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Short communication. Effective pollination period in 'RedChief' and 'Golden Delicious' apples (*Malus domestica* Borkh)

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Abstract

Due to the significant variation in the initial fruit set of apples among seasons and producing regions of Chihuahua, Mexico, it is important to determine the cause(s). Apple fruit yield is strongly determined by the initial fruit set, which is the result of a series of physiological events, such as pollination, pollen tube growth, ovule longevity and fertilisation. During one or two seasons, in the two main apple-producing regions in the state of Chihuahua, México, the effective pollination period (EPP) was evaluated in 'Golden Delicious', 'RedChief Delicious' and 'Golden Delicious Tardío,' a regional mutant of Golden Delicious. Daily manual pollinations after the full pink stage were made with 'Snow Drift' pollen to determine the EPP. The initial fruit set at 30 days after the last day of hand pollination was evaluated. A daily sampling of flowers was used to measure the pollen tube growth (average growth of 11.70 mm at 96 h) in the pistil and ovule viability in the ovary. The EPP lasted 6 days for RedChief Delicious, 4 days for Golden Delicious, and 10 days for Golden Delicious Tardío. The average ovule longevity of 63% seemed to be a limiting factor for Golden Delicious, leading to a reduced initial fruit set.

Additional key words: fruit set, ovule longevity, pollen tube growth.

Resumen

Comunicación corta. Periodo de polinización efectiva en manzano (*Malus domestic*a Borkh) 'Red' y 'Golden Delicious'

En el estado de Chihuahua, México, existe una gran variación en el amarre inicial del fruto de manzana entre los diferentes ciclos y regiones productoras, por lo que es importante establecer alguna(s) causa o causas de ello. La producción en el manzano está determinada fuertemente por el amarre inicial de frutos, y éste es el resultado de una serie de eventos fisiológicos como polinización, crecimiento del tubo polínico, longevidad del óvulo y fecundación. Durante uno o dos ciclos, el periodo de polinización efectiva (EPP) se evaluó en 'Golden Delicious', 'RedChief Delicious' y 'Golden Delicious Tardío', un mutante regional de Golden Delicious, en las regiones de mayor producción de manzana en el Estado de Chihuahua, México. Para determinar el EPP, se llevaron a cabo polinizaciones manuales diarias, después del estadio de rosa completo, con polen de Snow Drift. Se evaluó el amarre inicial de frutos a los 30 días después del último día de polinización manual. Se tomaron muestras diarias de flores para determinar el crecimiento del tubo polínico (crecimiento promedio de 11,70 mm a las 96 h) en el pistilo y la viabilidad de los óvulos en el ovario. El EPP observado fue de 6 días para 'RedChief Delicious', 4 días en 'Golden Delicious' y 10 días en 'Golden Delicious Tardío'. La viabilidad del óvulo, un promedio de 63%, pudo ser un factor limitante para 'Golden Delicious', el cual redujo el amarre inicial de fruto.

Palabras clave adicionales: amarre de frutos, crecimiento del tubo polínico, longevidad del óvulo.

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Apple (*Malus domestica* Borkh) production in Mexico is currently 605,000 tons per season on 60,000 ha of land (www.faostat.fao.org). The state of Chihuahua has a production of 431,340 tons, which represents approximately 70% of the nation's total. Apple production is a result of a series of physiological events (Sanzol and Herrero, 2001), including fruit set. In the geographical regions of the present study, the initial fruit set is highly variable from year to year and is a factor that directly affects the harvest. Compatible pollen transfer to a receptive stigmatic surface (Sanzol and Herrero, 2007), pollen germination and pollen tube growth are necessary for reaching viable ovules (Kaufmane and Rumpunen, 2002), and fertilisation is required to obtain an adequate initial fruit set (Soltész, 1997; Sanzol and Herrero, 2001; Schneider *et al*., 2001).

The objectives for this study were as follows: 1) to determine the effective pollination period (EPP) of 'Golden Delicious' (main apple cultivar in the state and country), 'RedChief Delicious' and 'Golden Delicious Tardío' apples; and 2) to determine if the evaluated Snow Drift pollen tube growth and/or ovule longevity (viability) of the cultivars were key factors affecting the EPP.

Research was carried out at two apple-growing areas in the state of Chihuahua: a) Cuauhtémoc, located at 28° 24' LN and 106° 52' LW, 2,060 m above sea level, with a semi-dry temperate climate, 400 to 600 mm rainfall and a mean annual temperature of 12 to 18°C; and b) Guerrero, located at 28° 33' LN and 107° 30' LW, 2,100 m above sea level, with a semi-cold subhumid climate, a summer rain period with an annual rainfall of 600 to 1,200 mm, and a mean annual temperature of 8 to 12°C (INEGI, 2000).

RedChief Delicious apple, a cultivar reported as having fruit set problems due to pollination (Guerrero-Prieto *et al*., 2006), was evaluated during one season, only in Cuauhtemoc, due to late frost damage in the flowers. Golden Delicious and a regional mutant of it, characterised by its late (month of May) blooming period, called Golden Delicious Tardío (late Golden Delicious), were evaluated only in the Guerrero locality during two seasons. Golden Delicious Tardío is known and used only in the Guerrero area of the study. Snow Drift pollen was used as the sole source of pollen since its blooming period overlaps those of the cultivars evaluated and it is available and used by the apple growers in both areas of the study as a pollination variety (Guerrero-Prieto *et al*., 2006). Pollen from the evaluated cultivars was not used due to either self-incompatibility or incompatibility among themselves or a low fruit set (Soltész, 1997; Schneider *et al*., 2001). A sample of 50 apple flowers was collected from the Snow Drift pollinator at the pink stage (Guerrero-Prieto *et al*., 2006). Flower petals were completely removed, and the collected anthers were rubbed against a mesh and then air-dried for 24 h at room temperature to collect the pollen. Pollen was stored at 4°C. Snow Drift pollen was used to hand pollinate flowers to measure pollen tube growth and ovule viability (Guerrero-Prieto *et al*., 1985). Before Snow Drift pollen was used, a germination test was done with a solution of 15% sucrose plus 15 mg L^{-1} of boric acid. After 2 h, germination percent was evaluated under a light microscope, but only considering a minimum of 70% germination (Guerrero-Prieto *et al*., 1985). Germination tests were done only in the sucrose solution and not on the flower stigmatic surface since germinating pollen grains were evaluated through their pollen tube growth. Five trees were used for each cultivar evaluated. On each tree, four cardinally-oriented branches, each with 100 flower clusters, were selected and marked. The flowers were emasculated for the first season and covered with a cloth veil during the second season. Hand pollinations with Snow Drift pollen were done daily on 20 flowers on each marked branch. Day zero was considered when 40 to 50% of the flower clusters were in the «pink» stage. The initial fruit set was evaluated after 30 days after the last day of hand pollination and was expressed as the number of fruits set per each 100 flower clusters (Williams, 1965). On day «zero», 250 flowers were hand pollinated on each labelled tree branch, from which 20 flowers were sampled daily until day 10. Collected flowers were killed and fixed in an FAA solution (formalin, acetic acid and 70% ethyl alcohol, 5:5:90, respectively), vacuumed for an hour, and stored in 70% ethyl alcohol until preparation for squashing. Flower pistils were softened in a 5% sodium sulphite solution and autoclaved at 90°C for 20 min (Guerrero-Prieto *et al*., 1985). The pistils were squashed in a 0.1% aniline blue, 0.1N K_3PO_4 buffer solution. Squashed pistils were observed under fluorescent light on a microscope. Snow Drift pollen tube growth was measured by the length of the fluorescent pollen tubes growing into the style (Guerrero-Prieto *et al*., 1985). The average length between the stigmatic surface and the middle part of

Abbreviations used: EPP (effective pollination period), FAA (formalin-aceto-alcohol).

the ovary in the flowers of the three evaluated cultivars ranged between 12.50 to 13.00 mm. Ovule longevity was evaluated by ovule fluorescence, and non-fluorescent ovules were considered viable (Guerrero-Prieto *et al*., 1985). Standard deviations were calculated for the pollen tube growth means. Snow Drift pollen germination gave an average of 86% germination throughout these experiments.

The longest pollen tube growth, 11.70 mm, was obtained at 96 h into the second season of evaluation; these pollen tubes had already reached the ovary base. By contrast, the pollen tubes were almost at the base of the ovary during the first season, with a pollen tube length of 9.80 mm. Golden Delicious, in the Guerrero locality, showed the lowest values (1.10 mm, at 24 h) for pollen tube growth, while Golden Delicious Tardío in Guerrero had the highest pollen tube growth (11.63 mm, at 96 h) (Table 1).

During the first season in Guerrero, in RedChief Delicious after six days, percentages went from 100 to 28% of initial fruit set. After 4 days of the second season, the initial fruit set ranged from 73 to 25%. Fruit set values of zero in Guerrero for the first season were due to lack of fruit. In Cuauhtémoc, the initial fruit set varied from 38 to 57% after 6 days of the second season (Fig. 1a). The Golden Delicious EPP was variable for both seasons. For the first season, the EPP lasted for five days, starting from 78, increasing to 100, and finishing with 22% of the initial fruit set. For the second season, the initial fruit set showed a continuous decrease, going from 78 to 5% over the course of 8 days (Fig. 1b). On day six, an initial fruit set of zero was due to a lack of fruit. During the first season, the Golden Delicious Tardío EPP lasted five days, beginning with 78%, then remaining constant at a 100% fruit set for 3 days, and finishing with 40% of the initial fruit set. There was also a drop on day four to 0% due to a lack of fruit. For the second season, the EPP lasted 10 days, with an initial fruit set of 88% at day one and a final of 18% (Fig. 1c). A lack of fruit in all of the cultivars for all of the evaluated seasons and localities corresponded to a reading of zero for the fruit set.

RedChief Delicious in Guerrero had an ovule viability of 100% during both seasons and throughout the evaluation period. In the Cuauhtemoc locality, the ovule viability decreased from 100% at 24 h to 80% at 96 h for the second season. For the first season, Golden Delicious had a variable percentage of ovule viability with the highest being 81% at 48 h, whereas during the second season the ovule viability remained at 100% at 48 and 72 h and declined to 88% after 96 h. During the first season, the ovule viability in Golden Delicious Tardío was 100% for 4 days (96 h) but was only 62% after 72 h in the second season (Table 2).

The percent of pollen germination was sufficient not to interfere with the initial fruit set results. Ortega *et al*. (2004) had similar results to ours**.**

Pollen tube growth (Table 1) was not considered to be a limiting factor for the EPP since the pollen tubes reached the ovary base during the time frame (in days) for the EPP with each cultivar and season evaluated, which is in agreement with previous results (Sanzol and Herrero, 2001).

Fruit set values in the evaluated cultivars, seasons and localities allowed for what is considered a commercial level of fruit production (Vicens and Bosch, 2000). The EPP values in apples can vary from two to six days, depending on several factors, such as nutritional status of the flowers (Williams, 1965) and temperature

Table 1. Snow Drift pollen tube growth means[a] (mm) for two seasons in three apple cultivars in Guerrero and Cuauhtémoc (Chih., Mexico), at 24, 48, 72 and 96 hours after pollination

	24 h	48 h	72 h	96 h
Season				
First	0.96 (0.6)[b]	3.60 (2.0)	5.70 (3.7)	9.80 (2.9)
Second	3.45 (2.3)	4.50 (0.7)	10.08 (3.1)	11.70 (2.5)
Cultivar and locality				
RedChief Delicious (Guerrero)	2.77 (2.7)[b]	2.15 (1.7)	6.97 (4.2)	10.05 (1.6)
Golden Delicious (Guerrero)	1.10 (0.7)	3.97 (1.7)	6.90 (4.6)	11.02 (3.5)
Golden Delicious Tardío (Guerrero)	1.10 (0.7)	5.30 (1.0)	10.62 (2.4)	11.63 (2.1)
RedChief Delicious (Cuauhtémoc)	2.05 (1.2)	—[c]	8.60 (2.9)	11.05 (3.5)

[a] 20 flowers. [b] Standard deviation. [c] Lack of sample.

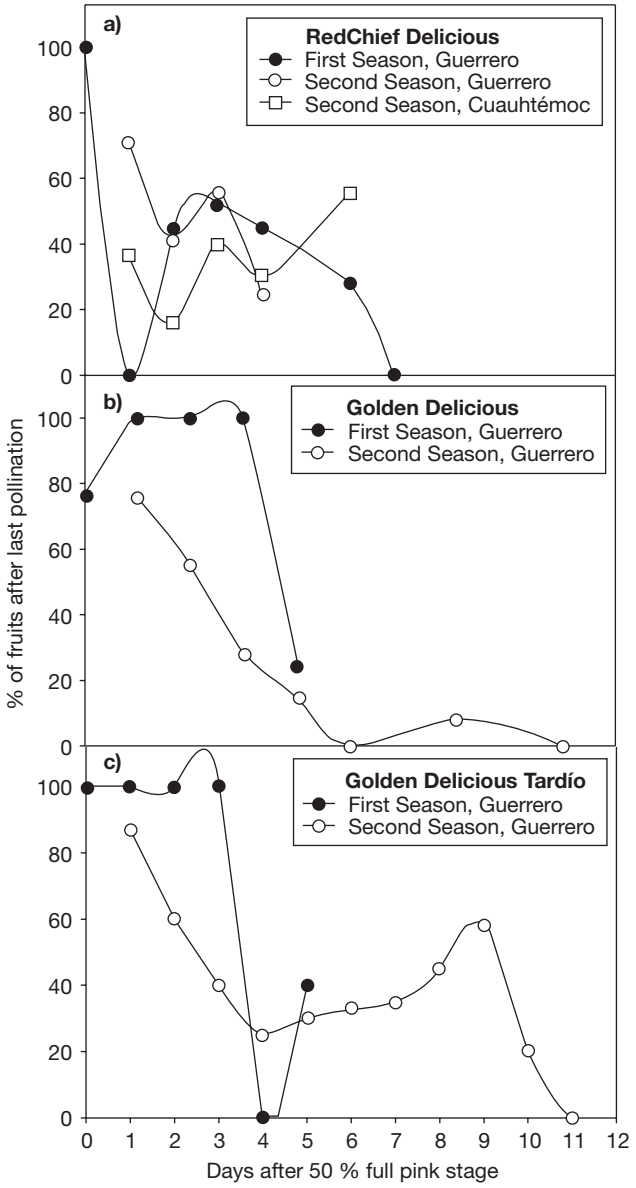

Figure 1. Effective pollination period (EPP) in a) RedChief Delicious, b) Golden Delicious and c) Golden Delicious Tardío apples in Guerrero and Cuauhtémoc (Chih., Mexico).

following the blooming period (Tromp and Borsboom, 1994). The length of the EPP can also be influenced by stigmatic receptivity in apples (Tromp and Borsboom, 1994). The flower emasculation during the first season for manual pollination could have been another factor influencing our EPP results, since this practice could damage the flowers or causing flower or fruitlet drops, which could have lowered the initial fruit set. Fruit set differences among the evaluated cultivars could have been due to temperature differences during the blooming time, since RedChief Delicious blooms first, with an average temperature lower than those for Golden Delicious, which blooms after RedChief Delicious but before Golden Delicious Tardío. Higher mean temperatures during the blooming times for Golden Delicious and Golden Delicious Tardío could have reduced the stigmatic receptivity and ovule longevity period (Sanzol and Herrero, 2001; Guerrero-Prieto *et al*., 2006). Also, windy weather during the blooming time, which is common for the localities studied, restricts bee flight and thereby restricts pollination efficiency (Benedek, 1996).

Only Golden Delicious showed a reduction of EPP as a result of ovule viability since after 96 h the viability was reduced. The variation of the climatic conditions during the previous winter, which affected all evaluated cultivars and established a variable blooming pattern from season to season, also influenced the length of the blooming period. The longer than normal blooming period forced flowers to be exposed to higher temperatures (Guerrero-Prieto *et al*., 2006). Ovule longevity was considered a limiting factor for Golden Delicious EPP. Pollen germination, pollen tube growth and ovule viability in all of the evaluated cultivars, localities and seasons affected the initial fruit set. Our results showed that ovule longevity had a negative effect on the initial fruit set for Golden Delicious, Mexico's main apple cultivar.

Table 2. Ovule longevity (viability, %)[a] for two seasons in three apple cultivars in Guerrero and Cuauhtémoc (Chih., Mexico) at 24, 48, 72 and 96 hours after pollination

	First season				Second season			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
RedChief Delicious (Guerrero)	100	100	—[b]	100	100	—	—	100
Golden Delicious (Guerrero)	35	81	71	66	—	100	100	88
Golden Delicious Tardío (Guerrero)	100	100	100	100	—	—	62	—
RedChief Delicious (Cuauhtémoc)	—	—	—	—	100	—	—	80

[a] 20 flowers. [b] Lack of sample.

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