Effect of Indaziflam Application and Soil Manipulations on Pecan Evapotranspiration and Gas Exchange Parameters

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Abstract. Appropriate soil management practices and correct use of agrochemicals for crop protection are essential to alleviate stresses that affect the quality and yield of pecans [*Carya illinoensis* (Wangenh.) K. Koch]. A greenhouse study was conducted to evaluate the effect of soil surface manipulation and indaziflam application on evapotranspiration (ET) and gas exchange parameters of pecan trees, and phytotoxicity effects of indaziflam on pecan trees. Trees were planted in large pots with a homogeneous porous media (HM), including the controls (C), preferential flow channels open at the soil surface (PF), and preferential flow channels with surface soil manually tilled to 5 cm depth [shallow tillage (ST)]. Trees with HM, PF, and ST were treated with 50 g a.i./ha of indaziflam in 2014 and 2015, whereas an application rate of 150 g a.i./ha was used for trees with HM and ST in 2016. All trees were irrigated about every 14 days with 7 L of water in 2014 and 2015, and 5 L in 2016. A water balance analysis determined the ET in different treatments in 2014 and 2015. Gas exchange parameters were measured before and after irrigation in 2015 and 2016. Photosynthetic rates in C, HM, PF, and ST were consistently significantly lower before than after irrigation. PF and ST did not decrease the available water content of the soil because there was no significant difference in the volume of effluent, ET, and gas exchange parameters among the treatments. No herbicide injury symptoms and no influence on gas exchange parameters and ET were observed after using both application rates of indaziflam.

Pecan [*Carya illinoensis* (Wangenh.) K. Koch] is an important crop of southern New Mexico, USA. Appropriate management practices are required to maintain the quality and yield of pecan while conserving water resources (Andales et al., 2006; Deb et al., 2011; Wang et al., 2007). Garrot et al. (1993) reported that pecan trees subjected to moderate water stress before irrigation showed reduced yield, nut weight, and tree growth. Plant water stress is influenced by weather, soil water availability, and tolerance capacity of species. Deb et al. (2012) reported that the stem water potential of pecan trees decreased with decreasing soil moisture content and

increasing air temperature. Similarly, lower photosynthetic rate was observed in blue oak leaves (*Quercus douglasii* H. & A.) in mid-summer as a result of seasonal changes in soil water content and temperature (Xu and Baldocchi, 2003). A previous study reported that maximum ET in a flood-irrigated pecan orchard was observed after budbreak during early summer (Sammis et al., 2004). However, evaporation was accounted for most of the water lost from the soil until 50% budbreak.

Plant water stress as a result of water deficit lowers photosynthetic rate during the growing season. High vapor pressure deficit between the plant and atmosphere combined with low soil water availability promotes the decline in plant water potential and stomatal conductance, and subsequently reduces the photosynthetic rate and transpiration (Sasaki et al., 1997; Turner et al., 1985). In addition, poor soil drainage, which can reduce soil oxygen levels, can exacerbate the decline in photosynthetic rate for pecans (Kallestad et al., 2007; Sparks, 2005). An increase of

intercellular CO₂ concentration in pecan trees subjected to prolonged flooding leads to decreases in photosynthetic rates (Kallestad et al., 2007).

Soil tillage practices have an important influence on soil hydraulic properties (Shukla et al., 2003; Strudley et al., 2008). Schwen et al. (2011) evaluated the impact of conventional, reduced, and no-tillage on the soil hydraulic properties and found greater bulk density, as result of natural compaction, and soil moisture content for no-tillage. Similarly, Shipitalo et al. (2000) reported higher macropore flow in no-tillage than conventional tillage. Macropores play an important role in the movement and distribution of water through the soil profile, as well as ecological interactions such as plant and soil relationship (Beven and Germann, 1982; Kramer and Boyer, 1995). Several studies have reported deeper than expected herbicide leaching through preferential flow channels (Flury et al., 1995; Rao et al., 1974; Shukla, 2014).

The correct use of agrochemicals applied for crop protection is beneficial for controlling crop pests, minimizing the entry of agrochemicals into water resources, and avoiding crop damage. Previous studies reported that the application of fungicides and insecticides temporarily reduced the photosynthetic rate of pecan under greenhouse and field conditions (Wood et al., 1984, 1985). Therefore, inappropriate application of pesticides could significantly reduce the tree energy reserves affecting the productivity of pecans (Wood et al., 1985). Indaziflam is a cellulose biosynthesis inhibitor registered with Environmental Protection Agency in 2012 and is used for preemergence control of annual grass and broadleaf weeds. Indaziflam is a new herbicide, and there is limited information available on its fate and transport under laboratory and field conditions (González-Delgado et al., 2017; Guerra et al., 2014; Trigo et al., 2014). Information on phytotoxicity effects of indaziflam on pecan is especially limited (González-Delgado et al., 2015). It is important to monitor the influence of indaziflam application, soil, and irrigation management practices on pecan ET and photosynthetic rates in arid regions with limited water resources because of their vital influence on quality and yield of pecan. The objectives of this study were to evaluate the effect of soil surface manipulation and indaziflam application on ET, gas exchange parameters of pecan trees, and phytotoxicity effects of indaziflam on pecan trees.

Materials and Methods

Surface soil conditions. This greenhouse study started in July 2014 and was continued until Nov. 2016 at the New Mexico State University's Fabian Garcia Science Center in Las Cruces, NM. The greenhouse was equipped with an exhaust, evaporative cooler pads on the east side, a heating system, and an automatic temperature control unit. In 2014,

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2-year-old pecan trees of the variety “Wichita” (Archer Farms, Inc., Las Cruces, NM) were planted in 0.035 m³ pots with an inner diameter and height of 36 cm. Before tree planting, holes on the bottom of empty pots were covered with metal mesh to prevent soil loss. A 5-cm layer of gravel was placed on the bottom of the pots to facilitate free drainage. Sandy loam soil was air-dried and sieved through a 2-mm sieve. The organic matter content ($1.5 \pm 0.2\%$) and pH (8.4 ± 0.08) of the sandy loam soil were determined using the loss on ignition method and a 1:1 soil to water ratio with a pH meter, respectively. Pots were subsequently packed with a 10-cm layer of sandy loam soil, a pecan tree was placed at the center of the pot and sandy loam soil was packed around it.

Three different soil surface manipulations were created in 18 pots. A set of six trees were planted in pots with a HM, another six with PF channels open to the soil surface, and the remaining pots with the combination of preferential flow channels and shallow tillage up to 5 cm depth from soil surface (ST). Preferential flow channels were created by placing four 0.64-cm diameter wooden sticks at opposite sides of the pots at a 45-degree angle toward the roots. The lower ends of the wooden sticks were near the cluster of roots at ≈ 17 cm depths and were left for 2 weeks to allow for stabilization of preferential flow channels. Preferential flow channels were intended to facilitate indaziflam and water movement toward the roots. After 2 weeks, the wooden sticks were removed from the pots, and the soil surface up to a 5 cm depth was manually tilled to interrupt the connection of the preferential flow channels at the soil surface in 6 of the 12 pots (ST treatment). The PF channels were created because injury to some pecan trees of the variety “Wichita” was reported in some orchards treated with indaziflam. It was widely hypothesized that indaziflam movement toward roots via PF channels caused the injury. Three additional trees planted in HM were used as control (not treated with indaziflam).

The trees were arranged using a completely randomized design with replications. During the study, trees were irrigated about every 14 d with 7 L (0.007 m³) of water and once a month with 2 L of water during the dormant period using a watering container.

Indaziflam application. A small hand pump sprayer was used to spray indaziflam at different application rates directly over the soil. Care was taken to contain the spray plumes within the pot and as far as possible to not cause contamination. Indaziflam was applied using an application rate of 50 g a.i./ha on 18 Aug. 2014 and 27 July 2015 when all trees had fully expanded leaves after the end of the dormant periods. This rate is slightly lower than field application rate considering the lower dissipation rate of herbicide in greenhouse than in the field. The pots were irrigated with 7 L of water after 24 h of indaziflam application and every 2 weeks until the end of the study. Plastic containers were placed below the pots to

collect the leachate after irrigation for water balance analysis (below). Necrosis of leaves and varying trunk injuries were herbicide injury symptoms observed in two pecan orchards located in Arizona and New Mexico 3–4 months after indaziflam application (González-Delgado et al., 2015). Therefore in this study, trees were evaluated visually for injury symptoms because of indaziflam after the applications. A visual evaluation for the development of necrotic tissue in the leaves was performed weekly until the dormant period. At the end of the dormant period, the trees were evaluated visually for injury symptoms again. On 25 July 2016, only HM and ST pots were treated with 150 g a.i./ha of indaziflam and irrigated with 5 L of water 48 h after indaziflam application. The amount of irrigated water was reduced, whereas the applied indaziflam rate was three times greater than the rate used in 2014 and 2015. No leachate was observed in the plastic containers after irrigation; therefore, pecan root exposure to indaziflam was higher in 2016 compared with the previous years. The aphids were treated with soap solution in 2014, Sivanto (Propylene carbonate) in 2015, and Admire Pro (Imidacloprid) in 2016.

Water balance. A water balance analysis was conducted to determine the ET in 2014 and 2015. The change in storage (ΔS) was obtained from soil water content measured using Hydra Probes (Stevens Water Monitoring System, Inc., Portland, OR) at 12 and 22 cm depths from the soil surface. The total depth of irrigation water (I), depth of deep percolation, and ΔS were used to determine the ET using Eq. [1] (Shukla, 2014).

$$ET = P + I - DP - \Delta S - RO \quad [1]$$

There were no precipitation (P) and runoff (RO) because the trees were grown in a pot inside the greenhouse.

On 5 Nov. 2014, 10 Nov. 2015, and 2 Nov. 2016, trees were moved to an outside facility to induce the dormancy stage. During the dormancy stage, trees were irrigated with 2 L of water, and no effluent came out of the pot bottom after irrigation. By 6 May, the 25% budbreak (end of dormancy stage) in the trees was observed, and all the trees were moved back to the greenhouse.

Gas exchange parameters of pecan trees. Gas exchange parameters [(photosynthetic rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), intercellular CO₂ concentration ($\mu\text{mol}\cdot\text{mol}^{-1}$), vapor pressure deficit (kPa), transpiration ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), leaf temperature (°C), and relative humidity (%)] were measured before and after irrigation using a LI-COR 6400-portable gas exchange system (LI-COR Biosciences, Inc., Lincoln, NE). The LI-COR 6400-portable gas exchange system was adjusted at 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ for both control reference CO₂ and flow rate. Gas exchange parameters were measured between 11:00 AM and 2:00 PM about every 15 d from 13 July to 9 Nov. 2015 and from 12 July to 24 Sept. 2016. No data were collected between 21 Aug. and

8 Oct. 2015 because of adverse weather conditions and unavailability of the LI-COR 6400-portable gas exchange system. Photosynthetic active radiation was consistently between 998 and 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using the 6400-02B light source. Measurements were made on fully expanded leaves to cover the entire chamber of the portable gas exchange system on sunny days. One-way analysis of variance was performed to determine the difference in ET, effluent, and gas exchange parameters among treatments using SAS 9.2 (SAS Institute Inc., Cary, NC).

Results and Discussion

The average cumulative effluent from the pots of the C, HM, PF, and ST treatments ranged from 3.51 ± 0.65 to 7.73 ± 2.2 cm in 2014 and from 6.64 ± 0.8 to 8.85 ± 1.2 cm in 2015 (Fig. 1A). For both years, HM had the highest cumulative volume of effluent compared with the C, PF, and ST treatments. However, no significant differences in leachate volumes were seen among the treatments.

Macropores are reported to increase deep percolation (Beven and Germann, 1982; Mori and Hirai, 2014; Shipitalo et al., 2000; Zhou et al., 2012). In this study, the absence or presence of PF channels did not show an increase on the cumulative volume of effluent. No increases in the deep percolation in the PF and ST treatments could be due to the PF channels being only up to 17 cm depth from surface, smaller total volume of PF channels, likely collapse of the PF channels, and predominant lateral infiltration into the soil matrix. Mori and Hirai (2014) reported that empty macropores collapsed during the infiltration process after conducting a solute transport experiment with and without fibrous materials in the macropores. Zhou et al. (2012) reported higher infiltration in soils with macropores open at the soil surface than those closed at the surface or no macropores.

Average cumulative ET values for the C, HM, PF, and ST treatments ranged from 21 $\pm$ 3.8 to 28 $\pm$ 1.1 cm in 2014 and from 55 $\pm$ 6.7 to 75 $\pm$ 2.4 cm in 2015 (Fig. 1B). Cumulative ET in 2015 was ≈ 3 times greater than the cumulative ET in 2014 because six more ET measurements were included in the water balance analysis conducted in 2015. The C and HM treatments had lower cumulative ET than PF and ST treatments; however, cumulative ET was not significantly different among the C, HM, PF, and ST treatments. Soil evaporation could have increased by the subsoil exposed to the atmosphere in plots with PF channels and tillage (Cattle, 1999).

PF flow channels were expected to increase pecan root exposure to indaziflam and cause phytotoxicity. However, no visual phytotoxicity effects on pecan trees were observed in 2014. Gas exchange parameters were also measured in 2015 to determine if they were influenced by the second indaziflam application (50 g a.i./ha). But no phytotoxicity effects were observed in 2015 also using an application rate of 50 g a.i./ha.

Fig. 1. Cumulative volume of effluent (A) and evapotranspiration (B) for control (C), homogeneous porous media (HM), preferential flow channels open at the soil surface (PF), and preferential flow channels with shallow tillage (ST) treatments for 2014 and 2015. Error bars represent standard error. Letters indicate significant difference among treatments based on the least significant difference test, $P < 0.05$.

Therefore, in 2016, the pecan trees with HM and ST were treated with 150 g a.i./ha of indaziflam and irrigated with 5 L of water to prevent the leaching of indaziflam and increase in the exposure time. No phytotoxicity effects were seen even after increasing the application rate from 50 to 150 g a.i./ha and decreasing the leaching to zero. The absence of phytotoxicity effects could not explain the sporadic injury symptoms reported by González-Delgado et al. (2015) for an application rate of 73.1 g a.i./ha. González-Delgado et al. (2015) reported that indaziflam was mostly detected in the unimpacted areas (no injury observed on pecan trees) of two pecan orchards 1 year after indaziflam application. A second field study reported that the percent mass recoveries of indaziflam could have been influenced by the organic matter content in the New Mexico pecan orchard where sporadic injury symptoms were observed in 2012 (González-Delgado et al., 2017). Percent mass recoveries of indaziflam were mostly higher in the unimpacted areas where the organic matter content was greater

than that in the impacted area. This greenhouse study selected a sandy loam soil similar to the one found in the New Mexico pecan orchard in González-Delgado et al. (2017). Soil pH was also similar but organic matter content in the pots was greater ($1.5 \pm 0.2\%$) than that in field ($0.60 \pm 0.03\%$). This small difference could partially explain the absence of phytotoxicity effects in young pecan trees exposed even to an application rate two times greater than the recommended application field rate of indaziflam. Schneider et al. (2015) reported increased sorption of indaziflam with increasing organic matter content after evaluating the phytotoxicity of indaziflam on bermudagrass.

Another soil condition that might have been different between the greenhouse and field was the absence of any material greater than 2 mm in greenhouse soil. Therefore, the macroporosity was expected to be greater in the pecan orchard than the greenhouse soil. In contrast to the first field study, no further phytotoxicity effects were observed for the application rates of 36.5

and 73.1 g a.i./ha during the second field study. The exact causes of the phytotoxicity observed after the first indaziflam applications in the two orchards are not fully known. However, root exposure to the indaziflam concentration which was much higher than the recommended rate likely caused injury.

The photosynthetic rate, transpiration, stomatal conductance, and relative humidity were higher after irrigation than before (Fig. 2). However, the opposite trend was observed for vapor pressure deficit from 13 July to 9 Nov. 2015 and from 12 July to 24 Sept. 2016 (Fig. 3). Studies have also reported increases in photosynthetic rate, transpiration rate, and stomatal conductance with irrigation (Asseng et al., 1998; Reed and Loik, 2016; Shem et al., 2009; Toselli et al., 2014; Xue et al., 2003). Increases in the relative humidity at the leaf surface after irrigation was the result of an increase of leaf water content (Fig. 3). Low soil moisture content increased vapor pressure deficit, decreased the leaf water potential, and promoted stomata closure (Comstock and Mencuccini, 1998; Gunderson et al., 2002; Turner et al., 1984). Under water stress conditions, the decrease in stomatal opening limits CO_2 uptake that causes a reduction in photosynthetic activity and intercellular CO_2 concentration (Anderson et al., 1995; Gunderson et al., 2002; Osakabe et al., 2014; Sharma et al., 2017; Turner et al., 1984). In contrast, some other studies reported an increase of the intercellular CO_2 concentration in different tree species subjected to drought conditions (Brodribb, 1996; Kubiske et al., 1996). In this study, no clear trends in intercellular CO_2 concentrations were observed for treatments ≈ 14 d after the previous irrigation in 2015 and 2016. Higher intercellular CO_2 concentrations with increasing water stress were observed in some treatments before irrigation in 2015 on 13 July, 27 July, and 19 Oct., and also in 2016 on 26 July, 10 Aug., and 22 Sept. These could be explained by the reduction of carboxylation efficiency in the photosynthetic apparatus (Farquhar and Sharkey, 1982; Gomes et al., 2004). In 2015, leaf temperature did not show a clear trend with increasing water stress, but leaf temperature values were consistently higher 1 d before irrigation during 2016 (Fig. 3).

Photosynthetic rates 1 d before irrigation corresponded to the time of maximum water stress. Mostly, these values did not significantly decrease after indaziflam application on 27 July 2015 and 25 July 2016 and remained consistent with those collected before the herbicide applications. The data suggested that indaziflam applications had no permanent effects on gas exchange parameters, except on 12 Aug. 2016 when photosynthetic rate was lower than expected. The reduction in the photosynthetic rate was most likely the result of treating the leaves of the pecan trees with an insecticide “Admire Pro” (Imidacloprid) on 8 Aug. 2016 for aphid control. It was observed that the leaves were

Fig. 2. Photosynthetic rate (A and B), transpiration (C and D), stomatal conductance (E and F), intercellular CO₂ concentration (G and H) for homogeneous porous media (HM), preferential flow channels open at the soil surface (PF) and preferential flow channels with shallow tillage (ST) treatments in 2015 and for control (C), HM, and preferential flow channels with ST treatments in 2016. Error bars represent standard error. Letters indicate significant difference among treatments based on the least significant difference test, $P < 0.05$.

covered by the protective coating of the insecticide for a longer period of time in 2016. However, photosynthetic rate values recovered to levels before the insecticide application after 6 weeks (Fig. 2B). Although trees were also sprayed with Sivanto (Propylene

carbonate) on 23 June 2015 for aphid control, but no reduction in photosynthetic rate was noted at that time. Wood et al. (1997) reported reductions in the photosynthetic rate of pecan for about 14 d after treating with an organo-silicon surfactant. Generally, trees planted

in indaziflam sprayed pots with HM, PF, and ST did not show a significant difference in the measured gas exchange parameters for most days. However, the photosynthetic rate was mostly lower in the HM treatment than in other treatments in 2015. Similarly, the

Fig. 3. Vapor pressure deficit (A and B), leaf temperature (C and D) and relative humidity (E and F) for homogeneous porous media (HM), preferential flow channels open at the soil surface (PF) and preferential flow channels with shallow tillage (ST) treatments in 2015 and for control (C), HM, and preferential flow channels with ST treatments in 2016. Error bars represent standard error. Letters indicate significant difference among treatments based on the least significant difference test, $P < 0.05$.

photosynthetic rate was higher in ST than those with HM on 12 July, 12 Aug., 22 Sept., and 24 Sept. 2016. Greater plant biomass is reported in plots with artificial macropores than in control plots as a result of enhanced infiltration, nutrients, and oxygen (Mori

et al., 2014). In this study, pots were filled with a sandy loam soil previously air-dried and sieved through a 2-mm sieve; therefore, the soil was free of organic debris and rocks that would normally influence the aeration process. The location of the preferential flow channels

in pots with the PF and ST treatments might have contributed to a lower degree of stress as the result of greater aeration and easier root water uptake immediately after irrigation. Pecan trees are sensitive to poor soil drainage, and their productivity tends to decrease

in areas under prolonged flooding conditions (Sparks, 2005).

Conclusions

There was no significant difference in the cumulative volume of effluent among the HM, PF, and ST treatments probably because of some degree of failure of the artificial preferential flow channels. Similarly, there was no significant difference in cumulative ET among treatments. Macropore channels did not seem to change soil water dynamics likely being present up to the partial soil depth. Less water stress was observed for pecan in ST and PF treatments likely because of quicker drainage and higher aeration than HM treatment. No phytotoxicity in pecan trees treated with 50 and 150 g a.i./ha of indaziflam suggested that indaziflam did not affect pecan trees subjected to water stress in this study under greenhouse conditions.

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