Additional Nitrogen Application Reduced White Drupelet Disorder in ‘Sweetie Pie’ Blackberry

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SUMMARY. White drupelet disorder (WDD) in blackberry (*Rubus* subgenus *Rubus*) is an abiotic condition resulting from a cultivar and environment interaction. Although high temperatures and light intensities have been implicated, little is known why this disorder manifests. Other factors, such as overall plant stress, may be contributing influences. In this study, three treatments were applied to examine whether the addition of nitrogen (N) can reduce WDD on ‘Sweetie Pie’ erect blackberry over three seasons. An initial 50 lb/acre (56.0 kg·ha⁻¹) N was applied to all plots at budbreak. Two additional N application treatments of 100 kg·ha⁻¹ were applied at one time (1×) or five, 20-kg·ha⁻¹ applications (5×), spaced 1 week apart for 5 weeks starting at bloom. One control treatment of no additional N (0×) was also included. Berries were harvested and weighed as a total, then berries with white drupelets were separated out and weighed. The two values were divided to create a proportion and were then multiplied by 100 to determine the percentage. Nitrogen application decreased the percentage of white drupelet berries from 13.0% (control) to 10.0% (one additional application) and 9.1% (five additional applications). WDD for the 0× treatment correlated negatively to maximum high daytime temperatures during May ($r = -0.58$, $P = 0.03$) over the three seasons. Occurrence of white drupelets by treatments 0×, 1×, and 5× correlated significantly with the cumulative number of rainfall events ($r = 0.49$, 0.47 , and 0.46 , respectively). Leaf chlorophyll index and photosynthesis measurements were unaffected by treatment. Although it is likely that multiple factors are involved in the development of white drupelets, additional N may reduce the problem.

White drupelet disorder is a problem in blackberry (*Rubus* subgenus *Rubus*) production, especially in regions where high

temperatures are experienced during fruit ripening. This disorder can be a significant issue for growers, especially if many berries and/or several drupelets per berry express the symptom, because the fruit are unattractive to buyers (Fig. 1). Many blackberry growers currently do not know which cultivars manifest the disorder nor how severe the problem can be, because it is a product of a genetic × environment interaction (Stafne et al., 2017). The complex genetic × environment interaction is not easy to predict or eliminate. Several cultivars are known to exhibit white drupelets, with some being more sensitive to WDD than others, and it can vary greatly from year to year.

Possible causes of WDD have been postulated, such as low humidity, wind, rainfall, high light intensities, ultraviolet

light, stinkbugs (Pentatomidae), redberry mite (*Acalitus essigi*), and various interactions of some or all these factors (Bolda, 2009; Clark, 2008, 2013; Fernandez, 2012); however, few studies have attempted to verify causes under experimental conditions. Thus, most of what is communicated about this disorder is observational conjecture.

Stafne et al. (2017) found that fruit harvested early in the season had the most WDD berries, but an increase in WDD was seen in the last harvest for all three cultivars (Chickasaw, Kiowa, and Sweetie Pie). The sugar content of extracted white drupelets was substantially less than normal drupelets. A 30% shade cloth treatment had a significantly positive impact by decreasing WDD symptoms in three cultivars by 63% when compared with unshaded plants (Stafne et al., 2017), a result that was consistent with that in a report by Takeda et al. (2013).

Nitrogen is an essential element for plants, but blackberry has a relatively low requirement compared with other fruit crops. Typical recommended application rates of N for blackberry are 40 to 80 lb/acre in mature plantings, and spring is the key time to apply for best results in fruit production and quality (Strik, 2008; Strik and Bryla, 2015). Fruit has been demonstrated to be the strongest sink for recently acquired N (Malik et al., 1991). Alleyne and Clark (1997) found that N applications increased fruit N and juice pH, but no other measures of fruit quality were impacted.

Quezada et al. (2007), working with ‘Heritage’ primocane-fruiting red raspberry (*Rubus idaeus*), stated N application through fertigation at 100 kg·ha⁻¹ reduced the percentage of white drupelets. No similar research has been done on the response of WDD in blackberry to fertilizer applications, although Edgley et al. (2019) reported that high N rates increased red drupelet reversion in ‘Ouachita’ erect blackberry grown under

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
25.4	inch(es)	mm	0.0394
1.1209	lb/acre	kg·ha ⁻¹	0.8922
1	ppm	μL·L ⁻¹	1
6.8948	psi	kPa	0.1450
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Fig. 1. White drupelet disorder on ‘Sweetie Pie’ blackberry can manifest on nonadjacent drupelets, sometimes on opposite sides of the berry, as seen in the image.

a high tunnel. The genetic mechanisms and environmental cues that induce these disorders are not well understood. Hence, the objective of our study was to determine whether supplemental N applications can reduce the incidence of WDD in ‘Sweetie Pie’ erect blackberry.

Materials and methods

PLANT MATERIAL AND CULTURE. The study was conducted in 2018–20 at the U.S. Department of Agriculture (USDA), Agricultural Research Service Thad Cochran Southern Horticulture Laboratory in Poplarville, MS (lat. 30°50′25″N, long. 89°32′03″W; elevation, 97 m; USDA hardiness zone, 8b; average annual extreme minimum temperature from 15 to 20 °F). The experimental design was a randomized complete block, with three blocks, with each block consisting

of one five-plant plot for each of the three treatments for a total of nine plots. The cultivar used in this study, ‘Sweetie Pie’ (‘Navaho’ × MSUS29), was released by the USDA, Agricultural Research Service (Stringer et al., 2017). Its pedigree includes ‘Brazos’ (43.75%), ‘Humble’ (25%), ‘Thornfree’ (18.75%), and ‘Darrow’ (12.5%). Plants were established in 2014.

TREATMENT APPLICATION. The equivalent of 50 lb/acre (56.0 kg·ha⁻¹) N was applied initially at budbreak to each plot as a broadcast top-dressing with 14N–6.1P–11.6K controlled-release fertilizer (Osmocote; Scotts Co., Marysville, OH). Treatment fertilizer applications consisted of N in the form of urea (46N–0P–0K or similar) equivalent to 100 kg·ha⁻¹ N (Quezada et al., 2007) in excess of initial annual fertilizer amounts starting in mid-April

(bloom) for the 1× treatment and weekly from mid-April to mid-May (5 consecutive weeks) for the 5× treatment (20 kg·ha⁻¹ N per application). Control (0×) plots received no additional fertilizer. The same plots were used for each treatment every year of the study. Daily weather measurements of maximum temperature, minimum temperature, rainfall and monthly averages were recorded at a nearby (≈500-ft) weather station placed 6 ft from ground level (Nimbus Max/Min Digital Thermometer; Sensor Instruments, Bow, NH) (Table 1).

MEASUREMENTS. Each cultivar was hand-harvested at the ripe stage (shiny to dull black) twice per week from 21 May to 11 June in 2018 (five harvests), from 28 May to 14 June in 2019 (seven harvests), and from 21 May to 18 June in 2020 (eight harvests). All plots were harvested completely, and harvested berries from each plot were separated into normal berries (no WDD) and berries with WDD. The total berry weight (2018–20), WDD berry weight (2018–20), and average berry weight for both WDD berries and normal berries (2019–20) were recorded for each plot at each harvest, then divided to obtain a proportion by weight of collected berries. After the fruiting season was complete, floricanes were removed from each plot and weighed in the field to obtain fresh weights, including remaining leaves (2019–20). Leaf chlorophyll index was measured in each treatment with a chlorophyll meter (SPAD-502; Konica Minolta Sensing, Osaka, Japan) on 10 June 2020. Ten random floricanes leaflets per plot were chosen based on appearance of being healthy, exposed to the sun, and fully expanded.

Gas exchange measurements were taken during red stage of berry development using a gas exchange analyzer (LI-COR 6800; LI-COR Biosciences, Lincoln, NE) with combined light source and measurement chamber (LI-COR 6800-01 A, LI-COR Biosciences). Plots contained five plants that were divided into canopy sections: top, middle, and bottom. The day before gas exchange measurements were taken, fully expanded leaves were selected and tagged at each section of canopy. One leaf per canopy section per plant was selected on both the east and west sides of the plant. This

Table 1. Average monthly maximum (max) and minimum (min) temperatures, cumulative rainfall, and cumulative number of rainfall events during the ‘Sweetie Pie’ blackberry harvest season for 3 years (2018–20) in Poplarville, MS.

Yr	Month	Avg max temp (°F) ^z	Avg min temp (°F)	Cumulative rainfall (inches) ^z	Cumulative rainfall events (no.)
2018	May	88.5	64.2	1.5	4
	June	91.0	71.4	4.2	8
2019	May	85.1	65.0	14.2	5
	June	89.3	69.2	9.8	12
2020	May	83.1	61.9	6.9	12
	June	87.6	69.7	7.8	16

^z(°F – 32) ÷ 1.8 = °C, 1 inch = 25.4 mm.

constituted 10 measurements per canopy section per plot. Gas exchange measurements were collected on 14 May 2020 between 0830 and 1130 HR, with the chamber set at a temperature equal to the outside ambient temperature (average, 27.1 °C), light levels of 800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a flow rate of 700 $\mu\text{mol}\cdot\text{s}^{-1}$, relative humidity of 65.2%, and reference carbon dioxide concentration of 400 ppm.

All proportional data were arcsine-transformed for statistical analysis and then converted to percentages. Nitrogen treatment and year were used as main effects. Data were analyzed by analysis of variance ($P < 0.05$) using statistical software (JMP version 12; SAS Institute, Cary, NC) with the Fit Model procedure. Means were compared by Tukey’s honestly significant difference at $P < 0.05$. Correlations of weather (cumulative rainfall total, number of rainfall events, and harvest day temperatures) and treatment (0x, 1x, and 5x at each harvest) were estimated by restricted maximum likelihood using the Multivariate procedure, and pairwise correlations were tested by Pearson’s correlation coefficient.

Results and discussion

The proportion of ‘Sweetie Pie’ blackberry fruit with white drupelets differed by fertilizer treatment ($P = 0.02$) and year ($P < 0.0001$) (Figs. 2 and 3); however, there was no interaction ($P = 0.80$). Bushes that received no additional N had a greater proportion of WDD berries (13.0%) than those that received the 1x or 5x treatment (Fig. 2). Although at first glance the differences were small in terms of percentage points (0x–1x = 3.0, 0x–5x = 3.9), the percentage decrease in WDD with supplemental N was 23% and 30%, respectively. A reduction of up to 30% could be a substantial difference if WDD berries are

rejected by consumers or wholesalers. The proportion of WDD also differed by year (Fig. 3), with 2020 having the greatest proportion of berries with white drupelets over the three seasons. Years 2018 and 2019 would be considered low-to-average WDD incidence for ‘Sweetie Pie’, whereas 2020 would be considered high based on a previous study (Stafne et al., 2017).

Berry weights were taken for both normal (all black) berries and those with WDD. Average berry weights did not differ among treatments for black ($P = 0.13$) and WDD berries ($P = 0.86$). Berry weights did differ by year for each type, with 2020 having larger berries for both black [4.5 vs. 4.0 g ($P < 0.0001$)] and WDD [4.1 vs. 3.7 g ($P = 0.0002$)] than 2019, respectively. This may be attributable to more rainfall events and more moderate temperatures compared with the previous year (Table 1).

One explanation as to why additional N applications may reduce

WDD is that more N leads to more vegetative growth, especially leaves, which could provide shading for berries during times of heat stress (Quezada et al., 2007). Although we did not measure total leaf weight, we did collect fresh weights of floricanes after harvest was completed in 2019 and 2020. Although the differences were not statistically significant ($P = 0.30$), both N treatments had two-thirds more fresh weight than the 0x treatment (4.2 vs. 2.8 kg). Presumably, there were more canes and more leaves to make up that difference as a result of previous years’ N applications (Naraguma and Clark, 1998). Although not statistically different, this increase in cane weight could be biologically important in the reduction of WDD. Ostensibly, this difference could provide additional shading, fitting the supposition of Quezada et al. (2007).

Additional N can reduce plant stress, specifically by reducing inhibition

Fig. 2. Percentage of blackberry fruit with white drupelets by nitrogen (N) fertilizer application rate averaged over 3 years (2018–20). Initial fertilizer application of 50 lb/acre (56.0 $\text{kg}\cdot\text{ha}^{-1}$) N was applied to all plots. Treatment 0x had no further N applied, whereas treatments 1x and 5x had 100 $\text{kg}\cdot\text{ha}^{-1}$ N applied in a single application at bloom and over 5 consecutive weeks (20 $\text{kg}\cdot\text{ha}^{-1}$ N per application). Different letters above the columns indicate significant differences at $P \leq 0.05$ as determined by Tukey’s honestly significant difference. 1 $\text{kg}\cdot\text{ha}^{-1} = 0.8922$ lb/acre.

Fig. 3. Percentage of blackberry fruit with white drupelets by year from 2018–20 for three nitrogen (N) application rates. Initial fertilizer application of 50 lb/acre (56.0 kg·ha⁻¹) N was applied to all plots. Treatment 0× had no further N applied, whereas treatments 1× and 5× had 100 kg·ha⁻¹ N applied in a single application at bloom and over 5 consecutive weeks (20 kg·ha⁻¹ N per application). Different letters above the columns indicate significant differences at $P \leq 0.05$ as determined by Tukey's honestly significant difference. 1 kg·ha⁻¹ = 0.8922 lb/acre.

of photosynthesis brought on by high-temperature stress (Huang et al., 2017); however, we did not find evidence of this from gas exchange measurements taken in mid-May. Photosynthesis measurements of carbon dioxide assimilation, transpiration, and stomatal conductance showed no significant differences among treatments ($P = 0.29$, $P = 0.50$, and $P = 0.61$, respectively). Readings were in the range or slightly greater than reported previously in blackberry (Lykins et al., 2021; Stafne et al., 2001). The only significantly different measurement was in the leaf vapor pressure deficit. The 0× treatment had a higher reading (1.22 kPa) vs. the other two additional N treatments (1.19 kPa). Although statistically different, the biological difference is minimal and therefore unlikely to have much overall effect on the blackberry plant or fruit.

Leaf chlorophyll index measured in 2020 did not differ among treatments ($P = 0.57$). Values obtained were slightly less than those reported by Stafne et al. (2017), but similar to those of Caillouet et al. (2016) and dos Santos Pereira et al. (2019). Therefore, these blackberry bushes were not likely stressed at the time of measurement based on leaf chlorophyll index and leaf photosynthesis measurements.

Changing weather conditions such as fluctuating temperatures and humidity, high temperatures, and rainfall have been associated with WDD, but mostly determined observationally. In this study, WDD for the 0× treatment correlated significantly and negatively to maximum high daytime temperatures during May (Table 2) over the three seasons—meaning that as temperatures increased, so did WDD. However, this

was not true for June (Table 2). There was no correlation between WDD and maximum daily temperature in May or June for the 1× or the 5× treatments (Table 2). May in South Mississippi is when spring-like conditions (early May) transition to more summer-like high daytime and nighttime temperatures, increased humidity, and higher solar radiation (late May). Lykins et al. (2021) suggested a mechanism by which blackberry leaves can adapt to high-irradiance conditions. There is some evidence recorded in our study to support that hypothesis (Table 2) and it does potentially help explain reductions in WDD over time observed by others (Fernandez, 2012). Huang et al. (2017) also stated that there is a photosynthetic lag when environmental conditions change from low irradiance to high irradiance; thus, activation of enzymes and stomatal function are not at optimal levels. They also concluded that high N applications can increase tolerance to high temperatures through better maintenance of stomatal conductance and leaf water potential. Combining these hypotheses could potentially clarify why WDD occurs and how its manifestation on berries changes as the harvest season progresses. In our study, gas exchange measurements were taken in mid-May and showed no significant differences among treatments. Perhaps measurements closer to harvest, when temperatures were hotter and the plants more stressed, would have led to different results that could explain the effect of N treatments. There were no significant correlations with minimum daily temperatures for any treatment (Table 2).

The only environmental variable that correlated significantly with WDD in all three treatments was the number of rainfall events during the harvest period over three harvest seasons. For

Table 2. Correlations of maximum (Max) and minimum (Min) daily temperatures during May and June with three nitrogen (N) fertilizer treatments (0×, 1×, and 5×) over three seasons (2018–20) for 'Sweetie Pie' blackberry in Poplarville, MS.

Treatment ^z	May				June			
	Max temp		Min temp		Max temp		Min temp	
	<i>r</i> ^y	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
0×	-0.76	0.03	0.19	0.65	-0.20	0.54	0.03	0.93
1×	-0.65	0.08	-0.12	0.78	-0.31	0.32	-0.20	0.53
5×	-0.48	0.23	-0.32	0.44	-0.19	0.55	0.01	0.97

^z0× = control, initial application of standard rate of 50 lb/acre (56.0 kg·ha⁻¹) N; 1× = 100 kg·ha⁻¹ N once at bloom in addition to the control rate; 5× = 20 kg·ha⁻¹ N starting at bloom and continuing for 5 consecutive weeks for a total of 100 kg·ha⁻¹ N in addition to the control rate. 1 kg·ha⁻¹ = 0.8922 lb/acre.

^yCorrelations were estimated by restricted maximum likelihood using the multivariate procedure; pairwise correlations were tested by Pearson's correlation coefficient (May, $n = 8$; June, $n = 12$) at $P \leq 0.05$.

0× ($r = 0.49$, $P = 0.03$), 1× ($r = 0.47$, $P = 0.04$), and 5× ($r = 0.46$, $P = 0.04$), the number of rainfall events all increased the percentage of berries showing WDD. However, rainfall amounts were not related to WDD. In theory, more rainfall events should lead to more cloud cover and lower temperatures, but humidity would be increased.

Overall, it is likely that multiple environmental factors are involved in creating white drupelets in blackberries and assigning cause and effect to one is difficult. This is also true in the case of other blackberry fruit disorders such as red drupelet reversion (Edgley et al., 2020). WDD expresses several stress response genes differentially (Fernandez et al., 2019), but more work is needed to narrow down the specific roles these genes play. In our study, increasing N decreased the presence of WDD; however, the economic and environmental costs must be weighed to determine whether additional applications are commercially beneficial.

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