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FLOWERING, POLLINATION AND FERTILIZATION IN VITIS

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Introduction

Although methods of modern biotechnology may produce new strategies of breeding procedures, conventional methods of hybridization will still be practiced by grape breeders in coming years. Therefore efforts should be made to obtain a thorough knowledge of all factors which deal with hybridization.

As a matter of fact, breeders engaged with hybridization of grape vines know how to make crossings. However do they know all steps of development of flowers, all steps of pollination, fertilization and embryo growth and their dependance on environmental conditions?

During the last few years, we did some experimental work in these fields from which we think that some results may be of interest for those who are engaged with grape vine breeding.

Results

Flowering and emasculation

During two years, flowering has been investigated in the

cultivars Müller-Thurgau and Blauer Spätburgunder. Ten inflorescences each, belonging to the same stage of development were selected, and the time of blooming of all flowers was recorded. From 5 a.m until 8 p.m. opened flowers were recorded, every 30 minutes, removed and put into a fixing solution immediately.

In both years about 95% of the flowers opened within 5 days. The remaining flowers began blooming over the following 6 days (Fig. 1). These flowers can certainly be neglected in further considerations because their opening was delayed due to abnormal conditions.

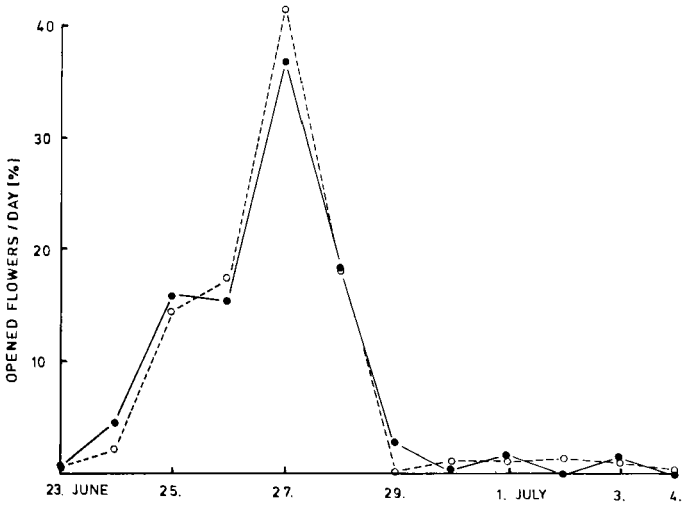

Fig. 1 - Opening of the flowers per inflorescence of Vitis vinifera cv. Müller-Thurgau (-) and cv. Blauer Spätburgunder (0-0) 1975.

Therefore, within one inflorescence the age of the single flowers differs up to 5 days.

All flowers fixed at the time of their opening were investigated for pollen germination and pollen tube growth. In both years a certain number of flowers had been pollinated before opening. Grape vines, therefore, can be called facultative cleistogamous.

From former investigations it is known that pollen germination and pollen tube growth depends on temperature (Staudt, 1981, 1982). Therefore, it was possible to calculate the probable time of pollination using the length of the pollen tubes and the corresponding temperature. Most flowers, pollinated before opening, were probably pollinated up to 4 hours before. But some must even have been pollinated up to 24 hours before opening.

There was a considerable difference in the extent of cleistogamy between the two cultivars, i.e. 60 * 1975 and 63% 1976 in Müller-Thurgau and 18% 1975 and 16% 1976 in Blauer Spätburgunder. This phenomenon and the fact of corresponding rates of cleistogamy between the years hint at a genetical basis of this character.

To prevent any self-pollination emasculation should be carried out at least one day before the first flower of an inflorescence comes to bloom.

Longevity of pollen grains

At a room temperature of about 22° C, pollen can be stored for 3 days without loss of viability. Within 30 days the original viability of 70% decreased close to 0 (Fig. 2). Viability was always proved by germination tests.

Pollen stored in an dessicator containing $CaCl_2$ at -5° C retained their original viability for 6 months. During the following 6 month it decreased to 50%.

In a long term experiment, pollen was stored in a dessicator containing $CaCl_2$ at -22° C. Germination capacity showed after

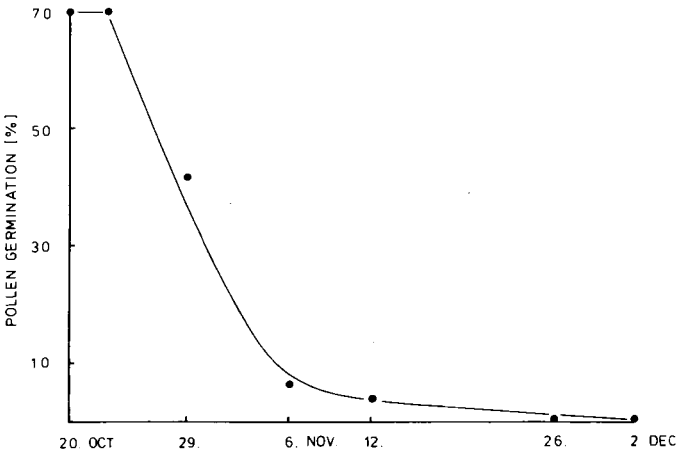

Fig. 2 - Course of germination capacity of pollen of Vitis vinifera stored at room temperature (ca. 22° C).

1 year continuous reduction. After 4 years, germination of 3,6% could still be observed. In this case the original germination capacity had been only 42%.

Viability of stored grape vine pollen is not only a question of the applied technique but also a question of the original viability and probably some other hitherto unknown causes.

For example, samples of pollen with the same original viability may not necessarily result in the same viability after a certain time under the same conditions of storage.

In areas near the northern borderline of vine growing, temperatures very often drop down near to 0° during the time of emasculation and pollination. It has therefore been investigated whether pollen germination and pollen tube growth are disturbed by low temperatures. Flowers were pollinated and subsequently kept up to 2 days under 2°, 5° and 10° C. To follow, they were brought back under normal conditions of 28° C. In all these experiments, pollen germinated after the cold treatment as normal and pollen tube growth was adequate (Tab. 1).

Longevity of stigmata and styles

Very often there is the question: within which time after emasculation should the flowes be pollinated. To solve this problem pistillate flowers of the same age, i.e. flowers which had opened on the same day, were pollinated 1, 3, 4, 5 and 6 days after opening. After 2 days respectively styles and stigmata were investigated for germinated pollen grains and pollen tube growth. In all flowers without those which were 5 and 6 days old when pollinated, pollen tube growth was found. Stigmata, therefore, retain their receptivity and can successfully ben pollinated up to the fourth day after opening.

When inflorescences were treated with different low temperatures immediately after pollination no pollen germination

Tab. 1 - Pollen tube growth after cold treatment for 48 h and subsequent growth at 28° C for 48 h

Treatment	Length of pollen tubes (µm) $\bar{x} \pm s$
2° C	2 502 ± 49
5° C	2 145 ± 143
10° C	2 265 ± 68
28° C (no cold treatment)	2 408 ± 41

could be observed after a treatment longer than 6 days with 5° C. In order to decide whether these low temperature conditions injure the pollen grains or the stigmata and styles, flowers from treatment after which no further pollen germination had been found, were pollinated for a second time and subsequently grown under 28° C. From the results of these experiments ist can be concluded that stigmata and styles may get over low temperatures down to 2° C for at least 2 days without any injury. Pollen germination and the ability of penetration into the style and pollen tube growth was normal if temperatures were raised subsequently to 28° C.

Longevity of egg cells

Under normal conditions, first symptoms of degeneration in egg cells of unpollinated flowers occurred from the third day after opening on (Kassemeyer and Staudt, 1981). This time corresponds with former results after which ovules of flowers grown under 10° C showed first signs of degeneration after about 4 days. Egg cells of grape vines therefore most probably must be fertilized within 2 days after opening of the flowers.

Pollination and fertilization

There are several notes in literature about grape vine flowers and their receptivity for pollen grains by producing droplets of secretion on their stigmata. From our investigations, especially those about successful pollination in unopened flowers, one can learn that receptivity does not necessarily depend on a visible droplet of secretion produced by the stigma.

The rate of pollen tube growth mainly depends on temperature. At a continuous temperature of 25° — 28° C, fertilization could be expected to take place from 12 hours after pollination on, at a temperature of 20° C after about 24 hours and at 15 ° C after about 48 hours. As a matter of fact, under in vitro conditions at 25° C, it has been found that fertilization occurred from 12 hours after pollination of (Roth and Staudt, unpublished). Under field conditions it has already been shown that fertilization occured 24 hours after opening of the flowers (Kassemeyer and Staudt, 1981, 1982).

LITERATURE CITED

1. Kassemeyer H. - H. and Staut G. (1981) - *Ober die Entwicklung des Embryosacks und die Befruchtung der Reben*. Vitis *20*, 202-210.
— 1982: Der Mitosezyklus der Zygotenkerne von Vitis vinifera. Ber. Deutsch. Bot. Ges. Bd. *95*, 449-455.
2. Staudt G. (1981) - *Die Abhängigkeit der Pollenkeimung und des Pollenschlauchwachstums von der Temperature bei Vitis rupestris in vitro*. Mitt. Klosterneuburg *31*, 233-230.
— 1982: *Pollenkeimung und Pollenschlauchwachstum in vivo bei Vitis und ie Abhängigkeit von der Temperatur*. Vitis *21*, 205-216.

SUMMARY

FLOWERING POLLINATION AND FERTILIZATION IN VITIS

From failures after crossings, which can be recognized by missing seed set or signs of self-pollination one may conclude that not all processes concerning emasculation and pollination are yet sufficiently known. In the course of our investigations about the flowering biology we made some observations which may be of use for all who are engaged in grape breeding.

1. Flowering and emasculation
Under normal weather conditions, average daily temperatures between 15-18° C, about 95% of the flowers of an iflorescence come to flower within 5 d. In a certain number of flowers anthers dehisce already before opening and germination of pollen grains and pollen tube growth may begin up to 24 h before that time.
In order to exclude any self-pollination emasculation must, therefore, precede the opening of the first flower of an inflorescence.

2. Storage of pollen
At room temperatures, 22° C, pollen can be stored without loss of viability for 3 d, at a temperature of -5 °C in an exsiccator with $CaCl_2$ for half a year and for one year at -21 °C.
After pollination pollen grains may survive temperature up to 25DC for at least two days without any injure of their ability of germination and pollen tube growth.

3. Viability of stigma and style
At normal temperatures the receptive stigma can successfully be pollinated up to the fourth day after blooming. Pollinated stigmata and the styles may survive temperatures up to 2° C for two days without any effect on germination of pollen grains, penetration of pollen tubes into the style and further growth.

4. Viability of egg cells
At normal temperatures first symptoms of degeneration occur in the egg cells of unpollinate flowers beginning with the third day after blooming. Considering ca. 24 hours for pollen tube growth, the stigmata, therefore, should be pollinated within two days after opening of the flowers.

5. Pollination and fertilization
The receptivity of the stigma does not necessarily depend upon a visible drop of secretion produced by the stigma. Egg cells are fertilized at normal temperatures within 24 hours after pollination. In inflorescences grown in vitro at permanent temperatures of 25° C the first fertilization could be observed 12 hours after pollination.