

# IN VITRO POLLEN GRAIN GERMINATION AND STARCH CONTENT IN SPECIES WITH DIFFERENT REPRODUCTIVE CYCLE II. *MALUS DOMESTICA* Borkh. CULTIVARS STARKRIMSON AND GOLDEN DELICIOUS

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## SUMMARY

*Malus domestica* Borkh., which has a short flowering period, presents mature pollen grains with or without starch.

During in vitro germination starch is always synthesized before tube emergence and hydrolysed when tube growth has come to its end.

The differences in the two cultivars' behaviour between the two considered years are explained on the basis of environmental factors.

## 1. INTRODUCTION

This second note deals with differences among pollen grains from two cultivars of *Malus domestica* Borkh., collected in two subsequent years (1982, 1983).

Starch behaviour and germination were taken into account, on the basis of previous experiments (AVANZI et al. 1980, FORINO & AVANZI 1981, BELLANI et al. 1983). These experiments, in fact, pointed out that mature pollen grains of cultivar Starkrimson show a higher percentage lacking starch grains and a higher germination rate than cultivar Golden Delicious.

## 2. MATERIALS AND METHODS

Pollen grains of *Malus domestica* Borkh., cv Starkrimson and cv Golden Delicious were gathered from plants growing at the Agricultural Experimental Farm of Bologna University. Pollen was collected in the two subsequent years 1982 and 1983.

- a) Viability, starch content of pollen and statistical analysis were accomplished as in note I (BELLANI et al. 1985).
- b) Germination was tested by the same medium of note I, tubes measured every hour until 24 hours on a sample of 500 grains. No influence seems to come from the sucrose in the medium; in fact no differences were detectable among

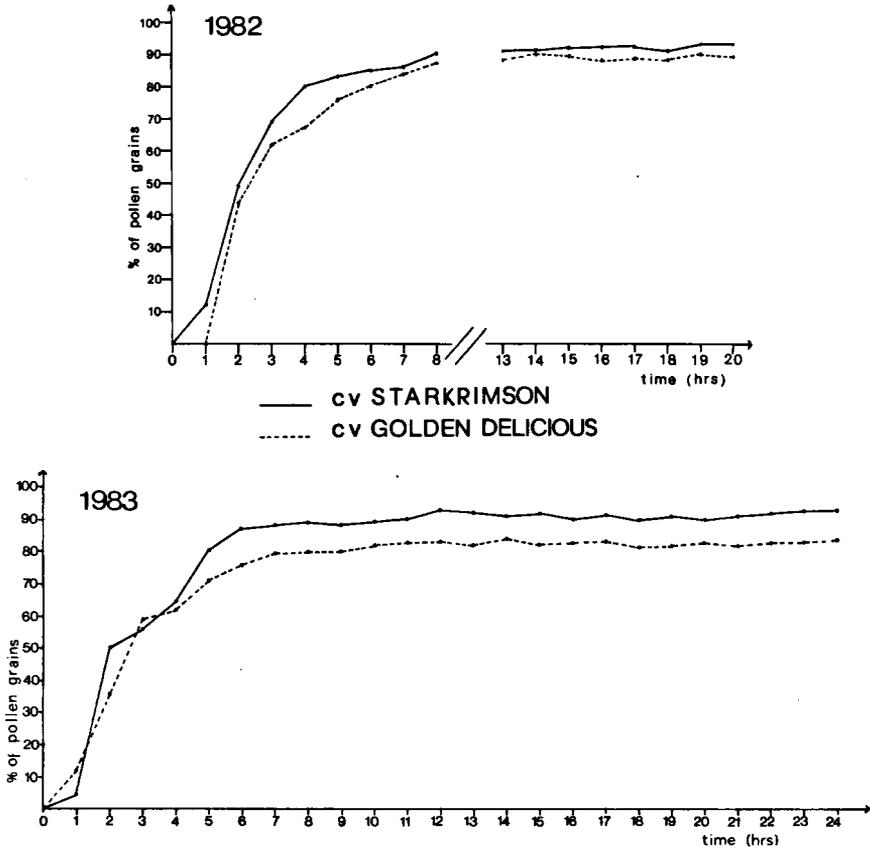


Fig. 1. *Malus domestica* Borkh. cultivars Starkrimson and Golden Delicious pollen grains. Germination percentage in two subsequent years. a) 1982, b) 1983.

three sucrose concentrations (5%, 10%, 15%) for this type of pollen (BELLANI unpublished) so that the middle concentration (10%) was chosen for this experiment.

Tests were effected few days after collecting the pollen, which was stored according to FIDIGHELLI (1968).

### 3. RESULTS

Cv Starkrimson pollen grains always show a higher percentage of viable grains than cv Golden Delicious with both methods used.

*Fig. 1a* shows the germination percentage of the first year. Cv Starkrimson pollen grains start earlier to germinate within the first hour and always keep a higher percentage of germinated grains. In the second year (*fig. 1b*) both culti-

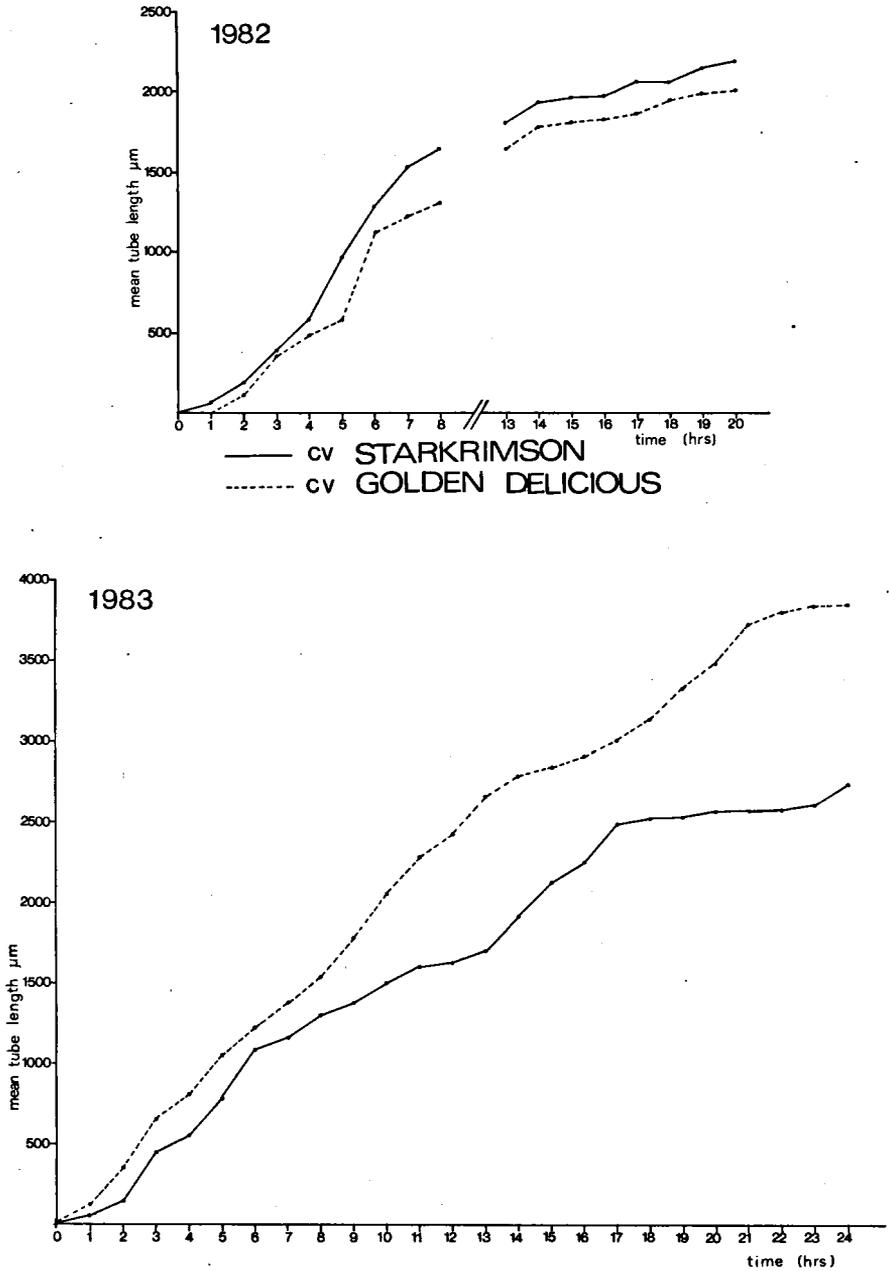
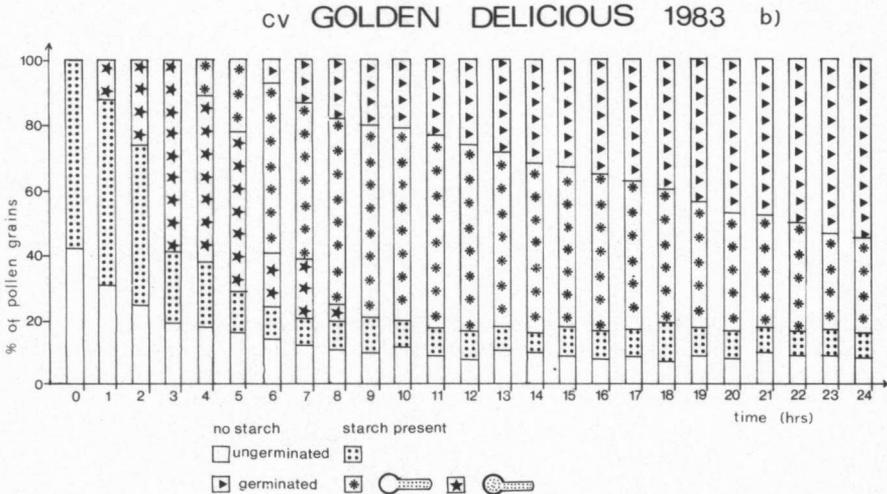
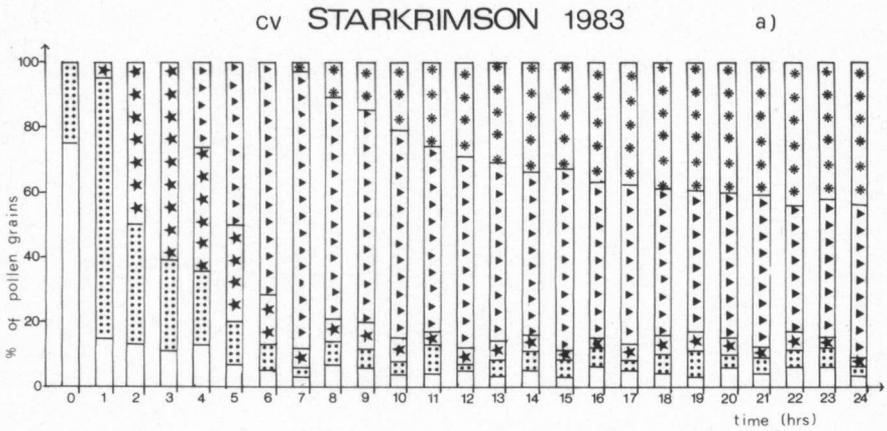
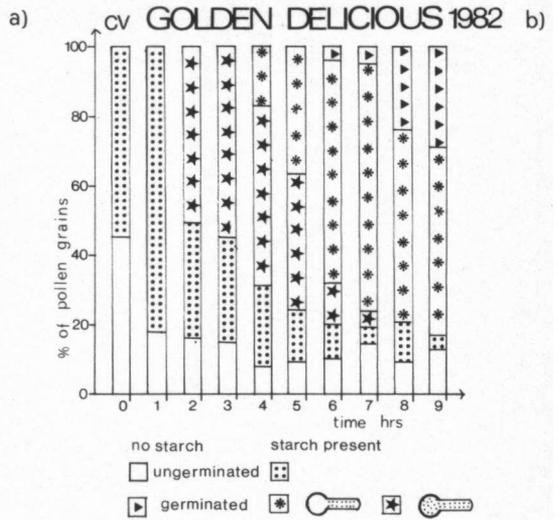
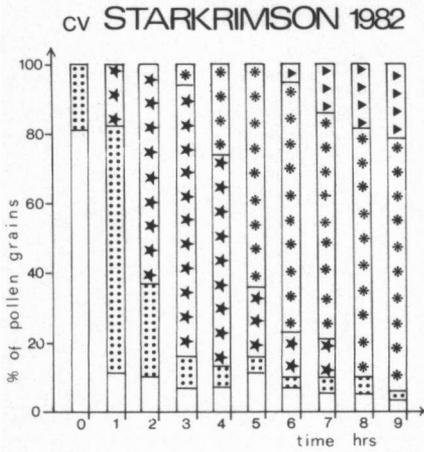


Fig. 2. *Malus domestica* Borkh. pollen grains: mean tube length in two subsequent years.  
 a) 1982. Cultivar Golden Delicious: standard error ranging from 5.0 to 6.7; cultivar Starkrimson: S.E. 6.0 to 8.8.  
 b) 1983. Cultivar Golden Delicious: S.E. 7.0 to 9.2; Cultivar Starkrimson: S.E. 6.3 to 9.3.



vars have started germination at the first hour, cv Golden Delicious having a higher percentage of germinated grains. For the following period, except the third hour, cv Starkrimson has a strong increase and always keeps a higher rate of germination.

*Figs. 2a and 2b* show the mean tube length in the two following years tested at a sucrose concentration of 10%. In the first year cv Starkrimson pollen grains always show a greater mean length for all the period considered. The second year cv Starkrimson grains follow almost the same pattern of growth and reach the same mean maximum length (about 2200  $\mu\text{m}$ ). Cv Golden Delicious grains, instead, have a far stronger growth, the mean length being always higher than that of cultivar "Starkrimson" and reaching the maximum mean length of nearly 4000  $\mu\text{m}$ , i.e. almost twice that of the previous year (nearly 2000  $\mu\text{m}$ ).

The test for starch content evidenced that in both years cv Starkrimson pollen grains show a little percentage of grains with starch, 19% in 1982; 25% in 1983 (*figs. 3a, 4a*). Starch however is soon synthesized within the first hour and no grain without starch was seen to germinate. During tube growth amyloplasts leave the grain cytoplasm and move into the tube, always towards the tip (BELLANI et al. 1983). After 7 hours the class of grains where starch has been digested is always increasing.

In cv Golden Delicious (*figs. 3b, 4b*) starch was present in a greater percentage of mature grains, 55% in 1982 and 58% in 1983. Apart from this initial difference the pattern of movement into the tube and of starch digestion is very much similar to the one described for cv Starkrimson.

#### 4. DISCUSSION

As to starch presence in mature pollen grains of the two cultivars considered, its amount is very variable in the single grain, but this variation has not been taken into account in the present study, because of the asynchrony of pollen ripening after meiosis (HESLOP-HARRISON 1968; FRANCHI & PACINI 1980). On the contrary, little difference is detectable, within each cultivar, in the percentage of pollen grains with starch in the cytoplasm, in the two years considered.

During the first hour in the germination medium cv Starkrimson pollen grains have a massive starch synthesis, and reach the percentage of grains with starch present in cv. Golden Delicious.

ROSEN et al. (1964) have already demonstrated that mature, not germinated pollen grain cytoplasm of *Lilium longiflorum* lacks starch, which is however demonstrable few minutes after the pollen has been placed in a germination medium. This rapid starch formation coincides with an initial period of high respiration (DICKINSON 1967; 1968), but no explanation has been given up to now to this starch synthesis.

*Figs. 3-4. Malus domestica* Borkh. cultivar Starkrimson and Golden Delicious pollen grains: histograms of starch presence in germinated and ungerminated grains. Dots mean starch present. 3) 1982, 4) 1983.

Cv Starkrimson pollen grains show a steady behaviour in the two years for the starting time of germination as well as for the maximum tube length reached, indicating also a faster metabolism than cv Golden Delicious pollen grains. In the second year, instead, "Golden Delicious" pollen grains start earlier to germinate than the first year and furthermore reach a tube length twice that of the first year.

It is known (ROSEN 1970) that growth *in vitro* is largely autotrophic, with new growth at the tip deriving from stored material, which is transformed and transferred to the wall via vesicles. This seems true at least for the first phase (LAFLEUR & MASCARENHAS 1978; MULCAHY & MULCAHY 1983). When the pollen tube growth switches to the second heterotrophic phase, substances from the medium are utilized (NAKAMURA 1978; MALIK *et al.* 1982). This switch occurs at the third hour at least for the *Malus domestica* pollen (TAGLIASACCHI, personal communication).

Moreover, the bulk of reserve material is imported into pollen during the maturation processes (LINSKENS 1973) and environmental factors, such as temperature, influence substrate metabolism in maturing anthers (KIYOSAWA 1962).

Pollen grains of cv Golden Delicious are reported to be more sensitive in respect to germination than grains of cv Starkrimson (CALZONI *et al.* 1979).

It seems however, difficult to explain on the basis of the considerations reported above why cv Golden Delicious pollen grains have a far longer tube in the second than in the first year. We can only say that, because in certain deciduous plants as *Malus domestica* the floral induction occurs the summer before the flowering, the influence of environmental factors on flower and pollen maturation could have occurred at that moment as well as during anthers ripening.

Viability, instead, does not appear influenced by environmental factors during pollen ripening, in agreement with previously reported results (CALZONI *et al.* 1979).

These two joint papers offer evidence that the reproductive cycle has a general pattern but that is, however, influenced by environmental factors, in different ways for plants with a continuous and long flowering period (*Lycopersicum peruvianum*), with many reproductive cycles in the same year (each cycle lasting from 32 to 36 days, PACINI & SARFATTI 1978) and for plants with a short flowering period (*Malus domestica*) and only one reproductive cycle every year.

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