

## Pollen hydration status at dispersal: cytophysiological features and strategies

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**Summary.** The aim of this paper is to draw attention to partially hydrated pollen, namely, pollen grains having a high water content (>30%); this type of pollen is more frequent than previously thought. Various cyto-physiological strategies are used to retain water during exposure and dispersal such as cytoplasm carbohydrates; in the absence of such strategies, fast pollination must be ensured, because uncontrolled loss of water leads to pollen death. On the other hand, a state of partial hydration allows a fast tube emission (even within 3–5 min). Several methods for determining the hydration status of pollen at anthesis are proposed.

**Keywords:** Carbohydrate reserve; Dehydration; Pollen tube emission; Pollen water content; Viability.

### Introduction

When the pollen is mature, the anther begins to dehydrate; this process involves the locular fluid, and in most cases also the pollen (Nepi and Pacini 1993, J. Heslop-Harrison et al. 1997). Partial dehydration causes a decrease in volume (Pyne 1981), in most cases with a change in pollen shape (Pacini 1990). Partially dehydrated pollen is in a “quiescent” state which is more fit to withstand the changes in environmental conditions that occur when the anther opens (Pacini 1990). Pollen volume may change during dispersal also, depending on the relative humidity (RH) of the environment (Lisci et al. 1994, Pacini et al. 1997). When pollen lands on a compatible stigma, it rehydrates and germinates (J. Heslop-Harrison 1987). The changes in pollen volume and shape during dehydration, dispersal, and rehydration are called harmo-

megathy (Wodehouse 1935) and cause mechanical stress that must be sustained by the pollen walls, plasma membrane, and protoplast (Blackmore and Barnes 1986).

The hydration status of pollen has been reported by many researchers, but its relationship with other factors has rarely been investigated. We refer to factors such as speed of pollen tube emission, germinability in time, pollen geometry, pollen wall structure, pore and furrow structure, and so forth (Nepi and Pacini 1993). In only a few instances, a slight dehydration was considered. For instance, J. Heslop-Harrison (1979: p. 737) reported that “the water content of pollen grains at the time of dispersal varies considerably among different families with most recorded values lying between 15–35% of fresh weight”. Grains instead which remain in a partly hydrated state, as it is the case of grass pollens, generally lack a dormancy period, remaining in a partly hydrated state in the anther after maturation (J. Heslop-Harrison et al. 1997); their cytoplasm shows organelles in active motion and an actin cytoskeleton still in the filamentous state, and devices for a rapid germination, such as wall precursor bodies (“P-particles”) in place for immediate tube tip growth, and even a callosic wall (J. Heslop-Harrison and Heslop-Harrison 1982, 1992; Nepi et al. 1995; J. Heslop-Harrison et al. 1997; Y. Heslop-Harrison 2000). On the other hand, some grains are reported to be strongly dehydrated as an adaptation to particular environments: for instance, the pollen of *Eucalyptus chlorantha* has a low water content and shows specific devices to resist heat stress well, maintaining a high viability in time (J. Heslop-Harrison and Heslop-Harrison 1985). Other researchers such as Gay et al.

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(1987) correlated the “absence” of dehydration with a rapid loss of viability.

Also Pyne (1981) reported that pollen of some species has a smaller percent reduction in volume (<10%) than that of others. Two species, *Cucurbita pepo* (Cucurbitaceae) and *Lavatera arborea* (Malvaceae), with pollen that is partially hydrated at dispersal, were recently reported (Nepi and Pacini 1993, 1999; Pacini 1996; Speranza et al. 1997). Besides reduced dehydration, the pollen of the two species shows many other morphological and functional similarities.

Since partially hydrated pollen may exist much more frequently than previously thought, the aim of the present study is to describe the phenomenon in its various aspects and consequences. For species having partially hydrated pollen, the most advantageous of these consequences is the brief interval between landing on a stigma and the production of a pollen tube; the main disadvantage is greater vulnerability to water loss. We also consider and compare ways of detecting reduced dehydration of pollen, with a view to develop standard methods to recognize nondehydrated or however partially hydrated pollen. Since in our experience, partially hydrated pollen shares characteristics such as large size and any number of pores but no furrows, in seeking further examples we favored species with pollen shown to have such characteristics in palynology atlases (Erdtman 1952, Kremp 1965) or according to personal observations. In this paper, we consider as “partially hydrated grains” those having a higher water content (over 30%, but in many cases even >50%), and as “partially dehydrated grains” those with a lower water percentage (<30%), having found that these groups behave differently.

## Material and methods

### Plant material

The pollen used for the various experiments was from plants growing spontaneously in the Siena area (Tuscany, central Italy) or cultivated in the botanical gardens of Siena University. Mature pollen was collected at anther dehiscence, sieved to remove any debris, and checked for viability. Only pollen with viability greater than 80% was used.

### Volume changes during pollen development

Anthers were squashed in acetic orcein to determine their developmental stage. Anthers at each stage of development were squashed in immersion oil and the diameter of the microspores measured with a micrometric ocular (the tetrads of the species exam-

ined were of the tetrahedral type and the form of each tetrad was roughly spherical).

For each developmental stage, five pollen samples were obtained from different flowers. They were placed on a microscope slide with a drop of immersion oil and covered with a cover slip. At least 100 pollen grains were measured in each sample.

### Dehydration status at anthesis

Pollen samples from newly open anthers were put on a slide and a drop of immersion oil was added to prevent exchange of water with the environment (Pacini 1990). The diameter of the pollen grains was measured with a micrometric ocular. In the case of elliptical pollen, we measured the longitudinal and equatorial axes. Pollen volume at anther dehiscence ( $V_{ad}$ ) was calculated. Another sample of pollen of newly open anthers was covered with a drop of water and immediately observed under the microscope. We measured the maximum diameter reached by the pollen and took the corresponding volume as complete hydration ( $V_{max}$ ). The difference ( $V_{max} - V_{ad}$ ) was taken to calculate the dehydration percentage.

For each species, three pollen samples for measurements in water and three for those in oil were obtained from flowers of different plants. 50 pollen grains were measured in each sample.

### Spontaneous changes in water content

In *Cucurbita pepo* pollen the water content was determined at different times after anther opening, using freshly dehiscent pollen. The change in water content was calculated from the percent weight loss of a sample of the same pollen kept at ambient temperature and RH ( $20 \pm 2^\circ\text{C}$ ,  $60\% \pm 15\%$  RH).

### Determination of mature pollen water content by drying

Pollen water content was determined from the difference between fresh and dry weight after drying to constant weight in an oven at  $105^\circ\text{C}$  for at least 48 h. It was expressed as a percentage of fresh pollen weight.

### Pollen viability

Pollen viability was tested by the fluorochromatic reaction described by J. Heslop-Harrison et al. (1984). 100 grains of each sample were counted.

### Time for pollen tube emission and germinability

The time required for germination was observed in vivo on the stigma and in vitro. Pollen was cultivated in vitro in Brewbaker medium (Brewbaker and Kwack 1964) with 15% sucrose. For each time interval, 100 pollen grains were scored. The pollen was regarded as germinated when the length of the pollen tube exceeded the pollen grain diameter.

### Sugar extraction and analysis

Sugars were extracted as described by Speranza et al. (1997) from dusty pollen, readily removed from anthers. In the case of sticky pollen, which remained attached to the anthers, a modification of the procedure was introduced. The anthers were gently squashed in 80% methanol in an Eppendorf tube and sonicated for 30 s. These treatments were repeated until the anthers were empty. The suspension was filtered and the grains ruptured in a Potter homogenizer. To obtain the weight of the pollen samples we weighed the empty tube and the tube after drying. The difference gives the

weight of the pollen sample. The dried material was resuspended in water and filtered (Sartorius, 0.2 µm diameter mesh).

Sucrose, D-glucose, and D-fructose concentrations (in µg/mg of pollen fresh weight) were determined in duplicate or triplicate with the Boehringer Mannheim test-combination kit nr. 716 260. The test is based on a UV method that measures the enzymatic reduction of NADH at 340 nm wavelength.

#### Polysaccharide histochemistry

Cytoplasmic starch and most polysaccharides can be detected by the periodic acid-Schiff stain (PAS) and Lugol tests as previously described (Franchi et al. 1996). Any callosic wall and/or cytoplasmic reserves of callose can be detected by fluorescence microscopy with aniline blue (O'Brien and McCully 1981), using whole pollen or sections.

## Results

### General pollen characteristics

Partially hydrated pollen has certain typical morphological characteristics and shares other features with partially dehydrated pollen. Typical properties are the generally spherical shape and the absence of furrows. Pollen such as that of the members of the family Poaceae, which has thin walls, loses water readily after anther opening and shrinks; other pollen, such as that of *Cucurbita pepo*, has thick walls and only decreases in volume (Nepi and Pacini 1993). Pores are not a distinctive character because some pollen is devoid of them (*Zantedeschia*), and some has one (Poaceae), three (*Urtica*, *Parietaria*), or many (*Cucurbita*, *Lavatera*, *Spinacia*).

The stage of maturation of the male gametophyte (two-celled and three-celled pollen) is another character which is not correlated with hydration status. Among partially hydrated pollen, there are species such as those of the Poaceae and *Spinacia oleracea* that are three-celled and species such as *Urtica pilulifera* and *Cucurbita pepo* that are two-celled; among partially dehydrated pollen there are likewise three-celled species such as *Helianthus annuus* and two-celled species such as *Chamaerops humilis* (Table 1).

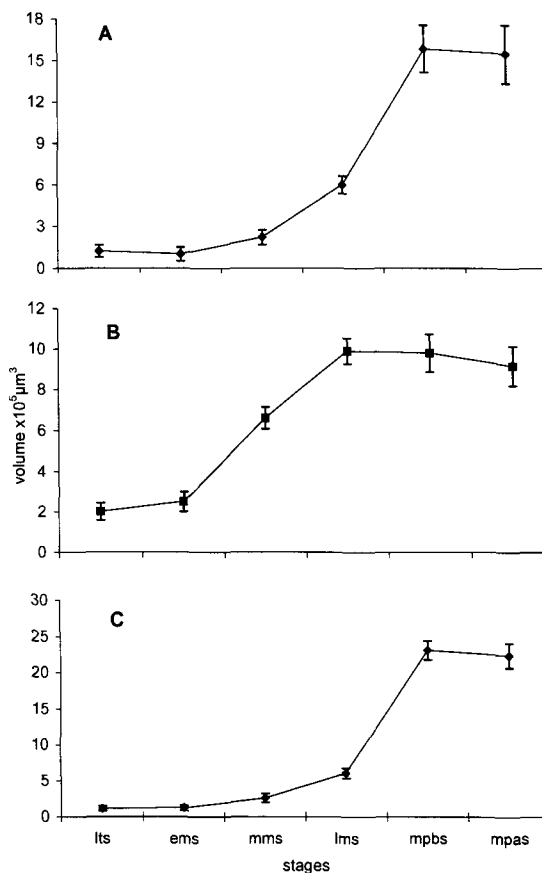
### Pollen growth during development and decrease in volume at anthesis

The increase in pollen volume was followed during development in three species with partially hydrated pollen: *Cucurbita pepo*, *Lavatera arborea*, and *Mirabilis jalapa* (Fig. 1). In *C. pepo*, observations were continued for 27 h after anthesis (Fig. 2), and viability and water content were also measured. Changes in

**Table 1.** Relative weight loss of desiccated pollen grains

Family and species	Pollen hydration status <sup>a</sup>	% Weight loss (mean ± SD) of pollen at 105 °C
Agavaceae		
<i>Dasyliiron acrotrichum</i> (Schiede) Zucc.	pd	1.7 ± 2.0
Amaryllidaceae		
<i>Zephyranthes candida</i> (Lindl.) Herb.	pd	5.4 ± 2.3
Araceae		
<i>Zantedeschia aethiopica</i> (L.) Spreng.	ph	58.8 ± 0.04
Asteraceae		
<i>Helianthus annuus</i> L. cv. Titan	pd	16.7 ± 0.4
<i>Helianthus tuberosus</i> L.	ph	43.7 ± 1.2
Bignoniaceae		
<i>Campsis radicans</i> (L.) Seem.	pd	8.3 ± 0.8
Cannabidaceae		
<i>Humulus japonicus</i> Sieb. & Zucc.	ph	65.5 ± 2.2
Convolvulaceae		
<i>Ipomoea purpurea</i> (L.) Roth.	ph	53.6 ± 7.1
Cucurbitaceae		
<i>Cucurbita moschata</i> (Duchesne ex Lam.) Duchesne ex Poir. cv. d'Albenga	ph	50.4 ± 0.8
Cucurbitaceae		
<i>Cucurbita pepo</i> L. cv. Greyzini F1	ph	44.6 ± 0.9
<i>Thladiantha dubia</i> Bunge	pd	7.9 ± 5.3
Euphorbiaceae		
<i>Ricinus communis</i> L.	pd	11.0 ± 3.8
Leguminosae		
(Caesalpinioideae)		
<i>Bauhinia forficata</i> Link.	ph	40.1 ± 18.1
Malvaceae		
<i>Hibiscus rosa-sinensis</i> L.	ph	57.4 ± 2.2
Myrtaceae		
<i>Feijoa sellowiana</i> O. Berg.	pd	9.9 ± 1.8
Onagraceae		
<i>Oenothera organensis</i> Munz.	ph	22.7 ± 2.1
Palmae		
<i>Chamaerops humilis</i> L.	pd	15.3 ± 0.9
<i>Trachycarpus fortunei</i> (Hook.) H. A. Wendl.	pd	11.9 ± 0.4
Papaveraceae		
<i>Papaver orientale</i> L.	ph	47.4 ± 2.0
Passifloraceae		
<i>Passiflora caerulea</i> L.	ph	46.3 ± 11.0
Poaceae		
<i>Coix lachryma jobi</i> L.	ph	31.3 ± 1.2
<i>Euchlaena mexicana</i> Schrad.	ph	32.2 ± 0.8
<i>Zea mays</i> L. cv. Majeur F1	ph	35.6 ± 0.2
Urticaceae		
<i>Urtica pilulifera</i> L.	ph	33.3 ± 5.5
Zingiberaceae		
<i>Hedychium coccineum</i> Sm.	ph	65.6 ± 4.2

<sup>a</sup> pd, partially dehydrated; ph, partially hydrated



**Fig. 1.** Pollen volume variations during development and at shedding in three species. **A** *C. pepo*, **B** *L. arborea*, **C** *M. jalapa*. The pattern is similar in *C. pepo* and *M. jalapa*, while in *L. arborea* the volume decrease begins earlier. *lts* Late-tetrad stage; *ems* early-microspore stage; *mms* mid-microspore stage; *lms* late-microspore stage; *mpbs* mature pollen before shedding; *mpas* mature pollen at shedding

volume after anther opening were followed up to 84 h in *L. arborea* (Fig. 3). Pollen volume at anthesis and at maximum hydration was measured in many other species (Table 2).

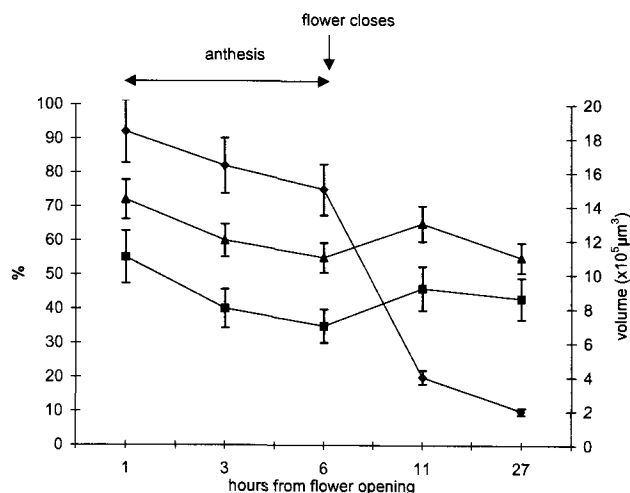
In *C. pepo*, the pollen volume increased constantly from early-microspore stage until anther opening. In *L. arborea* it increased until the first haploid mitosis and decreased slightly until maturity (Fig. 1). In *M. jalapa* the pattern is similar to *C. pepo* (Fig. 1).

In *C. pepo*, the pollen volume decreased constantly during anthesis (Fig. 2). 11 h after corolla closing the volume increased but decreased again in the hours that followed. The change in volume was closely correlated with water content; viability, which decreased gradually during exposure of the pollen, underwent a sharp decrease after the flower closed (Fig. 2). In *L. arborea*, the pollen volume decreased constantly, especially in the first 48 h (Fig. 3).

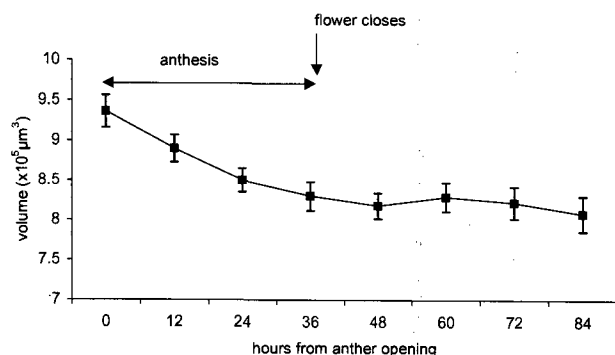
**Table 2.** Pollen volume and relative increase in volume of grains attaining the maximum hydration

Family and species	Pollen hydration status <sup>a</sup>	Pollen volume	
		at anthesis ( $10^3 \mu\text{m}^3$ )	% increase at max. hydration
Agavaceae			
<i>Dasyllirion acrotrichum</i> (Schiede) Zucc.	pd	$4.4 \pm 1.2$	$72.3 \pm 20.4$
Amaryllidaceae			
<i>Crinum zeylanicum</i> L.	pd	$88 \pm 9.8$	$67.2 \pm 18.2$
Cactaceae			
<i>Opuntia dillenii</i> Miller	ph	$877 \pm 41$	$35.5 \pm 7.3$
Caryophyllaceae			
<i>Silene dioica</i> (L.) Clairv.	ph	$31.7 \pm 4.2$	$19.9 \pm 3.1$
Chenopodiaceae			
<i>Spinacia oleracea</i> L.	ph	$37.9 \pm 4.3$	$34.2 \pm 4.2$
Cistaceae			
<i>Cistus incanus</i> L.	pd	$31.2 \pm 5.2$	$62.1 \pm 21.1$
Convolvulaceae			
<i>Ipomoea purpurea</i> (L.) Roth.	ph	$972.2 \pm 29$	$7.51 \pm 2.1$
Cruciferae			
<i>Capsella bursa pastoris</i> Moench	pd	$2.4 \pm 0.6$	$63.9 \pm 19.7$
<i>Diplotaxis eruroides</i> (L.) DC.	pd	$7.6 \pm 0.9$	$58.4 \pm 16.5$
<i>Isatis tinctoria</i> L.	pd	$4.8 \pm 1.8$	$74.9 \pm 14.8$
Cucurbitaceae			
<i>Cucumis melo</i> L. cv. Bush Star	ph	$65.4 \pm 8.9$	$58.6 \pm 18.3$
<i>Cucurbita pepo</i> L. cv. Greyzini F1	ph	$1465 \pm 55.1$	$28.9 \pm 5.4$
<i>Thladiantha dubia</i> Bunge	pd	$40.8 \pm 5.6$	$56.5 \pm 14.5$
Iridaceae			
<i>Iris triflora</i> Balbis	pd	$605 \pm 32.4$	$75 \pm 17.1$
Labiatae			
<i>Rosmarinus officinalis</i> L.	pd	$27.7 \pm 4.3$	$52.2 \pm 13.4$
Malvaceae			
<i>Althaea officinalis</i> L.	ph	$303.7 \pm 19.1$	$34.2 \pm 3.8$
<i>Hibiscus rosa-sinensis</i> L.	ph	$2424 \pm 98.2$	$29.8 \pm 6.7$
<i>Lavatera arborea</i> L.	ph	$936.4 \pm 33.3$	$38 \pm 5.7$
Nyctaginaceae			
<i>Mirabilis jalapa</i> L.	ph	$2247 \pm 107$	$34.8 \pm 5.3$
Palmae			
<i>Chamaerops humilis</i> L.	pd	$2.04 \pm 0.6$	$75 \pm 11.3$
Papaveraceae			
<i>Chelidonium majus</i> L.	pd	$7.5 \pm 1.3$	$72.8 \pm 13.4$
Poaceae			
<i>Zea mays</i> L.	ph	$287.4 \pm 21.3$	$50.1 \pm 11.6$
Portulacaceae			
<i>Portulaca oleracea</i> L.	pd	$195.9 \pm 18.6$	$20.1 \pm 6.2$
Ranunculaceae			
<i>Helleborus viridis</i> L.	pd	$25.4 \pm 3.8$	$61.2 \pm 13.4$
<i>Ranunculus ficaria</i> L.	pd	$13.6 \pm 1.3$	$57.8 \pm 16.4$
Rutaceae			
<i>Ruta chalepensis</i> L.	pd	$8.49 \pm 0.9$	$69.24 \pm 16.3$

<sup>a</sup> pd, partially dehydrated; ph, partially hydrated



**Fig. 2.** Pollen viability (%), water content (%), and volume ( $\Delta$ ) in *C. pepo* during and after anthesis. Pollen viability decreases slightly during anthesis and dramatically after flower closing. Water content and pollen volume have the same pattern, they decrease during anthesis, and there is a peak after flower closing, followed by a further slight decrease

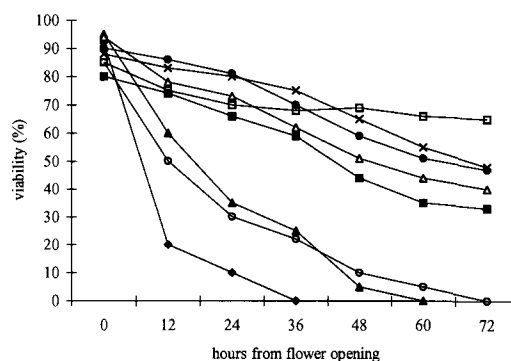


**Fig. 3.** Pollen volume variations in *L. arborea* during and after anthesis. The volume decreases during anthesis, and slight variations follow flower closing

The pollen volume measured at anthesis varied widely, ranging from  $4.4 \times 10^3$  to  $2.4 \times 10^6 \mu\text{m}^3$  (Table 2). With few exceptions, higher values belonged to partially hydrated pollen. These species showed the smallest increase in volume at complete hydration (Table 1).

#### Time courses of viability and germinability decrease and germination times

Pollen viability was around 92% in newly open flowers of *C. pepo* and about 75% when the flower closed (Fig. 2). It decreased rapidly in the afternoon, dropping to about 10% the next day (Fig. 2). In *L. arborea*, pollen viability was 88% just after anther opening and



**Fig. 4.** Patterns of viability loss in eight species with partially hydrated pollen grains, maintained in their environment, after anthesis.  $\blacklozenge$  *C. pepo*,  $\circ$  *Oenothera organensis*,  $\blacktriangle$  *Spinacia oleracea*,  $\blacksquare$  *Alcea rosea*,  $\triangle$  *M. jalapa*,  $\bullet$  *Althea officinalis*,  $\times$  *L. arborea*,  $\square$  *Opuntia dillenii*. In *C. pepo* all grains are dead after 36 h, while in *Opuntia dillenii* almost 70% are still viable at the same time

decreased constantly during and after anthesis, but was still as high as 50% 72 h after anther opening (Fig. 4). Pollen viability in other species with slight dehydration revealed different patterns (Fig. 4). In a first group (*C. pepo*, *Spinacia oleracea*, and *Oenothera organensis*), all pollen was dead within 72 h, viability plunging in the first hours. In a second group (*L. arborea*, *Althea officinalis*, *M. jalapa*, and *Alcea rosea*), viability decreased steadily to 40–60% after 72 h. A third pattern was that of *Opuntia dillenii* pollen, whose viability showed a small initial decrease, after which it remained almost constant, remaining as high as 70% at 72 h.

Germinability showed the same pattern as viability, determined by fluorochromatic reaction, but lower percentage values.

In *C. pepo*, germination occurs 3–5 min after pollination in vitro or in vivo (Nepi and Pacini 1993). In *L. arborea* (Nepi and Pacini 1999) germination in vivo takes 3–5 min and in vitro 15–30 min (Table 3). A grain of *L. arborea* pollen emits in vivo as well as in vitro 10–20 pollen tubes from a certain part of its surface (Nepi and Pacini 1999).

Fast germination seems to be a constant property of partially hydrated pollen. Table 3 shows some examples of germination times in vivo, obtained from literature and original observations. *Parietaria judaica* is an exception because, besides having small pollen grains, it takes about 2 h to germinate. Germination time is much longer in partially dehydrated pollen, depending, among other things, on whether the stigma is wet or dry. Typical times range from 20–30 min to 3 days, as in the case of the orchids *Oncidium* spp. See Owens (1992) for examples.

### Type of carbohydrate reserves

Pollen may contain the following types of carbohydrates: starch or similar polysaccharides (Franchi et al. 1996) in amyloplasts; PAS-positive cytoplasmic polysaccharides in small vesicles; callose in small vesicles; and sucrose, glucose, and fructose in the cytosol and/or membranes. Not all these types of carbohydrates are always present, nor are their proportions constant (Table 4 and Fig. 5). For example, the relative values of soluble sugars in 13 species, all with partially hydrated pollen, are shown in Fig. 5; the percentages and relative proportions of glucose, fructose, and sucrose differ widely.

The type of carbohydrate reserves in pollen does not seem to be related to hydration status. For example, *L. arborea* and *Hibiscus rosa-sinensis* have PAS-positive cytoplasmic vesicles like all partially dehydrated pollen (Table 4). Cytoplasmic vesicles containing callose are only found in a few species, such as *C. pepo* and *L. arborea*; all, however, have partially hydrated pollen and fast germination in vivo (Table 3). On the other hand,

pollen of *Parietaria judaica* has neither callosic walls nor cytoplasmic reserves of callose and therefore takes longer to germinate, although it is only partially hydrated (Tables 3 and 4).

### Spontaneous loss of water

Most partially hydrated pollen lacks mechanisms for retaining water. If it does not reach a stigma quickly, the water loss may be considerable if RH is low, such as on a normal sunny day. In *C. pepo*, the water content closely reflects the pollen volume, decreasing during anthesis and increasing in the first 5 h after anthesis (Fig. 2). At anther dehiscence, the water content is about 55%, dropping to 35% at the end of anthesis (Fig. 2).

### Determination of water content of mature pollen by desiccation

Spontaneous water loss can be determined by repeatedly weighing the pollen until constant weight is reached. Alternatively, total water content can be measured by drying the pollen in an oven. Some values are reported in Table 1. Partially hydrated pollen shows a greater water loss. A weight loss of 25–30% marks the limit between partially hydrated and partially dehydrated pollen. In *Oenothera organensis*, the weight loss is apparently less, probably due to the weight of the exine which has viscin threads.

## Discussion

### Cytological properties of fully or partially hydrated pollen

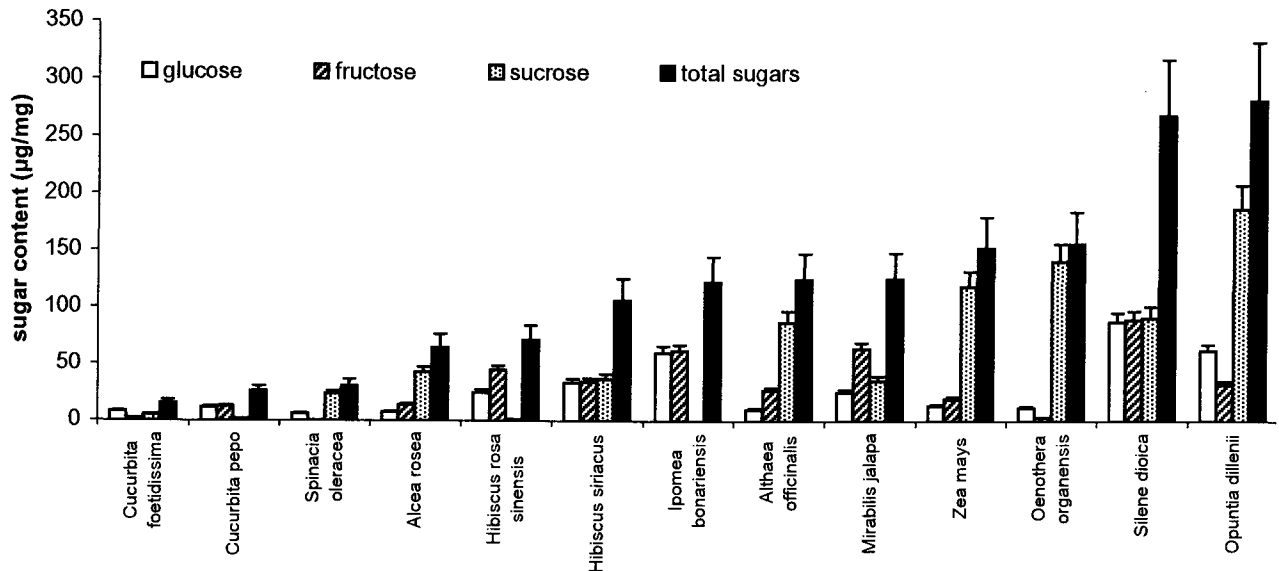
The pollen wall structure indicates at first sight whether the pollen dehydrates, without the need for any measurements. When the exine and intine are thin with respect to pollen diameter, as in the members of

**Table 3.** Time for pollen tube emission in several species with partially hydrated pollen grains at dispersal

Species	Time for in vivo pollen germination (min)	Source
<i>Cucurbita pepo</i>	3–5	Nepi and Pacini 1993
<i>Hibiscus rosa-sinensis</i>	10–15	our observations
<i>Lavatera arborea</i>	3–5	Nepi and Pacini 1999
<i>Mirabilis jalapa</i>	<30	Niesenbaum and Schueller 1997
<i>Oenothera organensis</i>	<40	Dickinson and Lawson 1975
<i>Parietaria judaica</i>	>120	our observations
<i>Portulaca oleracea</i>	7–10	our observations
<i>Silene vulgaris</i>	10	Heslop-Harrison 1987
<i>Sorghum bicolor</i>	5	Lansac et al. 1994
<i>Spinacia oleracea</i>	<30	our observations

**Table 4.** Reserve carbohydrates in species with partially hydrated pollen

Species	Starch	Cytoplasm		Callosic cytoplasmic reserves	Callosic wall
		PAS-pos.	PAS-neg.		
<i>Cucurbita pepo</i>	+		+	+	+
<i>Hibiscus rosa-sinensis</i>	+	+		+	+
<i>Lavatera arborea</i>	+	+		+	+
<i>Parietaria judaica</i>	+	+ (weakly)		–	–
<i>Spinacia oleracea</i>	+		+	+	+



**Fig. 5.** Histogram of glucose, fructose, sucrose, and total sugar content (means with standard deviations) in grains of 13 species with partially hydrated pollen. The amounts vary widely: contents are not correlated to dehydration extent but rather to viability preservation in time (cf. Fig. 4)

the family Poaceae, the pollen grain deforms as it loses water, because the walls do not adapt to the decrease in volume of the cytoplasm. On the other hand, when the exine is thick, as in *C. pepo* and some members of the Asteraceae, water loss does not lead to an evident deformation of the pollen, and it is better to detect dehydration by other parameters, for example, loss of weight or volume.

Most pollen of these types are large (80–200 µm in diameter), the only exceptions being those of *Parietaria judaica*, *Urtica pilulifera* and *Spinacia oleracea*, which are much smaller (<30 µm in diameter). Larger pollen can be regarded as favored, because greater water loss is possible without loss of viability; even limited water loss may be a significant percentage in small pollen, so that exposure and transport must be brief.

As far as the cytoplasm is concerned, the main differences between partially hydrated and dehydrated pollen, apart from water content, are the types of carbohydrate reserves. It is possible to distinguish species such as *C. pepo* that have a very high content of starch and a very low content of mono- and disaccharides, and species with a PAS-positive cytoplasm and variable quantities of cytoplasmic mono- and disaccharides. The disaccharide sucrose protects pollen membranes during dehydration and helps to maintain the partially dehydrated state (Hoekstra and Van Roekel 1988, Speranza et al. 1997).

#### Hydration status and germination

Before and after anther dehiscence, pollen volume and percentage of water are graded. The maximum quantity of water that partially hydrated pollen can absorb from the environment causes an increase in volume that goes from less than 10% to about 40% (Table 2) and is usually less than that of partially dehydrated pollen, in which the increase is between 50% and 75% (Table 2). There is a narrow overlap of values between these two types of pollen, but in these cases, attribution to one or the other category can be made on the basis of ecological or morphological parameters. The reasons for the different degree of dehydration in the two groups may depend on characteristics of individual species, the environment in which they grow, or reproductive strategies. For example, maize and melons have partially hydrated pollen in spite of a large capacity to absorb water, not only considering their different morphologies, in both cases without harmomegathic adaptability, but also considering that these species flower in a warm humid environment and that pollen is transported over a short distance, even if the types of pollination are different. Differences in the same family (e.g., Cucurbitaceae) or genus can also be explained in the same way. For example, *Helianthus annuus* flowers in midsummer, when the humidity may be low; its pollen is partially dehydrated and can therefore adapt to water loss imposed by environmental

conditions, whereas *H. tuberosus*, which flowers in autumn, when the weather is wet, has partially hydrated pollen because it has less need to prevent water loss or to survive dehydration.

The main advantage of partially hydrated pollen is its capacity to emit pollen tubes very quickly, i.e., within 1–15 min. Reduced dehydration enables the cytoskeleton to remain organized (active) and the time required for reorganization is saved (J. Heslop-Harrison et al. 1997). Activity is easily observed as cyclosis in the cytoplasm (J. Heslop-Harrison et al. 1997). Fast germination also depends on any callosic reserves in the pollen and the presence of a callosic wall. *Parietaria judaica* has neither and takes 2 h to germinate, the time needed to synthesize callose (our unpubl. data). In partially dehydrated pollen such as *Lycopersicon peruvianum*, a callosic wall appears just before pollen tube emission (Cresti et al. 1977); if the cytoplasm contains callosic reserves, this clearly accelerates pollen tube emission. In this species, the tube takes 3 h 30 min to be emitted in vivo, and only 45 min in vitro. In *C. pepo*, in vitro and in vivo germination times are the same, because the pollen is only partially hydrated and callose is already present. A quick germination may be considered as advantageous in the cases of male competition, which is especially high in grasses (Ottaviano and Mulcahy 1989). However, it has never been determined whether partially and fully hydrated pollen is subject to the mass effect or a pollen population effect (Brewbaker and Majumder 1961, Stanley and Linskens 1974) since germination should happen at once, without having to wait for further pollen, due to its high speed. This eliminates one of the occasions for male competition (Pacini and Franchi 1999). Examples of a pollen population effect reported so far all concern species with partially dehydrated pollen.

#### *Viability in time and cytoplasmic carbohydrates*

Not all pollen can sustain the same stress, and viability patterns may not depend on pollination type but on RH and water retention capacity (Bassani et al. 1994, Pacini et al. 1997).

Cytoplasmic carbohydrates reflect water retention capacity and may explain the longer survival of, for example, *L. arborea* pollen with respect to *C. pepo* pollen.

The duration of viability may be regarded as depending on two factors: the possibility of retaining

water and the possibility of maintaining plasma membranes intact and hence efficient. From the present study and those of Hoekstra and Van Roekel (1988), Hoekstra (1992), Franchi et al. (1996), and Speranza et al. (1997), it appears that PAS positivity of the cytoplasm is correlated with water retention and that the presence of sucrose is correlated with the efficiency of the plasma membrane. Partially dehydrated pollen retains water for longer, by virtue of the carbohydrates it contains. It can therefore survive longer exposure to low air relative humidities. This is not generally true for partially hydrated pollen, which dies quickly, except in cases such as that of *L. arborea*.

In the past, much importance was attributed to the state of maturation of the male gametophyte (two- or three-celled) at anthesis as a parameter indicative of viability in time (Hoekstra 1972, Stanley and Linskens 1974). The present study shows, however, that the duration of viability is due to other factors.

Contrary to initial belief, not all partially hydrated pollen has brief viability. The viability of partially hydrated pollen may be correlated with its sucrose content; the higher the sucrose content, the longer the pollen remains viable. *Opuntia dillenii* has a high sucrose content and pollen viability is more than 70% 72 h after flower opening. It is interesting that *Oenothera* pollen, which also has a high sucrose content, has brief viability. This is probably due to the fact that the pollen (triporate) has an intine exposed at the pores, which protrude (Hesse 1978) and through which water may be easily lost. In *Alcea rosea*, pollen sucrose content is not as high as in *Opuntia dillenii*, but viability is just below 50% 72 h after flower opening. The pattern of *L. arborea* pollen viability is quite similar to that of *Alcea rosea*: the sucrose content has not been measured, but since the pollen cytoplasm is PAS-positive, and since this positivity is consistently associated with the presence of sucrose, it can be supposed that the sucrose content is high. Pollen of *Spina-cia oleracea* and *C. pepo* has the lowest sucrose content and perishes most readily.

The sucrose content is always high in partially dehydrated pollen (Speranza et al. 1997) because the pollen undergoes a sharp loss of water in the last stage of its development in the anther. Hence the protective effect of this disaccharide on the plasma membrane already acts before the anther opens (partially dehydrated pollen) or once the pollen is exposed to the external environment (partially hydrated and some cases of partially dehydrated pollen). Sucrose content is one



of many biochemical, cytological, and physiological factors influencing the viability of partially or fully hydrated pollen.

#### *Methods for recognizing partially hydrated pollen*

Depending on pollen structure and the abundance of pollen for experiments, the dehydration status of pollen can be recognized by different methods. If pollen is abundant, as in many anemophilous species, water content can be determined by weighing and drying, when the anther opens and after different periods of exposure to environmental or controlled temperature and RH conditions. When not much pollen is available, changes in volume can be observed when the pollen is brought to complete hydration. This works when the exine is not too thick and pollen geometry is relatively simple (i.e., volume can be calculated to a good approximation). This method is very successful for partially dehydrated pollen, where increases in volume are mainly evident in the furrow area.

Finally, changes in the volume of developing pollen can also be measured, to check for an increase and subsequent decrease before or during anthesis. In partially hydrated pollen, the decrease in volume after anthesis is often associated with a decrease in viability (see also Bassani et al. 1994, Pacini et al. 1997).

A good guess is also possible on the basis of pollen morphology.

#### *Conclusions*

This study demonstrates that pollen grains are not all the same as far as their resistance to the external environment is concerned. This depends on their hydration status and on whether they have biochemical or physiological mechanisms of protection. These differences have practical implications: depending on when pollination occurs, the effects and results may be completely different for a given time interval. It is therefore important to know the hydration status of pollen of plants of commercial interest, especially if hand pollination is contemplated, or when pollen is stored for breeding purposes. A high percentage of water should probably be incompatible with pollen freezing. In fact, maize pollen, for example, can be stored successfully at  $-196^{\circ}\text{C}$  or  $-75^{\circ}\text{C}$  only if its water content is reduced to an adequate extent by gentle drying (Barnabás and Rajki 1981).

We have shown that the pollen hydration status can be evaluated in different ways. Some of these methods provide a quantitative measure of dehydration, but often simple morphological examination reliably indicates the type of pollen.

#### **References**

- Barnabás B, Rajki E (1981) Fertility of deep-frozen maize (*Zea mays* L.) pollen. *Ann Bot* 48: 861–864
- Bassani M, Pacini E, Franchi GG (1994) Humidity stress responses in pollen of anemophilous and entomophilous species. *Grana* 33: 146–150
- Blackmore S, Barnes SH (1986) Harmomegathic mechanisms in pollen grains. In: Blackmore S, Ferguson IK (eds) *Pollen and spores: form and function*. Academic Press, London, pp 137–149
- Bonner LJ, Dickinson HG (1989) Anther dehiscence in *Lycopersicon esculentum* Mill. I: structural aspects. *New Phytol* 113: 97–115
- Brewbaker JL, Kwack BH (1964) The calcium ion and substances influencing pollen growth. In: Linskens HF (ed) *Pollen physiology and fertilization*. North-Holland, Amsterdam, pp 143–151
- Majumder SK (1961) Cultural studies on the pollen population effect and the self-incompatibility inhibition. *Am J Bot* 48: 457–464
- Cresti M, Pacini E, Ciampolini F, Sarfatti G (1977) Germination and pollen tube development in vitro of *Lycopersicon peruvianum* pollen: ultrastructural features. *Planta* 136: 239–247
- Dickinson HG, Lawson J (1975) Pollen tube growth in the stigma of *Oenothera organensis* following compatible and incompatible intraspecific pollination. *Proc R Soc Lond B* 188: 327–344
- Erdtman G (1952) *Pollen morphology and plant taxonomy: angiosperms*. Almqvist & Wiksell, Stockholm
- Franchi GG, Bellani L, Nepi M, Pacini E (1996) Types of carbohydrate reserves in pollen: localization, systematic distribution and ecophysiological significance. *Flora* 191: 143–159
- Gay G, Kerhoas C, Dumas C (1987) Quality of stress-sensitive *Cucurbita pepo* L. pollen. *Planta* 171: 82–87
- Heslop-Harrison J (1979) An interpretation of the hydrodynamics of pollen. *Am J Bot* 66: 737–743
- (1987) Pollen germination and pollen tube growth. *Int Rev Cytol* 107: 1–78
- Heslop-Harrison Y (1982) The growth of the grass pollen tube 1: characteristics of the polysaccharide particles (P-particles) associated with apical growth. *Protoplasma* 112: 71–80
- (1985) Germination of stress-tolerant *Eucalyptus* pollen. *J Cell Sci* 73: 125–137
- (1992) Intracellular motility, the actin cytoskeleton and germinability in the pollen of wheat (*Triticum aestivum* L.). *Sex Plant Reprod* 5: 247–255
- Shivanna KR (1984) Evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. *Theor Appl Genet* 67: 367–375
- Heslop-Harrison JS (1997) Motility in ungerminated grass pollen: association of myosin with polysaccharide-containing wall-precursors bodies (P-particles). *Sex Plant Reprod* 10: 65–66
- Heslop-Harrison Y (2000) Control gates and micro-ecology: the pollen-stigma interaction in perspective. *Ann Bot* 85 Suppl A: 5–13

- Hesse M (1978) Zweierlei Formen der Pollenverkittung bei den Onagraceae. *Naturkundl Jahrb Stadt Linz* 23: 9–16
- Hoekstra FA (1972) Ademhaling en vitaliteit van bijen trinukleaat pollen. In: thesis, Agricultural University of Wageningen, Wageningen, the Netherlands
- (1992) Stress effects on the male gametophyte. In: Cresti M, Tiezzi A (eds) *Sexual plant reproduction*. Springer, Berlin Heidelberg New York Tokyo, pp 193–201
- van Roekel T (1988) Desiccation tolerance of *Papaver dubium* L. pollen during its development in the anther: possible role of phospholipid composition and sucrose content. *Plant Physiol* 88: 626–632
- Keijzer CJ (1987) The process of anther dehiscence and pollen dispersal 1: the opening mechanism of longitudinally dehiscing anther. *New Phytol* 105: 487–498
- (1999) Mechanisms of angiosperm anther dehiscence: a historical review. In: Clément C, Pacini E, Audran J-C (eds) *Anther and pollen: from biology to biotechnology*. Springer, Berlin Heidelberg New York Tokyo, pp 54–67
- Kremp GOW (1965) *Morphologic encyclopedia of palynology*. The University of Arizona Press, Tucson, Ariz
- Kress WJ (1986) Exineless pollen structure and pollination systems of tropical *Heliconia* (Heliconiaceae). In: Blackmore S, Ferguson IK (eds) *Pollen and spores: form and function*. Academic Press, London, pp 329–345
- Stone DE (1982) Nature of the sporoderm in monocotyledons, with special reference to the pollen grains of *Canna* and *Heliconia*. *Grana* 21: 129–148
- Lansac AR, Sullivan CY, Johnson BE, Lee KW (1994) Viability and germination of the pollen of sorghum [*Sorghum bicolor* (L.) Moench]. *Ann Bot* 74: 27–33
- Lisci M, Tanda C, Pacini E (1994) Pollination ecophysiology of *Mercurialis annua* L. (Euphorbiaceae), an anemophilous species flowering all year round. *Ann Bot* 74: 125–135
- Nepi M, Pacini E (1993) Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Ann Bot* 72: 526–536
- (1999) What may be the significance of polysiphony in *Lavatera arborea*? In: Clément C, Pacini E, Audran J-C (eds) *Anther and pollen: from biology to biotechnology*. Springer, Berlin Heidelberg New York Tokyo, pp 13–20
- Ciampolini F, Pacini E (1995) Development of *Cucurbita pepo* pollen: ultrastructure and histochemistry of the sporoderm. *Can J Bot* 73: 1046–1057
- Niesenbaum RA, Schueller SK (1997) Effects of pollen competitive environment on pollen performance in *Mirabilis jalapa* (Nyctaginaceae). *Sex Plant Reprod* 10: 101–106
- O'Brien TP, McCully ME (1981) *The study of plant structure: principles and selected methods*. Termarcaphi Pty, Melbourne
- Ottaviano E, Mulcahy DL (1989) Genetics of angiosperm pollen. *Adv Genet* 26: 1–64
- Owens SJ (1992) Pollination and fertilization in higher plants. In: Marshall C, Grace J (eds) *Fruit and seed production: aspects of development, environmental physiology and ecology*. Cambridge University Press, Cambridge, pp 33–55
- Pacini E (1990) Harmomegathic characters of pteridophyta spores and spermatophyta pollen. *Plant Syst Evol Suppl* 5: 53–59
- (1996) Types and meaning of pollen carbohydrate reserves. *Sex Plant Reprod* 9: 362–366
- Franchi GG (1999) Types of pollen dispersal units and pollen competition. In: Clément C, Pacini E, Audran J-C (eds) *Anther and pollen: from biology to biotechnology*. Springer, Berlin Heidelberg New York Tokyo, pp 1–9
- Lisci M, Nepi M (1997) Pollen viability related to type of pollination in six angiosperm species. *Ann Bot* 80: 83–87
- Pyne WW (1981) Structure and function in angiosperm pollen wall evolution. *Rev Paleobot Palinol* 35: 39–59
- Speranza A, Calzoni GL, Pacini E (1997) Occurrence of mono- or disaccharides and polysaccharide reserves in mature pollen grains. *Sex Plant Reprod* 10: 110–115
- Stanley RG, Linskens HF (1974) *Pollen: biology, biochemistry, management*. Springer, Berlin Heidelberg New York
- Wodehouse RP (1935) *Pollen grains*. McGraw-Hill, New York