Effect of Irrigation Frequency and Shade Levels on Vegetative Growth, Yield, and Fruit Quality of Piquin Pepper (Capsicum annuum L. var. glabriusculum)

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Additional index words. bird pepper, capsaiacinoids, chiletep, pungency, water deficit

Abstract. In Mexico, piquin peppers are highly valued horticultural products with limited cultivated production due to low seed germination, morphologic and genetic variability, disease susceptibility, and limited environmental physiology information. The objective of this study was to evaluate the effects of irrigation frequency and shade level treatments on vegetative growth, yield, and fruit quality of a commercial ecotype of piquin pepper. The study was conducted during two consecutive years using a hierarchical linear mixed-effects model design, with yearly data of irrigation frequency as main treatment blocks and shade levels as secondary blocks (nested within irrigation frequency treatments). Our results indicate that more frequent irrigation and increased shade levels favored vegetative growth. In addition, moderate shade levels (interception of 35% of full sunlight) and daily irrigation provided the best conditions for fruit production. This effect could be attributed to an increase in vegetative growth (thus higher photosynthetic and crop load capacity); more moderate conditions (temperatures and relative humidity) that favored flowering and fruit set, or a combination of these factors. Fruit size and pungency were not significantly affected by the treatments. Our results provide basic information for the development of guidelines for the cultivation of piquin pepper plants.

The Capsicum genus consists of at least 32 wild and 5 domesticated species with more than 2000 cultivars of economic importance distributed throughout the world (Bosland and Votava, 2012). Mexico is one of the sites of origin and domestication of Capsicum plants (Chiou and Hastorf, 2014; Kraft et al., 2013), and is the second largest producer of peppers in the world (FAOSTAT, 2012). However, Mexican pepper production is limited to a small number of varieties that includes jalapeño, bell, serrano, poblano, New Mexican (all Capsicum annuum L.), habanero (Capsicum chinense, Jacq.), and manzano (Capsicum pubescens R. & P.) (SIACON, 2014). Piquin peppers (C. annuum L. var. glabriusculum (Dunal) Heiser & Pickersgill) are highly appreciated in Mexico due to their unique characteristics of flavor and pungency (Rodríguez-del-Bosque, 2005). Piquin pepper plants have not been fully domesticated because they encounter problems related to low and erratic seed germination, morphologic and genetic variability, and limited environmental physiology information (García-Federico et al., 2010). High consumer preference and limited cultivation can cause fresh piquin peppers to reach more than 10 times the retail value of other peppers (J.I. Valiente, personal observation).

Piquin pepper plants are herbaceous with heights of 0.2 to 2.6 m (Fig. 1). Stems are upright and extended. Fruits are round or oval (diameter of 4 to 7.5 mm) and are positioned at the junction of the stems. Fruits are green when unripe, turning to orange and red when mature. Although natural seed germination averages only 15% (range 0% to 66%), plant propagation is primarily by seeds (Cano-Vázquez et al., 2015).

Phenology of piquin pepper plants varies according to the environmental conditions in which they are grown. Wild populations of piquin pepper plants are perennial and are commonly associated with partially shaded conditions in the understory of trees. When cultivated at full sun, piquin pepper plants can be considered annual crops as frost, disease, and pest susceptibilities limit the productive cycle (Rodríguez et al., 2003). Under cultivation, seed germination requires 7 to 28 d (J.I. Valiente, unpublished data), 60 to 90 d for seedling development, and 70 to 80 d after transplant for the onset of flowering, and 30 d for the first commercial fruits (Almanza-Enríquez, 1998; E. Puga, personal observations).

Water availability affects vegetative and reproductive development of Capsicum plants (Sung et al., 2005). Habanero pepper plants grown under daily irrigation increased fruit yield and vegetative growth when compared with water-stressed plants (Borges-Gómez et al., 2010; Ruiz-Lau et al., 2011). Deficit irrigation (at 70% of field capacity every 3 d) reduced fruit size and yield of bird pepper plants (C. annuum L.) (Ismaíl, 2010). In hot pepper plants (C. annuum L. var. annuum), water deficit conditions reduced photosynthetic levels in comparison with plants growing under daily irrigation (Sung et al., 2005). Water stress also reduced photosynthesis, vegetative growth, and fruit set of habanero pepper plants (Alvino et al., 1994; Rodríguez-del-Bosque et al., 2003).

The response of Capsicum plants to light intensity varies by species and cultivars. Yield of sweet pepper plants cv. Pepón (C. chinense Jacq.) was higher at 40% shade level (1100 to 1250 μmol·m⁻²·s⁻¹) than at direct sunlight and 60% shade (800 μmol·m⁻²·s⁻¹) (Jaimez and Rada, 2011). At low irradiance levels (200 μmol·m⁻²·s⁻¹) bell pepper plants had reduced vegetative growth and increased floral abscission (Aloni et al., 1996). In addition, the interaction of light intensity with temperature can also affect reproductive development of Capsicum plants. In bell pepper plants, the combination of high irradiance with average temperatures of 26 °C increased dry weight, leaf area, and reduced stem elongation. Temperatures above 36 °C caused flower drop, as young leaves presented a greater ability to obtain phloem photosynthates than did the developing fruitlets (Aloni et al., 1996).

Environmental conditions can affect yield and fruit quality attributes of Capsicum plants. In ‘Beauty zest’ and bird pepper plants (both C. annuum L.), water deficit reduced fruit size and yield (Ismaíl, 2010; Sung et al., 2005). By contrast, in habanero peppers, irrigation treatments did not affect fruit weight. Fruit pungency varied by cultivar and was affected by water availability. In habanero and ‘Beauty zest’ fruits, capsaicinoids increased when plants were exposed to water deficit (Harvell and Bosland, 1997; Ruiz-Lau et al., 2011; Sung et al., 2005). The effect of water deficit on capsaicinoid
levels seems to be related to fluctuations in soil-water availability. In habanero peppers, pungency was unaffected when plants were maintained at reduced water availability (Borges-Gómez et al., 2010). Water stress increased capsaicin synthesis enzymes and reduced the catabolic agents (Contreras-Padilla and Yahia, 1998; Sung et al., 2005).

Capsaicinoids are the alkaloid compounds responsible for the pungency of hot peppers and their levels vary during fruit development and maturation. In ‘Beauty zest’ peppers, capsaicin levels reached maximum concentrations at 30–50 d after flowering. Similarly, de Arbol, habanero, and piquin peppers reached maximum capsaicinoid levels at 40–45–50, and 50 d after fruit set, respectively (Contreras-Padilla and Yahia, 1998; Sung et al., 2005).

In summary, environmental factors can have major effects on Capsicum plants and the lack of reliable environmental physiology information has limited the cultivation of piquin pepper plants. Therefore, the objective of this study was to evaluate the response of piquin pepper plants to irrigation frequency and shade level treatments on vegetative growth, fruit yield, and fruit quality attributes. This information is needed to develop reliable guidelines for the production of piquin peppers under cultivated conditions.

Materials and Methods

Study site. The study was conducted under field conditions in Marín, Nuevo León, México (25.8581 N, 100.0663 W, altitude 393 m) during 2011 and 2012. Marín has a subtropical dry semiarid climate according to the Köppen climate classification system. The experimental site had a kastanozem soil type according to the FAO/UNESCO soil classification system (INEGI, 2013). In addition, soil particle analysis indicates that the study was conducted in a sandy clay loam soil containing 48.58% sand, 27.02% silt, and 24.4% clay (Fertilab, 2011, Laboratorio de Nutrición Vegetal, Celaya, Guanajuato, Mexico).

Plant materials. The piquin pepper plants used for both years of the study were from the same commercial ecotype originally from San Fernando, Tamaulipas, México. Before the study, the ecotype had been selected during 3 years based on productivity and biophysical attributes of plants and fruits. Ecotype characteristics included plant height (80 to 120 cm), canopy width (70 to 100 cm), and round to slightly oval fruits (Fig. 1).

Plant propagation. Seeds of mature piquin peppers were treated in a 500 ppm gibberellic acid solution (Cyto-gibb®; Mexico City, Mexico) for 24 h at 28 °C to enhance germination (García-Federico et al., 2010). The seeds were subsequently planted in trays with 10 peatmoss: 1 perlite (by volume) under greenhouse conditions. Seedlings were transplanted to the field in rows (in a 1 × 1 m arrangement) when they reached an average height of 30 cm (≈120 d later). Plants were left to establish under direct sunlight and daily irrigation for 30 d before the application of treatments (Fig. 2). The experimental site was covered with a polyethylene cover to reduce water infiltration from rainfall. Plants were placed at the center of 30-cm diameter openings.

Fertilization. Plants were supplemented with granular fertilizers following the guidelines recommended for serrano peppers. Total amounts of fertilizer applied were 18 g N, 8 g P, and 8 g K per plant. Fertilizer applications per plant were 30 g of ammonium phosphate nitrate (33-3-0), 45.1 g of diammonium phosphate (18-46-0), and 18.6 g of potassium sulphate (0-0-51-18S). Fertilizer applications were divided at transplant, vegetative growth, anthesis, fruit ripening, and after the first harvest (Azofeifa and Moreira, 2008; Macías-Valdez and Valdez-Marín, 1999).

Fig. 1. Images of piquin pepper plant and fruits.

Fig. 2. Meteorological conditions and experimental activities of the 2011 and 2012 production cycles.
were separated by 2 m to minimize possible interactions among them. Data loggers (HOBOM® models U23-001 and UA-002-64; Bourne, MA) were placed at each shade level to monitor temperature, relative humidity, and light intensity. Daily light integral (DLI) was estimated by averaging all light intensity measurements and adjusting them to 24 h (Eq. [1]). Additional weather data were obtained from a meteorological station of the Mexican National Institute of Forestry, Agricultural and Livestock Research located at 2500 m from the experimental site.

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DLI = \left(\frac{\sum_{i=1}^{n} LI_i}{n}\right) \times 0.0864
\]

where

- DLI = Daily light integral (mol·m⁻²·d⁻¹)
- LI = light intensity (µmol·m⁻²·s⁻¹) at time i.
- \(n\) = number of measurements per day.

Units conversion factor (s⁻¹·1,000,000⁻¹) = 0.0864.

Response variables to irrigation frequency and shade level treatments were vegetative growth, fruit yield, and fruit quality attributes. Vegetative growth was determined every 30 d by measuring height from the ground to the uppermost aeriel organ, and lateral growth (width) was obtained by averaging the length and width of the plants. Fruit yield was measured as total fresh fruit weight and number of fruits per plant. Fruit quality attributes included individual fruit weight and pungency. Fresh fruit weight was obtained by weighing a sample of 25 individual peppers per plant in an analytical scale. Pungency (measured as capsaicinoids levels) was determined using the spectrophotometry method described by Sadasivam and Manickam (2008). For this purpose, we used a Beckman DU-800 spectrophotometer (Fullerton, CA) and the levels of capsaicinoids were determined using two standards: capsaicin (Sigma® No.12084; 99% purity, St. Louis, MO) and dihydrocapsaicin (Sigma® No. M1022; 90% purity).

Statistical analysis. Vegetative growth, fruit yield, and fruit quality attributes were analyzed as response variables to irrigation frequency, shade level, and their interaction in a hierarchical linear mixed-effects model. In this model, the effect of irrigation frequency was analyzed using yearly data as replicated units of the main blocks. The effect of the shade levels was determined by considering shade as subplots of the irrigation frequency treatments. Statistical analyses included nested analysis of variance of linear mixed-effects models with irrigation frequency and shade levels as main effects. In addition, contrast procedures were performed to compare individual combinations of treatments. Significance level was set at 5% (P ≤ 0.05) throughout the study. The statistical analyses were performed using R v. 3.1.2 (R Core Team, 2014) with the linear mixed-effects models (nlme) (Pinheiro et al., 2015), and the contrast methods packages (Kuhn et al., 2013).

Results

Weather conditions. Results were affected by contrasting environmental conditions of the production years. The year 2011 was warmer with periods of more than 40 d in which daily highest temperatures were above 40 °C and average daily temperatures were over 32 °C. In 2012, temperatures were more moderate (daily highest was below 40 °C and average temperatures were 30 °C). DLI was 16.8% higher in 2011 (78.4 mol·m⁻²) in comparison with 2012 (67.1 mol·m⁻²). Cumulative rainfall from May to August was 346.3 mm in 2011 and 170 mm in 2012. On 28 and 29 Nov. 2011, an early frost (with temperatures reaching −0.1 °C during 4 h) caused plant death and early termination of the production cycle (Fig. 2).

The use of shade nets over the individual plots caused changes in their microclimate conditions (reduced DLI and temperatures as well as increased relative humidity). Shade conditions of 35%, 50%, and 80% reduced average temperatures by 1.5, 2.25, and 2.8 °C, and average maximum temperatures by 2.2, 4.35, and 6.3 °C, respectively. Similarly, the effect of the shades increased relative humidity levels by an average of 0.6%, 1.6%, and 3.9% (Table 1). Rates and volumes of irrigation water applied at all irrigation frequency treatments during the entire production cycles are presented in Table 2. Total volumes of irrigation water were considered equivalent, since the differences in applied volume were less than 4.5% during the entire production cycles.

Vegetative growth. In 2011, plant vegetative height was 27.1% greater than the values obtained for 2012. This effect was consistent across treatments and could be related to higher DLI and temperatures of 2011 (Fig. 3). In both years, irrigation frequency (R) shade level (L), and their interaction (R × L) significantly affected vegetative growth of plants (P ≤ 0.05). The main effects of irrigation frequency and shade level treatments were that more frequent irrigation and increased shade favored vegetative growth. At every shade level, the tallest plants developed with daily irrigation, and less frequent irrigation reduced plant size. Similarly, the most compact plants were obtained at direct sunlight and the tallest at 80% shade level. Shade level had a greater effect on plant height in comparison with plant width. Piquin pepper plants exposed to 50% and 80% shade levels showed rapid growth from the early stages. Apparent inconsistencies in this tendency can be explained by factors not related to the treatments. In 2011, high-speed winds (26.4 km·h⁻¹) caused plant lodging. Partial height measurements obtained during vegetative growth (not shown) indicated that plant lodging only occurred in the tallest plants at the time when winds occurred. Lodging occurred in 30% and 100% of plants growing under 50% and 80% shade, respectively (data not shown). In 2012, all plants grown at 50% shade and R5 showed leaf curling symptoms associated with virus infections that curtailed their growth (Fig. 3).

Yield per plant. In 2012, total yield of peppers (g) was 227% higher than in 2011 (Fig. 4). This difference could be attributed to more moderate environmental conditions (lower DLI and temperatures) in 2012 than...
in 2011. In addition, harvesting in 2012 could be performed twice, whereas in 2011, an early frost in Nov. 2011 caused an early termination of the production cycle and prevented a second harvest. Nevertheless, each individual harvest of 2012 had higher yields than the single harvest of 2011.

Fruit yield per plant in both years (measured as total fruit weight and number of fruits) was significantly affected by irrigation frequency (R), shade level (L), and their interaction (R × L) (P ≤ 0.05). The main effects of irrigation frequency on fruit yield per plant were that more frequent irrigation (R1) consistently maintained the highest yields. Productivity was significantly reduced as the plants were irrigated less frequently (R10 and R15 when compared with R1) (Fig. 4). As for shade level treatments, at the 0% shade level (DLI of 78.1 mol·m⁻²·s⁻¹), fruit yield was significantly lower than at 35%. Shade levels of 50% showed a small, nonsignificant increment in relation to the 0%, and yield was significantly reduced at 80% shade (DLI of 6.7 mol·m⁻²·s⁻¹). Therefore, for shade level and irrigation frequency treatments, the main effect for maximum fruit production was at the 35% shade levels and daily irrigation (P ≤ 0.001). Regarding the interaction of the factors, the main interactions in fruit production occurred at the combination of 35% shade level with R5, R10, and R15, when compared with the R1 and 0% shade levels.

**Fruit quality attributes.** Production years did not have a significant effect (P > 0.05) on average fruit weight (99.3 to 157.2 mg per plant in 2011 and 80.8 to 134.5 mg per plant in 2012) and total capsaicinoids (0.67 to 2.23 mg·g⁻¹ in 2011 and 1.83 to 3.13 mg·g⁻¹ in 2012).

### Discussion

The increase in vegetative growth and yield of piquin pepper plants caused by frequent irrigation confirms the importance of water availability for *Capsicum* plants. Water availability has proven crucial for increased vegetative growth and productivity in a number of *Capsicum* cultivars including ‘Beauty zest’ and bell pepper (Sung et al., 2005). Increased water availability promotes higher uptake of water and nutrients (Borges-Gómez et al., 2010) and higher photosynthetic rates (Alvino et al., 1994).

Shade levels also had a significant effect on vegetative growth of piquin plants. The most compact plants were obtained at direct sunlight and the largest at 80% shade. Exposure of piquin pepper plants to increased shade levels caused plants to grow more rapidly from the early stages (data not shown). The response of *Capsicum* plants to different irradiance conditions can vary by cultivar. Bell pepper plants exposed to low irradiance (DLI of 4.14 mol·m⁻²) increased plant height with thin and fragile stems (Zulkifli-Jaafar, 1995), but when plants were grown at high irradiance (~2100 μmol·m⁻²·s⁻¹) they increased vegetative growth with greater stem density (Díaz-Pérez, 2013). Our results indicate that in the case of piquin pepper, as with bell pepper (Díaz-Pérez, 2013), increased vegetative growth seems to be a response to shaded conditions to improve light interception and capture.

Intermediate shade increased vegetative growth and improved fruit yield of piquin plants. Higher productivity under moderate shade could be related to an increase in total leaf area, greater crop load capacity, lower temperatures that did not cause flower or fruit abscission, or a combination of these factors (Aloni et al., 1996; Bazan-Tene et al., 2005; Díaz-Pérez, 2013). Since previous reports have established that photosynthetic levels were unaffected by moderate shade, increased vegetative growth could result in higher yields by maintaining photosynthetic capacity on a larger number of leaves. In addition, *Capsicum* plants have been reported to adjust stomatal density in response to different light conditions, which gives them a higher degree of plasticity to changes in irradiance (Fu et al., 2010).

The increase in yield caused by partially shaded treatments could also be related to changes in the microclimate conditions of the shaded plots. In addition to reduced DLI, shade levels also caused a reduction in temperature and an increase in relative humidity in comparison with the plots at full sun (Table 1). More extreme conditions under direct sunlight could have caused flower drop and reduced fruit set. Yield reduction at an 80% shade level (which correspond to a DLI of 10.8 and 8.7 mol·m⁻², in 2011 and 2012, respectively) seems to be related to a reduction in photoassimilates due to the effects of low irradiance on photosynthetic capacity and increased allocation to vegetative organs (Díaz-Pérez, 2014). Our results concur with previous reports for piquin and ‘Chameleon’ peppers (also *C. annuum* L.) in which the highest yields were obtained under partial shade, whereas full sunlight reduced productivity (Milenkovic et al., 2012; Rodriguez-del-Bosque et al., 2005).

The higher yield at the best combination of conditions for fruit production (daily irrigation and 35% shade) was primarily related to a higher number of fruits. Although a trend of larger fruits could be observed at these conditions, fruit weight increase was not consistent across treatments. Our results are comparable to a similar study on bell pepper in which yield (measured as total number of fruits and fruit size) was increased by partially shaded conditions (Díaz-Pérez, 2014). In this study, shade improved overall plant water status by reducing temperature and evapotranspiration demand. In our study, the 35% shaded conditions could have also increased photoassimilate availability by increasing vegetative growth while maintaining photosynthetic rates (Díaz-Pérez, 2013).
While this effect could also be observed at the R5 irrigation frequencies, the effect on yield increase was reduced in comparison with that observed for R1. The nonsignificant effect of 50% and 80% shade conditions could be related to the allocation of photoassimilates to vegetative growth.

Fruit quality attributes were not significantly affected by irrigation frequency and shade level treatments. Variability of average fruit weight data caused inconsistencies in the observed effects of the treatments. The effect of water deficit on fruit weight has also been reported inconsistent in other pepper cultivars. For instance, habanero pepper weights were apparently unaffected by water deficit treatments (Ruiz-Lau et al., 2011), whereas ‘Beauty zest’ and bird pepper plants reduced fruit size and weight in response to water stress (Ismail, 2010; Sung et al., 2005).

Even though soil water availability has been reported to influence capsaicinoid levels of other peppers (Ismail, 2010; Ruiz-Lau et al., 2011; Sung et al., 2005), in our study, the treatments did not have a significant effect on capsaicinoid levels ($P = 0.126$). However, the overall trend seemed to be that capsaicinoid levels increased at moderate water deficit (R5 and R10). Similarly, for the shaded treatments the overall trend seemed to be that capsaicinoids varied reflecting changes in yield per plant. Thus, we hypothesize that pungency levels could be affected by the overall factors that determine yield. While for both treatments, the nonsignificant results could be related to data variability, further studies are needed to evaluate this possible effect.

The treatments in this study were designed to obtain the response of piquin pepper plants to a wide range of conditions, 0 to 80% shade, and irrigation frequency from daily to every 15 d. In both cases, the most extreme conditions (80% shade and R10 and R15) turned out to be of little value and should not be considered. Fruit production at the best combination of conditions (35% shade and daily irrigation)
could still be considered low. To achieve better economic profitability of piquin peppers further work should focus on plant selection, fertilization, as well as on pest and disease management strategies.

Conclusions

Shade level and irrigation frequency treatments had a direct effect on vegetative growth and fruit yield of piquin peppers. Higher irrigation frequency and increased shade levels favored vegetative growth. The best results for fruit yield were obtained at the combination of daily irrigation at 35% shade level. In addition to the effects of the individual treatments (daily irrigation and moderate shade increased yield), this combination of treatments provided the best conditions to increase fruit yield per plant without compromising fruit quality. We believe these results provide reliable environmental physiology information needed to develop guidelines for the cultivated production of piquin peppers.

Literature Cited


