Patterns of Floral Nectar Production of Onion (*Allium cepa* L.) and the Effects of Environmental Conditions

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ABSTRACT. Successful pollination of onion (*Allium cepa* L.) flowers greatly depends on adequate nectar production. In order to understand the nectar production dynamics of onion flowers, nectar was collected at regular intervals during a 24-hour period. Hourly nectar volumes were compared to a variety of environmental conditions, including amount of solar radiation, relative humidity, temperature, wind speed, and evapotranspiration. Production patterns showed mid- to late-morning peaks and late evening peaks in nectar volume. Nectar appeared to be reabsorbed by the flowers during the afternoon and overnight hours. Individual flowers produced the highest amount of nectar several days after initially opening. Nectar production was significantly and inversely related to relative humidity while the effects of temperature, evapotranspiration, wind speed and solar radiation on nectar production were not significant in this study.

Pollination is necessary for propagation of hybrid onion seed. Successful seed production depends on honey bees for cross-pollination of male and female genetic lines. Seed production is inconsistent and may fail due to lack of pollination (Shaw and Bourne, 1936). Honeybees appear to have distaste for flowers of certain varieties of onions and avoid visits to them. It has been found that the onion flowers which bees consistently avoid produce little or no nectar while those flowers that do attract bees produce a greater volume of nectar (Silva and Dean, 2000). Thus, the quantity of this carbohydrate-rich food reward appears beneficial in ensuring honeybee visitation to the flowers.

Nectar production is a multigene trait that can be impacted by selective breeding programs (Bond and Fyfe, 1968; Murrell et al., 1982; Pederson, 1953). However, in addition to this genetic component, environmental factors also have a significant impact on the quantity and quality of the nectar produced by the flower. Temperature, solar radiation, vapor pressure, and soil moisture all may affect nectar production (Corbet, 1990; Michaud, 1990; Nicolson, 1995; Pleasants, 1983; Shuel, 1952). These effects may be further heightened depending on the genotype by environment interactions (Jakobsen and Kristjansson, 1994; Rathcke, 1992). In order to maximize selection efforts for high nectar production, it is important to understand the degree to which these environmental factors affect production.

The rate and amount of nectar produced daily varies considerably within and among species (Burquez and Corbet, 1991; Gilbert et al., 1991; Mohr and Jay, 1990; Pham-Delegue et al., 1991; Stern et al., 1996). Many different 24-hour nectar production patterns have been demonstrated. In addition to characteristic 24-h nectar production rates, several species show a relationship

between nectar production and flower age (Bernardello et al., 1994; Cocucci et al., 1992; Galetto and Bernardello, 1993; Galetto et al., 1994; Jakobsen and Kristjansson, 1994; Kakutani et al., 1989; Real and Rathcke, 1991).

The goal of this study was to characterize the 24-hour and lifetime nectar production pattern of hybrid onion flowers as well as characterize the specific effects of environmental factors on onion nectar production in order to assist in the improvement of the standing nectar of the crop. With these data, a clearer picture of the physiology behind nectar production and the behavior of onion pollinators may be elucidated.

Materials and Methods

FIELD EXPERIMENT. Two onion inbred lines obtained from Seminis Seed (Woodland, Calif.) and one open-pollinated cultivar of onion ('Walla Walla Sweet') were planted on 4 Aug. 1998 at the Washington State University Irrigated Agriculture Research and Extension Center in Prosser, Wash. in three plots (2.4 × 4.9 m), each plot containing one of the genotypes. Preplant fertilizer (16N–7P–13.3K) (Simplot, Prosser, Wash.) was applied at a rate of 56 kg·ha⁻¹ N. The plants were overwintered in the field. A postplant application of ammonium nitrate at a rate of 112 kg·ha⁻¹ was made the following March. Crops were irrigated as needed using drip tape. Fungicide applications of chlorothalonil (Bravo 720, ISK Biosciences, Mentor, Ohio) were applied four times throughout June and July 1999 at a rate of 2.32 kg·ha⁻¹.

Only the 'Walla Walla Sweet' onions were planted in 1999 for the study due to the fact there were no significant differences in nectar production patterns between genotypes in 1998. Three replicate plots (2.4 × 4.9 m) were planted and cultivated as in the 1998-planted plots.

NECTAR COLLECTION: TIME OF DAY. Nectar volumes were measured in order to observe changes in production throughout the day as well as to correlate production with environmental factors. Plastic bags were placed over the umbels 24 h before nectar collection to minimize nectar evaporation and prevent

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insect foraging. Paper bags were placed over the plastic bags to minimize temperature increases. Umbels were harvested and individual flowers at the pollen-shed stage were immediately placed in 1.5-mL microcentrifuge tubes. Samples were centrifuged at a rate of 1398 g_n for 5 min. After centrifugation, individual flowers were removed from the tubes and nectar was measured using calibrated pipets. Nectar was subsequently stored at -20°C in the microcentrifuge tubes sealed with parafilm for subsequent analysis. In 1999, nectar was collected from individual flowers on three different plants (one plant per replicated block) from the three genotypes previously described at five sampling times (0800, 1000, 1200, 1400, and 1600 HR). The sampling was repeated in this manner over the six days during which the onion seed crop was at full bloom. For analysis, if no block $\times$ time, cultivar $\times$ time, or day $\times$ time interactions were found, samples collected over the six days were pooled and considered replicates of the specific sampling time to elucidate the nectar production patterns.

In 2000, only ‘Walla Walla Sweet’ onions were used in the study due to the fact that in the previous year no significant differences in the nectar production patterns of the genotypes were seen ($P = 0.56$). Flowers from three different plants (one per replicated block) were taken at pollen shed. Nectar was collected at eight sampling times in order to quantify nectar production during the overnight hours: 0300, 0600, 0900, 1200, 1500, 1800, 2100, and 2400 HR. Sampling was limited to five days due to the faster maturation of the crop. As in the previous year, if no block $\times$ time or day $\times$ time interactions were found, samples collected over the five days were pooled and considered replicates of the specific sampling time. Nectar concentrations were determined by HPLC (described below).

NECTAR COLLECTION: STAGE OF BLOOM. A second set of nectar samples were taken to determine nectar production changes as individual flowers on the umbel aged. Nine individual flowers of the appropriate developmental stage age (prepollen shed, pollen shed, and postpollen shed) were immediately placed in 1.5 mL microcentrifuge tubes, three florets per tube. Samples were centrifuged at a rate of 1398 g_n for 5 min. After centrifugation, individual flowers were removed from the tubes and nectar was measured using calibrated pipets. Nectar was subsequently stored at -20°C in the microcentrifuge tubes sealed with parafilm for subsequent analysis. Nectar concentrations were determined by HPLC (described below).

WEATHER ANALYSIS. Weather data for relative humidity, temperature, solar radiation, wind speed and evapotranspiration were obtained on both an hourly and daily basis using the Public Agricultural Weather System (PAWS) (Washington State University, Prosser, Wash.) and correlated with nectar produc-

tion of the ‘Walla Walla Sweet’ onion. Evapotranspiration was calculated using the Penman-Monteith method ($\text{ETc} = \text{ETo} \times \text{Kc}$), using grass evapotranspiration values as ETo and the crop constant (Kc) for irrigated onions of 1.05 (FAO, 1998). These values were correlated with the hourly nectar data.

ANALYSIS OF NECTAR SUGAR CONTENT. Nectar concentrations were measured to determine if the concentration of sugar secreted in the nectar (sucrose + glucose + fructose) changed throughout the course of the day. Concentrations for the nectar produced at each of the sampling times was determined from the samples collected for nectar volume determinations. Replicates of each sampling time on each day were pooled to obtain a large enough volume for analysis, diluted with deionized water at a 1:5 ratio, and analyzed for sugar composition and concentration by high performance liquid chromatography (HPLC). The HPLC used for analysis included a Waters 510 pump (Waters, Milford, Mass.) for solvent delivery and a Waters 712 WISP injection system with a Waters 410 differential refractometer. Separation of carbohydrates was achieved using a Waters carbohydrate analysis column ($3.9 \times 300\text{ nm}$, $125\text{ \AA}$, $10\text{ }\mu\text{m}$). The operating conditions consisted of a flow rate of $2\text{ mL}\cdot\text{min}^{-1}$ and a run time of 25 min. An 80:20 solution of acetonitrile: water was used as the mobile phase. Analysis was conducted with the coupled Maxima 820 data system (Waters).

STATISTICAL ANALYSES. Analysis of variance and simple linear regression was conducted with Statview (SAS Institute, Inc., 1998). The Fisher least significant differences test (LSD) was used for all a posteriori comparisons, with $P < 0.05$.

Results

HOURLY DATA COLLECTION, 1999. During the 1999 experiment, significant differences were shown between the amounts of nectar produced at different sampling times as shown in Table 1. Cultivar $\times$ time ($P = 0.56$), day $\times$ time ($P = 0.26$), and block $\times$ time ($P = 0.94$) for nectar volume were not significant. Nectar production reached a peak of approximately 8 μL /individual flower during mid- to late-morning, declining to a level of about half that volume by mid afternoon. During late afternoon, nectar production began to increase again. No significant differences were seen in the concentration of nectar sugars during the course of the day ($n = 90$, $F_{df=4,85} = 1.515$, $P = 0.20$). Cultivar $\times$ time ($P = 0.35$) and day $\times$ time ($P = 0.55$) interactions with nectar concentration were not significant.

Significant differences in hourly nectar production were found in the 2000 experiment (Table 1). Nectar production reached a first peak of 14 μL /individual flower at late-morning. Volumes

Table 1. Mean nectar volumes per hour ($\pm$ SE) and coefficient of variation for hourly sampling times. Nectar volumes were obtained by averaging the nectar volumes produced by individual flowers over a 5-d (2000) or 6-d (1999) period.

1999				2000			
Hour	n	Volume (μL /individual flower) ^a	CV	Hour	n	Volume (μL /individual flower)	CV
0800	54	7 $\pm$ 1ab	0.56	0300	20	13 $\pm$ 1ab	0.29
1000	54	9 $\pm$ 1b	0.50	0600	20	9 $\pm$ 1a	0.59
1200	54	7 $\pm$ 1ab	0.59	0900	20	9 $\pm$ 1a	0.50
1400	54	4 $\pm$ 1a	0.78	1200	20	14 $\pm$ 1b	0.37
1600	54	5 $\pm$ 1ab	0.99	1500	20	12 $\pm$ 2a	0.68
				1800	20	14 $\pm$ 1b	0.42
				2100	20	18 $\pm$ 2b	0.44
				2400	20	17 $\pm$ 1b	0.75

^aMean separation in columns by Fisher’s protected LSD test at $P \leq 0.05$.

Table 2. R-values of the relationships between hourly/daily onion floral nectar production and temperature, solar radiation, wind, and relative humidity, 1999 and 2000. Nectar volumes are average values taken over a 24-h period. Weather values were obtained directly from the Public Agricultural Weather System (PAWS).

Data collected	Temp (°C)	Solar radiation (MJ m ⁻² ·d ⁻¹)	Wind (m·s ⁻¹ at 2 m)	Evapotranspiration ^z (kg·m ⁻² ·s ⁻¹) (10 ⁻⁶)	Relative humidity (%)
1999 Hourly	0.21				-0.20
2000 Hourly	0.14				-0.39*
1999 Daily	0.04	-0.78	-0.28	-0.59	-0.80*
2000 Daily	0.52	0.72	0.12	0.38	-0.92*

^zEvapotranspiration calculated using Penman-Montheith method and grass Eto.

*Significant at $P < 0.05$.

began to decrease until mid-afternoon and subsequently began to increase again, reaching a second peak of 18 μ L/individual flower at 2100HR. Nectar volumes then declined steadily until daybreak. Like the previous year, there was not a significant difference between nectar concentrations at the various times ($n = 40$, $F_{df=7,32} = 1.244$, $P = 0.33$) and cultivar $\times$ time ($P = 0.35$) and day $\times$ time ($P = 0.55$) interactions with nectar concentration were not significant.

HOURLY NECTAR VOLUMES VS. HOURLY WEATHER DATA, 1999 AND 2000. Hourly nectar volumes produced by individual flowers in 1999 were not statistically correlated at the 5% level with solar radiation, temperature, or relative humidity (Table 2). Hourly nectar volumes in 2000 were inversely related to relative humidity ($P = 0.004$, $r = -0.39$). No correlation was found between solar radiation or temperature and nectar volumes in 2000 (Table 2). The concentration of nectar was not correlated with any weather factor.

DAILY NECTAR VOLUMES AND WEATHER FACTORS, 1999 AND 2000. Average daily nectar production was not statistically correlated with temperature, solar radiation, evapotranspiration or wind speed (Table 2). Nectar production was negatively correlated with average relative humidity (Table 2). Nectar concentration was not correlated with any weather factor.

NECTAR PRODUCTION PATTERNS: STAGE OF BLOOM. Nectar production differed significantly at each stage of bloom ($n = 236$, $F_{df=2,233} = 40.94$, $P < 0.0001$). During the stage of prepollen shed, nectar production was at an intermediate level (6 μ L/individual flower). At pollen-shed, nectar levels were greatest (9 μ L/individual flower). As the anthers dehiscid, nectar production declined (4 μ L/individual flower). Nectar carbohydrate concentration was not significantly different over time.

Discussion

The secretion of floral nectar is essential to plants dependent on insect pollination for reproduction (Shuel, 1992). Despite its importance, there are still many unanswered questions about this physiological process. Although potential nectar yield may be set by heredity, the extent to which the maximum production potential of the plant is realized depends upon the environmental conditions (Shuel, 1992). Thus, to better succeed in efforts to select for high nectar production, environmental effects on both short and long time scales should be understood.

Onion floral nectar production was found to change on both an hourly and daily basis. Throughout the day, characteristic patterns of high and low production rates were followed, with peaks in the morning and evening. These patterns were the same with the three genotypes of onions that were studied. According to Wainselboim et al. (2003), the most relevant parameter in

the honey bee's assessment of the profitability of a crop is the volume of nectar that the bee has been able to collect in a certain period of time. Thus, periods of low standing nectar volume in the afternoon could have a detrimental effect on pollination due to the fact that pollinators of the onion flower (specifically honey bees) are active at this time. Foraging upon the onion crop during times of low nectar flow may result in communication to the hive that it is a poor food source.

The pattern of nectar production throughout the life of the flower shows that most nectar is produced at pollen shed. Thus, the greatest number of pollinators should be attracted to the flower at the time when they would likely distribute pollen. Although the ovaries of the onion flowers are receptive to pollen before this time, this should not affect overall pollination of the crop due to the fact that florets of the umbel bloom at a random sequence throughout the umbel. This encourages pollinators to investigate the entire umbel regardless of individual floret bloom stage.

Many of the studies regarding nectar production patterns on a daily scale have shown the phenomenon of nectar reabsorption. This can be described as nectar solutes disappearing from an unvisited flower (Burquez and Corbet, 1991). A study of *Grevillea robusta* showed that reabsorption occurs at both a high nectar volume and a high nectar concentration (Nicolson, 1995). The author speculates that reabsorption may have the function of maintaining a constant low nectar concentration in spite of evaporation. Burquez and Corbet (1991) suggest that reabsorption does not occur in flowers in which nectar accumulates at a site remote from the nectary. This could be an important evolutionary modification for the plant; a flower that reabsorbs uncollected nectar reclaims a part of the energy allocated to nectar production if the cost of reabsorption is less than production. The 1999 and 2000 data suggest that nectar reabsorption may be occurring in onion flowers, since nectar concentration remains constant across sampling time despite significant differences in nectar volumes.

Environmental factors have a significant effect on the nectar production of plants. Since the plant's response to environmental changes can be accomplished on a relatively rapid scale, nectar production can also change over a short time period. Temperature and relative humidity have been most consistently shown to impact nectar production. With onion floral nectar production, relative humidity was correlated with nectar production on both hourly and daily bases. Bertsh (1983), studying *Epilobium angustifolium*, found that relative humidity was positively correlated with nectar volume. Similarly, cotton nectar production was found to decline with decreasing relative humidity which the authors attribute to increasing moisture stress (Butler et al., 1972). However, onion nectar production showed an opposite relationship to relative humidity; at higher relative humidities, nectar production declines. This may be due to increased move-

ment of sap through the xylem; a smaller percentage of water vapor in the air may lead to a greater rate of transpiration and thus more water movement throughout the plant. Since nectaries are supplied by both xylem and phloem, more sap moving through the xylem would result in greater nectar volume. Shuel (1992) notes that in the absence of moisture stress, lower humidity may enhance the flow of solution to the nectaries. The fact that the onion nectar collected in this study is relatively dilute compared to other reported nectar concentrations (4% to 60%) also suggests a high proportion of xylem connections to the nectary and possible increased flow of xylem sap at low relative humidity. Further studies need to be performed in order to correlate the moisture status of the onion plant with nectar production and the effects of environmental conditions. It is also worthwhile to emphasize that the nature in which nectar was collected in this experiment prevented evaporation of water from the nectar. In the field, increases in nectar production due to increased transpiration by the plant at lower relative humidities may be negated by increased evaporation of the nectar. Thus, although gross nectar production in dry environments may be high due to water loss from the plant, net nectar production may be less than in humid environments where evaporation of nectar is minimized.

Enhancement of our understanding of nectar production could help in efforts to improve the bee attractiveness of pollination-poor crops. By understanding the environmental effects that influence nectar productions, plant breeders could more readily select plants that will be more likely to produce an adequate seed crop.

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