Effect of environmental factors on bee activity and onion (*Allium cepa* L.) seed yield

Efecto de factores ambientales en la actividad de la abeja y en el rendimiento de semilla de cebolla (*Allium cepa* L.)

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Abstract

Pollinators are required to produce onion seeds. This specie is one of the main vegetable crops. Two types of onion varieties are mainly grown worldwide: hybrids and open pollination (OP) cultivars. Although hybrids offer advantages to bulb growers, seed yields of hybrids are lower than OP cultivars and that is a significant problem. The influence of environmental factors (temperature, radiation, rainfall, relative humidity (RH) and wind speed) was determined, as well as bee attraction and seed production in three locations of the main onion seed production area in Argentina. Nine male sterile lines (MSL) and one OP were used. The results obtained showed a marked variability in the attraction of bees and seed production between the OP and MSL and within MLS. In addition, environmental factors such as minimun temperature or RH were determinant to modify bee foraging behavior, where values lower than 9°C and 22%, respectively, caused that bees stop their activity.

Keywords
Allium cepa L. • seed production • pollination • *Apis mellifera* L.

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Resumen

Los polinizadores son necesarios para producir semillas de cebolla. Esta especie es uno de los principales cultivos hortícolas. Dos tipos de variedades de cebolla se cultivan principalmente en todo el mundo: híbridos y cultivares de polinización abierta (PA). Aunque los híbridos ofrecen ventajas a los cultivadores de bulbos, los rendimientos de semillas de los híbridos son más bajos que los cultivares PA y eso es un problema importante. Se determinó la influencia de los factores ambientales (temperatura, radiación, precipitación, humedad relativa (HR) y velocidad del viento), así como la atracción de abejas y la producción de semillas en tres localidades, de principal zona productora de semillas de cebolla en Argentina. Se utilizaron nueve líneas androestériles (LAE) y una PA. Los resultados obtenidos mostraron una marcada variabilidad en la atracción de abejas y la producción de semillas entre la variedad PA y las LAE y dentro de las LAE. Además, los factores ambientales, como la temperatura mínima o HR, fueron determinantes para modificar el comportamiento de alimentación de las abejas, donde valores inferiores a 9°C y 22%, respectivamente, hicieron que las abejas detuvieran su actividad.

Palabras clave

Allium cepa L • producción de semilla • polinización • *Apis mellifera* L.

Introduction

Onion (*Allium cepa* L.) the most cultivated specie of the subgenus *Allium*, from the Alliaceae family (33). Onion seed production is very important in Argentina. Around 200 t per year are produced mainly in the provinces of Mendoza and San Juan. Half of the production is exported. Onion is an allogamous specie with protandria, anthers release pollen before the stigma is receptive, promoting the out-crossing among onion plants (12). In the world, two types of onion varieties are usually grown: open pollinated (OP) and hybrids (33). Hybrids offer greater vigor and uniformity than the OP materials (12, 14). However, reports from seed companies and previous studies have reported that hybrid seed yield is erratic and considerably lower compared to OP varieties (37).

For hybrid seeds production, systems based on cytoplasmic male sterility (CMS) are used throughout the world. Two main sources of CMS have been genetically characterized, identified as S and T. Type S is the most used due to the usual occurrence of the recessive allele in Ms (20). To obtain these seeds, it is necessary to cross a sterile male line (MSL) with a fertile one. Almost two thirds of the onion cultivars of the most important seed companies' catalogs belong to the hybrid category (12). Very variable seed yield has been observed among the different onion hybrids. Poor seed yields are often due to poor pollination (19, 24, 34, 35, 36). Therefore, increasing seed yield depends on increasing the activity of the cross-pollinating insects (3).

There are at least 276 species of insects identified as pollinators of onion flowers, however bees (*Apis mellifera* L.), are the most efficient and handling them in the crops is easier, compared to other insects used, as flies (8). A distinctive characteristic of bees with respect to other insects is that bees visit only one type of flower at a time, this fact that some authors call floral constancy, makes the transfer of pollen from flower to flower more efficient, making possible the pollination (22).

Onion seed yield of hybrid varieties mainly depends on bee activity (25), the frequency at which they move and carry the pollen from the fertile plants to the sterile ones, as well as the proportion of fertile plants and their distribution in the crop (13, 28, 41). A poor pollination is one of the main causes of low yield; therefore, bees are necessary for a good seed set. However, the widespread use of bees as a pollinator does not always bring expected results because of the onion nectar is not particularly attractive to bees (7, 19, 40).

Around 10 beehives per hectare are used for onion pollination. Beehives usually are not introduced at the same time, starting when a 10% of fertile flowers are opened (40) until 50% of flowering (10). The flowers open irregularly during a period that lasts between two and four weeks (32).

Another important factor to consider in hybrid materials is the synchronization in flowering between the parental lines "Nicking", *i.e.* the coincidence between the period in which the pollen is viable and the stigma is receptive (28).

Environmental factors influence both plant and pollinators. An onion bulb can have more than one floral scape, each scape can have an umbel, and therefore, a plant that produces several inflorescences can open its flowers for a month or more. Each umbel has hundreds of perfect flowers (18). The production of onion flowers is induced by environmental factors. The abundance and viability of pollen is low at 14°C, but it is higher at 23°C. Pollen tube growth and seed formation are negatively affected by temperatures above 40°C (10).

On the other hand, bee behavior is controlled by both environmental and genetic factors (38). The influence of climatic conditions on bee flight activity has been reported (29, 31). Radiation and temperature are the most important factors on both, flight and communication between bees (1, 23, 31). Although, other studies have shown that with humidity higher than 75%, or precipitations, bee foraging activity is almost null, as well as when the wind speed exceeds 40 $km.h^{-1}$ (30). The aim of this work is to determine the influence of environmental factors (temperature, humidity, rainfall, wind and cloudiness) on the attraction of bees and the production of onion seed in the Cuyo region.

Materials and methods

Plant material

Ten onion lines, 9 male sterile lines (LAE) and one fertile line (OP) were selected, looking for materials that differed in their genetic characteristics and harvest yields. On April 8, 2015 bulbs produced in San Juan from each of these lines were planted in identical conditions in three locations. The management of the crop was the same for the three farms, no pesticides were used throughout the experiment, and the plants were drip irrigated. A number from 1 to 10 was assigned to each line, being number 4 the OP variety (table 1, page XXX). All the lines were provided by Bayer Argentina.

Locations where trials and experimental design were implemented

Three locations were selected in different environments within the onion seed productive area.

Table 1. Characteristics of the lines and seed yield for the three studied areas (g. umbel^{-1}).

Tabla 1. Características de las líneas y rendimientos de semilla para las tres localidades (g.umbela^{-1}).

LINE	Bulb color	Bolting	Bulb shape	Luján de Cuyo	Zonda	Media Agua
♀ 1	Red	Early	Globe	4.03 ab	3.86 b	0.72 ab
♀ 2	Yellow	Medium	Flat	3.96 ab	3.76 b	1.45 acd
♀ 3	Yellow	Late	Flat	4.06 ab	3.89 b	5.43 d
♂ 4	White	Late	Globe	5.09 c	5.39 c	3.23 dc
♀ 5	White	Late	Globe	3.79 ab	3.70 ab	2.85 dc
♀ 6	Yellow	Early	Globe	4.06 ab	3.87 b	0.53 a
♀ 7	Yellow	Medium	Globe	4.08 ab	3.79 b	0.92 ab
♀ 8	Yellow	Early	Globe	4.22 b	3.97 b	1.64 abc
♀ 9	White	Late	Globe	3.54 a	3.53 ab	2.58 bcd
♀ 10	Yellow	Late	Globe	3.62 a	3.04 a	3.27 dc
			Average:	4.04	3.88	2.26

Los valores representan la media de tres repeticiones. Los valores en la misma columna con diferente letra representan diferencias significativas P ≤ 0,05.

represent mean of three determinations. Values in the same column with different letters present significant differences P ≤ 0.05.

One of the trials was located at INTA Mendoza Agricultural Experimental Station in Lujan de Cuyo, Province of Mendoza (33°00'22 "S- 68°51'35,15" W), the other two were located in the province of San Juan, one of them in the department of Sarmiento (32°00'23" S- 68°21'38" W) and another in a farm located towards the northwest of San Juan in the Valle de Zonda (31°32'11" S- 68°42'19" W).

A randomized complete block design was used, with three repetitions and 150 bulbs in each of them. The bulbs were distributed in 3 rows of 5 meters long and a density of 10 bulbs per meter. Rows were about one meter apart. The ratio between sterile and fertile plants was 3: 1, which is the usual ratio used in commercial crops. Among all fertile and sterile lines there was 1 meter of distance.

Bee foraging behavior evaluation

Two bee hives (*Apis mellifera* L.) were placed in each experimental plot. Other insects also visited the flowers, but their work on the umbels was almost insignificant, with bees mainly found on the umbels during the trial period. At 10% of the anthesis of the fertile flowers (Line 4) the hives were introduced in the experimental parcels. Each hive had 4 open breeding frames and 2 frames with operculated brood. The queens came from an apiculture located in the Valle de Uco that has the Proapi certification. These bees had as remarkable characteristics, meekness and very good foraging ability.

An observation area was delimited, taking into account that there was the greatest homogeneity possible among them; each area had a length of 1 m. Visits were count visually; the observation was made alongside the group of umbels, during a period of two minutes per repetition, following the methodology proposed by Maldonado (2014). A visit was recorded when a bee stayed more than 3 seconds on the umbel. The number of bee visits was registered during seven days from 50% of flowering, defining as reading schedules from 12:00 a.m. to 2:00 p.m. The only days that no readings were taken were the days with strong wind or rain.

Flowering period

To evaluate the flowering percentage, the number of bloomed flowers per umbel inside the marked area was recorded daily, considering as an open umbel the one that had at least one flower with anthesis between the months of October and December. Then an average was calculated between the three repetitions of each of the experimental plots.

Environmental parameters

Air temperature and humidity were recorded in the 3 locations with temperature and humidity sensors (Onset® Mark, Model Pro v2). This instrument took readings every 15 minutes through the entire crop cycle, storing more than 9000 readings in each experimental plot. Sensors were place on a wooden stake at umbels height. Precipitation, wind and cloud data were extracted from nearby meteorological stations.

Seed harvest

The umbels were harvested manually between 15th and 20th January of 2016, taking as an optimum point of maturity when black seeds were shown in the umbels, then they were placed in mesh bags to allow drying. The bags were placed in dryers that allowed air circulation and exposure to the Sun. The moisture loss was controlled every two days with a John Deer moisture sampler calibrated for onion, when the average humidity reached 14%, the samples were milled, approximately 15 days after harvesting. The seed was processed in a sieve machine and then by hand the remaining impurities were extracted. The seeds obtained in each of the repetitions were weighed separately.

Statistical analysis

The statistical analysis of data was carried out using the analysis of variance (ANOVA) and means were compared using the Tukey significance test. The results were considered significant at $P \leq 0.05$ unless specified otherwise. Principal component analysis was applied to highlight the data structure and to find the overall relationships between environmental parameters and the effectiveness of MSL pollination for the production of hybrid onion seeds. Basic statistics and multivariate analysis were performed with the statistical package InfoStat2016 for Windows (Córdoba, Argentina). All data were reported as the mean ± standard deviation (SD) of three replicates.

Results

Seed production

Seed production was variable, there were significant differences among lines and locations, and there was interaction between lines and location. The line * location interaction observed was due to the fact that Media Agua suffered a special situation due to the fact that bees stopped visiting the crop after the third week of pollination, yields of Media Agua lines were very erratic, therefore, to determine the source of the variability associated with the production, this locality was removed from the analysis. When Media Agua was eliminated, we observed that there was no significant interaction between line * locality. The major source of variability was explained (81.42%) by the difference among the studied lines. In Luján de Cuyo were obtained the highest yields for all the lines compared to the yields obtained by the same lines in San Juan. The line with the highest yield per umbel was line N°4 (OP) in both Luján de Cuyo and Zonda (table 1, page XXX). In both locations, the OP line (L4) differed significantly from the MSL. Among the MSL, differences were also observed, line 10 had the lowest seed production in the two locations and line 8 was the most productive.

Relationship between onion lines and bee activity

The blooming period in the onion lines cultivated in Luján de Cuyo was about 10 and 15 days later than the lines that were cultivated in the Zonda and Media Agua localities. The most precocious variety was MSL N° 10 while the latest one was MSL N° 6, this pattern was repeated in the three locations, while the open pollination line N° 4 had an intermediate behavior (figure 1).

At 50% of flowering the bee activity in Luján de Cuyo, was always higher than the observed in Zonda. This behavior was observed through all the flowering period and was related to seed yields. The OP line (L4) was the most visited in both locations.

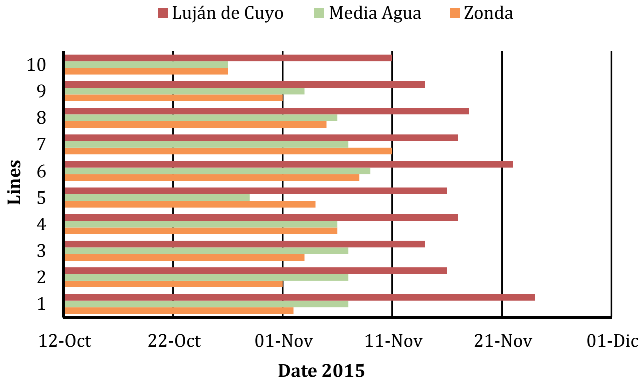


Figure 1. Date when the different onion lines reached 50% of flowering in the three studied locations.

Figura 1. Fecha en la que diferentes líneas de cebolla alcanzaron el 50% de floración en las tres localidades de estudio.

Within the MSL, L8 was the most visited and L10 the least visited in Luján de Cuyo whereas in Zonda the least visited for that percentage of flowering was L6. On the other hand, in Media Agua, it was observed that those lines that had more visits did not have the highest seed yield, for the reasons previously mentioned (figure 2). When we analyzed the source of variation, it was observed that these differences were explained by the difference between the locations in 11.43% and by the line factor in 47.40%. Bee visits at 50% flowering in all the onion lines in Luján de Cuyo and Zonda showed a high correlation with seed yields (r = 0.69).

Bee activity varied through the flowering period as well as along the day. In those days when the weather conditions were not favorable for bees at noon, no insects were observed on the umbels and bee increased during the afternoon. This behavior was similar for all the onion lines. For line 4 (OP) from Luján de Cuyo, during the first four days of readings, more bee visits were counted in the afternoon hours (5:00 p.m. to 6:00 p.m.) than during midday (12:00 p.m. 2:00 p.m.). The highest number of bees for this line was registered at 90% of flowering. The same was observed in male sterile lines. Bee activity at 50% of flowering was low and increased until it reached 85%, remaining constant until the end of flowering (figure 3, page XXX).

Effect of environmental factors on bee visits

The temperature values registered in Luján de Cuyo, during the observation days, reached a maximum of 38.5°C and a minimum of 7.3°C and the values of relative humidity varied from 10% to 100% on days when rainfall was recorded; while in Zonda, San Juan the minimum and maximum temperatures were 16 and 38.8°C respectively and the recorded humidity varied between 18% and 100%. The maximum humidity values, as well as the precipitations were the environmental factors that had more influence on the bee activity. Although the most extreme values were observed in Media Agua (Max Temp 50°C and Min Temp -6,5°C), if we consider the mean values between the three locations there were no significant differences.

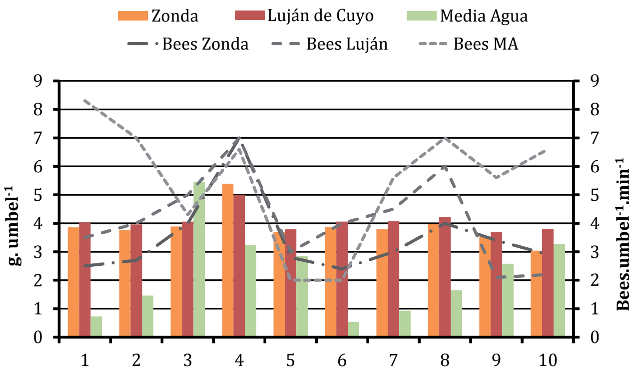
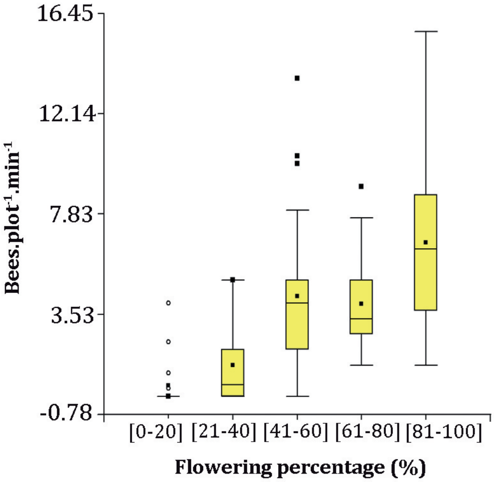


Figure 2. Seed yield per umbel and bee activity at 50% of flowering.

Figura 2. Rendimiento por umbela y actividad de abeja al 50% de floración.



Horizontal bars represent medians, box plots represent interquartiles and bars represent minimum and maximum values.

Las barras horizontales representan las medianas, los diagramas de caja representan intercuartiles y las barras representan valores mínimos y máximos.

Figure 3. Bee activity according to the flowering percentage in Zonda and Luján de Cuyo.

Figura 3. Actividad de la abeja de acuerdo con el porcentaje de floración en la localidad de Zonda y Luján de Cuyo.

The flowering percentage as well as the temperature influenced positively on bee activity under open field conditions; on the other hand, RH (Max and Min), cloudiness and precipitation are the parameters that most is negatively influenced bees (figure 4, page XXX).

On cloudy days (6-8 oktas) or when the wind speed was higher than 50 km.h^{-1}, bees did not visit onion flowers. In addition, in the presence of precipitations bees reduced the frequency of visits or it became null. If rainy days are not taken into account, between 50% and 100% of flowering, the factor that has greatest influence in each locality was the minimum RH. In days where the minimum percentage of HR was low (less than 20%) or it was very high (above 55%), bees did not fly. Analyzing those days, with an optimal range of humidity, radiation and without wind, the factor that influenced the most, is temperature; the activity of bees decreased below 9°C and the highest frequency of visits was recorded between 22°C and 33°C. As an example, in figure 5 (page XXX), Luján de Cuyo and Zonda locations are represented and it shows how the environmental factors influenced in the total number of visits of bees per plot. In addition, it was observed that not only the climatic conditions were very variable during the flowering period, but also through the day (figure 6, page XXX).

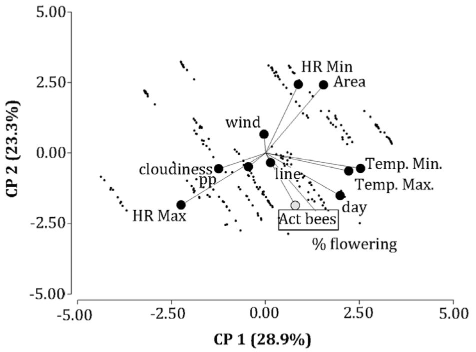


Figure 4. Principal components analysis graph: interaction between climatic factors with the bee activity, lines and the percentage of flowering for the three studied areas.

Figura 4. Gráfico de componentes principales: interacción entre todos los factores climáticos, la actividad de la abeja y las líneas y el porcentaje de floración para las tres localidades estudiadas.

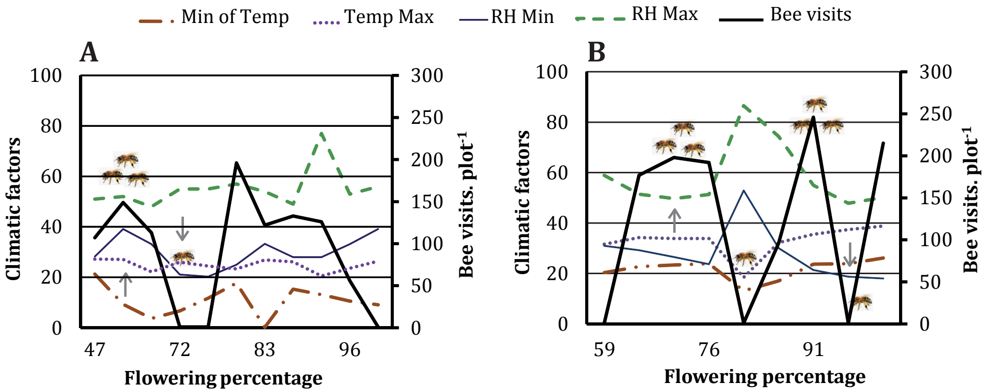


Figure 5. Influence of climatic factors on bee activity between 50 y 100% of blooming. A: Luján de Cuyo; B: Zonda.

Figura 5. Influencia de los factores climáticos en la actividad de las abejas entre el 50 y 100% de floración. A: Luján de Cuyo; B: Zonda.

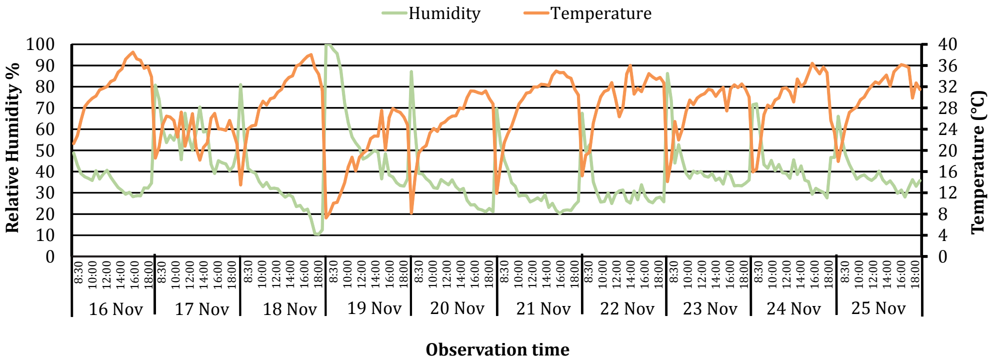


Figure 6. Temperature and humidity record during 10 days in Luján de Cuyo.

Figura 6. Registro de temperatura y humedad durante 10 días en la localidad de Luján de Cuyo.

Discussion

Seed yield differed between the OP variety and the studied hybrids, in addition differences were observed among the MSL. Although an interaction between location and seed yield was observed, the differences were maintained in the locations of Luján de Cuyo and Zonda. Media Agua was affected by the natural flora that attracted more the bees than onion flowers. The OP variety produced 1.4 folds more seeds than the N° 10 line, which had the lowest seed yield among all male sterile lines. Silva and Dean (2000) and Ahmad *et al.* (2003) reported differences in the seed production per umbel among the different hybrids. These results are in concordance with those reported by Maldonado (2014) who observed lower yield in male sterile lines than the OP in cage conditions.

The seed production of the OP line as well as the MSL was correlated with bee visits. These results are in agreement with other studies where 2 fold more bees on the OP lines than in MSL were observed (9, 34), which is expected since in pollination open lines also have pollen (4, 10). Other authors consider that this difference is due to a greater amount of nectar and concentration of sugars in the fertile lines (9, 26, 41). Silva and Dean (2000) reported that the average amount of nectar produced in onion flowers has a significant positive correlation with the number of bee visits.

In an average umbel that can have up to 600 flowers, 20 to 30% of the flowers have receptive stigmas. Benedek (1977) suggested that there should be between 5 to 8 bee visits on an umbel to have a satisfactory setting through the flowering season. When the lines were close to 50% flowering bee activity was register and the recording time was past noon between 2:00 p.m. and 4:00 p.m. Maldonado (2014) observed 1.5 times more bees at 50% of flowering than at 30% or 70% of flowering, furthermore reported that the highest foraging activity occurred at 3:00 pm, finding up to 1.5 times more bees at that time than at 9:30 am. It should be noted that these results were obtained under experimental conditions in a cage. However, several studies report that the highest activity of bees occurs from late morning to early afternoon, both in the field (16) and cage (42). Our results showed that the highest bee activity occurs when the climatic conditions were ideal for the pollinator. According to reports by Silva and Dean (2004), the highest bee activity is observed in the afternoon, which is negative for the pollination of onion flowers because they produce more nectar in the middle and late morning. However, other authors say that the lowest forage occurs from 10:00 a.m. to 5:00 p.m. (6).

Bee activity was very erratic through the flowering period even when the climatic conditions were favorable for foraging, sometimes no bees were seen visiting the flowers. Some days, the weather conditions at the recording time were not ideal for bees, therefore the activity was almost null, but this situation was solve in the course of the afternoon when conditions improved, increasing the bee activity on the umbels. Neupane *et al.* (2006), reported that there are several factors involved in the preference of bees and that the frequency of their visits varies through the day, as well as with the flowering stages. The seed yields found in the different lines indicate that the climatic conditions may have improved at certain times of the day, making it possible for the bee to efficiently perform its work.

The bee activity increased when the temperature was between 22 and 33°C and the day was sunny, conditions that only occurred in the afternoon some days. The differences in the attraction according to the time of the day could be attributed to variations in temperature and cloudiness, which are the most influential parameters in the flight of bees, when rain and strong winds (greater than 80 $km.h^{-1}$) are not consider since under this situation bees are tent to stop all activity outside the hive. Our results are in agreement with observations previously made by other authors, if the temperature is below 9°C, bees do not fly (21, 23). The flight and temperature are linearly correlated in the range of 14-22°C under field conditions, but the residence time per onion umbel is not influenced by the air temperature (7). Tan *et al.* (2012) observed that at 20°C the bee activity was the highest, while at 43°C the activity was reduced almost completely, the same as at temperatures below 10°C. Although, the most precocious lines yield better in warmer locations, like San Juan, onion seed production for all lines was higher in Mendoza. Temperature had the same effect in the attraction of bees for the different lines; therefore, the differences of attraction of bees would be explained by the differences among the genetic materials used.

In Media Agua it was observed that during the days when the temperature exceeded 38°C the bee stopped visiting the umbels regardless of the line observed, nor were observed foraging flowers of other species, but they could be grouped in large numbers on the puddles of water that remained after irrigation. Some studies have reported that a high temperature (higher than 40°C) increases bees water collection to the detriment of foraging or pollen collection (15, 17) and that foraging activity in the field correlates negatively with the increase in temperature (6). Voss *et al.* (1999) also determined that with temperature increases the onion nectar becomes more viscous and therefore less attractive for the bee.

Humidity and cloudiness influenced negatively in bee activity. Even when there are adequate temperatures, bees do not fly if there is not enough light, on cloudy days they stay near the hive. According to the results obtained, the minimum RH had a great influence on the behavior of the bees. At values lower than 20% or higher than 55% bees drop their activity. These results are in agreetment with Puškadija *et al.* (2007), who reported that the highest bee activity for sunflower pollination is between 40 and 50% of humidity, but they are lower than those reported by other authors (2, 3). Burill and Dietz (1981) found that, bee foraging increased when air temperature rises, but was not correlate to changes in atmospheric pressure and relative humidity. So far in most of the studies reported, it was found that the combination of several meteorological parameters control the foraging pattern of pollinators and that the cessation of activity seems to be governed by the rapid decrease in the intensity of light. In our work we observed that climatic factors affected the frequency of visits for all lines in the same way, therefore, the differences between the visits are explained by the variability between the genetic materials used and not by the meteorological parameters.

Conclusions

Onion seed production for all lines was higher in Mendoza than in San Juan. It would not be convenient to carry out an onion seed production in areas where the temperatures are very high during the month of November, month where the highest frequency of bee visits occurs between 50 and 85% of flowering.

The results showed a marked variability in the attraction of bees and seed production between the OP and MSL and within the MSL. In addition, it was shown that environmental factors such as temperature and humidity modify the rate of bee visits on the flowers.

This study contributes to the understanding of the factors that affect the bees foraging behaviour for the production of onion seeds. According to the results obtained, it is presume that the variability in the bee attraction by the studied lines would be influenced by the climatic factors; however, these would not explain the total variability in the performance of the different studied lines. Therefore, the study of other factors such as the floral traits of OP and MSL and the composition of the nectar, which directly or indirectly affect the bee attraction, would be necessary.

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