



Pollen Viability Related to Type of Pollination in Six Angiosperm Species

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Pollen viability was tested at various times during anther opening in three anemophilous (*Chamaerops humilis*, *Festuca arundinacea*, *Mercurialis annua*) and three entomophilous (*Acanthus mollis*, *Cucurbita pepo*, *Spartium junceum*) species. In certain species of both pollination types (*Cucurbita pepo*, *Festuca arundinacea*, *Mercurialis annua*), all pollen grains were dead 72 h after anthesis, and in others (*Spartium junceum*, *Acanthus mollis*, *Chamaerops humilis*) viability lasted longer. The results are discussed in terms of the reproductive ecophysiology of the species.

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INTRODUCTION

Anthers may open before or after the flower according to the sequence of male and female receptivity in a given species (Pacini, 1992). The pollen may leave the anthers as soon as they open or be held in the anther by devices such as pollenkitt, tryphine or elastoviscin (fluids of different viscosity and ontogenesis), viscin threads or sporopollenin filaments (exine extensions that tangle with the pollen). Pollenkitt, tryphine, elastoviscin and viscin threads are all tapetal products (Pacini and Franchi, 1993). In gymnosperms, Poaceae, Cupuliferae and Urticaceae, which have anemophilous pollination, the pollen is dispersed in individual grains because there is nothing to hold it together or to keep it in the anther. In entomophilous and some anemophilous angiosperms, the pollen is held in the anther until the arrival of a pollinator or wind sufficiently strong to blow it away (Lisci, Cardinali and Pacini, 1996). Irrespective of the type of pollination, pollenkitt, tryphine, elastoviscin and viscin threads have the task of forming pollen clumps or massulae which reach the stigma together if the distance to travel is short, or break up to some extent if the distance is long. In many anemophilous species the distance is short, as is the time elapsing between anther opening and pollination, because these plants often grow close together. In entomophilous species, the pollen must await the pollinator, and the possibility of compatible pollination depends on pollinator efficiency and on chance.

In a previous study we examined the effect of humidity stress on pollen viability in anemophilous and entomophilous species (Bassani, Pacini and Franchi, 1994). The aim of the present study is to determine whether pollen viability is related in some way to the type of pollination.

There are various methods of determining whether a pollen grain can emit a pollen tube and fertilize (Stone, Thomson and Dent-Acosta, 1995). The most direct is to see whether the pollen effectively induces the ovary to grow and a fruit to develop when placed on a stigma. Another is to see whether the pollen germinates when placed on a stigma. A third is to see whether the pollen grows *in vitro* (Shivanna and Johri, 1985). There are also indirect methods based on cytological parameters. One is the fluorochromatic reaction, which determines the state of the pollen plasma membrane (Heslop-Harrison and Heslop-Harrison, 1970; Shivanna and Johri, 1985). This method is used when viability has to be measured in many samples; it provides results that agree well with those obtained by germination *in vitro* (Heslop-Harrison, Heslop-Harrison and Shivanna, 1984).

MATERIALS AND METHODS

The pollen of four species of dicotyledons: *Spartium junceum* L. (Leguminosae), *Mercurialis annua* L. (Euphorbiaceae), *Cucurbita pepo* L. (Cucurbitaceae), *Acanthus mollis* L. (Acanthaceae), and two monocotyledons: *Festuca arundinacea* Schreber (Gramineae) and *Chamaerops humilis* L. (Arecaceae) growing in the Botanical Gardens of Siena University, was used. *S. junceum*, *C. pepo* and *A. mollis* are entomophilous, while the other species are anemophilous.

To determine the manner in which pollen viability decreases after anther opening, we first observed the time of day that the anthers opened and whether or not the pollen was dispersed immediately. For *Spartium junceum* (which has explosive anther dehiscence), *Mercurialis annua* (in which the pollen falls from the anthers about 6 h after anthesis in the absence of sufficient wind) (Lisci, Tanda and Pacini, 1994) and *Festuca arundinacea* (the pollen of which is released as the anther opens) (Bassani *et al.*, 1994), some

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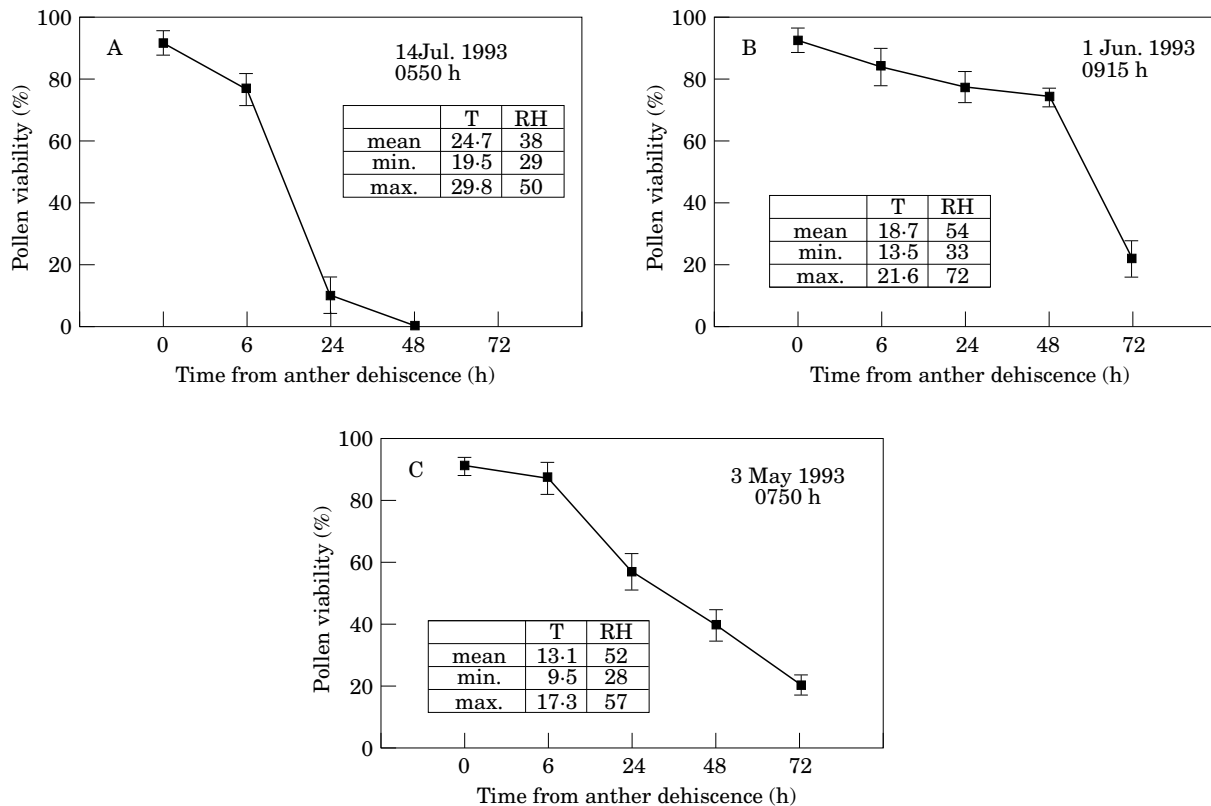


FIG. 1. Decline in pollen viability in three entomophilous species, *Cucurbita pepo* (A), *Spartium junceum* (B) and *Acanthus mollis* (C). In *Cucurbita pepo*, all pollen was dead 48 h after opening of the anther; in *Spartium junceum* and *Acanthus mollis* about 20% of the pollen was still viable 72 h after anther opening. The date of the experiment and the starting time (which was when the anthers opened) are indicated, together with the mean, maximum and minimum temperature and relative humidity (RH) measured during the experiment. Bars represent s.d.

pollen was collected on a microscope slide, covered with closely woven gauze and left near the plant until sampling. In the other plants, the pollen was left in the anther.

To avoid intraspecific differences, the pollen was always collected from the same plant. Plants with a pollen viability of at least 90% at anthesis were always chosen. Temperature and relative humidity (RH) were measured near the plant with a portable instrument; minimum, maximum and mean values are given in Figs 1 and 2. Five tests were performed simultaneously for each species, at the dates and times indicated in Figs 1 and 2. This was done to exclude the influence of changes in temperature and relative humidity between one test and another. It is impossible to measure changes in the viability of airborne pollen. Our system of keeping pollen on a slide in the open air (rather than in the laboratory) should, however, approximate the situation of airborne pollen.

Pollen viability was determined by the fluorochromatic reaction (FCR) (Shivanna and Johri, 1985) at the start of the experiment and at different times until a drastic reduction was observed. After each time lapse, pollen grains were collected from anthers or slides with an eyelash and dusted onto a slide for viability determinations. To avoid false negatives, we left pollen grains to rehydrate in a humid atmosphere before testing according to Shivanna, Linskens and Cresti (1991). Two hundred grains were monitored each time.

RESULTS AND DISCUSSION

In all species pollen viability decreased with time. All *Cucurbita pepo* pollen was dead after 48 h (Fig. 1A) and that of *Mercurialis annua* and *Festuca arundinacea* after 72 h (Fig. 2A and B). About 20% of the pollen grains of *Spartium junceum* and *Acanthus mollis* were still alive after 72 h (Fig. 1B and C); in *Chamaerops humilis* this figure was about 70% after the same period (Fig. 2C). The pattern of decline varied from species to species: in *Spartium junceum* (Fig. 1B) and *Festuca arundinacea* (Fig. 2B), there was a sharp decrease in viability between 48 and 72 h, in *Cucurbita pepo* (Fig. 1A) between 6 and 24 h, and in the other species the decline was gradual (Figs. 1C, 2A and C).

Two patterns of pollen viability were apparent in entomophilous species: a rapid decline as in *Cucurbita pepo*, the species with the most transient pollen viability, and a gradual decline as in *Spartium junceum* and *Acanthus mollis* in which there was still viable pollen 72 h after the opening of the anthers (Fig. 1). The rapid decrease in pollen viability in *Cucurbita pepo* is related to the fact that the flowers of both sexes of this monoecious plant are accessible to insects (bees and bumble bees) for only 6 h and the pollen is only partially dehydrated (Nepi and Pacini, 1993). After this period the flower closes and wilts. There is no adaptive advantage to be gained by the pollen remaining viable for longer than the pollinators have access to the flower. In the

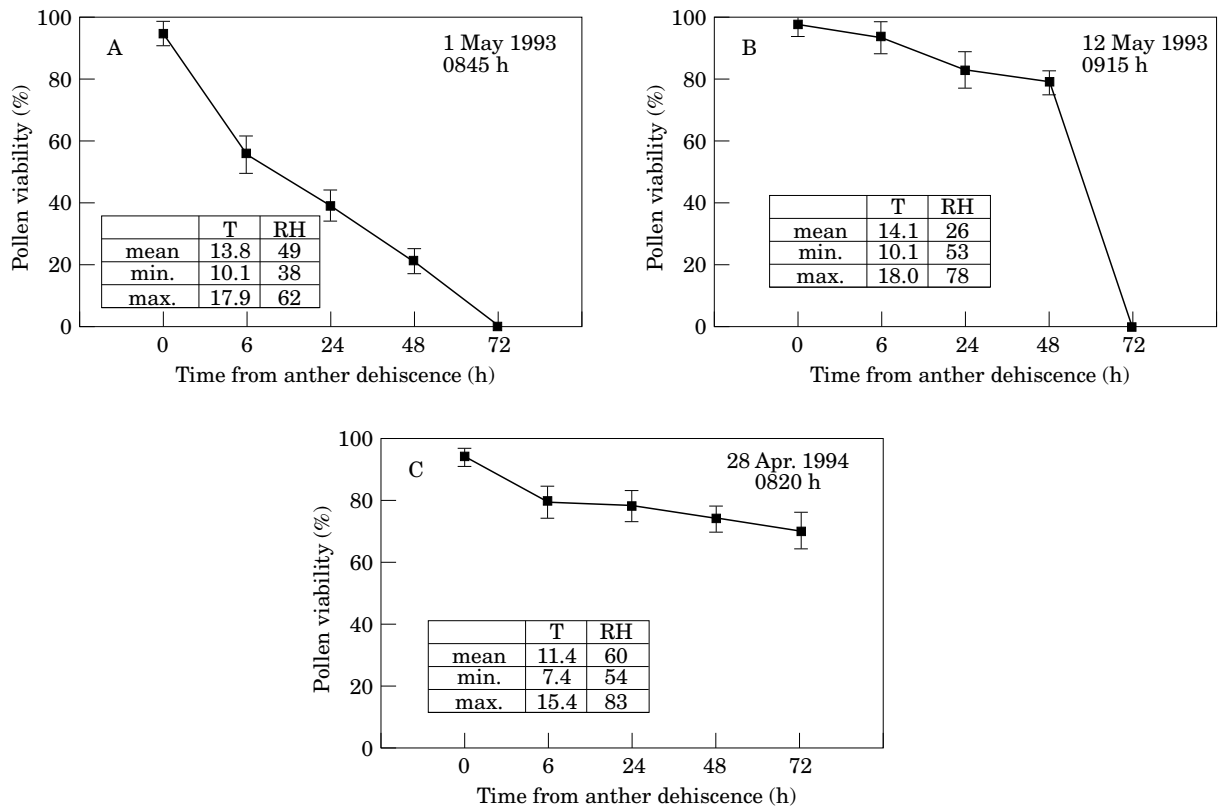


FIG. 2. Decline in pollen viability in three anemophilous species, *Mercurialis annua* (A), *Festuca arundinacea* (B) and *Chamaerops humilis* (C). In *Mercurialis annua* and *Festuca arundinacea*, all pollen was dead 72 h after opening of the anthers; in *Chamaerops humilis* more than 70% of the pollen was still viable more than 72 h after anther opening. The date of the experiment and the starting time (which was when the anthers opened) are indicated, together with the mean, maximum and minimum temperature and relative humidity (RH) measured during the experiment. Bars represent s.d.

other two entomophilous species, pollen survived for longer than in *Cucurbita pepo*. The explosive dehiscence of *Spartium junceum* anthers means that insects (bumble bees according to Faegri and van der Pijl, 1979) are dusted with pollen; the rest may be carried by the wind to a compatible stigma. The pollen of *Acanthus mollis* survives for a long period of time: it is protected inside the anthers at the mouth of the corolla tube. Insects, attracted by nectar, brush past the anthers and are dusted with pollen.

The pattern of pollen viability also varied among the anemophilous species. *Mercurialis annua* and *Festuca arundinacea* pollen was completely dead after 72 h, and the rates of viability decrease are different (Fig. 2). In fact, *Festuca arundinacea*, like most Poaceae, has pollen which is only partially dehydrated (Heslop-Harrison, 1979, 1982; Dumas *et al.*, 1983; Pacini, 1996) and a large percentage of it survives longer than that of *M. annua*, but between 48 and 72 h viability suddenly drops to zero. On the other hand *Mercurialis annua* pollen dehydrates faster, and the loss of viability is proportional to time. Both species are herbaceous and the fact that they grow crowded together may justify the fairly rapid decline in pollen viability: this close crowding and the consequent short pollen flight mean that pollen longevity is unimportant because there is little selection pressure. *Mercurialis annua* is dioecious, but its peculiar manner of seed dispersal favours seed germination in a

small area (Pacini, 1990a). The relationship of plants of the two sexes in a given area is dictated by chance.

The pollen of *Chamaerops humilis*, on the other hand, remained viable for an exceptionally long time. This is not surprising, because the pollen of this palm, like that of other palms, is resistant to thermal (Al-Helal, Basalah and Mohammed, 1988) and water stress (Bassani *et al.*, 1994). *Chamaerops humilis* is the only palm indigenous to Europe; it is dioecious and flowers between April and May when temperatures and relative humidity can be quite high in Mediterranean seaside environments.

The duration of pollen viability has been studied before (for a survey of the literature see Stanley and Linskens, 1974). Although pollen viability had not previously been correlated with the type of pollination, Ottaviano and Mulcahy (1989) observed that pollen must be programmed to have high viability in the habitat of the species. Previous studies correlated the decrease in viability with the stage of development of the male gametophyte, binucleate pollen generally surviving longer than trinucleate (Stanley and Linskens, 1974). Among the latter, however, at least in the Gramineae, some differences had been noted (Chaudhury and Shivanna, 1986). *Mercurialis annua*, one of the plants considered in the present study, flowers all year round high in the Mediterranean area, but the flowers and anthers open at different times of day, according to the season, and the

pollen adapts to some extent to changes in relative humidity (Lisci *et al.*, 1994).

Pollen develops in the locule and is nourished by locular fluid. This liquid is reabsorbed or evaporates, partially dehydrating the anther and causing it to open (Pacini, 1994; Keijzer *et al.*, 1995). In some species, however, it has been proposed that the mechanisms of anther opening are rather different (Keijzer 1987*a, b*; Bonner and Dickinson, 1989, 1990). The pollen grain withstands changes in volume due to variations in water content, relative humidity and temperature by virtue of harmomegathy (Pacini and Franchi, 1984; Pacini, 1990*b*; Bassani *et al.*, 1994; Lisci *et al.*, 1994).

Pollen resistance to dehydration is thought to be due to cytoplasmic disaccharides and oligosaccharides that act as membrane stabilizers (Hoekstra and Van Roekel, 1988; Hoekstra, Crowe and Crowe, 1989; Buitink *et al.*, 1996; Speranza, Calzoni and Pacini, 1997). *Cucurbita pepo*, the species with the shortest pollen viability of those considered here, has none of these carbohydrates, unlike *Mercurialis annua* (Franchi *et al.*, 1996; Speranza *et al.*, 1997).

Another facet of pollen dehydration/rehydration is plasma membrane structure; this aspect has only been investigated in angiosperms. Shivanna and Heslop-Harrison (1981) suggested that in the partly dehydrated grain at the time of dispersal the membranes are largely dissociated and do not form an osmotic barrier. Platt-Aloia *et al.* (1986) did not find any ultrastructural difference in membrane bilayer organization between dry and hydrated pollen grains of *Phoenix*, *Collomia* and *Zea*. Tiwari, Polito and Webster (1990) found multilamellate membranes in dry pear pollen and a normal unilamellate structure upon hydration. The question of ultrastructural and physiological changes in the plasma membranes and cytoplasm of pollen during dehydration and hydration is still much debated (Heslop-Harrison, 1979; Kerhoas, Gay and Dumas, 1987; Buitink *et al.*, 1996).

The loss of pollen viability in the different species has been correlated with water loss and maintenance of the dehydrated state both in nature and the laboratory (Heslop-Harrison and Heslop-Harrison, 1985; Gay, Kerhoas and Dumas, 1987; Linskens and Cresti, 1988; Nepi and Pacini, 1993; Lisci *et al.*, 1994). In extreme cases, water loss occurs at the same pace as loss of volume, leading to death, as in *Cucurbita pepo* (Nepi and Pacini, 1993), or the pollen can be stored for long periods at low temperature after controlled drying, as in the Gramineae (Barnabas and Rajki, 1981). There are exceptions, however, among the Gramineae: the pollen of *Pennisetum typhoides*, for example, can survive for 2 weeks under laboratory conditions (Chaudhury and Shivanna, 1986). In cases in which partial dehydration of the pollen facilitates its conservation, the mechanisms and rates at which rehydration occurs are important factors in determining the percentage of pollen germination (Shivanna *et al.*, 1991; Yates and Spark, 1993). In the same way, species sensitive to dehydration show an increased viability after controlled hydration (Shivanna and Heslop-Harrison, 1981). Rehydration at high humidity (RH 85–95%) increased the percentage of pollen grains of *Epilobium angustifolium* that germinated *in vitro* (Heslop-Harrison and

Heslop-Harrison, 1993). Heslop-Harrison and Heslop-Harrison (1992, 1993) showed that when *Hyacinthus orientalis* pollen was subjected to cycles of dehydration and hydration, the number of grains that germinated was markedly reduced and the cytoskeleton was damaged irreparably.

The results obtained by keeping pollen at, or near, its site of production were similar to the results of a previous study on pollen and humidity (Bassani *et al.*, 1994). In the species studied, we found short and long pollen viability both in anemophilous and entomophilous species. Within these two categories, however, we found that individual species may have pollen with longer or shorter viability depending on features such as life form, sex expression, number of individuals in a certain area etc. Although entomophilous species are generally considered to have longer pollen viability because the grains must wait for pollinators, *Cucurbita pepo* can be regarded as an exception because it has large pollen grains which do not dehydrate before dispersal. The fact that both male and female flowers are only accessible to insects for 6 h is a strategy to prevent insects from circulating with unviable pollen. In the same way, *Chamaerops humilis* is an exception among the anemophilous plants studied because it is dioecious with widely spaced plants. We now need to know how common these exceptions are, and whether they are linked to the type of environment in which the plant grows and the plant's respective cytophysiological adaptations.

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