

Phenology, pollen synchronization and fruit characteristics of european hazelnut (*Corylus avellana* L.) cv. "Tonda de Giffoni" in three sites of central Chile

Fenología, sincronización polínica y características frutales de avellano europeo (*Corylus avellana* L.) cv. "Tonda de gifoni" en tres localidades de Chile central

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ABSTRACT

Phenology, pollen synchronization and fruit characteristics were studied during the season 2011-2012 for European hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" and three of its pollinizers ("Tonda Romana", "Tonda Gentile delle Langhe" and "Barcelona") in three agro-ecological conditions of central Chile. Male and female blooms occurred from June through August, with a flowering span ranging between two to two and a half months depending on the cultivar, pollinizer and study sites. "Tonda di Giffoni" male flowering onset occurred during the first week of June, up to two weeks earlier than female flowers (271 to 417 chilling hours) showing a marked protandrus dichogamy. "Tonda Gentile delle Langhe" and "Barcelona" pollinizers completely covered the female flowering period of "Tonda di Giffoni", while "Tonda Romana" fail to cover the first flowering week. In general, starting dates for the different phenological stages were directly and significantly ($P < 0.05$) correlated with chilling hour accumulation and growing degree days. Fruit set (34.1%) and maximum fruit diameter (16.6 mm) were significantly lower in the case of "Tonda Gentile delle Langhe" compared to "Tonda Romana" (82.2%, 17.3 mm) and "Barcelona" (74.7%, 17.4 mm).

Keywords

filbert • chilling hours • growing degree days • pollen compatibility

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RESUMEN

Se estudió la fenología, sincronización polínica y características frutales de avellano europeo (*Corylus avellana* L.) cv. "Tonda di Giffoni" y tres polinizantes ("Tonda Romana", "Tonda Gentile delle Langhe" y "Barcelona") bajo tres condiciones agroecológicas de Chile central durante la temporada 2011-2012. Las floraciones masculina y femenina ocurrieron entre junio a agosto y la floración varió entre dos y dos meses y medio dependiendo del cultivar, polinizante y sitios de estudio. El inicio de la floración masculina de "Tonda di Giffoni" ocurrió durante la primera semana de junio, hasta dos semanas antes que las flores femeninas (271 y 417 horas frío), manifestando una marcada dicogamia de tipo protándrica. "Tonda Gentile delle Langhe" y "Barcelona" cubrieron por completo el período de floración femenina de "Tonda di Giffoni", mientras que "Tonda Romana" no cubrió su primera semana de floración. En promedio, las fechas de inicio para las diferentes etapas fenológicas se correlacionaron directa y significativamente ($P < 0,05$) con la acumulación de horas frío y días grado. El porcentaje de cuaja de frutos (34,1%) y el diámetro máximo de frutos (16,6 mm) fueron significativamente menores cuando se comparó el polinizante "Tonda Gentile delle Langhe" con "Tonda Romana" (82,2%, 17,3 mm) y "Barcelona" (74,7%, 17,4 mm).

Palabras clave

avellano • horas frío • días grado • compatibilidad polínica

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is one of the oldest and most important dried fruit bushes (3, 8), holding the second place in the nut global market, with productions exceeding 800,000 metric t per year (24). This fruit has been labeled as a functional food and its consumption has been associated to several human health benefits due to the high concentration of bioactive compounds, mainly tocopherol and phenols (9, 11, 13). In recent years the demand for this species has risen significantly, mostly driven by a fastly growing functional food, chocolate and confectionery industries. The market is currently dominated by Turkey (75% of world production), followed by Italy and the United States (10). Chile, with an approximated cropping area of 18,000 ha, is the main Southern hemisphere supplier,

and it is expected that by 2025 the country will become the third world producer, with a cultivated area of ~30,000 ha. Regarding its floral biology, it is a monoecious plant with female (glomeruli) and male flowers (catkins), although most varieties and cultivars are self-incompatible (19, 30). This sporophytic incompatibility is controlled by a single S locus with multiple alleles (31). The pollination is anemophilic, and in many cases there is dichogamy, where there is a lack in synchronization between pollen release and stigma receptivity from the female flower. Pollination and fruit setting are strongly conditioned by ecological and environmental factors, such as climate and soil type where the species develops (6, 27). Pollination occurs during winter, when female flowers emerge for several

weeks and pistils can be receptive in alternating periods of time (32). Factors such as average temperature, chilling hours, degree days, solar radiation, rainfall, wind speed and relative humidity, among others, strongly affect the occurrence of different phenological stages, as well as pollination and fruit set success (18). Thus, having a compatible and synchronized pollinizer and main variety combination becomes a requirement to achieve sufficient viable pollen production during the long flowering period (16). Several authors (11, 23) have studied the effects of different pollen origins on fruit set, yield and various quality parameters (weight of the nut, proportion of skin / fruit, proportion of fruit, etc.).

All have concluded that selecting the right pollinizer and main variety combination adapted to a specific area is a requirement to achieve sustainable and economic productions (11, 23). Despite a wide agroclimatic cultivation area, there are no systematic studies in Chile concerning the effect of agroclimatic conditions on phenological development, pollinizer compatibility and synchronization, and yield and fruit quality.

In addition, climate change adds an extra layer of complexity to hazelnut cropping, given its direct effect on pollen synchronization; thus, making floral phenology monitoring a permanent task to achieve high fruit set, yield and quality. "Tonda di Giffoni" is the second main cultivar for the Chilean hazelnut industry, and is characterized by showing medium vigor and yields ranging between 2,000 and 3,000 kg/ha. In addition, its medium fruit size (14mm) and excellent organoleptic characteristics (10) contribute to a high demand from the chocolate industry.

The main goal of this study was to characterize the phenology, pollen

synchronization, and fruit characteristics for European hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" and three pollinizers: "Tonda Romana", "Tonda Gentile delle Langhe" and "Barcelona" in three agro-ecological conditions of central Chile.

MATERIALS AND METHODS

Study site characterization

The study was carried out during the years 2011 and 2012, at three different sites located in the Maule Region, central zone of Chile (table 1, page 58).

Plant material

Six-year-old hazelnut trees of cv. "Tonda di Giffoni" and three pollinizers (11%) were selected: "Tonda Romana" (TR), "Tonda Gentile delle Langhe" and "Barcelona" (table 1, page 58).

In each study site the plantation framework was 5 x 4 m in north-south oriented rows. Trees were trained to a multi-axis system and watered weekly to water balance from November to the end of March with a micro-sprinkler system (40 L/h). Conventional cultivation practices (fertilization, phytosanitary management, weed control and pruning) were carried out, avoiding the use of growth regulators.

Floral phenology

A phenological monitoring was carried out weekly during the reproductive stage (From May 1, 2011 to the end of March 2012). In each study site (table 1, page 58) five uniform trees corresponding to "Tonda di Giffoni" and five nearby pollinating trees were selected.

Table 1. Study site and orchard specifications for Hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" and its pollinizers (11%).**Tabla 1.** Sitios de estudio y características de los huertos de avellano europeo (*Corylus avellana* L.) cv. "Tonda di Giffoni" y sus polinizantes (11%).

Study site	Coordinates	Soil type (5)	Climate (29)	Pollinizers (11%)
Los Niches	35°3'522" S; 71°7'3127" W	Huecán soil series. Sandy clay loam.	Average maximum temperature during January: 27.5°C. Average minimum temperature during July: 4.0°C, 1380 degree days (base 10°C) and 1472 chilling hours (base 7°C).	"Tonda Romana"
Camarico	35°16'16 82" S; 71°22'13 82" W	San Rafael soil series. Silt loam (FL). Soil phase SRF-6.	Average maximum temperature during January: 30.1°C. Average minimum temperature during July: 4.0°C, 1788 degree days (base 10°C) and 1283 chilling hours (base 7°C).	"Tonda Gentile delle Langhe"
San Rafael	35°15'34 70" S; 71°33'24 65" W	San Rafael soil series. Silt loam (FL). Soil phase SRF-7.	Average maximum temperature during January: 31.4°C. Average minimum temperature during July: 5.5°C, 2228 degree days (base 10°C) and 536 chilling hours (base 7°C).	"Barcelona"

In each tree, 50 cm long segments located in the middle third of five homogeneous branches, with current season's shoots, were selected and marked. In these segments the phenology of catkins and glomeruli ("Tonda di Giffoni"), or only catkins in the case of pollinizers, was studied (2).

The occurrence of phenological stages was compared with accumulated growing degree days (GDD) and chilling hours (CH) records. Climatic records were obtained from nearby meteorological stations belonging to the Chilean national agroclimatic network (www.agromet.cl).

For the calculation of GDD, the thermal average method (average daily temperature-base temperature) was utilized.

$$GGD = \sum_{i=1}^n (Ta - Tb) \quad (1)$$

where:

n = number of days from May 1 (biofix) to the date of occurrence of each phenological stage.

Ta = average daily temperature and Tb is the base temperature (10°C). The Weinberger method (14, 32), still in frequent use (17), was used to estimate the accumulation of CH.

$$CH = \sum_{i=1}^n H; \text{ if } 0^{\circ}\text{C} < T < 7^{\circ}\text{C}, 1 \text{ is added, if not, } 0 \quad (2)$$

where:

H = number of hours in which temperature (T) is between 0°C and 7°C, from May 1 (biofix) and the date of occurrence of each phenological stage.

Fruit characteristics

The following evaluations were carried out in the same "Tonda di Giffoni" marked trees and shoot segments utilized in the phenological monitoring.

Fruit setting

Fruit setting percentage was calculated based on the total number of fruits related to the previously counted female flowers for each shoot segment.

Fruit weight and diameter and seed yield

Seed yield (%) = (Seed weight/fruit weight)*100 (3)

Data analysis

Floral phenograms and graphs comparing accumulated GDD and CH among localities were built to describe phenological stages and its relationships with these temperature parameters. Pearson correlations and linear regression analyses were conducted for thermal accumulation and the occurrence of phenological events. Analyses of variances (ANOVA) were conducted to examine variation among study sites for all productive parameters (percentage of fruit set, diameter and weight of fruits and seeds). Means were compared using Tukey's multiple comparison test ($P < 0.05$). All statistical analyses were carried out using the statistical software program SPSS 15.0® Inc 2006.

RESULTS

Floral phenology

Differences in phenological stages for hazelnut cv. "Tonda di Giffoni" across study sites are shown in figures 1 and 2 (page 60) to figures 3 and 4 (page 61).

During the season 2011-2012, male and female bloom occurred from June to August, with a span varying between two and two and a half months according to the main cultivar, pollinizers and study sites (figure 1, page 60). "Tonda di Giffoni" presented a marked protandrous dichogamy in the three study sites. Male flowering began during the first days of June and lasted on average 64 days and male full blooming (more than 50% of the catkins releasing pollen), lasted 43 days in Los Niches and San Rafael and 49 days in Camarico. Female flowering (pistillated flowers) began the second and third week of June (up to two weeks later than the staminate) and lasted for 59 days. "Tonda Romana", "Tonda Gentile delle Langhe" and "Barcelona", flowering lasted on average 71 days, with "Tonda Romana" being 10 days shorter than "Tonda Gentile delle Langhe" and "Barcelona". Pollen release occurred at the three sites while "Tonda di Giffoni" stigmas were receptive (pollen synchrony), although there were differences for full female flower coverage. "Tonda Gentile delle Langhe" and "Barcelona" completely covered "Tonda di Giffoni", however, "Tonda Romana" did not release pollen the first week of "Tonda di Giffoni" full blooming. The evolution of the phenological stages varied across study sites (figures 1 and 2, page 60; to figures 3 and 4, page 61).

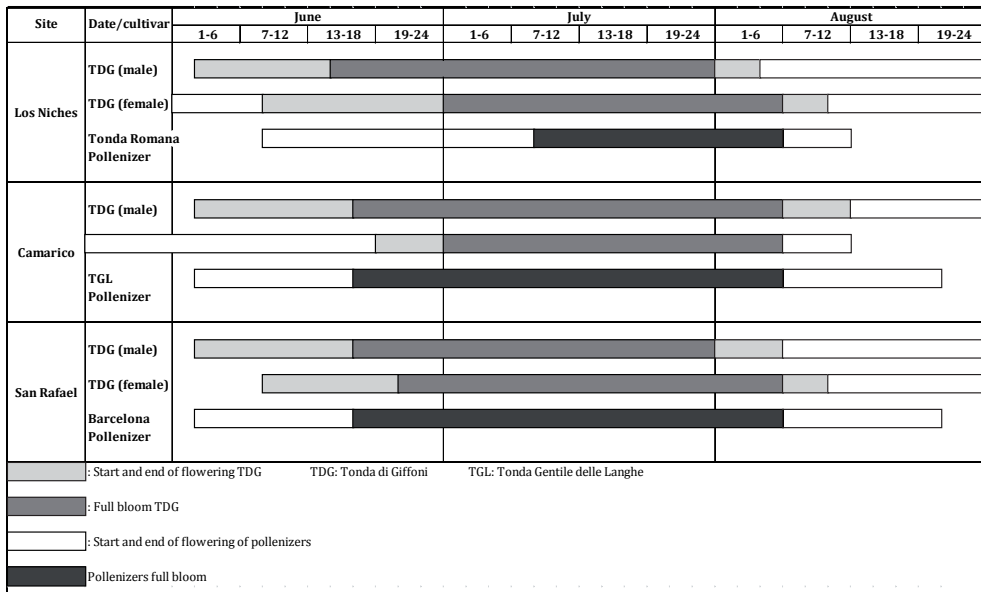


Figure 1. Floral phenology for hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" and their pollenizers at study sites.

Figura 1. Fenología floral para avellano europeo (*Corylus avellana* L.) cv. "Tonda di Giffoni" y sus polinizantes en los sitios de estudio.

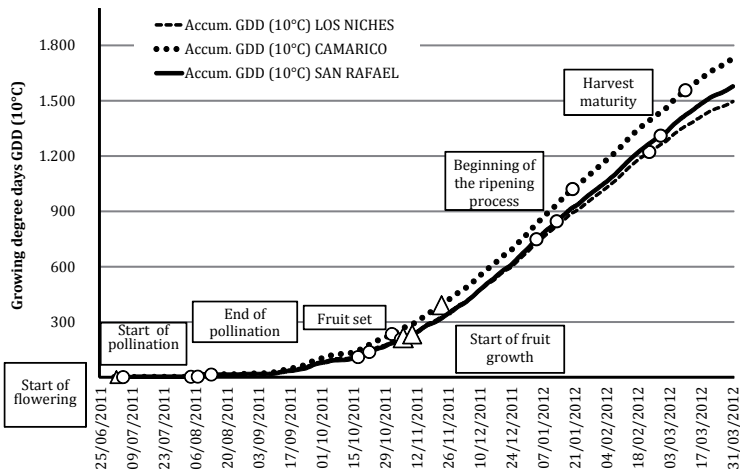


Figure 2. Floral phenology of Hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" according to accumulated growing degree days (GDD) at study sites.

Figura 2. Fenología floral de avellano europeo (*Corylus avellana* L.) cv. "Tonda di Giffoni" de acuerdo con la acumulación de días grado (DG) en los sitios de estudio.

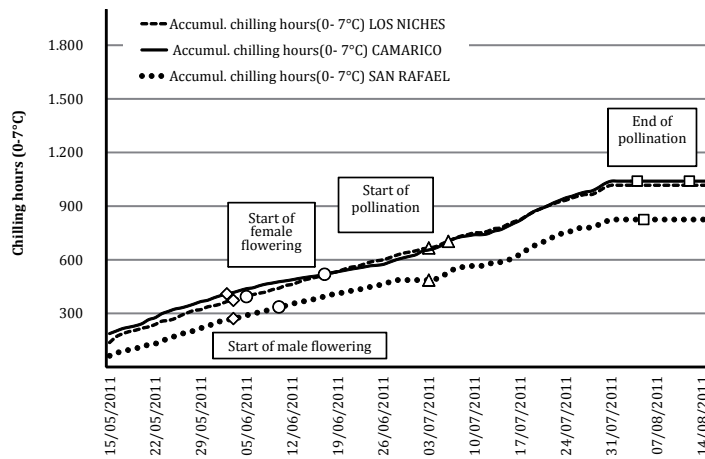


Figure 3. Floral phenology of hazelnut (*Corylus avellana* L.) cv. "Tonda di Giffoni" at study sites according to accumulated chilling hours.

Figura 3. Fenología floral de avellano europeo (*Corylus avellana* L.) cv. "Tonda di Giffoni" de acuerdo con la acumulación de horas frío en los sitios de estudio.

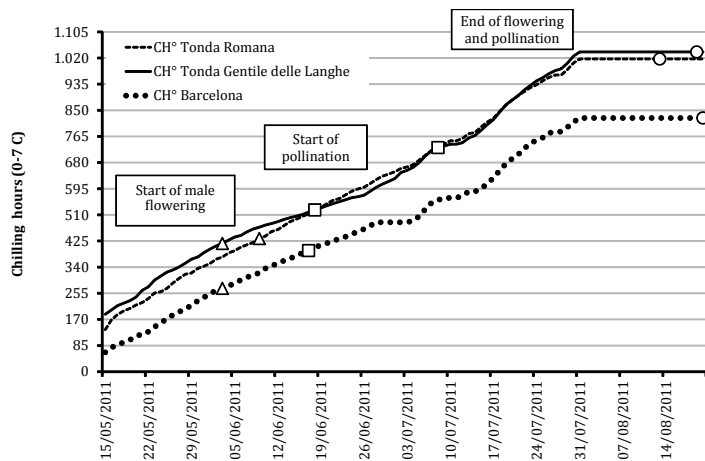


Figure 4. Floral phenology of hazelnut (*Corylus avellana* L.) pollinizers at study sites

Figura 4. Fenología floral de los polinizantes de avellano europeo (*Corylus avellana* L.) en los sitios de estudio.

Los Niches proved to be the earliest site, ahead for most phenological stages harvesting when compared to other productive areas. This site was followed very closely by San Rafael and finally by Camarico, which showed the latest harvest.

Correlation of phenological stages and accumulation of growing degree days and chilling hours

The occurrence of phenological stages during the season 2011-2012 was positively and significantly correlated ($P < 0.05$) with CH and GDD accumulation, with coefficients of determination (R^2) of 0.81 and 0.82, respectively (figure 5).

However, important differences between study sites were observed. This is especially evident in the case of Camarico, where in spite of a greater accumulation of CH and GDD, the occurrence of phenological stages was clearly delayed when compared to Los Niches and San Rafael (figures 1 and 2, page 60; to figure 3 and 4, page 61).

Chilling hours

"Tonda Di Giffoni" male bloom begun at same time (3.6.11) at the three study sites, up to two weeks earlier than female flowers (protandrous-type dichogamy), with an accumulation of chilling hours that varied between 271 and 417 CH.

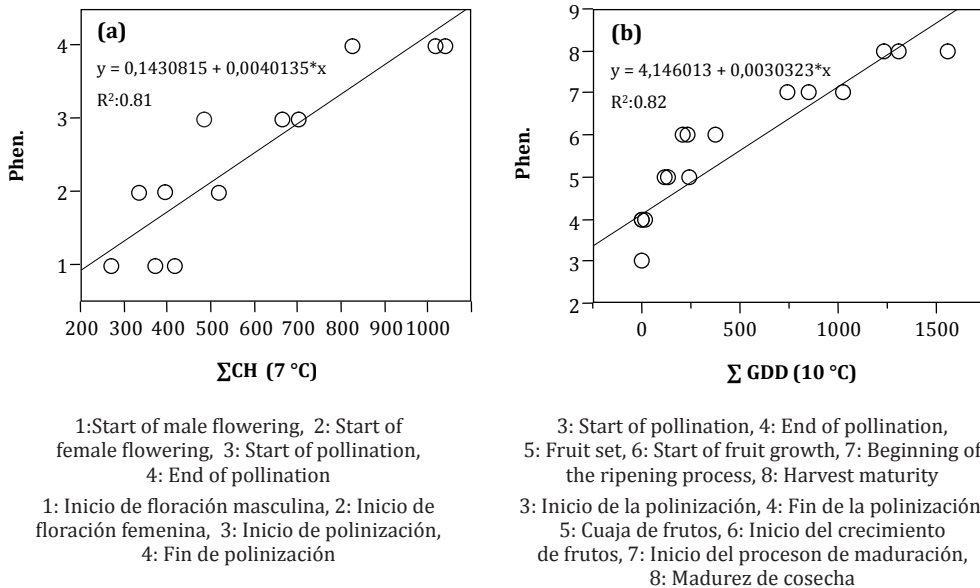


Figure 5. Linear adjustment for the relationship between phenological development (Phen.), accumulated degree days (ΣDG) (a) and chilling hours (ΣHF) (b) for hazelnut cv. "Tonda di Giffoni" and their pollinizers at study sites (2011-2012).

Figura 5. Ajuste lineal para la relación entre desarrollo fenológico, acumulación de días grado (ΣDG) (a) y horas frío (ΣHF) (b) para avellano europeo cv. "Tonda di Giffoni" y sus polinizantes en los sitios de estudio (2011-2012).

The earliest onset of female flowering occurred in Los Niches (5.6.11), with an accumulation of 394 CH and the latest in Camarico (17.6.11), with an accumulation of 519 CH. When "Tonda Di Giffoni" floral phenological stages are compared with the accumulation of chilling hours for each locality (figure 3, page 61), it can be observed that in Los Niches, "Tonda Di Giffoni" accumulated 1,017 CH, in Camarico, 1,040 CH, and in San Rafael, 825 CH. In the case of the pollinizers (figure 4, page 61), "Tonda Romana" (Los Niches) accumulated 433 CH at the beginning of male bloom, 733 CH at the beginning of pollination, and 1,017 CH at the end of flowering and pollination. "Tonda Gentile delle Langhe" (Camarico) accumulated 417 CH at the beginning of male bloom, 526 CH at the beginning of pollination and 1,040 CH at the end of flowering. "Barcelona" (San Rafael) accumulated 271 CH at the beginning of flowering, 394 CH at the beginning of pollination and 825 CH at the end of flowering. Thus, the highest accumulation of CH occurred for "Tonda Romana" and "Tonda Gentile delle Langhe", while "Barcelona" showed the lowest.

Growing degree days

Camarico produced the highest values for each phenological stage, followed by San Rafael and then Los Niches. The accumulation of growing degree days was 1556.9 GDD in Camarico, 1310.5 GDD in San Rafael and 1229.2 GDD for Los Niches. In Camarico, phenological stages showed, in general, two weeks of delay compared to Los Niches and San Rafael. Fruit set occurred with an accumulation range of 115 and 239 GDD. The beginning of fruit growth ranged between 205.7 and 374.1 GDD, the beginning of maturity between 740.6 and 1028.8 GDD, and harvest maturity between 1229.2 and 1556.9 GDD.

Productive parameters

From all the evaluated parameters, only fruit setting and fruit equatorial diameter were significantly different among studies sites (table 2).

Los Niches and San Rafael showed statistically similar percentages of fruit setting (82.2% vs. 74.7%, respectively). In contrast, Camarico, produced the lowest fruit setting (34.1%) and equatorial diameter (16.6 mm), compared to Los Niches (82.2%, 7.3 mm) and San Rafael (74.7%, 17.4 mm).

Table 2. Productive parameters for hazelnut cv. "Tonda di Giffoni" at study sites.

Tabla 2. Parámetros productivos para avellano europeo cv. "Tonda di Giffoni" en los sitios de estudio.

Location	Fruit setting (%)	Fruit ecuatorial diameter (mm)	Fruit weigh (with peel) (g)	Seed weight (g)	Seed yield (%)
Los Niches	82.2 b	17.3 b	3.1 a	1.4 a	44.3 a
Camarico	34.1 a	16.6 a	2.9 a	1.3 a	45.0 a
San Rafael	74.7 b	17.4 b	3.1 a	1.4 a	43.8 a

* Different letters for each column indicate statistical differences according to Tukey (p < 0.05).

* Diferencias en las letras para cada columna indicant diferencias significativas según Tukey (p < 0,05).

DISCUSSION

The strong protandric dichogamy showed by "Tonda di Giffoni" at the three study locations coincides with previous reports concerning this cultivar, which generally displays varying degrees of protandric dichogamy across seasons, mostly as a response to fall and winter temperatures (27, 29). Capik and Molnar (2014) found that European hazelnuts behave as protandrous or protogynous depending on their genetic makeup and the regional climate conditions where they are grown (15, 17). In regions with Mediterranean-type climates, such as those like the present study, the protandric dichogamy tends to be more common, while protogynous tends to predominate in areas with cold and long winters (20, 21, 26).

Grau (2014) in a study conducted in the province of Ñuble (Chile), observed that only 60% of hazelnut varieties were protandrous, while the rest was protogynous. In addition, "Tonda di Giffoni" evaluations conducted in the Chilean Bio-Bio region reported that male flowering occurred between the end of May and July, while female flowering was observed between the middle of June to the end of July (1). Thus, pollen availability to fully cover "Tonda di Giffoni" female flowering becomes a limiting factor, especially when using a single pollinator, a practice commonly used in Chile (ranging between 5% and 11%).

The risk of limited pollen synchrony even increases when considering seasonal climate variations, given the direct effect of climatic variables, such as temperature, humidity, rainfall, and wind, on floral staminate and glomeruli behavior. Undoubtedly, an inefficient coverage of feminine flowers will affect fruit setting

and therefore fruit yields. Thus, two or more pollinators are recommended per main cultivar, and has become a standard practice among the main hazelnut-producing countries (16). Hazelnut trees tolerate extreme cold, including temperatures as low as -15°C , being CH requirements an adaptive condition for preventing development when flowers or leaves are likely to be damaged by frost (7). Estimated CH accumulations observed in this study (between 825 and 1040 CH) is in agreement with previously reported values for "Tonda di Giffoni" (7). Differences observed for male and female flowers CH accumulation were also described in previous studies (4), with staminate flowers typically showing lower CH requirements compared to glomeruli flowers to break fall dormancy. Positive and significant correlations ($P < 0.05$) between phenological stage dates and CH ($R^2=0.81$) and GDD ($R^2=0.82$) accumulations points out a strong relationship between these variables, in agreement with Črepinšek *et al.* (2012). Even though temperature accumulation (CH and GDD)/phenological evolution trend was similar in all study sites, Camarico showed higher CH and GDD accumulations, but not an earlier phenological development compared to the other two study sites. This behavior has been observed in previous studies (18, 33). Differences among sites, in terms of phenological evolution and CH and GDD accumulations, have been explained by factors, such as relative humidity, photoperiod, solar radiation, cultivar genetics, cropping and soil types (18, 33).

Pollinizers are essential to ensure pollen availability, fruit setting and yield. However, there must exist synchrony between

pollinizers and main cultivar flowering windows. "Tonda Gentile delle Langhe" showed the earliest flowering and pollen release when compared to the rest of the evaluated pollinizers. This fast flowering behavior has been reported previously (12), and it has been explained by a lower CH requirement. Phenological variation for "Tonda di Giffoni" and its pollinizers at different study sites agrees with previous findings, where synchronization between glomeruli opening and pollinizer's pollen release has been associated to genetic and cultural factors, photoperiod and solar radiation, among others (18, 22, 28, 33).

Differences for fruit setting and diameter depended on the pollen source and agrees with descriptions by various authors (11, 25), who found that several fruit quality and yield traits, such as nut weight, husk/fruit ratio, fruit diameter, etc., were affected by the pollen source. "Tonda Gentile delle Langhe" showed the earliest flowering and was the least CH demanding pollinizer, in agreement with previous reports (12). The phenological variation observed for "Tonda di Giffoni" and its pollinizers at the three study sites is in accordance with similar studies (18, 22, 28, 33), which also reported that the synchronization between glomeruli aperture and pollen release depended on genetic and cropping factors, photoperiod, and solar radiation among others.

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CONCLUSIONS

During the season 2011-2012 "Tonda di Giffoni" male and female flowering occurred during June through August, with flowering spans ranging from two to two and a half months, depending on the specific location and pollinizers, showing a clear dichogamous behavior (protandric).

The studied pollinizers displayed differences on pollen synchrony. In general, the beginning of phenological stages were significant and positively correlated ($P < 0.05$) with CH and GDD accumulation. Differences in CH and GDD accumulations were detected across study sites. Camarico showed the greater CH and GDD accumulations but the most delayed phenology compared to Los Niches and San Rafael. Fruit setting and diameter were significantly influenced by the pollinizer. Results generated by this study may aid the decision-making when selecting cropping areas for hazelnut "Tonda di Giffoni" in similar climates. An adequate selection of type, number and proportion of pollinizers under specific CH and GDD accumulation conditions may significantly increase yields and fruit quality.

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