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SHORT COMMUNICATION

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Micropylar exudates in Douglas fir – timing and volume of production

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Abstract In *Pseudotsuga menziesii*, a secretion fills the micropylar canal about 7 weeks postpollination until fertilization. Micropylar volumes were measured and found to show variation. Dissection of the ovuliferous scales caused excess fluid to be exuded from the micropylar canal, forming a drop at the tip of the micropyle. This drop was collected, and its production quantified in three trees. Volume and percentage of ovules with drops were greatest when the archegonia in the female gametophyte were at central cell and/or egg cell stage. The volume of exuded drops far exceeded that of the micropyle. Production of subsequent drops by the ovules further confirms that the fluid is actively produced upon dissection

Key words *Pseudotsuga menziesii* · Ovule secretions · Micropyle volume

Introduction

How sexually reproductive organisms select gametes is of fundamental importance. Haig and Westoby (1989) have speculated that the process of pollination in plants is one of the major driving forces in evolution of seed plants. From a less theoretical perspective, once sexual barriers are bypassed, opportunities exist for creation of novel genotypes with economic potential such as those provided by in vitro fertilization. Basic to all application and manipulation is a sound understanding of these barriers. In Douglas fir, wind-blown pollen grains arrive at the tip of the micropyle where extensions of the integument direct the pollen to the entrance to the ovule (Owens et al. 1981). The pollen then need only penetrate the nucellus to reach the megagametophyte, which houses the eggs. The pollen path is considered to be spatially shorter and developmentally less complex than that found in flowering plants. However, gymnosperm pollen generally spends more time than angiosperm pollen in the sporophytic (maternal) tissue. Pollen durability itself becomes an issue. Douglas fir pollen may spend up to 10 weeks in the micropyle before fertilization is completed. In contrast, the egg is viable for little more than a week (Fernando et al. 1996). The coordination of pollen growth is, therefore, of critical importance. Secretions play a role in triggering pollen germination (Said et al. 1991; Takaso et al. 1996). Furthermore, this fluid secretion is developmentally restricted, implying a role in coordinating prefertilization events.

Neither the origins of the secretion nor its contents are understood although there have been suggestions that it may originate in the megagametophyte (Takaso and Owens 1996). Towards more fully understanding the role this fluid plays in reproduction we have undertaken to establish the time-course of the fluid drop and to quantify this secretion. Our second goal is to verify the presence of the post-pollination secretion within the micropylar canal in vivo.

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Materials and methods

Cones of Douglas fir, *Pseudotsuga menziesii*, were collected from the University of Victoria campus. Three different trees were used and collections were made weekly for 6 weeks following pollination, which had been closely monitored earlier in the season. More intensive collection was done daily for the next 4 weeks. Weekly collections were again carried out during later stages of seed development.

Ovuliferous scales were dissected from cones and placed in wet petri dishes. At 15 min after dissection, the drops were collected from the micropylar tips of the exposed ovules on a dissecting microscope (Wild) fitted with a fibre optic system using a micropipette tip. Once the drops were collected, the ovules were left in the petri dish to monitor for the production of subsequent drops. The number of drops collected per number of ovules was recorded and used to calculate the average drop volume. Five megagametophytes were cleared daily with methyl salicylate (Fernando et al. 1996) to determine the developmental stage of the female gametophyte.

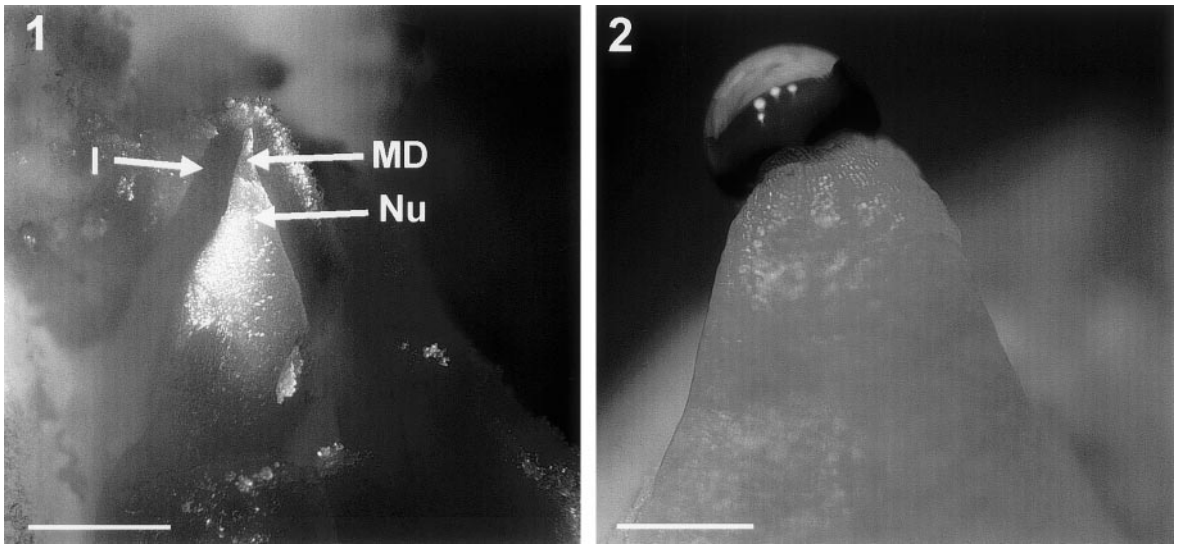

Fig. 1, 2 *I* Integument, *MD* micropylar drop, *Nu* nucellus. **Fig. 1** Photomicrograph of ovule frozen in liquid nitrogen in situ and dissected to reveal the frozen micropylar drop (*MD*), located between the integuments (*I*) above the nucellus (*Nu*). *Bar* 1 mm. **Fig. 2** Photomicrograph of a drop formed at the apex of an intact ovule following dissection. *Bar* 500 μm

To verify the presence of the secretion drop in vivo, *Pseudotsuga* cones were submerged in liquid nitrogen while still attached to the tree. The frozen cones were then severed, and stored in liquid nitrogen until dissected under a microscope to reveal the micropylar canal and the presence or absence of the drop frozen within. Dry ice was used to keep the cones frozen during dissection. The volume of the micropylar canal was calculated using the formula for a truncated cone,

$$V=1/3\ \pi(r_B r_T+r_T^2+r_B^2)h,$$

where h is the distance from the top of the nucellus to the tip of the micropyle and r_T and r_B correspond to one half of the top and bottom widths of the micropylar canal, respectively. By calculating the volume of the micropylar canal, the volume of the secretion in vivo could be estimated and compared to the volume of the exuded dissection drop collected in vitro.

Four or five cones were collected daily from the three *Pseudotsuga* trees selected. All bracts were removed from the cones. The mean number of bracts per cone for these trees is 40±8.5, resulting in collection of approximately 320 ovules per tree each day.

Results and discussion

It was possible to find secretions for a 2-week period covering stages of late central cell and egg cell development. Ovules frozen on the tree and then dissected over dry ice contained secreted liquid which occupied all or most of the micropyle (Fig. 1). All trees examined during the period of drop production had liquid in the micropyle. These drops were never found extruded outside the apex of the micropyle on intact cones. When ovules attached to the ovuliferous scale/bract complex were removed from cones that had not been frozen and placed on petri dishes, they produced secretions beyond the apex of the micropyle (Fig. 2), confirming previous observations of dissection-related drops in larch (Barner

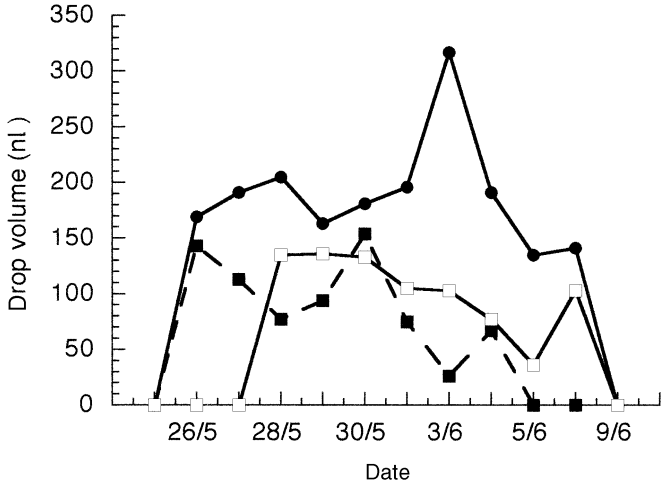

Fig. 3 Graph of the percentage of ovules from trees 1 (●) and 2 (□) and 3 (■) during the period of exudation.

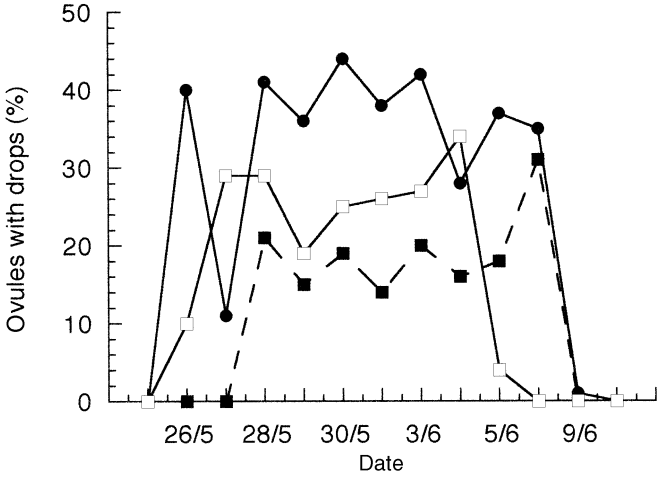

Fig. 4 Graph of mean nl/drop in ovules from trees 1 (●), 2 (□) and 3 (■) during the period of exudation

and Christianson 1960) and Douglas fir (Barner and Christianson 1962). Removal of the drop emptied the micropylar chamber as immediate freezing and dissection revealed (not shown). Once removed, the ovules were able to produce up to three drops.

Production was measured by calculating, the percentage of ovules producing drops (Fig. 3) and the volume of drops (Fig. 4) produced. There was variation between trees. Tree 1 produced the largest drops, averaging 196 nl each. The average volume of the micropylar canals ranged from 24±5.6 nl (tree 3) to 58±14 nl (tree 1). The volume of the exuded drops was found to be 1–4 times the micropylar volume.

Our results confirm the hypothesis that this secretion is a postpollination phenomenon (Villar et al. 1984), unrelated to pollination drop mechanisms found in some other conifers (Owens and Morris 1990). The drop has been implicated in the induction of in ovulo pollen germination (Said et al. 1991; Takaso et al 1996), as all pollen germination occurs during the same 2-week period during which drop secretion occurs. The predictable occurrence and abundance of this post-pollination/pre-fertilization drop is coincident with egg-ripening, which suggests that in *Pseudotsuga* it plays a significant role in prefertilization events, such as male selection (Said et al. 1991).

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