treatmer | 1t    |       |       |       |       |       |                              |
| $I_1$   | 2519    | 2780 | 0.0      | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0                          |
| $I_2$   | 2035    | 2296 | -19.2    | -17.4 | -10.7 | 7.9   | -11.3 | 7.1   | -8.2  | 10.9                         |
| $I_3$   | 1588    | 1849 | -37.0    | -33.5 | -34.1 | 1.2   | -27.4 | 10.7  | -32.6 | 1.8                          |
| $I_4$   | 1602    | 1863 | -36.4    | -33.0 | -15.6 | 26.0  | -15.4 | 28.6  | -15.4 | 27.3                         |
| $I_5$   | 1105    | 1366 | -56.1    | -50.9 | -39.5 | 23.2  | -30.6 | 39.3  | -39.1 | 25.5                         |
| $I_6$   | 822     | 1084 | -67.4    | -61.0 | -55.6 | 13.4  | -51.9 | 25.0  | -56.5 | 10.9                         |

<sup>a</sup> Total irrigation (Total I), total water applied (TWA) is total I plus precipitation, dry biomass (DB), rubber yield (RY), resin yield (ReY), and DB-WP, R-WP, and ReY-WP, are the water productivities (based on TWA) for DB, RY, and ReY, respectively.

TWA. The percent increases in the WP of the yield parameters were the most for the  $I_4$  and  $I_5$  treatments and were generally similar between the two treatments (Table 5). However, given the greater reduction in TWA for the  $I_5$  than  $I_4$ , the relative gain in WP was more appreciable.

## 4. Discussion

This experiment was conducted to evaluate yield responses to six different irrigation strategies for two direct-seeded guayule cultivars grown in the U.S. Southwest desert climate. For the study, we focused on the most common irrigation system (furrow) that is used for field crops in Pinal County, Arizona, where the study was conducted. Limited past research suggested that irrigation water use could be substantially reduced for guayule grown in heavier soils, which hold more available water than lighter soils, while maintaining or increasing the RY generally associated with fully irrigated guayule. Thus, the experiment was conducted on a heavier silty clay loam soil in Eloy. Consequently, the results are limited to the soil type, planting period, and irrigation method used in the experiment. Additional research and analysis would be needed to evaluate deficit irrigation regimes for guayule grown in similar and lighter soils with pressurized and non-pressurized irrigation systems in the region.

For direct-seeded guayule, there is need to keep the soil surface layer

wet during the first two weeks for optimum germination and to then to provide moist soil conditions for seedlings over the next  $\approx$ six weeks until stand establishment is complete (Bucks et al., 1986; Jorge et al., 2006; Dissanayake et al., 2008). In this experiment, a total of 511 mm was applied to plots for the two months following planting to meet the germination/establishment soil water conditions. Over one-half the amount was applied with sprinklers for the first two weeks, while the remainder was applied by furrow irrigation. The total applied was  $\approx$  25% less than what was reported by Elshikha et al. (2021) for the first two months in earlier direct-seeded studies with sprinkler/furrow irrigation at the Eloy location. However, in the earlier Eloy study, more irrigation was provided by furrow during the establishment period compared to the present study. In direct-seeded field germination experiments on a sand soil in Yuma, Arizona, Bucks et al. (1986) applied daily irrigation for 14-day germination periods for early-Apr. plantings, followed by irrigation about every three days during the subsequent establishment period. In that study, between 100 and 150 mm of total irrigation for germination and  $\approx$  215–250 mm total irrigation for the next two months was applied as an overhead mist using a lateral move sprinkler system. Although the direct-seeded guayule germination and survival rates in that study were quite poor, this earlier work shows that germination/establishment irrigation use could be considerably lower than 500 mm. The present study and Elshikha et al. (2021) have clearly

demonstrated that high guayule plant populations are now attainable with direct seeding in both heavy and light soils. However, a major gap still exists on how to appropriately manage direct-seeded guayule irrigation during germination/establishment. More research should be conducted to determine optimum frequencies and amounts of irrigation that satisfy the needed soil moisture levels under various soil types, irrigation system methods, planting dates, and locations.

The cumulative irrigation applied to the I<sub>1</sub> treatment after 22.5 months (2519 mm) was about 700 mm less (or 22% less) than applied to the spring-planted level furrow treatment after 23 months in the earlier direct-seeded study at Eloy (Elshikha et al., 2021). Compared to the latter study, the lower amount for I<sub>1</sub> was due to less germination/establishment irrigation, significantly lower irrigation requirements during the second-year summer due to frequent precipitation, and that no irrigation was needed during the final four months before harvest. Other studies in central Arizona using transplants and furrow irrigation reported cumulative irrigation amounts of 3360 mm after 21 months (Bucks et al., 1985a) and 3570 mm after 24 months (Hunsaker and Elshikha, 2017) for well-watered treatments.

Some general outcomes of DB and rubber production can be related to the soil water depletion levels of the treatments in the study. During the first year of the experiment, the average measured %SWD was similar for the I1-I4 treatments (47-53%) and higher, though not extreme, for I<sub>5</sub> and I<sub>6</sub> (67% and 59%, respectively). However, during the second year, %SWD averaged 54%, 69%, 79%, 72%, 80%, and 81% for I1-I6, respectively. The results show that DB was significantly reduced for treatments with 79% or greater average %SWD in the second year (i.e., I<sub>3</sub>, I<sub>5</sub>, and I<sub>6</sub>), while %R was also significantly increased at the higher % SWD levels. However, the increase in %R for those treatments was farless than that needed to achieve RY comparable to treatments with lower average %SWD in the second year. The %R results for the I5 and I6 differ considerably from the Hunter and Kelley (1946) findings, also on a heavier soil type. The I5 and I6 obtained about a 20% increase in rubber content over the well-watered treatment, whereas Hunter and Kelley (1946) found about a 50% increase in %R for extremely "dry" treatments over %R obtained for well-watered treatments. The irrigation regime of the I<sub>6</sub> treatment in the present study was very similar to the number 4 treatment of Hunter and Kelley, where only a spring irrigation was given in the second year. The average %SWD of I<sub>6</sub> in the second year was less than that estimated for the number 4 treatment (>90%), as precipitation was significant at Eloy in Jul.-Aug. of the second year. It is possible, however, that the R would have been higher in I<sub>6</sub> (and I<sub>5</sub>) had there been no rain in summer to decrease the treatment's soil water stress level. The responses in DB and %R using alternating periods of high and low %SWD for the I4 treatment was consistent with the treatment of every-other irrigation studied by Veihmeyer and Hendrickson (1961). After two years of growth in each study, there was no difference between the alternate irrigation and the fully-irrigated treatment in %R nor DB. For a treatment with a single spring irrigation in the second year (similar to number 4 treatment of Taylor and Kelley and the present  $I_6$ ), Veihmeyer and Hendrickson (1961) found a significant increase in %R over the well-watered but it was only 20% higher, like the %R gain for I<sub>6</sub>. For the same soil type in earlier studies at Eloy (Elshikha et al., 2021; Wang et al., 2022), applying about one half the irrigation applied to fully-irrigated furrow plots did not increase the %R nor significantly reduce the DB of guayule. This was consistent with the  $\mathrm{I}_4$  results, where the average %SWD was maintained at about 75% during the second year of growth in the earlier Eloy studies.

Elshikha et al. (2021) reported ET<sub>c</sub> data from a direct-seeded guayule experiment in 2018–2020 at the same location and  $\approx$ planting date as the present study. For the well-watered level furrow treatment in that study, they indicate a maximum ET<sub>c</sub> rate of 10.8 mm d<sup>-1</sup> occurred in Jun. of the second year. Bucks et al. (1985b) determined maximum ET<sub>c</sub> rates were  $\approx$  7.0 mm d<sup>-1</sup> in the first year during Aug.-Sep. and were about 10.0 mm d<sup>-1</sup> in the second year during Jun.-Jul. for a well-watered furrow irrigation treatment with spring-transplanted guayule. In good

agreement with the current and previous studies, Hunsaker and Elshikha (2017) reported well-watered, fall-transplanted, guayule using level furrow irrigation had  $ET_c$  rates of 6.4 mm d<sup>-1</sup> in Aug. and Sep. of the first year and 9.8 mm  $d^{-1}$  in Jun.-Jul of the second year. Relevant literature on guayule K<sub>c</sub> estimation based on ET<sub>o</sub> was provided by Elshikha et al. (2021). That study indicated average monthly K<sub>c</sub> values for the well-watered level furrow treatment were somewhat higher during the first summer Jun.-Sep. (0.74-1.23) compared to the those in present study. In the second year, monthly K<sub>c</sub> were higher in Jun. (1.23) but lower in Jul. and Aug. (1.17 and 1.22, respectively) than the Kc for those months during the present study. Cumulative  $ET_c$  for the  $I_1$ treatment (3165 mm) was about 5% lower than that for the well-watered level furrow treatment in Elshikha et al. (2021). For approximately the same guayule growing period in other studies, cumulative ET<sub>c</sub> was 3000 and 3275 mm (Bucks et al., 1985b and Hunsaker and Elshikha, 2017, respectively), indicating similar crop water use for well-watered direct-seeded and transplanted guayule.

Linear relationships of guayule DB, RY, and ReY regressed by cumulative ET<sub>c</sub> or by total TWA have been reported in the literature for  $\approx$ two-year and four-year, transplanted crops (Miyamoto et al., 1984; Bucks et al., 1985c; Nakavama, 1991; Hunsaker et al., 2019; Hunsaker and Elshikha, 2017). In the cited studies, the r<sup>2</sup> for DB versus the water variable were somewhat higher (0.90-0.98) than the r<sup>2</sup> obtained for DB by TWA in the present study (0.85). As pointed out years ago by Miyamoto et al. (1984), guayule DB production is driven by water input and, thus, DB generally responds to water in a similar manner as that observed for many vegetative crops. On the other hand, relationships of RY to water can be more variable than for DB, particularly if there is a pronounced decline in %R at higher water input. The range of r<sup>2</sup> for RY versus water input from the transplanted studies above varied from 0.48 to 0.91 and averaged 0.77, while it was lower (0.59) in the present study. For ReY versus water, r<sup>2</sup> values were 0.90 or higher from the literature but 0.69 in the present study. However, the lower  $r^2$  in the present study were greatly affected by significant differences in RY and ReY between AZ2 and AZ6. The RY and ReY with TWA were highly linear when regressions considered each cultivar separately, giving  $r^2$ values more in line with the literature. Current results suggest that linear relationships between direct-seeded guayule yield parameters with water will hold up even with extremely different irrigation timing, though different cultivars may have different linear relationships.

The mean DB for the AZ2 cultivar within the I<sub>1</sub> treatment was about 21% lower than that obtained with direct-seeded AZ2 grown at the same location for a well-watered furrow irrigation treatment (Elshikha et al., 2021). However, the earlier-study treatment received 24% more TWA. Consequently, DB-WP was about the same for the I<sub>1</sub> treatment and the well-watered furrow treatment in the earlier study at Eloy (0.75 and 0.74 kg m<sup>-3</sup>, respectively). The DB for I<sub>1</sub> was comparable to well-watered guayule transplants grown with furrow irrigation in other Arizona studies on lighter soils (Bucks et al., 1985b; Coffelt and Ray, 2010; Hunsaker and Elshikha, 2017). Earlier data for well-watered guayule on the silty clay loam soil in California indicate DB about 40% lower than the I<sub>1</sub>.

Although the AZ2 cultivar within the I<sub>1</sub> treatment achieved less DB than the furrow treatment of Elshikha et al. (2021), due to the higher R and R in the present study, RY and ReY were similar in the studies, where RY and ReY for I<sub>1</sub> was 11% higher and 5% lower than those in the earlier study, respectively. In regard to rubber and resin contents, the AZ2 cultivar in the present study achieved R and R that averaged 4.5% and 8.1%, respectively, compared to 2.9% and 7.1% for the furrow treatment reported by Elshikha et al. (2021). The present study, Elshikha et al. (2021), and Wang et al. (2022) used a different laboratory analytic method to determine the R and R (i.e., the Soxhlet-based NIR method; Placido et al., 2020) than that used in the cited earlier guayule studies (i.e., accelerated solvent extraction [ASE] method; Pearson et al., 2013). While the two methods are well-correlated, R by ASE is notably higher than that by NIR (Placido et al., 2020). Therefore, R, R, R, R,

and ReY for present and earlier studies cannot be compared without bias. In the present study, as evidenced by the significant irrigation by cultivar interaction for %Re, %Re response to irrigation was different between the AZ2 and AZ6 cultivars. While %Re for AZ2 generally increased with the irrigation treatment TWA, it generally decreased with TWA for AZ6. Discrepancies in the final %R and %Re also occurred between the AZ2 (significantly greater) and AZ6 cultivars. Although mean DB for AZ2 was only 9% greater than for AZ6, cultivar differences in %R and %Re resulted 28% and 24% higher mean RY and ReY for AZ2 than AZ6, respectively, which resulted in significantly greater mean RY-WP and ReY-WP for AZ2 of about the same magnitude. Reasons for the different cultivar %R responses are unclear and differ from Ray et al. (1999), who indicated significantly higher %R for AZ6 than AZ2 after two years. However, the significantly greater %Re for AZ2 than AZ6 was consistent with Ray et al. (1999), though less extreme in the latter. The plant height and cover data indicate much more rapid development occurred for the AZ2 than AZ6 within each irrigation level. Because AZ6 plants were significantly shorter than AZ2, it is possible that lower %R for AZ6 occurred because those plants may have experienced lower water stress effects than those experienced by the AZ2 plants within the same irrigation treatment.

In comparing the different water deficit strategies to I<sub>1</sub>, it appears that the  $I_4$  treatment (every other irrigation of  $I_1$ ) approach is the best option to substantially reduce guayule irrigation water use in this soil type. This was most evident in AZ2, where final DB, RY, and ReY were not significantly lower than those for I1 even though TWA was curtailed by 33% (and irrigation by 36%). The DB-WP, RY-WP, and ReY-WP for AZ2 within I<sub>4</sub> were maximum, or near maximum, and always significantly greater than those for I1. The AZ6 cultivar within the I4 treatment had significantly greater DB-WP and ReY-WP than for AZ6 within  $\mathrm{I}_1$  but also had lower DB and RY than the I1. Terminating irrigation in the second year at mid-summer, seven months before the final harvest (I2), resulted in a 17% reduction in TWA (19% for irrigation), which did not significantly reduce DB, RY, and ReY compared to I1. However, unlike the I<sub>4</sub>, the reduction in TWA for I<sub>2</sub> did not significantly increase the WP above those for I1. This was the case for either cultivar. Terminating irrigation during summer months in the second guayule year (I<sub>3</sub>) cut TWA to the same extent as for I<sub>4</sub>. However, the effects of this water deficit period significantly reduced DB and yields compared to I1 and did not increase WP. While the I<sub>3</sub> treatment had somewhat higher %R than for I<sub>1</sub>, the difference was not significant. Thus, it appears that imposing severe water stress during the entire second summer of guayule when ET<sub>c</sub> demand is highest was harmful rather than beneficial. For the I<sub>5</sub> and I<sub>6</sub> treatments having extremely limited irrigation after establishment, the I<sub>5</sub> (two irrigations per year) appeared to fare better than the I<sub>6</sub> (one irrigation per year) with regard to yields and WP. However, while the DB, RY, ReY, and the WP were always higher for I<sub>5</sub> than I<sub>6</sub>, they were not significantly greater, except in the case of DB for the AZ6 cultivar. The I5 treatment did achieve significantly greater %R than I1 in AZ2 but the low DB production in  $I_5$  cut short attaining RY sufficiently close to  $I_1$ .

Considering that the DB and yield responses for the I<sub>3</sub> and I<sub>5</sub> treatments were not significantly different, and that both treatments had severe water deficit during the second summer, it is inferred that both treatment approaches could achieve higher DB without significantly changing the total irrigation given to the treatments during the experiment. In other guayule studies, biomass for well-watered guayule rapidly increased from early spring to near maximum levels in late summer during the second year (Hunsaker and Elshikha, 2017; Hunsaker et al., 2019; Wang et al., 2022). Severely limited irrigation treatments during this period, however, had much slower DB accumulation, as shown in Hunsaker and Elshikha (2017) and Hunsaker et al. (2019). For the I<sub>3</sub> and I<sub>5</sub> treatment approaches, providing one summer irrigation and deleting the fall irrigation might significantly increase the DB with a potential gain in the final %R that those treatments achieved in the experiment. Thus, TWA for the two treatment approaches would not have to change, only irrigation timing. The same irrigation timing

switch is foreseen as also improving the I<sub>6</sub> treatment approach.

In Pinal County, growers are presently reducing the number of hectares that they planted in the past because Colorado River water is no longer available for irrigation. Traditional crops, such as cotton and alfalfa, grown in the county and much of the U.S. Southwest desert require about 1450-1900 mm of irrigation per year (NASS, 2010). However, yields for such crops typically decline rapidly when water stress occurs due to limited irrigation (Bronson et al., 2019; Hunsaker et al., 2002). Guayule is seen as a viable crop alternative in the region because it can withstand significant water stress and, as the I4 treatment indicates, needs substantially less irrigation per year ( $\approx$ 800 mm) to achieve maximum yields. Significant reductions in irrigation needed in germination/establishment of guayule may also be likely with additional research focus, as mentioned earlier. Considering the possibility that future water allotment cutbacks will be insufficient for even the I4 schedule, e.g., on the order of 500 mm year, then the guayule crop would have to be managed similar to the I5 scheme. However, based on the poor yield for I<sub>5</sub>, it would probably be more effective to provide the allotted water in spring and summer where it can be utilized by the plant for growth.

### 5. Conclusions

Direct-seeded guayule cultivars AZ6 and AZ2 were grown in a field experiment conducted for 22.5 months in central Arizona on a silty loam to silty clay loam soil using level furrow irrigation. The aim was to determine effective deficit irrigation strategies that could be used to reduce irrigation water use, while maintaining guayule biomass and rubber and resin yields comparable to those attained with a fullyirrigated control treatment. Clearly, the best deficit irrigation strategy found was applying irrigation every other time the fully-irrigated treatment was irrigated. In this study, fully-irrigated guayule required irrigation about every 10 days during summer months and about every 30 days during spring and fall. Deficit irrigation strategies that imposed high soil water deficits during the summer of the second year of guayule were deemed to be detrimental even though they significantly reduced irrigation water use. We conclude that subjecting guayule to severe water deficits during the second summer was not a good strategy. Future research should evaluate deficit irrigation strategies that focuses on fall rather than summer soil water deficits during the second year of guayule. In this regard, it may be possible to boost the rubber yields to some extent under extremely limited irrigation if only a single irrigation can be applied during the second summer. Because AZ2 outperformed AZ6 for all yield and water productivity criteria, AZ2 should be the cultivar focus for guayule commercialization efforts. Future research is also needed to determine best management practices and more efficient early season irrigation practices for establishing direct-seeded guayule crops. Using surface drip irrigation instead of sprinklers for germination and establishment might result in saving significant amounts of water, cost, and labor during this short period of time.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

# Acknowledgments

The authors thank Bridgestone Americas, Inc. for providing the guayule seeds and assistance in planting and harvesting. We thank Stefan Dittmar and Russell Prock, with Bridgestone Americas, for field technical support and help with field irrigation, and Theresa Sullivan, Bridgestone Americas for shrub processing and rubber, resin, and moisture analysis. This project was supported by Sustainable Bioeconomy for Arid Regions (SBAR), USDA National Institute of Food and Agriculture (NIFA) Grant no. 2017-68005-26867.

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