

## Wettable and Unsinkable: The Hydrodynamics of Saccate Pollen Grains in Relation to the Pollination Mechanism in the Two New Zealand Species of *Prumnopitys* Phil. (Podocarpaceae)

JOSHUA SALTER\*, BRIAN G. MURRAY and JOHN E. BRAGGINS

School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

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The pollination mechanism of most genera of the Podocarpaceae involves inverted ovules, a pollination drop and bisaccate pollen grains. Saccate grains have sometimes been referred to as 'non-wettable' due to their buoyant properties, while non-saccate pollen grains have been described as 'wettable'. The hydrodynamic properties of saccate pollen grains of seven podocarp species in five genera, *Dacrydium* Sol. ex G. Forst., *Dacrycarpus* (Endl.) de Laub., *Manoao* Molloy, *Podocarpus* L'Hér. ex Pers. and *Prumnopitys* Phil. have been tested in water, together with saccate and non-saccate pollen of four other conifer genera, *Cedrus* Trew (Pinaceae), *Cephalotaxus* Siebold & Zucc. ex Endl. (Cephalotaxaceae), *Cupressus* L. (Cupressaceae) and *Phyllocladus* Rich. ex Mirb. (Phyllocladaceae), and spores of three fern species and one lycopod species. All four spore types studied were non-wettable, whereas the bisaccate and trisaccate pollen types, like all other conifer pollen types, were wettable, enabling the grains to cross the surface tension barrier of water. Once past this barrier, grain behaviour was governed by presence or absence of sacchi. Non-saccate and vestigially saccate grains sank, whereas saccate grains behaved like air bubbles, floating up to the highest point. In addition, the grains were observed to float in water with sacchi uppermost, consistent with the suggestion that distally placed sacchi serve to orientate the germinal furrow of the pollen grain towards the nucellus of an inverted ovule. Observations of pollen grains in the pollen chambers of naturally pollinated *Prumnopitys* ovules confirmed this. The combination of buoyancy and wettability in saccate pollen has implications for the efficiency of the typical podocarp pollination mechanism.

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**Key words:** Podocarpaceae, *Prumnopitys*, *Prumnopitys ferruginea* (G. Benn. ex D. Don) de Laub., *Prumnopitys taxifolia* (Sol. ex D. Don) de Laub., saccate pollen, pollen hydrodynamics, pollination mechanisms, conifer pollen, fern spores, lycopod spores.

### INTRODUCTION

The mature pollen grains of *Prumnopitys taxifolia* (Sol. ex D. Don) de Laub. and *P. ferruginea* (G. Benn. ex D. Don) de Laub. (Podocarpaceae), like those of most other podocarps, resemble the bisaccate grains of the genus *Pinus* L. (Pinaceae) in that the outer layer of the exine, the ektexine, balloons out from the body of the grain to form two hollow sacchi, located on either side of the distal germinal furrow (Pocknall, 1981a). In *Pinus* and in the saccate podocarp taxa observed to date, the spore walls are formed while the microspores are still in tetrads with their proximal poles tightly appressed. As a result, the sacchi develop towards the distal pole of each grain (Dickinson and Bell, 1970; Singh, 1978; Huynh and Sampson, 1983; Tomlinson, 1994; del Fueyo, 1996).

Doyle and O'Leary (1935) were the first to suggest that the primary function of the sacchi in bisaccate pollen grains of *Pinus* is as flotation devices in water, allowing the pollen grains to float upwards in the pollination drop inside the inverted ovule, and furthermore, serving to orientate the pollen grain with its germinal furrow facing the apex of the nucellus. From observations in *Podocarpus* L'Hér. ex Pers. and *Prumnopitys* Phil., Doyle (1945) predicted that the

correlation of bisaccate pollen grains with inverted ovule orientation and the production of a pollination drop would also be found in many other genera in the Podocarpaceae. He also suggested that the diverse pollination mechanisms in extant Pinaceae and Podocarpaceae have evolved independently from an ancestral type such as the Upper Permian conifer, *Ullmannia* Göppert, which had inverted ovules. While revised interpretations and recent discoveries have negated some of Doyle's (1945) ideas on evolution of the pollination mechanism in conifers, they have shown that most extinct conifers appear to have had inverted ovules (Mapes, 1987; Clement-Westerhoff, 1988; Miller, 1999), and that saccoid or saccate pollen (or prepollen possibly carrying motile antherozoids) was widespread among fossil gymnosperms (Kurmman and Zavada, 1994; Poort *et al.*, 1996), reinforcing Doyle's (1945) suggestion that the pollination drop was basal in the conifers.

It is now well recognized that there is considerable diversity among conifers in the details of their pollination mechanisms (Owens *et al.*, 1998; Tomlinson and Takaso, 1998). Detailed studies of conifers from both the Northern (e.g. Owens *et al.*, 1981, 1987; Runions *et al.*, 1999) and Southern hemisphere (e.g. Tomlinson *et al.*, 1991, 1997; Tomlinson, 1994; Möller *et al.*, 2000; Mill *et al.*, 2001), while adding to our understanding of the various mechan-

\* For correspondence. Fax 64 93737416, e-mail j.salter@auckland.ac.nz

isms, have by-and-large confirmed Doyle's implied hypothesis of coevolution between pollen grain and ovule (Doyle, 1945). Tomlinson (1991) and Tomlinson *et al.* (1991) have shown that in at least 12 podocarp taxa (belonging to seven genera), the pollination mechanism is dependent on a suite of characters: an inverted ovule or at least a downward-pointing micropyle, producing an inverted pollination drop, and saccate pollen. They added another character to the suite characterizing the podocarp pollination mechanism, namely a wettable zone on the cone axis, just beneath the mouth of the micropyle, which serves to extend the surface area of the pollination drop, and furthermore allows for 'scavenging' of pollen grains which arrive prior to exudation of the drop. They suggested that this suite of characters is an adaptation which increases the efficiency of wind-pollination while ensuring the exclusion of foreign particles (at least non-buoyant ones) from the pollen chamber.

In another pollination study, Tomlinson *et al.* (1997) highlighted important differences between the pollination mechanism of New Zealand species of *Phyllocladus* Rich. ex Mirb. and the podocarp mechanism, adding to the evidence for a separate family, Phyllocladaceae. More recently, Möller *et al.* (2000, p. 158) have reported observations which, they claim, 'invalidated' the taxonomic value of the pollination mechanism character suite when used 'on its own'. However, it should be noted that the separation of the Phyllocladaceae from Podocarpaceae, first proposed in 1907 by Bessey (Molloy, 1996) and independently by Keng (1975), has been based on a wide range of characters, and that Tomlinson *et al.* (1997) made no taxonomic claims on the strength of one character. Notwithstanding the contrary interpretation of Möller *et al.* (2000), Tomlinson *et al.* (1997) have now confirmed that the tropical species of *Phyllocladus* Rich. ex Mirb., *P. hypophyllus* Hook. f., shares the same features that characterize the pollination mechanism of the Phyllocladaceae, namely erect ovules, functionally non-saccate pollen and drop retraction in response to pollen.

The pollination mechanism of another unusual conifer, *Acmopyle* Pilg. (Podocarpaceae) has now been elucidated (Möller *et al.*, 2000). In 1945, Doyle was only aware of the morphology of the mature erect seed of *Acmopyle*, stating that 'the form and lie of the young ovule of this rare plant are unknown' (Doyle, 1945, p. 57). Nevertheless, on the basis of its bisaccate pollen, Doyle (1945, p. 57) suggested that 'the young [*Acmopyle*] ovule may well be strictly inverted, assuming the maturer position by later uneven growth, as so commonly occurs in *Dacrydium* [Forst. f.]'. Doyle may have been referring to *Lepidothamnus* Phil., formerly included in *Dacrydium* s.l., but the statement still holds for *Dacrydium* s.s. with an ovule that is obliquely inverted at the time of pollination becoming obliquely erect at maturity (Quinn, 1969; Tomlinson *et al.*, 1991; Tomlinson, 1992). Although Doyle's (1945) suggestion of an inverted young *Acmopyle* ovule has been shown to be mistaken, his implied prediction of a podocarp-type pollination mechanism (buoyant pollen and inverted pollination drop) in *Acmopyle* has been confirmed (Möller *et al.*, 2000; Mill *et al.*, 2001). At pollination, the morphologically erect ovule

is neither erect nor inverted with respect to the cone axis, but is more or less perpendicular to it, with its micropyle bent downwards 90° so that the emerging pollination drop is inverted. In this respect it resembles *Lepidothamnus*, which also has a 'hooked' micropyle instead of a fully inverted ovule (Tomlinson, 1994, Table 2). In addition to the suite of characters it shares with other podocarps, namely buoyant saccate pollen, inverted pollination drop and pollen scavenging, the pollination mechanism in *Acmopyle* involves rapid drop retraction in response to pollen, a phenomenon observed in *Phyllocladus* (Tomlinson *et al.*, 1997; Möller *et al.*, 2000). However, since this phenomenon has been reported in the Pinaceae, with inverted ovules (Doyle and O'Leary, 1935), as well as in the six families typified by morphologically erect ovules, Cephalotaxaceae, Cupressaceae, Phyllocladaceae, Sciadopytaxaceae, Taxaceae and Taxodiaceae (Tomlinson and Takaso, 1998), drop retraction is clearly not confined to taxa with erect pollination drops. Furthermore, within the Podocarpaceae the occurrence of drop retraction, or slower drop resorption (as distinct from simple evaporation) (Tomlinson *et al.*, 1991) is as yet incompletely documented, and may not be unusual in this family. The related phenomenon of re-secretion of the drop after reception of pollen is likewise poorly documented, although repeated secretion prior to pollination has been reported (Tomlinson *et al.*, 1997).

Doyle and O'Leary (1935, p. 185) were the first to refer to the bisaccate pollen of *Pinus* as 'readily wettable'. Their suggestion that one of the functions of the sacchi is to orient the germinal furrow towards the nucellus depends on the grains being both buoyant and wettable, so that they can float freely in the pollination drop and move directly up the micropyle to the pollen chamber. However, it has been regarded as 'physically impossible' (Tomlinson, 1994, p. 709) or at least 'unlikely' (Tomlinson and Takaso, 1998, p. 270) for saccate pollen to float freely within the pollination drop because saccate pollen was observed to float only on or in the surface of the drop. Bisaccate pollen grains which contacted the pollination drop were said to float up the meniscus of the inverted drop until they reached the mouth of the micropyle, finally entering the ovule on the surface of the receding drop. Tomlinson (1994) and Tomlinson *et al.* (1997) referred to such grains as 'non-wettable' because of their buoyant properties, to contrast them with non-saccate grains, and *Phyllocladus* grains with very small sacchi (Ueno, 1960; Pocknall, 1981b), which were termed 'wetable' because they sink in an upright pollination drop. Although Tomlinson and Takaso (1998) have suggested alternative terms ('non-sinkable' for 'non-wettable', and 'sinkable' for 'wetable'), Tomlinson (2000) has retained the terms 'non-wettable' and 'wetable' to distinguish saccate from non-saccate pollen, and stated that saccate pollen can only be incorporated in a drop of water if the meniscus is inverted.

Other studies have also been unclear on this point. For example, Owens *et al.* (1981, p. 1834) stated that in *Pinus contorta* Douglas ex Loudon the pollen 'appeared to float on the surface of the pollination drop with the sacchi oriented outward', yet earlier in the same paragraph the pollen 'directly entered the pollination drops', suggestive of

TABLE 1. Pollen and spore types used in the ten tests, together with general results

| Taxa  | Grain type | Test no. |   |   |    |    |    |    |   |   |    |
|---|------------|----------|---|---|----|----|----|----|---|---|----|
|   |            | 1        | 2 | 3 | 4  | 5  | 6  | 7  | 8 | 9 | 10 |
| Coniferophyta (pollen grains)   |            |          |   |   |    |    |    |    |   |   |    |
| <i>Cedrus deodara</i> (Roxb.) G. Don  | S          | +        | + | + | +  | +  | +  | +  | + | + | o  |
| <i>Cephalotaxus harringtonii</i> var. <i>drupacea</i> (Siebold et Zucc.) Koidz. | NS         | –        | – | o | nt | nt | nt | nt | o | o | nt |
| <i>Cupressus macrocarpa</i> Hartw. ex Gordon                                    | NS         | –        | – | – | nt | nt | nt | nt | – | – | nt |
| <i>Dacrycarpus dacrydioides</i> (A.Rich.) de Laub.                              | S(3)       | +        | + | + | +  | +  | +  | +  | + | + | o  |
| <i>Dacrydium cupressinum</i> Sol. ex G. Forst.                                  | S          | +        | + | + | +  | +  | +  | +  | + | + | o  |
| <i>Manoao colensoi</i> (Hook.) Molloy   | S          | +        | + | o | o  | o  | o  | o  | o | o | o  |
| <i>Phyllocladus trichomanoides</i> D. Don                                       | VS         | –        | – | o | nt | nt | nt | nt | – | – | o  |
| <i>Phyllocladus toatoa</i> Molloy   | VS         | –        | – | – | nt | nt | nt | nt | – | – | nt |
| <i>Podocarpus henkelii</i> Stapf. ex Dallim. et A. B. Jacks.                    | S          | +        | + | o | o  | o  | o  | o  | + | + | nt |
| <i>Podocarpus totara</i> D. Don   | S          | +        | + | o | o  | o  | o  | o  | o | o | o  |
| <i>Prumnopitys ferruginea</i> (G. Benn. ex D. Don) de Laub.                     | S          | +        | + | o | o  | o  | o  | o  | o | o | A  |
| <i>Prumnopitys taxifolia</i> (Sol. ex D. Don) de Laub.                          | S          | +        | + | + | +  | +  | +  | +  | + | + | A  |
| Pterophyta  |            |          |   |   |    |    |    |    |   |   |    |
| <i>Blechnum novae-zelandiae</i> T.C.Chambers et P.A.Farrant (monolete spores)   | NS         | #        | # | # | nt | nt | nt | nt | # | # | nt |
| <i>Cyathea medullaris</i> (Forst. f.) Swartz (trilete)                          | NS         | #        | # | # | nt | nt | nt | nt | # | # | nt |
| <i>Dicksonia fibrosa</i> Colenso (trilete)                                      | NS         | #        | # | # | nt | nt | nt | nt | # | # | nt |
| Lycophyta   |            |          |   |   |    |    |    |    |   |   |    |
| <i>Lycopodium volubile</i> Forst. f. (trilete spores)                           | NS         | #        | # | # | nt | nt | nt | nt | # | # | nt |

Test 1: Dry pollen/spores on to upright drop. Test 2: Dry pollen/spores at edge of upright drop. Test 3: Dry pollen/spores onto upright drop, then drop inverted. Test 4: Dry pollen/spores touched to inverted drop with scalpel. Test 5: Hydrated pollen touched to inverted drop with scalpel. Test 6: Hydrated pollen to inverted drop from upright drop. Test 7: Hydrated pollen to inverted drop from elongated drop. Test 8: Dry pollen/spores to top of water column. Test 9: Dry pollen/spores to bottom of water column. Test 10: Pollen angles at nucellus.

S, Bisaccate; S(3), trisaccate; NS, non-saccate; VS, vestigial sacchi.

nt, Not tested because grain type inapplicable; o, not tested because pollen unavailable; wettable, floated (+); wettable, sank (–); non-wettable (#); pollen angles measured (A).

wettability in these grains. The saccate pollen of *Picea engelmannii* Parry ex Englm. was similarly described as ‘entering the drop’ (Owens *et al.*, 1987, Fig. 12).

Ultrastructural studies of saccate pollen in Pinaceae and Podocarpaceae show the presence of micropores (or micropunctae) which penetrate the ectexine of the saccus (e.g. Pocknall, 1981a, b; Runions *et al.*, 1999; Tomlinson, 2000), and recent work has focused on the role played by the micropores in buoyancy. For instance, *Picea orientalis* (L.) Link (Pinaceae) has pollen grains that sink in the pollination drop despite their well-developed sacchi. In a study comparing *P. orientalis* pollen with the buoyant bisaccate pollen of *P. glauca* (Moench) Voss, Runions *et al.* (1999) have shown that buoyancy is governed by the degree of porosity of the sacchi and the relative volumes of air remaining trapped in the endoreticulations of the sacchi after hydration of the grains. Tomlinson (2000) has shown that air probably enters the sacchi through the micropores as the pollen dries out before its release. When the grain is in water the air is trapped by surface tension at the micropore opening. Tomlinson (2000) found that saccate grains which normally float in water can be caused to sink when treated with organic solvents or detergent under vacuum, and interpreted this as evidence for a hydrophobic surface on the micropore walls which prevents water from displacing the air in the sacchi. Such studies, while providing explanations for the buoyancy of saccate pollen, have not yet addressed the separate issue of their wettability, a property which would

seem to be necessary for saccate pollen grains to float within the pollination drop up the micropyle, whatever their orientation (Doyle and O’Leary, 1935).

In a later study of pollination in *Pinus*, McWilliam (1958) reported that pollen showed no preferred orientation on the nucellus and concluded that drop absorption was more important than flotation in delivering pollen to the nucellus. The similar conclusions of Tomlinson and coworkers (Tomlinson *et al.*, 1991; Tomlinson, 1994, 2000; Tomlinson and Takaso, 1998) have been reached on the basis of observations of the behaviour of saccate pollen when added to pollination drops of several different podocarp genera, and from observations of dissected ovules. However, while observing sections of pollinated ovules during our studies of embryological development in *Prumnopitys* (Podocarpaceae), it seemed that in the majority of cases the germinal furrow of the pollen grains in the pollen chamber faced the nucellus, albeit at various angles and sometimes at a distance. If it were correct that saccate pollen grains remained trapped by the meniscus and were pulled into the micropyle by the receding pollination drop, the pollen grains would not be expected to arrive at the nucellus in any preferred orientation. If, on the other hand, saccate pollen were wettable and could freely enter the pollination drop, the grains would be expected to float up the micropyle and arrive sacchi-uppermost at the nucellus. A re-examination

TABLE 2. Percentage of pollen grains with germinal furrow facing towards or away from the nucellus in ovules of *Prumnopitys taxifolia* and *P. ferruginea*

| Taxa                 | Pollinated ovules | Grains observed | Grains measured | Grains at 0–88° | Grains at 89–91° | Grains at 92–180° | Grains facing towards nucellus (%) | $\chi^2$ |
|----------------------|-------------------|-----------------|-----------------|-----------------|------------------|-------------------|------------------------------------|----------|
| <i>P. taxifolia</i>  | 29                | 83              | 52              | 38              | 4                | 10                | 73.1                               | 16.354   |
| <i>P. ferruginea</i> | 10                | 43              | 29              | 25              | 0                | 4                 | 86.2                               | 15.241   |
| Totals               | 39                | 128             | 81              | 63              | 4                | 14                | 77.8                               | 31.195   |

All chi-squared values had  $P < 0.001$  (1 d.f.), indicating that a significant majority of the grains were 'facing towards the nucellus'.

of the sections of pollinated ovules was therefore undertaken to determine more accurately the positions of pollen grains inside the pollen chamber.

The objectives of the present study were two-fold: first, to test the hydrodynamic properties of bisaccate pollen from the two species of *Prumnopitys* in New Zealand, *P. taxifolia* and *P. ferruginea*, in comparison with several other pollen and spore types; and secondly, to test Doyle's hypothesis that bisaccate pollen grains would tend to come to rest in the pollen chamber with the germinal furrow oriented towards the apex of the nucellus, by measuring the angles of pollen grains in pollinated ovules of *P. taxifolia* and *P. ferruginea*.

#### MATERIALS AND METHODS

Pollen of 12 conifer species and spores of three fern species and one lycopod were used (Table 1). Table 1 indicates which species were used in each of the experiments and investigations. Cones or sporangia were collected just before pollen or spore dispersal, and dried in paper packets until the pollen or spores were shed. Dried pollen and spores were then stored in glass vials at room temperature until required.

##### *Hydrodynamic properties of selected pollen and spores*

Various tests were tried to determine the hydrodynamic properties such as wettability of the various grains and spores. These involved observations of grain and spore behaviour in upright drops of tap water (tests 1 and 2), in inverted drops of water (tests 3–7) and in water columns (tests 8 and 9). In addition, the hydration process was observed for most grain types, and hydration times were recorded for individual pollen grains of *Prumnopitys taxifolia* and *Cedrus deodara* (Roxb.) G. Don added to an upright drop on a glass slide.

##### *(a) Tests with upright water drops on glass slides*

*Test 1.* A small amount of dry pollen/spores was sprinkled onto the surface of a drop of tap water on a glass slide.

*Test 2.* A drop of tap water was placed beside dry pollen/spores on a glass slide and single grains were moved towards the edge of the drop with a scalpel. Observations were made using a Leica Wild M3C dissecting microscope, with illumination from above or below. Where necessary, bright reflected light was used to reveal the positions of grains and spores in relation to the drop surface.

##### *(b) Inverted water drops on glass slides*

Inverted water drops were obtained by placing a drop of tap water on a glass slide and quickly inverting it so that the drop was not disturbed or allowed to run. The glass slide was then supported at each end so that the inverted drop could be observed from above with a dissecting microscope. Some pollen was tested dry, and some was hydrated prior to testing. After some tests the glass slide was left undisturbed to allow the drop to dry, and then photographed. Observations were made using a Wild Photomakroskop M100.

*Test 3.* Several hundred dry pollen grains or spores were dropped onto an upright drop and time allowed for hydration. The drop was then inverted.

*Test 4.* While observing from above, several hundred dry grains were transferred to the centre of an inverted drop from the tip of a scalpel blade.

*Test 5.* Several hundred hydrated grains were transferred to an inverted drop from the tip of a scalpel blade.

*Test 6.* Several hundred hydrated grains were transferred to an inverted drop from an upright drop. First, dry grains were sprinkled onto an upright drop on another glass slide and allowed to hydrate for 1 or 2 min. Then, while observing from above, the upright drop was gently brought into contact with the inverted drop, until the drops touched at their centres.

*Test 7.* Several hundred hydrated grains were transferred to the centre of an inverted drop from an elongated drop on the side of a horizontal needle. First, a large number of grains were hydrated in an upright drop. These were then picked up on the side of a steel needle by revolving it while drawing it across the surface of the drop. The resulting elongated drop saturated with hydrated grains was then applied to the centre of the inverted drop while keeping the needle horizontal.

##### *(c) Water column tests*

Glass pipettes were used to form narrow vertical water columns to facilitate observations of grain behaviour in deeper water (approx. 5 mm diameter  $\times$  100 mm deep).

*Test 8.* Glass pipettes (two for each grain type) were filled with tap water and positioned vertically, with their narrow tips blocked. Dry grains, scooped up with a small square of paper, were tapped gently on the water surface, and their behaviour observed from the side.

*Test 9.* A small quantity of each pollen or spore type was placed in the centre of a Petri dish. A glass pipette was filled with water and inverted, with the narrow tip upper-

most, blocked by a finger so that the water column was held with a convex meniscus just protruding below the open end of the pipette. The water column was lowered over the dry grains until the convex surface just touched them. Pollen and spore behaviour was observed from the side. For each pollen and spore type, two separate water columns were tested.

#### Orientation of pollen grains in naturally pollinated ovules

*Test 10.* As a test of the hypothesis that sacci enable bisaccate grains to reach the nucellus with the germinal furrow oriented towards it, the angles of pollen grains inside 39 ovules were measured. Longitudinal serial sections of naturally pollinated ovules (29 *Prumnopitys taxifolia* and ten *P. ferruginea* ovules), which had been prepared for embryological study, were available. They had been fixed in FAA, embedded in paraffin wax, sectioned at 7–10  $\mu\text{m}$ , and stained with haematoxylin and alcoholic light green. Slides were viewed with a Zeiss Photomicroscope III connected to a JVC video monitor. Two lines were drawn crossing at right angles on a sheet of transparent acetate, which was positioned over each image on the screen. The lines were used as reference points to measure from. The long axis was aligned as closely as possible to the central axis of the nucellus and micropyle, and the transverse axis was aligned tangentially to the surface of the nucellus. To measure the angle of each bisaccate pollen grain, another line was drawn touching both sacci and crossing the transverse line. A protractor was used to measure the angle of this line to the transverse line and hence the angle to the apex of the nucellus (see Fig. 3A). Pollen grains with the distal pole of the grain (i.e. the germinal furrow) nearest the nucellus and parallel to it were assigned an angle of  $0^\circ$ . Those with the proximal pole nearest the nucellus and parallel to it were assigned an angle of  $180^\circ$ . Grains with an angle ranging from  $0$  to  $89^\circ$  were deemed to be in the category 'facing towards the nucellus', while those in the range  $91$ – $180^\circ$  were deemed to be in the category 'facing away from the nucellus'. Grains in the range  $89$ – $91^\circ$  were recorded as ambivalent, but for chi-squared analysis were assigned to the category 'facing away from the nucellus' to reduce any inherent bias in the observations.

## RESULTS

### Hydrodynamics of selected pollen and spores

#### (a) Upright water drops

*Tests 1 and 2.* When dropped directly onto an upright water drop, the majority of pollen grains with well-developed sacci [*Cedrus*, *Dacrycarpus* (Endl.) de Laub., *Manoao*, *Podocarpus* and *Prumnopitys*] appeared to submerge rapidly the moment they contacted the drop surface (although this was difficult to see with ordinary top lighting) and then remained floating at the top of the drop with their sacci (distal poles) uppermost. When added to the edge of a drop, all the saccate pollen, except *Phyllocladus*, floated up to the highest point of the drop, again floating with distal poles uppermost. In contrast, the *Phyllocladus* species and

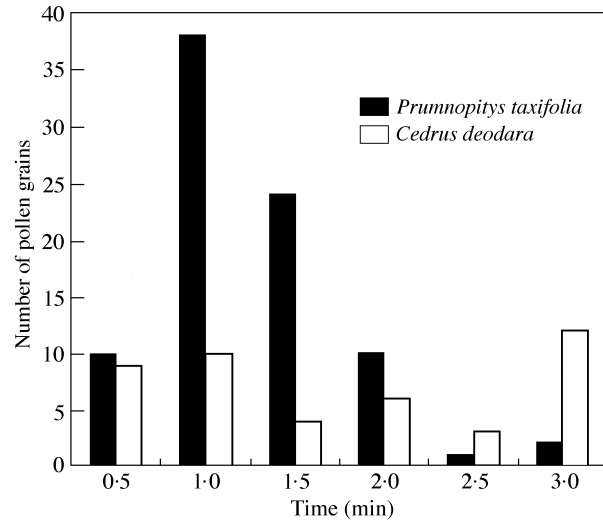


FIG. 1. Hydration times in tap water for bisaccate pollen grains of *Cedrus deodara* (44 grains) and *Prumnopitys taxifolia* (85 grains).

the non-saccate pollen grains all sank to the bottom of the drop when sprinkled on top, and when added at the edge of the drop, entered the drop and remained at the bottom. The spores of *Lycopodium* L. and the two fern species, when added to the top of a drop, tended to clump together on the surface. Clusters of spores then moved away from the centre of the drop, falling down the steep sides of the drop and accumulating at the drop's margin. When touched to the edge of a drop, they remained clustered at the edge without entering the drop.

Because grain positions in relation to the surface of the drops were difficult to see with top lighting or bottom lighting, they were also viewed with lighting arranged to cause reflections on the drops. A bright reflection on the drop surface permitted easy observation of any disruption to the surface contours of the drop. Grains sitting above the surface caused a dimple in the bright surface reflection, whereas grains below the surface, visible with normal top or bottom lighting, were not visible and had no effect on the smooth contour of the surface reflection.

Most of the pollen grains submerged immediately on encountering the drop, while some remained at the surface for a few seconds before submerging abruptly. Once submerged, all of the pollen grain types disappeared from view beneath the surface reflection. When ordinary top lighting was restored, the saccate grains (except those of *Phyllocladus*) could be seen at the top of the drop, while the non-saccate grains and grains of *Phyllocladus* were at the bottom. The *Lycopodium* spores and fern spores remained visible above the surface, whether at the centre or at the drop edge, as indicated by their effect on the surface reflection.

When dry, the body of bisaccate and trisaccate grains was contracted so that the sacci were compressed together over the germinal furrow. When hydrated, the body of the grain swelled and the sacci were spread apart. In some species, such as *Podocarpus totara* G. Benn. ex D. Don and *Prumnopitys taxifolia*, the fully expanded grains were twice

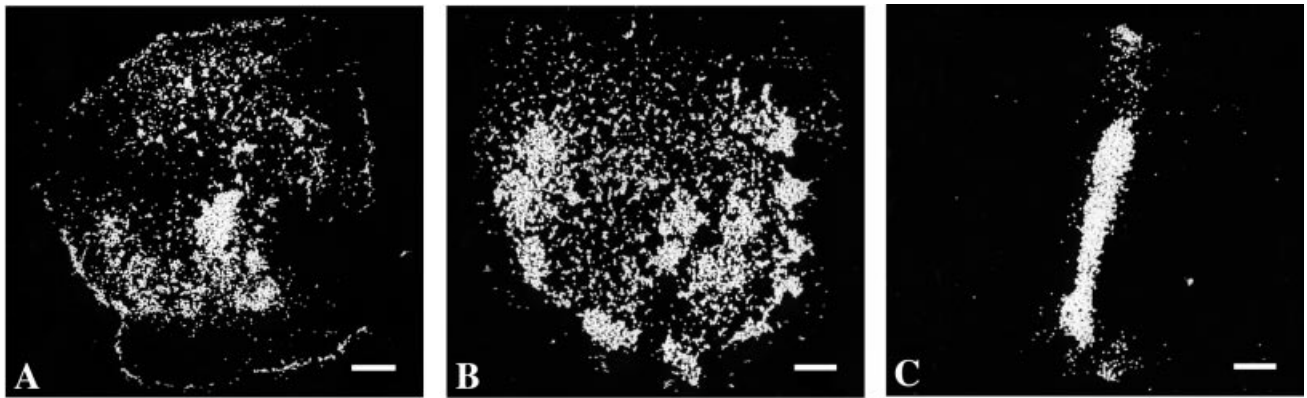


FIG. 2. Results of tests 4, 6 and 7, showing where saccate pollen grains of *Prumnopitys taxifolia* came to rest in inverted water drops, photographed after the drops had dried; viewed from above, with pollen grains on underside of glass slide. Bars = 1 mm. A, Result of test 4 (dry grains transferred from a scalpel blade): the majority of grains entered the drop and came to rest scattered over the drop area. Those grains which failed to enter the drop rode the drop surface to the margin. B, Result of test 6 (hydrated grains transferred from scalpel blade): almost all the grains entered the drop, but came to rest widely scattered due to turbulence in the drop. C, Result of test 7 (hydrated grains transferred from a needle): almost all the grains came to rest in a line above the needle.

the length of contracted grains. Hydration times were recorded for individual grains of *Cedrus deodara* (44 grains) and *Prumnopitys taxifolia* (85 grains). The majority of the grains took at least 1 min to hydrate fully, and many *Cedrus* grains were still not fully hydrated after 3 min (see Fig. 1).

The dry non-saccate grains of *Cephalotaxus* Siebold & Zucc. ex Endl. had an irregular shrunken appearance, and when hydrated they swelled to rounded or oval form. After a few minutes the exine split due to water uptake by the intine, and the microgametophyte, surrounded by a thickened intine, was released. Like the *Cephalotaxus* grains, the non-saccate grains of *Cupressus macrocarpa* Hartw. ex Gordon were shrunken when dry. When hydrated they swelled until spherical. A few grains were seen which had shed the exine and released a circular microgametophyte, although the actual process was not observed. The fern and *Lycopodium* spores showed no morphological responses to water, or any other signs of hydration, despite many minutes in water.

Whether the pollen grains were dry or hydrated, entry to the water drops was a sudden event with both saccate and non-saccate grains becoming fully submerged immediately on contact with the drop. In many cases dry grains at the edge of a drop appeared to ‘leap’ into the drop before it touched them. Once submerged, all saccate grains except *Phyllocladus* floated up the curved surface of the drop, reaching the highest point in a few seconds, and becoming fully hydrated more slowly. In some cases a few of the saccate grains remained above the surface or at the drop edge for several seconds or longer before abruptly submerging. In the case of *Phyllocladus* and non-saccate grains, entry to the drops was immediate.

#### (b) Inverted water drops

**Test 3.** In the upright drop the saccate grains behaved as in tests 1 and 2, submerging and remaining at the top of the drop. After allowing time for hydration the drop was inverted. The grains then moved more or less directly

through the drop up to the surface of the glass slide, although turbulence in the inverting drop prevented them from following a strictly vertical path. The non-saccate pollen grains also behaved as in tests 1 and 2, settling to the bottom of the upright drop. When the drop was inverted they sank again to the lowest point, accumulating in the centre of the drop. The four spore types likewise behaved as in tests 1 and 2, clustering together on the top of the upright drop and falling down the sides to the margins. On inversion of the drop, the spores fell from the drop margins to the centre of the drop.

**Test 4.** When a mass of dry saccate grains was touched to the fullest part of an inverted water drop, some of them immediately rode up the curved meniscus to the periphery of the drop, while the majority rose up through the drop to the underside of the glass slide, where they came to rest with distal poles uppermost. Grains in the central zone and the periphery were seen to hydrate after coming to rest. Figure 2A, photographed after the drop was dried, shows grains in the positions they first landed. The grains scattered over most of the area occupied by the drop are those which penetrated the surface layer and rose through the drop. The dense cluster of grains at the centre rose more or less vertically, while turbulence, due to disruption of the drop by the scalpel tip, caused the remainder to scatter before coming to rest. Those forming a line at the edge of the original drop are the grains which initially failed to enter the drop and rode up the surface meniscus.

**Test 5.** When a mass of saccate grains which had previously been hydrated for at least 1 min were touched to an inverted drop they behaved differently from dry grains. Few of them rode the meniscus to the drop periphery, and instead the vast majority of them moved rapidly up through the drop to meet the glass. As in tests 3 and 4, turbulence caused many of the grains to scatter before coming to rest on the underside of the glass slide.

**Test 6.** The hydrated grains began to rise rapidly through the inverted drop, but due to the initial turbulence

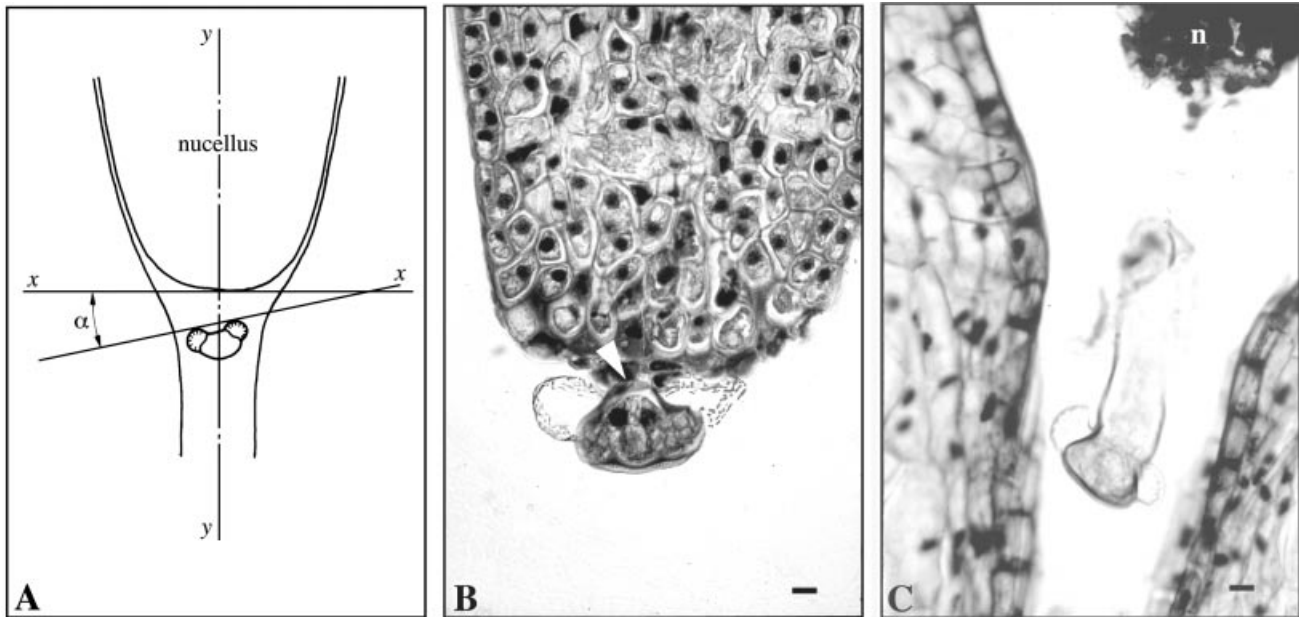


FIG. 3. Orientation of saccate pollen grains within naturally pollinated ovules of *Prumnopitys ferruginea* and *P. taxifolia*. Bars = 10  $\mu$ m. A, Method for measuring angle of pollen grain to nucellus tip. Axis  $y$ - $y$  aligned with micropyle; axis  $x$ - $x$ , perpendicular to  $y$ - $y$ , forms tangent to nucellus tip. The angle of the grain ( $\alpha$ ) is measured relative to the tangent of the nucellus. A pollen grain is shown with germinal furrow facing the nucellus. B, Saccate pollen grain of *P. ferruginea* with germinal furrow (arrowhead) in contact with tip of nucellus. (Ovule at free-nuclear gametophyte stage.) Adjacent sections show that this grain has germinated, and the pollen tube (approx. 225  $\mu$ m long) contains the tube nucleus and four to five prothallial nuclei. Two to four prothallial nuclei (two visible) remain in the grain with the body cell (not visible). C, Saccate pollen grain of *P. taxifolia* with pollen tube extending from germinal furrow towards nucellus (n). The grain is empty because the body cell and prothallial nuclei have moved out along the pollen tube towards the archegonia. (Ovule at fertilization stage.)

of the merging of two drops, the grains came to rest in scattered clusters more or less evenly spread over the area occupied by the drop (Fig. 2B). Almost no grains settled at the periphery.

*Test 7.* A needle was used to transfer the hydrated grains to reduce the volume of water involved and thereby reduce the turbulence. The elongated water drop, saturated with hydrated grains, was gently applied horizontally to the inverted drop. The grains moved vertically up from the needle through the inverted drop, forming a line of grains across the area of the drop (Fig. 2C). The reduced turbulence resulted in very few grains coming to rest elsewhere in the drop.

#### (c) Water column tests

*Test 8.* All of the grains with well-developed sacci (*Cedrus*, *Dacrycarpus*, *Dacrydium*, *Podocarpus* and *Prumnopitys*) showed the same behaviour. They sank under the surface, but remained at the top of the water column, vacating the centre of the concave meniscus and congregating around the periphery. None sank to the bottom of the tube. In contrast, most of the grains of both *Phyllocladus* species sank almost immediately. Some grains remained longer at the surface, resulting in a continuous fall of grains over several minutes. Eventually almost all the grains accumulated in the base of the water column. Those that did not sink remained as a faint yellowish tint at the surface. The grains of *Cupressus* L. submerged immediately on contact with the surface, and sank rapidly, accumulating

at the bottom. All the fern and *Lycopodium* spores remained at the surface, but unlike the saccate pollen, congregated at the lowest point of the concave meniscus.

*Test 9.* When touched by the base of the water column, all of the grains with well-developed sacci (*Cedrus*, *Dacrycarpus*, *Dacrydium*, *Podocarpus* and *Prumnopitys*) entered the water and moved up the column, accumulating in the peripheral meniscus. Grains of *Dacrydium cupressinum* Sol. ex G. Forst. floated up more slowly than the others, but eventually none remained at the bottom. Almost all the grains of both *Phyllocladus* species entered the water surface when touched by the base of the water column, accumulating in the convex meniscus protruding below the tube. The *Cupressus* grains showed similar behaviour to the *Phyllocladus* grains, massing at the bottom of the water column. When the pile of dry fern and *Lycopodium* spores was touched by the base of the water column the pile of spores was flattened, and the spores did not enter the water. On raising the water column, the spores from the surface of the pile adhered to the meniscus, and remained at the lowest point.

#### Orientation of pollen grains in naturally pollinated ovules

*Test 10.* Thirty-nine naturally pollinated ovules were examined (29 from *Prumnopitys taxifolia* and ten from *P. ferruginea*). Of 128 grains seen, 47 were unsuitable for measurement because they were too obscured by adjacent grains crowding or overlapping them, or were too crushed or

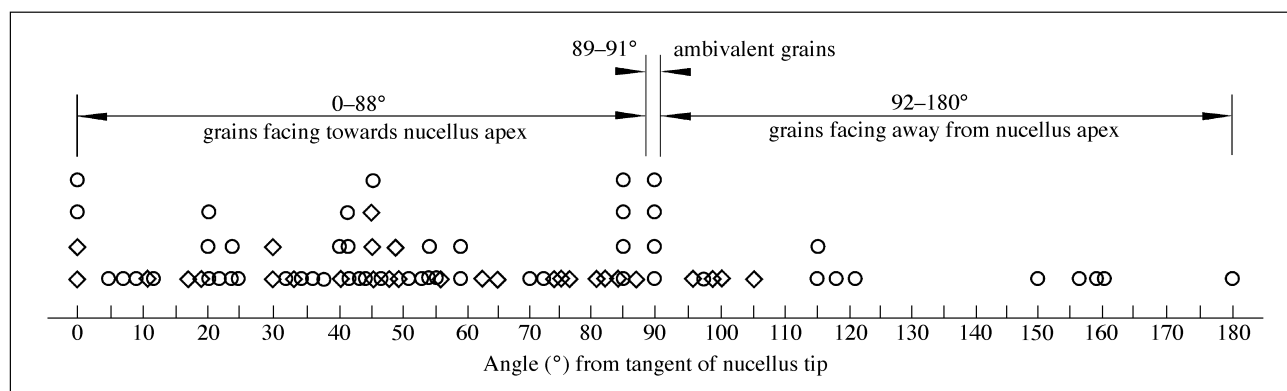


FIG. 4. Cumulative score of pollen grains observed at various angles to the nucellus, in 39 naturally pollinated ovules (*Prumnopitys ferruginea*: 10; *P. taxifolia*: 29). Each symbol represents an individual grain. *P. ferruginea* ovules: 43 grains present, 29 measured (open diamonds), 14 unmeasurable. *P. taxifolia* ovules: 83 grains present, 52 measured (open circles), 31 unmeasurable.

distorted by closure of the micropyle or general enlargement of the ovule. The remaining 81 grains were measurable. Two trisaccate grains of *Dacrycarpus* type and one of *Pinus* type were also observed, but were omitted from the counts because they were all unmeasurable. A number of grains were observed with emerging pollen tubes which had penetrated the nucellus tissue (Fig. 3C), while others showed no sign of tube growth. In all cases, the angle of the distal pole of the grain was determined relative to the tangent of the nucellus (Fig. 3A). In *P. taxifolia* ovules, four grains observed at 90° were included in the 'facing away' category. Thus, of the 52 measurable grains, 14 were facing away from the nucellus and 38 (73.1 %) were facing towards it. In the *P. ferruginea* ovules, no grains were at 90°, four were facing away from the nucellus and 25 (86.2 %) were facing towards it (Fig. 3B and C). In total, 77.8 % of the measurable pollen grains were facing the nucellus (Table 2, Fig. 4). Chi-squared tests of the total number and of the separate counts for *P. taxifolia* and *P. ferruginea* gave a very low probability ( $P < 0.001$ ) that these numbers could occur by chance. The results indicate that the pollen which entered these ovules tended to arrive with the germinal furrow facing the nucellus.

## DISCUSSION

### *Hydrodynamic properties of selected pollen and spores*

These experiments were conducted in relatively large volumes of ordinary tap water because the behaviour of pollen grains on or in a pollination drop is difficult to observe directly, even in the attenuated or reduced cones of podocarps.

Although surface tension forces are known to be affected by surface-to-volume ratio, and are therefore greater for very small drops than for larger ones, this effect acts at the molecular and colloid level (Everett, 1988). Therefore, at the scale of pollination drops vs. water drops on a slide, any variation is too small to affect the behaviour of objects as large as pollen grains, which range from 15–55  $\mu\text{m}$  in podocarps to 48–110  $\mu\text{m}$  in pines, excluding the sacci

(Ueno, 1958, 1960). Surface tension does, however, play a role in saccate pollen behaviour. Runions *et al.* (1999) and Tomlinson (2000) have shown that any buoyancy exhibited by saccate grains is probably due to a sufficient volume of air trapped in the sacci by surface tension forces at the entrance to the micropores in the ectexine. Such forces are unlikely to have any bearing on the wettability of the grains because they act at the air/water interface of the micropores of a pollen grain already immersed in water. Even if such forces do have an influence on the wettability of saccate grains, it is unlikely that there would be any detectable difference between 1 cm diameter water drops and 1 mm diameter pollination drops.

Pollination drops in a number of gymnosperms have variously been described as: 'mucilaginous' (Coulter and Chamberlain, 1917, cited in Doyle and O'Leary, 1935, p. 182), 'slightly mucilaginous' (McWilliam, 1958, p. 116), 'a viscous liquid' (Möller *et al.*, 2000, p. 149), 'more viscous than water' (Owens *et al.*, 1981, p. 1832), 'a drop of nectar' (Singh, 1978, p. 61) and 'a weak sugar solution' (Owens *et al.*, 1987, p. 1443), listed in descending order of viscosity. Analyses of pollination drops in a few conifers (reviewed by Chesnoy, 1993), indicate that sugars (especially fructose) are the main components, in amounts ranging from 1–10 % (in *Pinus*, *Picea* and *Taxus*). Other substances have been detected, such as galacturonic acids (1–4 % in *Thuja*, *Cephalotaxus* and *Taxus*) and small amounts of free amino acids. Such small quantities of solutes are inconsistent with the descriptive terms 'mucilaginous' or 'viscous'. This perception of conifer pollination drops may have arisen from misinterpretation of the resilience of the drops due to their surface tension. Descriptions of pollen behaviour (e.g. Doyle and O'Leary, 1935; Owens *et al.*, 1981, 1987, 1998; Tomlinson *et al.*, 1997; Möller *et al.*, 2000), and personal observations of pollen behaviour in pollination drops of *Phyllocladus toatoa* and *Prumnopitys taxifolia*, suggest that the pollination drop of conifers would be more accurately described as a weak sugar solution of a watery consistency (Owens *et al.*, 1987). Furthermore, these solutes, despite any biological role they may play in pollen grain germination, are unlikely to have a



TABLE 3. Behavioural properties in water for pollen grains of 12 conifers and spores of three ferns and one lycopod

|              | Unsinkable                      | Sinkable                           |
|--------------|---------------------------------|------------------------------------|
| Wettable     | <i>Cedrus deodara</i>           | <i>Cephalotaxus harringtonii</i>   |
|              | <i>Dacrycarpus dacrydioides</i> | var. <i>drupacea</i>               |
|              | <i>Dacrydium cupressinum</i>    | <i>Cupressus macrocarpa</i>        |
|              | <i>Manoao colensoi</i>          | <i>Phyllocladus toatoa</i>         |
|              | <i>Podocarpus henkelii</i>      | <i>Phyllocladus trichomanoides</i> |
|              | <i>Podocarpus totara</i>        |                                    |
|              | <i>Prumnopitys ferruginea</i>   |                                    |
|              | <i>Prumnopitys taxifolia</i>    |                                    |
| Non-wettable | <i>Blechnum novae-zelandiae</i> |                                    |
|              | <i>Cyathea medullaris</i>       |                                    |
|              | <i>Dicksonia fibrosa</i>        |                                    |
|              | <i>Lycopodium volubile</i>      |                                    |

significant effect on the strength of the surface tension forces. For the above reasons, it was considered that the qualitative and quantitative differences between pollination drops and tap water in drops or as columns in glass tubes, would not materially affect the hydrodynamic properties of the pollen grains or spores.

In the tests with upright drops, the majority of the bisaccate grains of *Cedrus*, *Dacrydium s.s.* and *Prumnopitys*, and the trisaccate ones of *Dacrycarpus*, floated as expected. They also appeared to be wettable, in that they rapidly entered the drops, whether dry or hydrated, and were seen to be floating beneath the surface with their distal poles uppermost.

In the tests in inverted water drops the bisaccate and trisaccate grains behaved essentially like small bubbles, as noted by Tomlinson and Takaso (1998, p. 268), ascending vertically through the drop except where the water was turbulent. They were also clearly wettable because they could cross the surface tension barrier. Most of the dry grains rose through the drop in a few seconds, becoming fully hydrated at least 1 min after coming to rest. Again, viewed from above, the grains were observed to float with distal pole uppermost. Only a few grains behaved initially as if unwettable, travelling up the drop surface to the periphery. This can be explained by differences in the morphologies of dry and hydrated saccate grains. When dry, the body of a saccate grain contracts so that the inflated sacchi press together over the germinal furrow (Tomlinson, 1994). When hydrated, the expansion of the body moves the sacchi apart exposing the germinal furrow. Some of the dry grains may have initially been hindered from being fully wettable by surface air trapped between the sacchi. They must have crossed the surface layer of the drop eventually, because they later hydrated like all the rest.

In the tests in water columns the bisaccate and trisaccate grains rapidly entered the water, then ascended through the water column, and came to rest floating under the water surface at the highest point (around the edge of the meniscus). The rate at which the saccate grains rose through the water column appeared to be affected by the relative size of the sacchi. For instance, *Dacrydium cupressinum* grains, with proportionately smaller sacchi in relation to body size

(Pocknall, 1981a), floated up more slowly than the other saccate grains. However, saccus size had no effect on the wettability of the grains.

In contrast, both *Phyllocladus* species behaved like the non-saccate wettable grains of *Cupressus macrocarpa* and *Cephalotaxus*, sinking rapidly in water drops and water columns. These observations confirm that the small sacchi of the *Phyllocladus* species are non-functional (at least as flotation devices), and, if saccate pollen is ancestral in the conifers (Tomlinson, 2000), probably vestigial. This pollen type should therefore be classed as wettable and functionally non-saccate. For the more or less upright ovules of the Phyllocladaceae, such pollen characteristics are appropriate for efficient pollination, as Tomlinson *et al.* (1997) and Möller *et al.* (2000) have shown.

The non-saccate fern spores and *Lycopodium* spores showed a tendency to adhere to the meniscus of inverted drops or columns, suggesting that they have some sort of electrostatic attraction to water. Nevertheless, they appeared to be the only non-wettable grains (in the sense that they were unable to cross the surface tension barrier), remaining on top of the water surface when dropped on it, and failing to enter the water column when placed at the bottom.

In conclusion, the term 'wetable' should be reserved for objects or surfaces with hydrophilic properties; likewise, 'non-wettable' applies to hydrophobic objects or surfaces. Thus all the pollen grains tested, even those which floated, were wettable, in the sense that they all became submerged beneath the surface layer of the water. Only after they had crossed the surface tension barrier did the grains sink or float, depending on absence or presence of functional sacchi. The truly saccate grains are more accurately described as 'wetable but unsinkable' rather than 'unwetable' because, although they readily submerge in water, the buoyancy of the air sacs prevents them from sinking. The conclusions are summarized in Table 3. The combination of 'non-wettable' with 'sinkable' is a logically impossible condition for such small particles as pollen grains or spores, as is reflected in the blank box of Table 3.

#### Orientation of pollen grains at the nucellus

A number of the grains were observed with emerging pollen tubes that had penetrated the nucellar tissue. Such grains frequently had a portion of the pollen tube outside the nucellar tissue so that the grains were held at an angle some distance from the nucellus (Fig. 3C). It was not possible to determine whether this was due to initial failure of the pollen tube to penetrate the nucellus (pollen tube growth pushing the grain 'backwards') or due to later shrinkage of the nucellus, or whether the grain came to rest in this position on arrival. This latter option seems most likely because extended pollen tube growth outside the nucellar tissue was most common in the more crowded cases where many grains were unable to reach the nucellus. Where grains were few or solitary, they tended to lie close to the nucellus so that either the pollen tube was not exposed at all (as in Fig. 3B) or the exposed portion was relatively short. The pollen tubes, by anchoring some grains, added to the confidence that grain positions were not artefacts of the

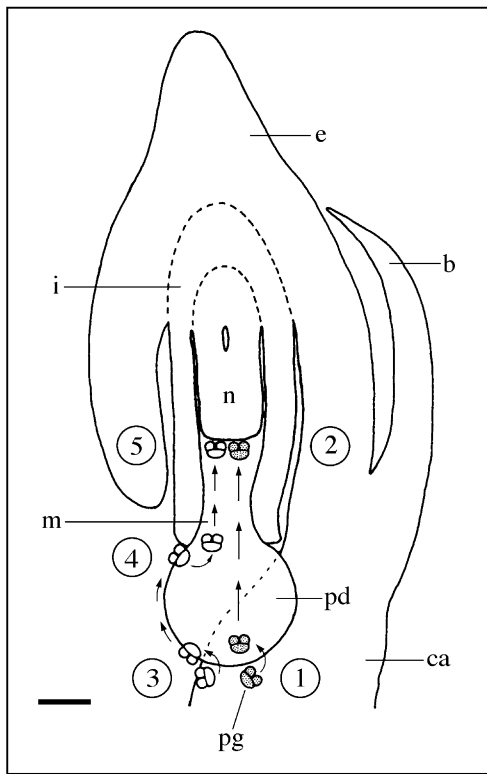


FIG. 5. Pollen capture in an ovule of *Prumnopitys ferruginea*. Stages 1 and 2 show the path taken by the majority of bisaccate pollen grains (shaded). Stages 3–5 show the path taken by grains (unshaded) which are initially trapped in the meniscus of the drop. Ovule and pollen grains drawn to scale; pollination drop and cone axis highly diagrammatic. b, bract; ca, cone axis; e, epimatium; i, integument; m, micropyle; n, nucellus; pd, pollination drop; pg, pollen grain. Stage 1, A dehydrated bisaccate grain (shaded) adhering to the cone axis, enters the pollination drop and begins to float up the micropyle, sacci-uppermost. Stage 2, A second or two later the bisaccate grain, just commencing hydration, comes to rest at the nucellus, sacci-uppermost. Hydration will continue until the grain is fully expanded in about 1 min. Stage 3, Another dehydrated bisaccate grain (unshaded) begins to enter the drop, but is initially held in the meniscus, unable to submerge due to surface air trapped between the sacci. It floats up the meniscus to the mouth of the micropyle. Stage 4, As soon as hydration begins to separate the sacci, the grain submerges and continues up the micropyle. Stage 5, This grain, despite the brief delay, still reaches the nucellus only partially hydrated. After a minute it too will be fully hydrated and expanded to full size.

preparation or sectioning processes. Many of the grains were compressed or distorted, either by closure of the micropyle or narrowing of the pollen chamber due to ovule development, but were still intact enough to be measured. Although a narrowing of the micropyle probably tilted some grains out of their original position, this would occur equally to grains facing towards or away from the nucellus at the time of arrival. It was considered that although distortion due to micropyle closure and ovule development would be likely to shift the average angle towards  $90^\circ$  this would not prevent detection of a tendency for grains to come to rest in a preferred position.

As shown in Table 2 and Fig. 4, the majority of grains were in the category 'facing the nucellus', suggesting that they had arrived at or near the nucellus with this orientation.

These results therefore support the hypothesis that bisaccate grains will tend to come to rest with the germinal furrow facing the nucellus. Some grains are affected by subsequent closure of the micropyle and growth of the ovule, which tend to alter the grain positions towards the  $90^\circ$  angle. Excess numbers of grains, or entry of grains to the micropyle from a peripheral position, may lead to grains bumping and jostling each other as they rise up the micropyle, resulting in grains not being in the optimal position. Furthermore, the pollen chamber can accommodate only a few grains (possibly five to six). Where grains are too abundant, late arrivals tend to accumulate along the micropyle, where they are likely to be crushed by growth of the closing cells.

## CONCLUSIONS

The experiments with water drops and water columns have provided useful information on the hydrodynamic properties of several pollen types which can help explain aspects of the pollination mechanisms. Only the fern and *Lycopodium* spores behaved as if unwettable, floating on top of the water surface, or resisting entry to a water column. All the conifer pollen grains tested, even those which floated, were wettable, in the sense that they all passed through the surface layer of the water. Once submerged beneath the surface layer, their different morphologies (functionally saccate or non-saccate) determined their behaviour in the variously shaped bodies of water, the functionally non-saccate grains sinking to the lowest point and the functionally saccate grains floating to the highest point of the water mass. Hydration was not necessary for pollen grains to enter the water, although saccate grain behaviour in inverted drops was found to vary depending on whether the grains were dehydrated when entering the drop (test 4, Fig. 2A) or hydrated (test 6, Fig. 2B). All the hydrated grains entered the drop without delay. Most of the dehydrated grains also entered the drop readily, but a small number initially failed to enter the drop and travelled up the drop surface to the drop margin (Fig. 2A) where they eventually hydrated. Such behaviour suggests an explanation for reports of grains floating on or in the meniscus of the drop (Tomlinson, 1994; Tomlinson and Takaso, 1998). The eventual hydration of these grains also indicates that such grains would also be able to float up the micropyle, that is, to reach the nucellus without the aid of a receding drop.

The non-random orientation of the pollen grains in ovules of *Prumnopitys taxifolia* and *P. ferruginea*, when viewed in combination with the hydrodynamic properties of the grains, has provided further evidence against the idea that bisaccate pollen is pulled into the ovule on a receding drop, at least in *Prumnopitys* (Tomlinson, 1994).

These results lead us to suggest, for *Prumnopitys* in particular, and probably for all podocarps with fully inverted ovules whether their pollen is bisaccate or trisaccate, a scenario that differs slightly from that proposed by Tomlinson (1994, Figs 20–25) for the behaviour of saccate pollen on encountering a pollination drop. Figure 5 shows a pollen capture sequence in an ovule of *Prumnopitys ferruginea*. When wind-borne bisaccate pollen grains arrive

and adhere to the cone axis, they are in the dehydrated condition, with sacci pressed together over the germinal furrow. Assuming it does not rain, they remain like this until they are captured by the pollination drop. As the pollination drop enlarges it fills the micropyle and then extends beyond the micropylar opening to the wettable surfaces of the cone axis. When contacted by the drop, a typical bisaccate pollen grain (shaded in Fig. 5) will immediately be submerged in the drop due to its wettability, and, floating sacci uppermost, will move rapidly up the micropyle towards the nucellus. The grain will arrive at the nucellus a few seconds after entering the drop and will come to rest, sacci uppermost. Since hydration takes about 1 min, the grain will reach the nucellus in a partially hydrated state. After a minute or so, the grain will be fully hydrated, with the sacci separated and the germinal furrow facing the nucellus. Some dehydrated bisaccate grains may initially be unable to enter the pollination drop because of external air trapped between the sacci folded over the distal pole. One such grain is shown (unshaded) in Fig. 5. On contact with the pollination drop this grain can only enter the drop surface, and it will therefore float sacci outwards up the meniscus until it lodges at the mouth of the micropyle, still in a dehydrated state. As soon as it becomes sufficiently hydrated for the sacci to start to separate, the trapped external air will be released and the grain will immediately submerge, whereupon it can continue up the micropyle, reaching the nucellus sacci uppermost. Since it has only been delayed a few seconds, the grain will still arrive at the nucellus in a partially hydrated state. After a minute it too will be fully hydrated and fully expanded, with germinal furrow facing the nucellus.

As is shown in Fig. 5, in the *P. ferruginea* ovule there is room for five or six grains at the nucellus in their dehydrated contracted state. The ovule of *P. taxifolia* has a similar capacity. If five or more grains arrive, expansion during hydration is likely to cause the grains to jostle and displace each other, so that very few retain their original position of arrival. They will mostly however retain their general orientation, and remain 'facing the nucellus'. The process in other podocarps with fully inverted ovules is likely to be similar. For instance, Tomlinson (2000, Figs 10 and 11) shows pollen grains germinating on the nucellus of *Podocarpus totara* D. Don ex Lamb. Despite the effects of crowding, most of the grains appear to be 'facing' the nucellus, especially those closest to it.

The hydrodynamic properties of the functionally saccate grains described here (namely buoyancy, wettability and a tendency to float distal pole uppermost) assist them in the pollination process, allowing rapid entry to an inverted ovule within a pollination drop, and arrival 'facing' the nucellus. Orientation of the grain is probably the least important of these, since pollen grains which land at a distance from the nucellus can readily grow pollen tubes (Fig. 3C; Tomlinson, 2000, Figs 10 and 11). This leads us to suggest that the suite of characters comprising the pollination mechanism of those podocarps possessing either morphologically or topographically inverted ovules act together to achieve three benefits, listed in order of importance: (1) the exclusion of foreign non-saccate pollen or spores, or foreign particles (e.g. dust) from the pollen

chamber (due to the combination of inverted ovule, pollination drop and saccate pollen); (2) rapid uptake of saccate pollen into the micropyle in a few seconds after entering the pollination drop (due to the wettability of the pollen); and (3) the preferred orientation of the bisaccate or trisaccate grain towards the apex of the nucellus (due to the distal position of the sacci, especially in the dehydrated grain, and the inverted ovule).

McWilliam (1958) observed that closure of the micropyle in *Pinus* occurred soon after pollination, and any pollen remaining in the micropyle was crushed. Micropyle closure is likely to be relatively rapid in *Prumnopitys* as well, because both open and closed ovules were collected at pollination time. Crushed pollen grains were seen in the micropyles of some ovules. Thus, although the position of the sacci is likely to cause the bisaccate grain to float germinal furrow upwards, the combination of functional sacci and wettability is more important, ensuring rapid entry of the grain to the pollination drop and rapid passage up through the micropyle to the pollen chamber, usually before being fully hydrated. Only a few of the grains that reach the pollen chamber are likely to germinate. Grains which land germinal furrow upwards would be likely to be the first to succeed, all other factors being equal.

These observations have implications for the pollination mechanisms in all conifers that produce inverted pollination drops. It is interesting to note that in *Picea orientalis* (L.) Link (Pinaceae) the apparent anomaly of sinking saccate pollen is correlated with unusual pendant ovulate cones in which the morphologically inverted ovules have topologically erect micropyles (Runions *et al.*, 1999). Clearly, for an understanding of the pollination mechanisms of species in Pinaceae, Podocarpaceae and Phyllocladaceae, an important distinction is not presence or absence of sacci, but whether or not any sacci are functional as flotation devices. Moreover, given the diversity of conifer pollination mechanisms already described, many other interesting variants of the pollination mechanism await study.

#### ACKNOWLEDGEMENTS

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