

THE EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE *IN VITRO* GERMINATION OF THE POLLEN OF AVOCADO

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Abstract

Optimum *in vitro* germination of the pollen of the avocado cultivars Fuerte, Nabal, Ettinger, Bacon and Zutano occurred at 25°C. Besides, significant differences among the cvs Fuerte, Nabal, Zutano and Hass regarding germination % and relative humidity requirements, for optimum pollen were shown. The most sensitive to relative humidity variation, was the pollen of Fuerte, the germination of which rose from 11.42%, when humidity was 40%, to about 50%, when kept for one hour at a relative humidity equal to 100%. In the cultivar Nabal, at variance, the germination % was already high at a relative humidity 40% and remained in the same high levels after exposure to high relative humidity. It was confirmed that increased relative humidity contributes to the preservation of avocado pollen viability.

Additional index words: cultivars, *Persea americana*, pretreatment humidity.

1. Introduction

Optimum temperature for pollen germination depends on the species and often varies between the cvs. Roberts and Struckmeyer (1948), for example, reported that optimum temperature for the germination of the pollen of the "McIntosh" apple cv was 20°C and 24°C for the cvs "Winesap" and "Wealthy". The same workers observed differences between cvs in respect with the lower temperature at which pollen germination occurs. Besides germination, pollen tube growth rate greatly depends on temperature (Porlingis and Sfakiotakis, 1968; Vasilakakis and Porlingis, 1985; Visser, 1955) while at high temperatures pollen tube burst can occur (Roberts and Struckmeyer, 1948; Visser, 1955). Sahar and Spiegel-Roy (1984), who first reported *in vitro* germination of avocado pollen, found that the optimum temperature for avocado pollen germination varied between 25 and 29°C. On the other hand the increase of the germination % of pollen, following the exposure to high RH before germination trials, has been reported for papaya (Cohen *et al.*, 1989), walnut (Luza and Polito, 1987), aglaonema (Henny, 1980, 1988), lily (Simons *et al.*, 1970) etc.

Avocado cultivation is expanding in southern areas of Greece and especially in Crete, where, both sub and supra-optimal temperature and humidity conditions for pollen germination that can affect fruit-set, often occur. The present study was conducted in order to evaluate the effect of

temperature and pre-treatment relative humidity conditions on the germination of the main commercial cvs of the avocado.

## 2. Material and methods

Pollen was germinated in a liquid medium containing, sucrose 15% and a mixture of mineral salts (100 mg H<sub>3</sub>BO<sub>4</sub>, 100 mg KNO<sub>3</sub>, 300 mg MgSO<sub>4</sub> and 1000 mg Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O per l), using the hanging drop technique. Small, inflorescence bearing twigs, kept in beakers in the laboratory provided the pollen required for the experiments. The pollen required was collected from newly dehisced anthers.

In temperature trials, the pollen was transferred to special germination slides immediately after collection from the dehisced anthers. The slides, subsequently, were incubated at 5, 10, 15, 20, 25, 30 and 40 ±0.1°C for three hours when most of the viable pollen had germinated. Pollen of Fuerte, Topa-Topa and Hass as well as of Ettinger, Nabal, Zutano and Bacon was tested for temperature requirements. Relative humidity conditions were adjusted to the desired levels (varying between 5 and 100%) by sulphuric acid solutions placed in glass desiccators (Lange, 1968). The desiccators with the desiccating solution were left several days for RH to equilibrate. RH level was verified using an electronic humidity meter (Vaisala HMP 35, Vaisala (UK) Ltd) by suspending the sensing probe through a rubber stopper in the desiccator lid.

## 3. Results and discussion

The optimum temperature for the *in vitro* germination of the pollen of the cvs; Ettinger, Nabal, Bacon and Zutano, incubated immediately after collection, was 25°C (Fig. 1). The higher germination rate was observed in Ettinger (64.64%) and the lower one in Zutano (47.69%). Nabal and Bacon had intermediate levels (48.54 and 58.89%, respectively). Important differences in the behaviour of the pollen of the cvs at supra and sub-optimal temperatures were found. Thus at 30°C an important drop of germination rates for the cvs Bacon and Zutano (37.91 and 27.48%) was found while Ettinger (51.58) and Nabal (48.44%) were less affected. Besides, as can be seen from Fig. 1, the pollen of the last two cvs (Ettinger and Nabal) kept to a satisfactory level (78 and 69% of the optimum) the ability to germinate even at 40°C. At 15°C the higher rate (17.22%) was shown by Zutano, a cv with significantly lower rates than the others at the optimum temperature (25°C). The germination of Bacon and Nabal at 20°C was unusually low (5.74 and 5.55%, respectively). Thus germination in Ettinger and Nabal seems to start at 15°C while in Bacon and Zutano, probably more heat requiring, at 20°C. Ettinger and Nabal had a satisfactory germination over a broader range (10 - 40°C) than the cvs Bacon and Zutano (20-30°C).

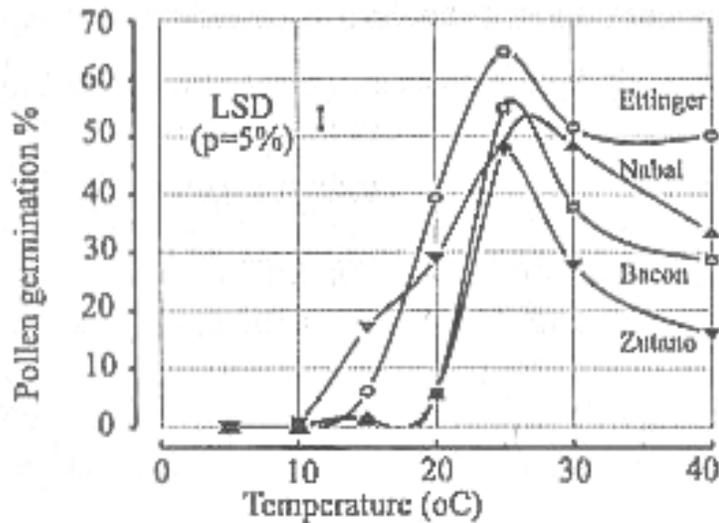


Fig. 1. The effect of temperature on the in vitro germination of the pollen of Ettinger, Nabal, Bacon & Zutano

In addition to the cvs shown in Fig. 1, pollen of the main commercial cvs Fuerte, Hass, Topa - Topa were tested for germination without pre-treatment. at 25 and 30°C. The last two cvs at 30°C had germination rates (48.7 and 41.32%) comparable to the ones found for the cvs shown in Fig. 1, and satisfactory germination at 20°C (20.71,18.81 %). Pollen of the cv Fuerte had a poor germination (7% at 25°C). This germination rate of Fuerte is about half of that reported by Sahar and Spiegel - Roy (1984). A satisfactory germination of Fuerte pollen was achieved, however, when the pollen was kept in a 100% R.H. environment, at room temperature, before incubation. Figure 2. shows the germination of pollen of Fuerte (kept for two hours at 100% RH), in relation to the incubation temperature. As can be seen from the Figure the germination rate achieved at 25°C (63.69%) is among the higher ones.

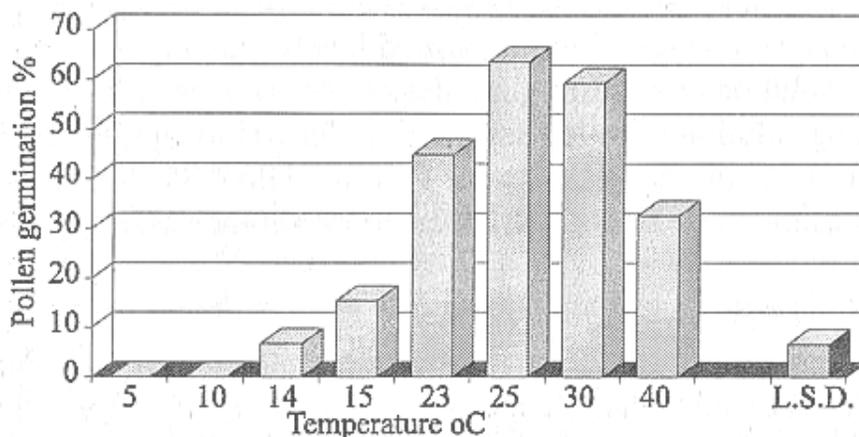


Figure 2. In vitro germination % of the pollen of Fuerte exposed for 2 hours to water vapour saturated atmosphere, in relation to temperature.

The temperature optimum for avocado pollen germination (25°C) found in this experiment is comparable to the temperatures reported for other species. Vasilakakis and Porlingis (1985) for example reported optimum range 15-23°C for the pear cv Tsakoniki, Visser (1955) gave 23 and 23 – 29°C for the apple cvs Brabante and Esterreinette, respectively.

As shown from the data described above environmental conditions and especially RH can affect the ability of the pollen of Fuerte to germinate. In Fig. 3. the effect of pre-incubation treatment of Fuerte and Nabal pollen with two levels of RH (40 and 100%) in relation to the

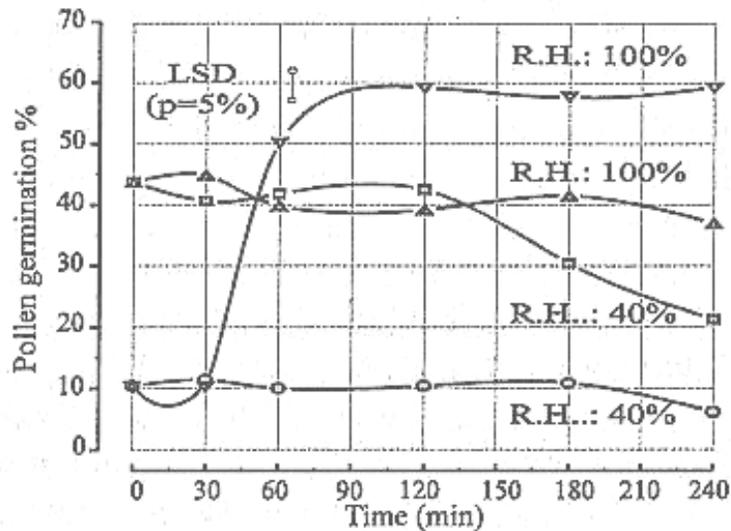


Figure 3. In vitro germination % of Fuerte and Nabal pollen exposed to 40 and 100% R.H., in relation to the duration of the exposure.

duration of this pre-incubation treatment (storage) is shown. It can be seen from the Figure that Nabal pollen had high germination rates immediately after collection from the anthers. By contrast Fuerte germination, initially very poor (about 10%), started rising only after exposure to water vapour saturated environment for 1 hour; peak germination (of Fuerte) was achieved after 2 hours exposure. Both cvs showed a drop of germination after prolonged exposure to 40% relative humidity. Thus it appears that for Fuerte an exposure of the pollen for 90 - 120 minutes to water vapour saturated environment is required for satisfactory germination, while in dry environments the ability of pollen to germinate deteriorates quickly. Additional pollen germination trials for Fuerte pollen kept at various moisture levels (5, 30, 55, 60, 70, 100%) for one and three hours showed that satisfactory germination rates were obtained when the pollen was placed at RE 55% or higher and maximum germination (71.58%) only after exposure to saturated atmosphere for one hour (Fig.4), storage under 30% RH quickly caused the decline of germination ability of the pollen.

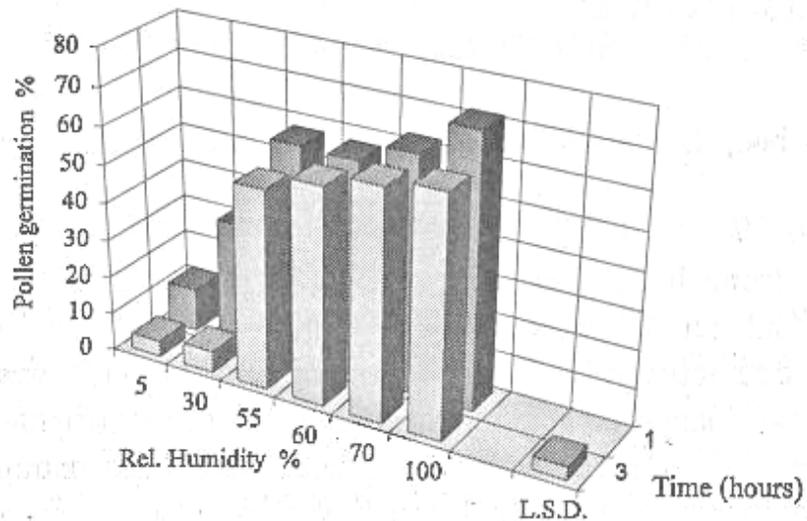


Figure 4. In vitro germination % of the pollen of Fuerte exposed to six R.H. levels for 1 and 3 hours.

### References

- Cohen, E., Lavee, S., and Spiegel Roy, P., 1989. Papaya pollen viability and storage. *Scientia Horticulturae*, 40: 317-324.
- Henny, R.J., 1980. Relative humidity affects *in vivo* pollen germination and seed production in *Dieffenbachia maculata* 'Perfection'. *J. Amer. Soc. Hort. Sci.* 105: 546-548.
- Henny, R.J., 1988. Pollen germination in aglaonema flowers of different ages. *HortScience* 213=218.
- Lange, N.A., 1967. Handbook of chemistry compiled and edited by Norbert Adolph Lange, pp2001.
- Loupassaki, M.H., 1995. The effect of girdling, of germination of pollen and of genotype on the fruit-set of avocado (*Persea Americana* Miller). Ph.D. Thesis, Aristotelian University of Thessaloniki,
- Luza, J.G., Polito, V.S., 1987. Effect of desiccation and controlled rehydration on germination in vitro of pollen of walnut (*Juglans* spp) *Plant Cell and Environment* 10:487-492.
- Porlingis, I.C., Sfakiotakis, E.M., 1968. Germination in vitro and storage of the olive pollen (tran. tittle). *Ann. Rep. Fac. Agr. and Forest., Arristotelian Univ. of Thessaloniki (Greece,)* 12:525-556..
- Roberts, R.H., Struckmeyer, B.E., 1948. Use of sprays to set greenhouse tomatoes. *Proc. Am. Soc. Hort. Sci.* 44: 417-427.
- Sahar, N., Spiegel-Roy, P., 1984. *In vitro* germination of avocado pollen. *Hort-Science*, 19: 886-888.
- Shivanna, K.R., Heslop-Harrison, J., 1981. Membrane state and pollen viability. *Ann. Bot.* 47:759-770.
- Simons, D. H., Sfakiotakis, E. and Dilley, D.R., 1972. Enhancement of *in vitro* pollen germination of lily with increased pre-inocubation humidity. *Hort. Science*, 7:556-557.
- Vasilakakis, M.D., Porlingis, I.C., 1984. Self-compatibility in Truito almond and the effect of temperature on selfed and crossed pollen tube growth. *HortScience*, 19: 659-661.
- Visser, T., 1955. Germination and storage of pollen. *Meded. Landbouwhoges., Wageningen*, 55:1-68.