Nectar and Flower Traits of Different Onion Male Sterile Lines Related to Pollination Efficiency and Seed Yield of F1 Hybrids

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ABSTRACT Honey bees are the main pollinators of onion crops for seed production, but owing to low attractiveness of flowers, pollination is often inadequate. Pollination problems result in low seed yields. This problem is accentuated when male sterile lines (MSL) are used to produce hybrid onion seeds. In this study, the effect of floral attributes and nectar composition on the preference of honey bees of four MSLs and one onion open pollinated cultivar were assessed. The chemical composition of nectar was described through the analysis of sugars, trace elements, volatile organic compounds, and phenol compounds. The samples studied showed qualitative and quantitative differences in the analyzed traits of flowers and nectar among the different lines. Furthermore, field observations showed a great difference on the number of bee visits and seed yield among the onion lines analyzed. For the first time, this study demonstrates that there are marked differences in the chemical composition of nectar and floral morphology between open pollinated and MSLs and also within MSLs. In addition, these differences were correlated with the number of visits and seed yield. Therefore, it would be possible to select indirectly the most promising productive MSL using simple determinations of chemical compounds or floral morphological characters.

KEY WORDS *Allium cepa* L., male sterility, floral traits, nectar composition, seed yield

Onion is considered a biennial crop that forms the bulb during the first growing season, and flowers at the second cropping cycle giving viable seeds. Seeds are the usual way that onion can be reproduced. The individual flowers of this species are hermaphrodite and fertile but exhibit protandry, that is, the anthers release pollen before the stigma is receptive (Acosta et al. 1993). Nevertheless, protandry does not prevent flowers of the same umbel to be selfed. Mainly bees bring pollen from other flowers on the same plant or other plants (Currah 1990).

Onion is an allogamous spice, which allow the exploitation of heterosis, and hybrids are increasingly used. Open-pollinated (OP) and first generation (F1) onion hybrids are usually grown around the world. Cytoplasmic-genic male sterility (CMS) systems are used to produce hybrid-onion seed (Brewster 1994).

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To produce F1 hybrid seed in onion, it is necessary to cross a male sterile line (MSL) with a fertile one. F1 hybrids have the advantage of greater uniformity and high yields in the production of bulbs; however, F1 hybrids normally produce lees seeds than OP cultivars. As a consequence, to be economically viable, they must have a good seed yield, otherwise become too expensive for growers. Moreover, highly variable seed yields between different hybrid seeds of onion have been observed (Silva and Dean 2000).

Cross-pollination of male and female genetic lines depends on honey bees (*Apis mellifera* L.) (Silva et al. 2004). Consequently, seed yield is closely correlated with the behavior of honey bees in seed onion fields (Benedek 1976).

Nevertheless, widespread use of the honey bee as pollinator not always bring about the expected results because the onion nectar is not particularly attractive for it. Honey bees have distaste for flowers of certain varieties of onion and avoid visits to them, and the appearance of different sources of nourishment can easily pull away these bees from plantations of flowering onion (Silva et al. 2004).

In animal-pollinated plants, fitness is influenced by floral traits that function as an advertisement and reward for pollinators (Poveda et al. 2005). Flowers have different structure that require different pattern to be learned through trial and error for acquiring food. All this information is used to choose between flowers of different species and to make foraging decisions

(Abrol 2006). Foraging pollinators typically visit flowers for nectar and/or pollen; these resources differ distinctly with respect to both foraging benefits and costs. Consequently, the choice within and between plant species varies with pollinator and floral morphology (Harder et al. 2011).

Flower attributes such as color, size, and specially nectar volume and composition are considered important factors for honey bee attraction. Minor components of nectar might directly affect bees foraging; therefore, pollination further accentuate the necessity for biological data on nectar. Nectar usually does not repel bees, but a particular nectar may be less attractive than nectar of competing flowers (Afik et al. 2006b). Some authors suggest that onion MSL flowers produce less nectar, as a consequence recommend doubling the number of pollinators for onion hybrid seed production (Wilkaniec et al. 2004).

It is assumed that the lower seed yield is given by a deficiency in pollination, related to the morphology of the flower or nectar composition, factors that influence the attraction exerted by different MSLs on bees. Little data are available concerning nectar properties as they relate to honey bee visitation and subsequent seed production in onion (Benedek 1976, Sajjad et al. 2008, Abrol 2010). Honey bees forage selectively among onion cultivars and are sensitive to qualitative and quantitative differences in floral cues and rewards. Development of hybrid onions may have altered flower nectar and aroma chemistry. Loss of attractiveness usually results in reduced pollinator visitation, reduced pollen transport, and reduced seed yields (Hagler et al. 1990).

Few studies have been done on the composition of onion nectar and its relationship with bee foraging behavior (Hagler 1990, Silva and Dean 2000); these works have focused on sugar and potassium concentration. It has been reported that potassium (K) levels in onion nectar has an effect on the attractiveness to honey bees; honey bee preferences for specific sugar solutions (fructose, glucose, and sucrose) and their correlation with onion flower attractiveness have been assessed as well (Silva and Dean 2000). However, phenolic substances are quite widespread in nectars (Torres and Galetto 1998). Their accumulation may turn the nectar toxic, becoming repellent to some visitors. Phenolic substances are also relatively common scent compounds of flowers. As well as attracting pollinators or repelling nectar thieves, these scent compounds may have a defensive function (Nicolson and Thornburg 2007). To our knowledge, there are no reports on the overall characterization of nectar and floral morphology of onion MSLs and their effect on bee preference and seed yield.

The aims of our work were as follows: 1) to describe qualitative and quantitative analyses of sugars, elemental species, volatile organic compounds (VOCs), and phenol compounds in onion nectar; 2) to analyze floral traits of onion flowers; 3) to compare between different onion MSLs the quantitative and qualitative profile; 4) to correlate nectar-analyzed compounds and floral traits with field observations of honey bee visits and seed yield. These data are intended to provide plant breeders a tool to select MSLs that may produce F1 hybrids higher seed yield.

Materials and Methods

Plant Materials. An OP onion cultivar, Valcatorce INTA (Galmarini 2000), as well as three MSLs (MSL 1, MSL 2, and MSL 3 from Enza Zaden) were cultivated in a randomized complete block design with three replicates for each cultivar under a cage (4 by 8 m) to isolate the materials from other pollinators, at the Institute of Horticulture (Agronomy Faculty, UNCuyo, Mendoza, Argentine). No pesticides were used along the experiment. The plants flowered from November to December, 2011. Flowers were picked at the middle of November, at 50% of flowering. At midday, 10 umbels per plot were randomly chosen to pick flower samples. Onion nectar was obtained in blossom.

Nectar Extraction. To obtain nectar in the most natural way, and preserve it in similar conditions as it is in the plant, we found that the most effective way of extraction was to separate freshly opened flowers from umbels, then anthers, filaments, and peduncle were removed, afterwards flowers were centrifuged (13,000 rpm, 30 min, 4°C) into a 1.5 ml microtube. From 10 umbels per plot, it was possible to extract around 100 μl of nectar.

Foraging Behavior of Bees. In the current study, *A. mellifera* L. was used as pollinator. At 10% of flowering, a hive with 10 nucs was placed in the cage. Since that time, the number of bees visiting each plot was recorded by a visual counting method for 1 min from each side of the plot. The mean of these three observations constituted a reading for each line. The number of bee visits per umbel per minute was recorded every day except cloudy days, three times a day at 9:00 a.m., 12:00 p.m., and 3:00 p.m., up to 100% bloom. Simultaneously, air temperature was recorded with sensors placed at the height of the inflorescences; solar radiation was recorded with a radiometer (Kavadivice).

When fruit set was accomplished, umbels were harvested and dried. Seeds were extracted manually and weighed to estimate seed yield. Relationships between seed yield and frequency of honey bee visits and volatile compounds were estimated.

Floral Traits. We selected five plants of each line, taking 10 flowers per plant. The flowers were kept in formalin–acetic acid–alcohol (FAA) until morphology measurements. For each flower, we measured receptacle and style length and also length and width of the ovary, tepal, anthers, and nectary. Ovary and anther area were calculated, and also nectary volume was estimated.

Chemical Analysis. *Sugar Content.* Sugar analysis of nectar was done on a Shimadzu HPLC, model 10 A, with a differential refractometer detector and a Rheodyne injector with 10 μl sample loop. RCM-Monosaccharide Ca^{+2} column (30 cm × 7.8 mm) was used with a mobile phase of EDTA-Ca 0.1 mM at 0.5 ml/min.

Table 1. Number of honeybee visits during day hours at 50% of blooming and seed yield per inflorescence in different onion lines

Line	Hours			Seed yield
	9:00 a.m.	12:00 p.m.	3:00 p.m.	
Valcatorce INTA (OP)	0.67 ± 0.07a	0.94 ± 0.08a	0.93 ± 0.10a	2.98 ± 0.53a
MSL 1	0.11 ± 0.05b	0.16 ± 0.07c	0.19 ± 0.05c	0.90 ± 0.42c
MSL 2	0.29 ± 0.05b	0.43 ± 0.07b	0.42 ± 0.08b	2.01 ± 0.20b
MSL 3	0.23 ± 0.04b	0.35 ± 0.09bc	0.34 ± 0.03bc	1.30 ± 0.06bc

Values represent mean ± SD of three determinations.
Number of visits during day is expressed as visits of honeybees per umbel per min.
Seed yield is expressed as grams per umbel.
Values in the same column with different letters present significant differences $P < 0.05$.

Concentration of sugars was expressed as grams per liter of nectar (IFUM 1996).

Mineral Composition. Twenty-two elements (B, Mg, K, Ti, V, Cr, Mn, Fe, Co, Ni, Ga, As, Se, Rb, Sr, Mo, Cd, Cs, Re, Tl, Pb, and U) were determined in each nectar sample. A transformation of nectar samples into homogenous liquid phase before sample introduction is rather required. To this aim, nectar samples (20 μl) were diluted with Milli-Q water (1:500 vol:vol) and acidified to 1% HNO_3. An inductively coupled plasma mass spectrometer, Perkin–Elmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used for element determinations. The argon gas with minimum purity of 99.99% was supplied by Air Liquid Corporation (Córdoba, Argentina). An HF-resistant and high performance perfluoracetate (PFA) nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC^3 system from ESI (Omaha, NE) was used. Tygon black/black 0.76 mm internal diameter (i.d.) and 40 cm length peristaltic pump tubing was used.

Taking into consideration that a nectar-certified reference material is not available, a recovery test was performed for method validation. Results were satisfactory; recoveries ranged from 92 to 104% in all cases.

Phenol Content. Phenolic compounds were analyzed by Capillary Zone Electrophoresis (CZE). CZE separations were carried out using a Capel 105M apparatus. Phenols were extracted from nectar samples (50 μg), which were homogenized in Milli-Q water (1:5, wt:vol) at pH 2 adjusted with HCl. The solutions were then filtered through a Sep-Pak C18 cartridge, which was previously activated with methanol (5 ml) followed by acidic water (5 ml). The phenolic compounds remain in the column while sugars and other polar compounds elute with the aqueous solvent, resulting in a flavonoid recovery of >95%. The column was rinsed with 1 ml of acidic water. The phenolic fraction was eluted with 500 μl of methanol. Conditions: 30 mM boric acid buffer, pH 9.50; capillary, 67 cm full length, 50 cm effective length, 75 μm i.d., 375 μm outside diameter; hydrodynamic injection at 30 mbar, 2 s; 25 kV constant voltage; 25°C, detection by ultra violet (UV) absorbance, electropherograms were recorded at 290 nm.

Volatile Compounds. The characterization of the volatile fraction of different cultivars under study was performed using headspace solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GCMS) and statistical analysis. Two hundred microliters of nectar were diluted (1:5) with pure water. Nectar samples were spiked with an internal standard solution to obtain a final concentration of 1 μg/ml. However, 500 μg of freshly opened flowers was sampled per replica. Both samples were placed into a 10-ml glass screw-top vial with polytetrafluoroethylene/silicone septa and placed on the magnetic stirrer (1,000 rpm). They were allowed to equilibrate for 30 min at 30°C. Then, the SPME fiber was exposed on headspace mode (2 cm) during 30 min. An 85-μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber was used. GC-MS analyses were performed on a Varian CP-3800 gas chromatograph with a Saturn 2200 Ion Trap Mass Spectrometric detector (Varian, Walnut Creek, CA). The system was operated by Saturn GC-MS Workstation software version 6.41. The column was a Factor Four capillary column VF-5MS (50 m by 0.25 mm i.d., with 0.25-μm film thickness; Varian, Lake Forest, CA). Qualitative analysis of the constituents was based on comparison of the obtained mass spectra with those of reference compounds in the NIST Mass Spectral Search Program (NIST version 2.0). Quantitative analysis was performed by means of the internal standard method.

Statistical Analysis. Statistical analysis was performed by analysis of variance (ANOVA), and means were compared using Tukey test. All the analyses were done in triplicate. The results were significant at $P < 0.05$ unless specified otherwise. To highlight the data structure and to find the overall relationships between nectar composition and flower morphology that condition the pollination efficiency of MSLs for onion hybrid seed production, principal component analysis (PCA) was used. Basic statistic and multivariate analysis were carried out using statistical package STATISTICA 7.0 for Windows (from StatSoft, Tulsa, OK).

Results and Discussion

Pollinator Preferences. There was a wide range of the number of bee visits among the onion lines analyzed. All MSLs had lower number of visits than the OP cultivar. In fact, MSL 1 had the lowest foraging population, which was sixfold lower than those observed in the OP line (Table 1). At 9:00 a.m., *A. mellifera* L. activity was very low when air temperature was 25°C for all the materials. In MSLs, the number of

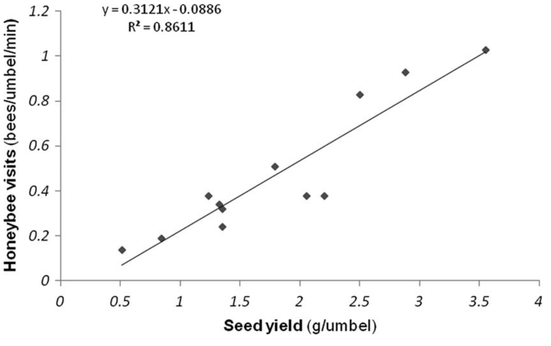

Fig. 1. Relationship between seed yield and the number of honey bee visits.

visits was between 0.11 and 0.29 visits/umbel/min, while for the OP cultivar was 0.67 visits/umbel/min. The foraging population increased thereafter, and maximum abundance was observed between 12:00 p.m. and 3:00 p.m. when the air temperature ranged between 30 and 35°C. The number of visits per umbel per minute at that time was 0.16 and 0.43; while for the OP cultivar was 0.94 visits/umbel/min (Table 1). These results are in agreement with those reported by Abrol (2010). As this author reported, different cultivars differ greatly in their attractiveness to pollinating insects; flower visitation rates differ at different times of the day depending on atmospheric conditions, availability of nectar, pollen, and bee species involved.

Seed Yield. The pollination effectiveness was determined on the basis of seed yield. A great difference was found between seed yield of the hybrids and the OP cultivar; the latter had threefold higher amount of seed than the F1 hybrid with lower yield. There were also seed yield differences among the hybrids. Frequency of honey bee visits and seed yield were highly related (Fig. 1). These results are in concordance with those reported by Wilkaniec et al. (2004). It is worth mentioning that the results obtained in the studied lines under a cage were consistent with data obtained for the same hybrids under open field conditions (Fig. 2).

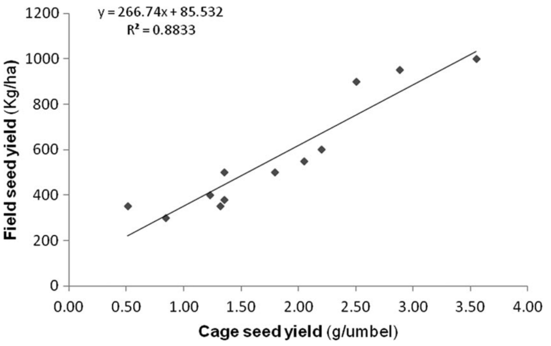

Fig. 2. Relationship between seed yield under controlled conditions and under field conditions of three F1 hybrids and one OP onion cultivar.

Floral Attributes. Tepal, internal and external anther, and style length showed significant differences between fertile and MSL and also among MSLs (Table 2). However, stamens and ovary measurements did not differ between lines. Nectary volume showed significant differences between fertile and male sterile line, as well as among male sterile lines.

Sugar Composition. Nectar sugar composition of all the onion cultivars showed hexose dominance. Nectars contained glucose and fructose; no other mono- or disaccharides were detected in appreciable quantities. These results are in concordance with those reported by Silva and Dean, 2000. Furthermore, Hagler et al. (1990) who studied the composition of onion nectar in six different cultivars, also reported that sucrose was the least abundant sugar present in all instances. The proportion of fructose was always higher than that of glucose (Table 3). OP cultivar had a higher amount of sugars than the MSLs. In MSLs, the fructose or glucose ratio was particularly unbalanced, as the proportion of fructose was twice than glucose. Despite MSL 2 having similar fructose or glucose ratio to MSL 3, MSL 2 had twice the amount of sugars than the other MSLs.

Mineral Content. Significant differences were observed among the studied lines (Table 4). OP onion cultivar was clearly different form MSLs. Among them, MSL 3 generally had differences with the other two lines, which were quite similar. The predominant elements found were K, Mg, B, Mn, and Fe. It is noteworthy the high amount of Mg found in the onion nectar samples analyzed, which have not been reported in other studies.

Phenol Content. There is a lack of knowledge about the profiles of phenolic substances in onion nectar samples. Analysis of phenolic compounds is usually carried out using HPLC, although gas chromatography and capillary electrophoresis have also been used in some instances (Biesaga and Pyrzynska 2009). Under optimum conditions, CZE allowed the simultaneous determination of 13 phenolic components: catechin, naringenin, rutin, cinnamic acid, syringic acid, chlorogenic acid, apigenin, vanillic acid, luteolin, quercetin, caffeic acid, 4-vinylphenol, and gallic acid.

The results of the quantitative determination of phenolic acids and flavonoids in nectar samples from different onion lines are presented in Table 5. Naringenin was found only in MSLs. The OP cultivar had a higher amount of luteolin than MSLs. Comparison with literature values is difficult, as previous studies have used different conditions for sample preparation, and most of them have studied these compounds in honey samples as a way of studying the floral and geographical origin of honeys. Most of phenolics found in our study are in agreement with those reported in honey; these could be explained because of their solubility in aqueous solutions and their production in the vicinity of the floral nectar; these compounds may also dissolve in nectar (Raguso 2004).

Volatile Compounds. In total, 108 volatile compounds were identified in flower and nectar onion samples, including esters, alcohols, alkenes, sulfides, heterocycles, carboxylic acids, ketones, and alde-

Table 2. Floral morphology characters of different onion lines

Floral traits	Variable	OP	MSL 1	MSL 2	MSL 3
Perianth					
Intern tepals	L	5.05 ± 0.08c	3.80 ± 0.20a	4.29 ± 0.19b	4.32 ± 0.15b
	W	2.40 ± 0.05ab	2.51 ± 0.04bc	2.70 ± 0.07d	2.62 ± 0.02cd
Outer tepals	L	4.77 ± 0.06b	3.72 ± 0.19a	3.89 ± 0.15a	4.03 ± 0.15a
	W	2.32 ± 0.03b	2.04 ± 0.07a	2.38 ± 0.14b	2.22 ± 0.03ab
	A	11.10 ± 0.30c	7.62 ± 0.63a	9.32 ± 0.94b	8.98 ± 0.43ab
Stamen					
Intern filament	L	5.03 ± 0.09bc	3.79 ± 0.40a	4.74 ± 0.10bc	4.39 ± 0.28ab
	W	2.29 ± 0.07b	1.85 ± 0.13a	2.28 ± 0.08b	2.14 ± 0.09b
Outer filament	L	3.76 ± 0.04b	2.99 ± 0.40a	4.07 ± 0.16b	3.75 ± 0.40b
	W	0.85 ± 0.08a	0.75 ± 0.03a	0.70 ± 0.03a	0.79 ± 0.08a
Intern anther	L	1.92 ± 0.01a	1.72 ± 0.01a	1.86 ± 0.22a	1.88 ± 0.04a
	W	0.93 ± 0.01b	0.74 ± 0.02a	0.74 ± 0.04a	0.74 ± 0.05a
	A	1.78 ± 0.01c	1.28 ± 0.04a	1.39 ± 0.24ab	1.40 ± 0.12ab
Outer anther	L	1.94 ± 0.03b	1.79 ± 0.06a	1.84 ± 0.09ab	1.95 ± 0.01b
	W	0.98 ± 0.00b	0.85 ± 0.03a	0.78 ± 0.04a	0.81 ± 0.02a
	A	1.90 ± 0.03b	1.52 ± 0.10a	1.45 ± 0.13a	1.58 ± 0.04a
Gynoecium					
Ovary	L	1.56 ± 0.09a	1.53 ± 0.03a	1.57 ± 0.07a	1.60 ± 0.06a
	W	2.79 ± 0.04a	2.91 ± 0.03ab	2.95 ± 0.10b	2.99 ± 0.01b
	A	4.41 ± 0.31a	4.46 ± 0.05a	4.65 ± 0.34a	4.79 ± 0.15a
Style	L	1.43 ± 0.06a	3.64 ± 0.32c	2.59 ± 0.18b	2.51 ± 0.13b
Receptacle	L	1.88 ± 0.01ab	1.84 ± 0.04a	1.96 ± 0.06b	1.94 ± 0.05ab
Nectary	L	0.53 ± 0.05c	0.27 ± 0.01a	0.35 ± 0.01b	0.31 ± 0.01ab
	W	0.51 ± 0.01d	0.31 ± 0.02a	0.35 ± 0.02b	0.33 ± 0.01ab
	V	0.07 ± 0.01c	0.01 ± 0.00a	0.03 ± 0.00b	0.02 ± 0.00ab

Values represent mean ± SD of three determinations.

Values of length and width are expressed as millimeters values of area are expressed as square millimeters and values of volume are expressed as cubic millimeters.

Values in the same file with different letters present significant differences $P < 0.05$.

hydes. The number of sulfur compounds is the largest among the detected compounds. The predominant volatile compound was dipropyl disulfide, followed by 1,3-dithiane, 2,2-dimethyl, dimethyl trisulfide, piperidine 4-methyl-3-pentenal, methyl(E)-1-propenyl sulfide, dipropyl trisulfide, and 3,4-dimethylthiophene.

There were marked differences in volatile sulfur compounds among examined onion plants. Variation among onion plants in their volatile sulfur compounds and their compositions have been reported; 1-propenyl and methyl groups are commonly found in onion, scallion, shallot, leek, and chive (Mochizuki et al. 1998). Dominant compounds of methyl and propyl groups in onion hybrids were observed in our study.

Comparing VOCs emitted from nectar and flowers, we found that most of these compounds are in lower concentration in nectar and many of the volatile compounds of flowers were not found in nectar. Only a small number of compounds were found merely in nectar, such as methyl sulfide or 2,4-nonadiyne. Other VOCs like dioxolanes were in higher concentration in nectar probably owing to their low volatility and high solubility in water. However, also higher concentration of alkenyl-sulfides were generally found in nectar (Fig. 3).

Table 3. Nectar sugar concentration of different onion lines

Line	Glucose	Fructose	R	Total sugar
OP	54.53 ± 0.32a	78.73 ± 1.45a	1.44a	133.26a
MSL 1	25.10 ± 0.95d	45.50 ± 2.52c	1.81b	70.60c
MSL 2	38.53 ± 0.65b	66.26 ± 2.17b	1.72b	104.80b
MSL 3	27.46 ± 0.64c	47.36 ± 1.01c	1.72b	74.83c

R, fructose/glucose ratio.

Values represent mean ± SD of three determinations.

Values in the same column with different letters present significant differences $P < 0.05$.

Sugar concentration is expressed as grams per liter of nectar.

Correlations Between Nectar Chemical Composition, Floral Traits, Pollination, and Seed Production. Hybrid onion low seed yield owing to poor pollination has previously been focused on three characteristics of onion flowers to explain their unattractiveness: nectar sugar composition, nectar potassium concentration, and nectar volume (Silva and Dean 2000). Nevertheless, so far little is known about the metabolites that are commonly found in onion nectar.

A positive correlation was found between bee visits and seed yield ($r = 0.86$) (Table 6). Taking in account the factors studied in this work, we found that there were positive and negative correlations with the number of bee visits and seed yield.

Among flower traits studied, only style length has a negative correlation with bee preference and seed yield ($r = -0.85$). MSLs with longer style had less visits of the pollinator. However, tepal and stamen length had a significant positive correlation with seed yield and the bee foraging behavior. Nectary volume was the most related trait with the onion fitness ($r = 0.93$). The higher the nectary volume, the greater the amount of bee visits and seed yield. Floral traits are crucial for a reproductive success of onion lines (Table 6), and can be considered as a component of a "floral integrated design," and therefore their size and vari-

Table 4. Nectar mineral content of different onion lines

Mineral	OP	MSL 1	MSL 2	MSL 3
B	8290.5 ± 270.5ab	7758 ± 436.0b	7734.5 ± 198.0b	8875.2 ± 323.3a
Mg	124279 ± 2765.5a	154333 ± 3310.0b	153555.5 ± 831.0b	157443 ± 3148.9b
K	3230988.5 ± 59373.5b	3330107 ± 11461.5ab	3358535 ± 57874.0a	2790578.7 ± 32568.9c
Ti	171.5 ± 22.0b	101.5 ± 12.5c	142 ± 32.0bc	265 ± 26.0a
V	11 ± 1.0a	8.5 ± 0.5b	6.5 ± 0.5b	12.2 ± 1.2a
Cr	599 ± 22.5a	451.5 ± 4.5c	446.5 ± 40.5c	520.6 ± 15.0b
Mn	1726 ± 42.5c	2609 ± 31.0b	2652 ± 30.0ab	2721 ± 26.0a
Fe	2030.4 ± 79.0d	2636 ± 114.5c	3168.5 ± 122.5b	4575.2 ± 326.8a
Co	10.5 ± 0.5b	8.6 ± 0.4c	9 ± 0.5c	19 ± 0.1a
Ni	389.5 ± 7.0a	210 ± 6.5c	217 ± 15.0c	320.8 ± 19.6b
Ga	40.3 ± 2.0a	12 ± 1.5b	12.5 ± 1.0b	13.8 ± 0.6b
As	32.0 ± 5.0a	15.5 ± 2.5b	17 ± 4.5b	23.5 ± 3.5ab
Se	62.3 ± 15.3a	68.5 ± 14.7a	86.5 ± 13.5a	59.5 ± 10.8a
Rb	1109 ± 40.0c	2416 ± 25.0a	2363.5 ± 23.0a	2194.5 ± 5.2b
Sr	950.5 ± 38.5c	1169.5 ± 8.0b	1162.5 ± 17.0b	1239.8 ± 16.2a
Mo	nd	23 ± 3.0b	23 ± 2.0b	28.2 ± 1.2a
Cd	18.5 ± 0.5a	6.5 ± 0.3c	7 ± 0.5c	11.2 ± 1.2b
Cs	8.5 ± 0.5a	4 ± 0.5b	3.5 ± 0.2b	4.3 ± 0.6b
Re	6.4 ± 0.1a	0.5 ± 0.1c	0.5 ± 0.1c	1.3 ± 0.6b
Tl	30.5 ± 1.5a	21 ± 1.4c	20.5 ± 0.5c	24.3 ± 0.5b
Pb	65 ± 2.0a	53 ± 1.5bc	49 ± 1.0c	56.5 ± 1.7b
U	10 ± 0.1a	1 ± 0.2c	1 ± 0.1c	3.3 ± 0.6b

nd, not detected.
Values represent mean ± SD of three determinations.
Values in the same file with different letters present significant differences $P < 0.05$.
Mineral composition is expressed as micrograms per liter of nectar.

ability can be related to the pollination success (Fernández et al. 2009).

Concentration and composition of nectar sugars has been often correlated with specific responses of nectar visitors (Bernardello et al. 1999, Perret et al. 2001, Afik et al. 2006a, Sala Junior et al. 2008, García and Gottsberger 2009). Among all the traits studied, nectar sugars were the most consistently related to bee visits and seed yield. A significant and positive correlation was found between these variables. The correlation with bee visits was higher for glucose than fructose ($r = 0.95$ and $r = 0.88$, respectively), while the correlation between glucose and seed yield was $r = 0.94$ (Table 6).

However, there is a clear relation between the phenolic profile and the honey bee behavior for each onion line. It is possible that the concentration of naringenin, only found in MSLs, might affect negatively honey bees foraging behavior, as the higher the concentration of naringenin, the less number of bee visits umbels had ($r = -0.78$) (Table 6). This compound may impart an unfavorable taste to nectar for pollinators. Phenolics have been reported as insect deterrents or repellents (Hagler and Buchmann 1993, Iwashina 2003, Ibanez et al. 2012); nevertheless, in onion the deterrent action has not been reported.

Nearly all previously reported works have attributed to high potassium concentrations the lack of pollination of onion crops (Hagler 1990, Brewster 1994, Nicolson and Thornburg 2007, Abrol 2010). An important result found in this study, in agreement with those reported by Silva and Dean 2000, is that the potassium concentration has no relationship with the onion fitness. However, we note that high concentra-

Table 5. Nectar phenolic content of different onion lines

Compound	Line			
	OP	MSL 1	MSL 2	MSL 3
Vinylphenol	nd	1.17 ± 0.03a	1.11 ± 0.11a	nd
Catechin	nd	nd	nd	2.57 ± 0.11
Naringenin	nd	8.01 ± 1.28a	2.57 ± 0.47b	2.52 ± 0.40b
Cinnamic acid	nd	nd	0.57 ± 0.01	nd
Chlorogenic acid	nd	nd	2.14 ± 0.10a	1.84 ± 0.08b
Quercetin	nd	1.04 ± 0.07a	0.19 ± 0.009b	nd
Vanillic acid	0.81 ± 0.03a	0.82 ± 0.03a	nd	nd
Luteolin	7.20 ± 0.23a	nd	nd	0.67 ± 0.03b
Caffeic acid	1.10 ± 0.01a	1.10 ± 0.01a	nd	0.76 ± 0.03b
TPC	9.12 ± 0.19b	11.78 ± 1.91a	6.52 ± 0.31c	8.18 ± 0.15bc

nd, not detected; TPC, total phenol content.
Values represent mean ± SD of three determinations.
Values in the same column with different letters present significant differences $P < 0.05$.
Phenolic content is expressed as milligrams per liter of nectar.

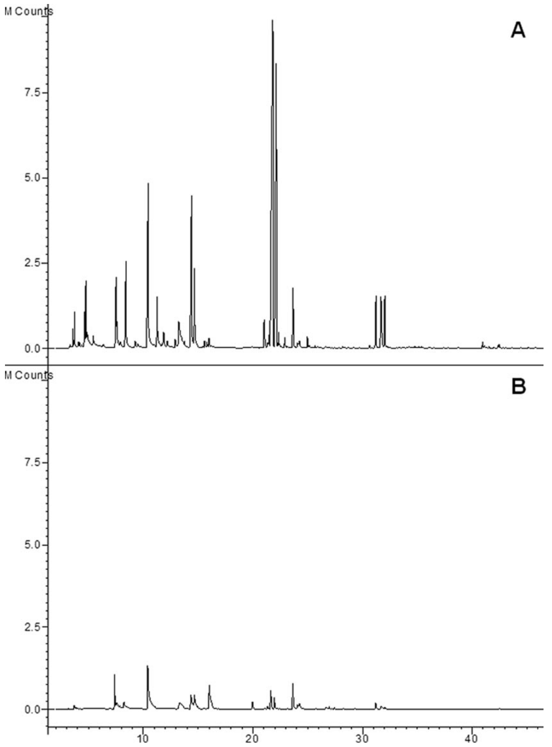

Fig. 3. Representative volatile compounds GC-MS chromatograms from flower (A) and nectar (B) of the same onion line.

tions of magnesium have a negative effect on the honey bee visits.

It is noteworthy that among VOCs, alkyl-sulfide compounds occur at higher amounts in the MSLs compared with the OP cultivar. In addition, alkyl-substituted piperidines and dioxolanes were higher in male sterile onions. Derivates of these compounds have been reported as insect repellents, even as insecticide (Xue et al. 2001, Scott et al. 2005, Chung et al. 2011, Murali and Chandrasekhar 2012, Dawid et al. 2012). Therefore, these compounds may lead to the difference in flower fragrance and nectar flavor of the sterile lines.

Table 6. Correlation between nectar, floral traits, frequency of bee visits, and seed yield

Trait	Bee visits	Seed yield
Outer anther area	0.854***	0.662*
Intern anther area	0.881***	0.725**
Outer tepal area	0.941***	0.879**
Intern tepal length	0.961***	0.881**
Ovary area	0.070^{ns}	0.090^{ns}
Style length	−0.916***	−0.854**
Nectary vol	0.983***	0.937***
Fructose concn	0.882***	0.925***
Glucose concn	0.951***	0.939***
Potassium concn	0.038^{ns}	0.138^{ns}
Magnesium concn	−0.911***	−0.798**
VOCs repellents concn	−0.661*	−0.759**
Naringenin concn	−0.786**	−0.815**
Luteolin concn	0.798**	0.936*
Bee visits		0.928***

Concn, concentration; vol, volume; ns, not significant.
The symbols *, **, and *** denote significance at $P < 0.05$, 0.01, and 0.001, respectively.

When the PCA was performed, the information about the samples contained in the original variables was projected onto three new variables called principal components (PCs). These three PCs were extracted explaining 94% of the total variance of nectar samples. The first principal component (PC 1) represented ≈56.3%, and the next PCs, 21.5 and 16.2%, respectively. Once we narrowed our attention to the main variables in the samples, the PCA applied shows that analyzed lines are separated in four groups. PCA results of analytical profile of nectar marked difference within MS lines and between them and the OP cultivar (Fig. 4).

There is a clear relation between the flower morphology, the nectar composition, and the honey bee behavior for each onion line. Among the morphological characteristics, the size of the nectary, anthers, and tepals play a key role in attracting bees. Within the analytical characterization, we found compounds that could act as attractants of bees, such as sugars, luteolin, or vanillic acid. Other factors may be acting negatively in pollinator attraction, such as style length; naringenin and vinylphenol concentration; volatile compounds such as dioxolanes, piperidines, and organosulfur compounds; and some metals like Mg, which were found in greater amounts in MSLs.

F1 hybrids seed yield is much lower than the open-pollinated variety, with a decrease of 60%. It is also highly variable the performance between different onion hybrids. Although F1 hybrids have greater uniformity and efficiency in the production of bulbs, they must have a good seed yield to be economically viable; otherwise, they become too expensive for producers, hence the importance of this study.

Silva and Dean (2000) reported that as long as some nectar is present, the quantity of nectar has little effect on the number of foragers and that the ratio of sugars found in onion nectar should not be discouraging honey bee foraging. Additionally, total nectar carbohydrate was shown not to affect the number of bee visits to the umbels. For pollinators, acquisition of energy rewards comes only with the costs. Time and energy are spent during all foraging activities. The optimal foraging theory assumes that animals will choose to behave in a manner that will minimize the amount of energy expended (Silva and Dean 2000, Abrol 2006). Thus, bees must prefer to visit the onion flowers that offer the greatest reward per visit, mainly represented by the volume of nectar and the sugars ingested. Our data somehow support this idea, as the quantity of nectar has great effect on the number of foragers, nectary volume and sugars ratio being the most important factors that influenced bee behavior. Furthermore, with these results, we demonstrate that a simple determination as the assessment of the nectary volume may facilitate selection of MSLs with

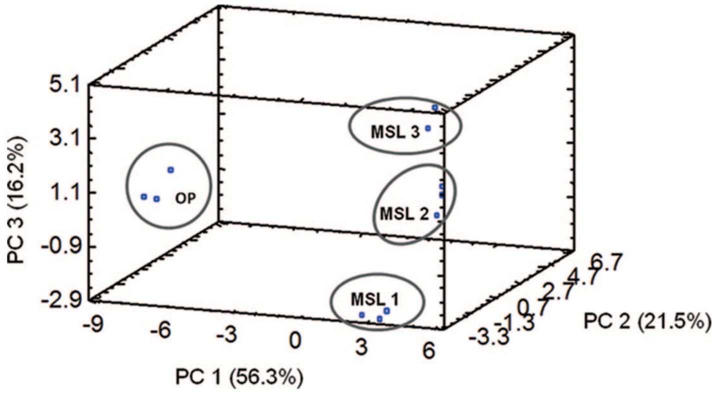

Fig. 4. PCA plot of four onion lines calculated on the basis of their chemical and morphological information from nectar and flowers. (Online figure in color.)

more attractive flowers to honey bees in a breeding program.

The experiments performed in this study clearly showed that the MSLs have nectar and floral traits profiles different from OP onion cultivars. Significant intervarietal differences between onion cultivars are common; this study proved that the combination of chemical and morphological information and statistical analysis is able to differentiate OP from onion MSLs as well as among MSLs.

The markedly qualitative and quantitative analytical differences in the profile of male sterile and open-pollinated lines found in this study contribute to the understanding of the factors that affect onion pollination for hybrid seed production. The correlated morphological and chemical traits with onion seed production are a great contribution for onion breeders, given the importance to select lines that have desirable traits for pollinator attraction, and as a consequence better seed yields. Particularly important are those chemical and morphological characters associated with pollination efficiency that are easy to assess in a breeding program.

The results obtained have attracted our interest for a more detailed study about onion flower morphology and nectar chemical composition in a greater number of onion MSLs.

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