Differential growth of almond pollen tubes in three environments

Socias i Company R.

in

Ak B.E. (ed.).
XI GREMPA Seminar on Pistachios and Almonds

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 56

2001
pages 59-64

Differential growth of almond pollen tubes in three environments

R. Socias i Company
Unidad de Fruticultura, SIA-DGA, Apartado 727, 50080 Zaragoza, Spain

SUMMARY – Pollen tube growth dynamics was followed in emasculated flowers maintained in three different environments: (i) on the original branches in the field; (ii) on cut branches placed at constant temperature in chambers; and (iii) on individual flowers placed on floating meshes in trays in the same constant temperature chambers. Self-compatible ('Cambra') and self-incompatible ('Bertina') cultivars were studied. Both were self- and cross-pollinated with 'Marcona' pollen that is inter-compatible with both of them. The results showed that the dynamics of pollen tube growth was the same in the three environments and that the only difference was due to the compatibility of the pollination. These results support the utilization of individual flowers on trays to study the compatibility relationships of almond pollination, thus resulting in freeing this study from the weather conditions in the field and in saving space in the chambers at constant temperatures.

Key words: Almond, pollination, compatibility, *Prunus amygdalus* Batsch.

RESUME *– "Croissance différentielle des tubes polliniques de l'amandier dans trois environnements". La dynamique de la croissance des tubes polliniques a été suivie chez des fleurs émasculées maintenues dans trois environnements différents : (i) sur les branches d'origine en plein champ ; (ii) sur des branches coupées et placées dans des chambres à température constante ; et (iii) sur des fleurs individuelles placées sur des mailles flottantes dans des plateaux dans les mêmes chambres à température constante. Un cultivar autocompatible ('Cambra') et un cultivar autoincompatible ('Bertina') ont été étudiés. Les deux ont été autopollinisés et pollinisés avec du pollen du cultivar 'Marcona' qui est intercompatible avec les deux. Les résultats ont montré que la dynamique de la croissance des tubes polliniques est la même dans les trois environnements et que la seule différence est due à la compatibilité de la pollinisation. Les résultats donnent un support à l'utilisation de fleurs individuelles sur des plateaux pour étudier les relations de compatibilité des pollinisations chez l'amandier, avec la conséquence d'affranchir cette étude des conditions atmosphériques en plein champ et d'économiser de l'espace dans les chambres à température constante.*

Mots-clés : *Amandier, pollinisation, compatibilité,* Prunus amygdalus *Batsch.*

Introduction

Compatibility of pollination is of paramount importance in almond growing because the commercial part of the crop is a seed, thus requiring a successful pollination and fertilization process. Compatibility relationships among pollinating cultivars are normally studied in the field, where always a final evaluation must be carried out, because the number of cultivars involved is not usually too high. However, when self-compatibility is evaluated in a breeding programme, a high number of seedlings must be tested and several procedures must be taken in order to make the high number of evaluations in an easy and quick way.

Pollen tube growth is a clear indication of the compatibility of the pollination and as a consequence it has been repeatedly used in compatibility determinations (Socias i Company, 1996). The flowers examined for assessing pollen tube growth can be kept in different environments as well as on the original branches or separated from them. Each system of maintaining the flowers offers advantages and disadvantages and caution must be taken in order to exactly interpret the results observed in each one.

Thus, studies conducted in the field show the most reliable response since they reflect the natural conditions of the pollination. However, these studies are subject to unpredictable weather conditions such as frosts. Frosts may destroy the pistil, but not so easily the pollen tubes, which growth is arrested by frosts, as well as by any low temperature (Socias i Company, 1982). These studies also require trees with enough flowers to conduct the assay, taking into account that the open flowers and the retarded buds are usually eliminated. As emasculation and pollination must be done in the open air, weather conditions are not always favourable to work because temperatures are usually very low at almond blooming time and if winds are blowing much attention must be paid to conduct the operations.

The problems to work in the field and the weather contingencies which could affect the study can be avoided by taking branches to the laboratory or greenhouse and conducting emasculation and pollination on them. In this case, branches must have a sufficient number of flowers, thus normally requiring a high flower density (Socias i Company, 1988) in order to reduce the space where the branches are maintained, especially if the branches must be kept at constant temperature chambers, a requirement when pollen tube growth rates need to be calculated. Compatibility relationships using cut branches are the same than in the field (Socias i Company *et al.*, 1976), with the only difference of growth rates related to the temperature differences among environments. However, if many tests must be conducted, the space required to keep the branches can be extremely large.

The utilization of individual flowers placed on floating meshes in trays with water can solve some of the mentioned problems. Even if the flower density is low, the flowers can be picked all over the tree, thus not requiring a high bud number. Besides, the required number of flowers can be collected in two times, allowing for the bud development, a case to consider when a young seedling from a breeding programme produces the first flowers and their number is low. Furthermore, a single tray can contain several samples and occupies a small pace, a case to be considered in constant temperature chambers.

The objective of the present study was to compare these three systems of studying pollen tube growth in order to consider the most reliable and easy way to conduct compatibility relationships of pollination in almond.

Materials and methods

Two cultivars were selected, one self-compatible and the other self-incompatible in order to follow the dynamics of pollen tube growth in two types of pollinations: self and cross. 'Cambra' is a self-compatible cultivar recently released from the breeding programme of the SIA of Zaragoza (Socias i Company and Felipe, 1999). 'Bertina' is a self-incompatible local cultivar introduced in the breeding programme because of its late blooming and large fruit size (Socias i Company *et al.*, 1999). Both were self- and cross-pollinated with 'Marcona' pollen that is intercompatible with both of them and was collected following the usual procedures (Kester and Asay, 1975) and kept in the refrigerator until use.

The trees were grown at the Spanish almond germplasm collection of the SIA of Zaragoza, grafted onto almond x peach hybrid rootstock, free from known viruses and maintained in the usual way. For the field treatment, several branches with enough flowers were selected around the trees and assigned randomly to the different treatments. The flowers that were ready, at stage D (Felipe, 1977), were emasculated and the open flowers and retarded buds were discarded.

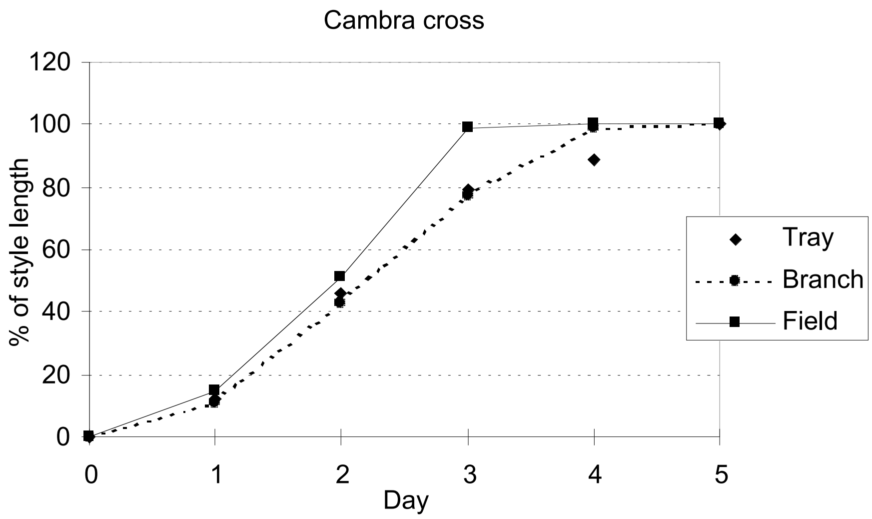
Other branches were cut and taken to the laboratory where they were placed in pots with running water. The base of the branches was cut under water to avoid cavitation as well as in alternate days when the water of the pots was changed. As in the field, flowers at stage D were emasculated and the rest were discarded. At the same time flowers were gathered from the field at stage D, emasculated and then floated in a tray on water with the peduncles passing through the holes of a plastic mesh screen.

Approximately two days after emasculation the pistils were self- or cross-pollinated with 'Marcona' pollen that is intercompatible with the two cultivars studied. The pots with the branches and the trays with the flowers were placed in dark chambers at 10°C. Samples of 10 pistils were collected for each treatment every 24 h. The pistils were fixed in FAA and kept until preparation for observation. Pistils were prepared by rinsing several times with water and autoclaved in a 5% solution of Na_2SO_3 for 10 min at 1.2 kg/cm^2. The outer part of the pistils was dissected (Socias i Company, 1979), leaving only the transmitting tissue through which pollen tubes grow. Pollen tube growth was assessed under an ultraviolet light microscope. In this method the callose deposits of the pollen tubes fluoresce following aniline blue staining of pistil squashes (Linskens and Esser, 1957).

Pollen tube growth was expressed according to Lewis (1942) as percentage of the total style length traversed by the longest tube. The calculation was made with eight out of the 10 samples after discarding both the lowest and the highest measurements. After analysis of variance means were compared for each time observation with the Duncan's test.

Results and discussion

Pollen tube progression through the style confirmed the compatibility of three of the pollinations: the two crosses with 'Marcona' pollen and the self-pollination of 'Cambra', whereas 'Bertina' showed the incompatibility of its self-pollination (Figs 1 and 2), reassuring that the compatibility of the pollinations was independent of the environment where the pistils were placed. However, in the compatible pollinations, pollen progression was not similar in all treatments and significant differences were observed at every time period (Table 1). These differences, however, do not affect the compatibility relationship of the pollinations neither allow to establish a consistent pattern of behaviour among treatments. As a consequence, the differences must be mostly attributed to the sampling of pistils or to their biological variability because, as they are living elements, they may react differently to the same treatment.



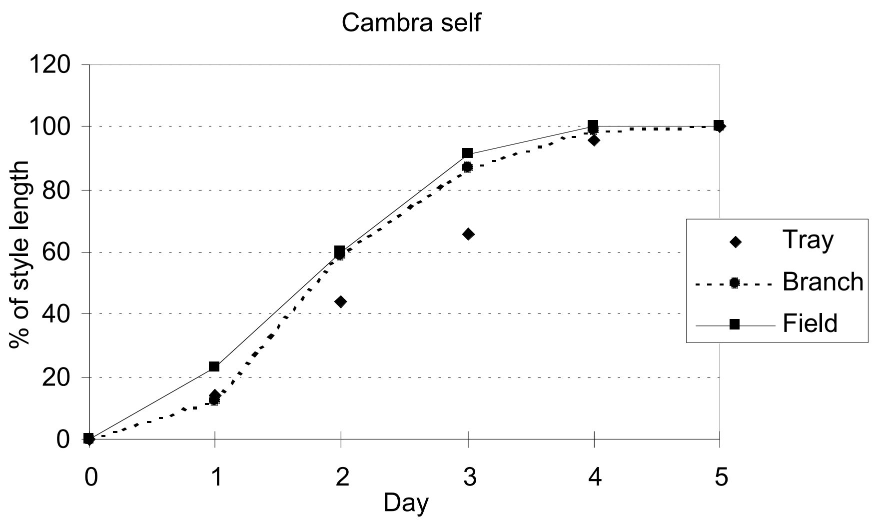
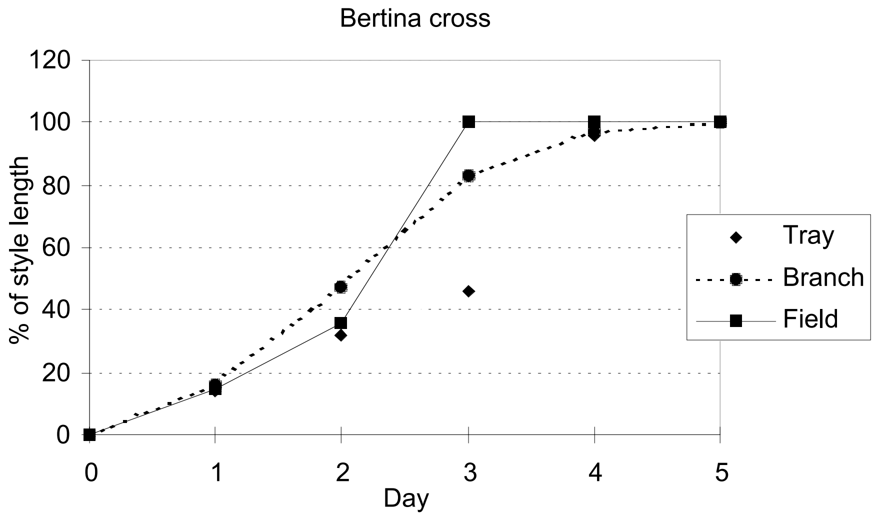


Fig. 1. Mean percentage of 'Cambra' styles traversed by the longest pollen tube after self- and cross-pollination in three environments.



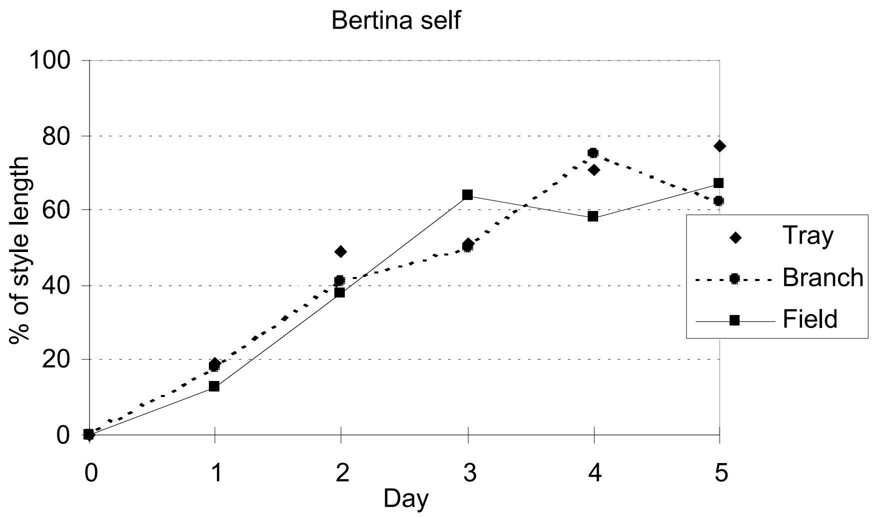


Fig. 2. Mean percentage of 'Bertina' styles traversed by the longest pollen tube after self- and cross-pollination in three environments.

Table 1. Percentage of style traversed by the longest pollen tube following self- or cross-pollination of 'Cambra' and 'Bertina' in the different treatments

Days after polli-nation	Cambra						Bertina					
	Tray		Branch		Field		Tray		Branch		Field	
	○	⊗	○	⊗	○	⊗	○	⊗	○	⊗	○	⊗
1	12 a	14 ab	11 a	12 a	15 abc	23 d	14 abc	17 cd	16 abc	18 bcd	15 abc	13 ab
2	46 bcd	44 bcd	43 bcd	59 e	51 de	60 e	32 a	49 d	47 cd	41 abcd	36 ab	38 abc
3	79 c	66 b	77 c	87 cd	98 e	91 de	46 a	51 a	83 cd	50 a	100 e	64 b
4	88 c	96 cd	99 e	99 e	100 e	100 e	96 cd	71 b	97 cd	75 b	100 e	58 a
5	100 d	100 d	100 d	100 d	100 d	100 d	100 d	77 c	100 d	62 a	100 d	67 b

[a,b,c,d,e]Mean percentages in each line separated by Duncan's test at P = 0.05.

A single conclusion can be drawn from these differences, as in most cases a slightly better growth is observed in the field treatments. This difference can be explained by the temperature effect on pollen tube growth (Socias i Company *et al.*, 1976). The two cultivars studied are late blooming and temperatures in the field are already high at this season and thus more favourable for pollen tube growth, a little better than the growth allowed by the 10°C at the constant temperature chambers.

Another consistent observation is that as a general rule pollen tube growth in 'Cambra' cross-pollinated is a little better than when self-pollinated, although not always significantly and not allowing to follow the suggestion of Godini (1981) that self-compatible pollen tubes grow more slowly than the foreign ones.

The tray treatment of 'Bertina' cross-pollinated showed a slower growth than the other treatments, but this is only seen at the sample of three days after pollination and could be due, as indicated, to the sampling of pistils or to their variability because the observation after four days agrees with those of the other treatments.

These results show that, despite these differences and punctual disagreements, there is a general trend followed by pollen tubes in their progression through the style: five days after pollination all the pistils showed pollen tubes at their base following compatible pollinations whereas not a single pollen tube was observed at the base of any pistil following self-pollination of 'Bertina'. For this reason, results are only shown for the first five days as growth of pollen tubes in 'Bertina' self-pollinated was arrested about 3-4 days after pollination in all treatments, having progressed between 2/3 to 3/4 of the style length. This observation agrees with the behaviour of self-incompatible pollen tubes at the temperature of these assays (Socias i Company *et al.*, 1976).

The conclusion of this study confirms the hypothesis that the environment where the study is conducted does not affect the compatibility relationship of the pollination and, as a consequence, the method can be selected depending on the conditions required. When a large number of seedlings from a breeding programme have to be tested, the use of individual flowers on trays is highly recommended because in this case the number of flowers required is easily accomplished collecting flower buds all over the seedling; furthermore, emasculation and pollen extraction from the same flowers are easily performed in the laboratory because the flower buds are all at the same stage and the conditions of work are more comfortable; finally, a large number of seedlings can be studied in the reduced space of a tray, an advantage which acquires more importance when pollinations are studied at constant temperature chambers, which volume may be small. A final consideration is that weather conditions in the field do not affect in any way this assay, thus avoiding the problem of frosts, a phenomenon quite common at blooming time.

Acknowledgements

Research conducted under project CICYT AGF-98-0211-C03-01. Comments by A.J. Felipe and technical assistance by Amalia Escota and J. Búbal are highly appreciated.

References

Felipe, A.J. (1977). Almendro. Estados fenológicos. *Inf. Técn. Econ. Agrar.*, 27: 8-9.

Godini, A. (1981). Observing pollen tube growth in self-compatible almond cultivars by means of fluorescence. *Options Méditerranéennes*, 81/I: 77-82.

Kester, D.E. and Asay, R. (1975). Almonds. In: *Advances in Fruit Breeding*, Janick, J. and Moore, J.N. (eds). Purdue Univ. Press, West Lafayette (IN), pp. 387-419.

Lewis, D. (1942). The physiology of incompatibility in plants. I. The effect of temperature. *Proc. R. Soc. London, Ser. B*, 131(862): 13-26.

Linskens, M.F. and Esser, K. (1957). Über eine spezifische Anfarbung der Pollenschlauche und die Zahl der Kallosepfropfen nach Selbstung und Fremdung. *Naturwissenchaften*, 44: 16.

Socias i Company, R. (1979). Aportación a las técnicas de observación de los tubos polínicos. Caso del almendro. *An. Inst. Nac. Invest. Agrar., Ser. Prod. Veg.*, 10: 233-236.

Socias i Company, R. (1982). Ensayo de polinización de diferentes clones del almendro 'Marcona'. *An. Inst. Nac. Invest. Agrar., Ser. Agric.*, 19: 11-21.

Socias i Company, R. (1988). La densité florale comme critère variétal chez l'amandier. In: VII Coll. GREMPA. *Rap. EUR*, 11557: 119-122.

Socias i Company, R. (1996). L'autogamia nel miglioramento genetico del mandorlo. *Frutticoltura*, 48(12): 67-70.
Socias i Company, R. and Felipe, A.J. (1999). 'Blanquerna', 'Cambra' y 'Felisia'. Tres nuevos cultivares autógamos de almendro. *Inf. Técn. Econ. Agrar.*, 95V: 111-117.
Socias i Company, R., Felipe, A.J. and Gómez Aparisi, J. (1999). A major gene for flowering time in almond. *Plant Breed.*, 118: 443-448.
Socias i Company, R., Kester, D.E. and Bradley, M.V. (1976). Effects of temperature and genotype on pollen tube growth in some self-compatible and self-incompatible almond cultivars. *J. Amer. Soc. Hort. Sci.*, 101: 490-493.