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STIGMA RECEPTIVITY DURING THE LIFE SPAN OF *PLATANTHERA CHLORANTHA* CUSTER (RCHB.) FLOWERS

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Stigma receptivity of *Platanthera chlorantha* was examined in a laboratory experiment. Flowers were hand-pollinated at bud stage and at different intervals between the beginning and the 14th day of anthesis. Afterwards, pollen tube growth was examined by fluorescence microscopy. Stigma receptivity was also tested for the presence of peroxidase using the Peroxtesmo Ko test. The efficiency of hand-pollination in flowers of varying ages was confirmed by capsule formation. Pollen tubes started to germinate 6 h after pollination. The stigma was overgrown with pollen tubes 24 h after pollination. The stigma became receptive at the bud stage. Receptivity lasted 15 days on average. Pollen did not germinate on stigmas with a dry surface, in flowers with a dehiscent perianth. Pollination did not affect stigma receptivity. Pollen tubes germinated from pollinaria deposited on the stigma additionally 6 days after the first pollination. Fluorescence microscope observations of pollen tube germination produced results corresponding to those obtained with the Pertexmo Ko test.

Key words: *Platanthera chlorantha*, Orchidaceae, stigma receptivity, pollen tube, pollination.

INTRODUCTION

A characteristic feature of orchid flowers is their longevity. This feature is connected with adaptation to low-level pollinator activity. Orchids often rely on specific, usually rare pollinators. Having long-lasting flowers, ready and waiting for possible pollination, seems a very efficient strategy (Ashman and Shoen, 1994). The effective pollination period is closely linked with the duration of stigmatic receptivity (Sanzol and Herrero, 2001).

The stigma in orchids occupies a shallow depression at the apex of the column beneath the anther cap. At anthesis it is filled with viscous fluid. According to Helsop-Harrison and Shivanna (1977), the Orchidaceae family shows a wet-type stigma with a receptive surface having low to medium papillae. However, observations by Calder and Slater (1985) and Slater and Calder (1990) on the stigma of *Dendrobium* revealed that detached cells are suspended in the viscous material filling the stigma and classified such stigma as "wet-detached-cellular" type. Similar stigmas were observed in Oncidinae (Clifford and Owens, 1990) and *Epidendrum* (Yeung, 1988).

The stigma of the investigated orchids may retain receptivity for a long time (up to 60 days). While the cuticular layer covering the stigma may protect and prolong the life of the stigmatic cells (Helsop-Harrison, 2000), the orchid stigma without a cuticular layer, as in *Dendrobium* (Calder and Slater, 1985), can also remain receptive over long periods.

The aim of the present work was (1) to determine the duration of stigma receptivity in *Platanthera chlorantha* flowers, (2) to check whether pollination stops receptivity, and (3) to compare the results on the presence of peroxidase with pollen germination ability in vivo in flowers of varying ages.

MATERIALS AND METHODS

Stigma receptivity in *Platanthera chlorantha* from a forest near Lublin was examined in 2001 and 2002. One week before flowering started, 23 plants were transplanted to pots and transported to the laboratory

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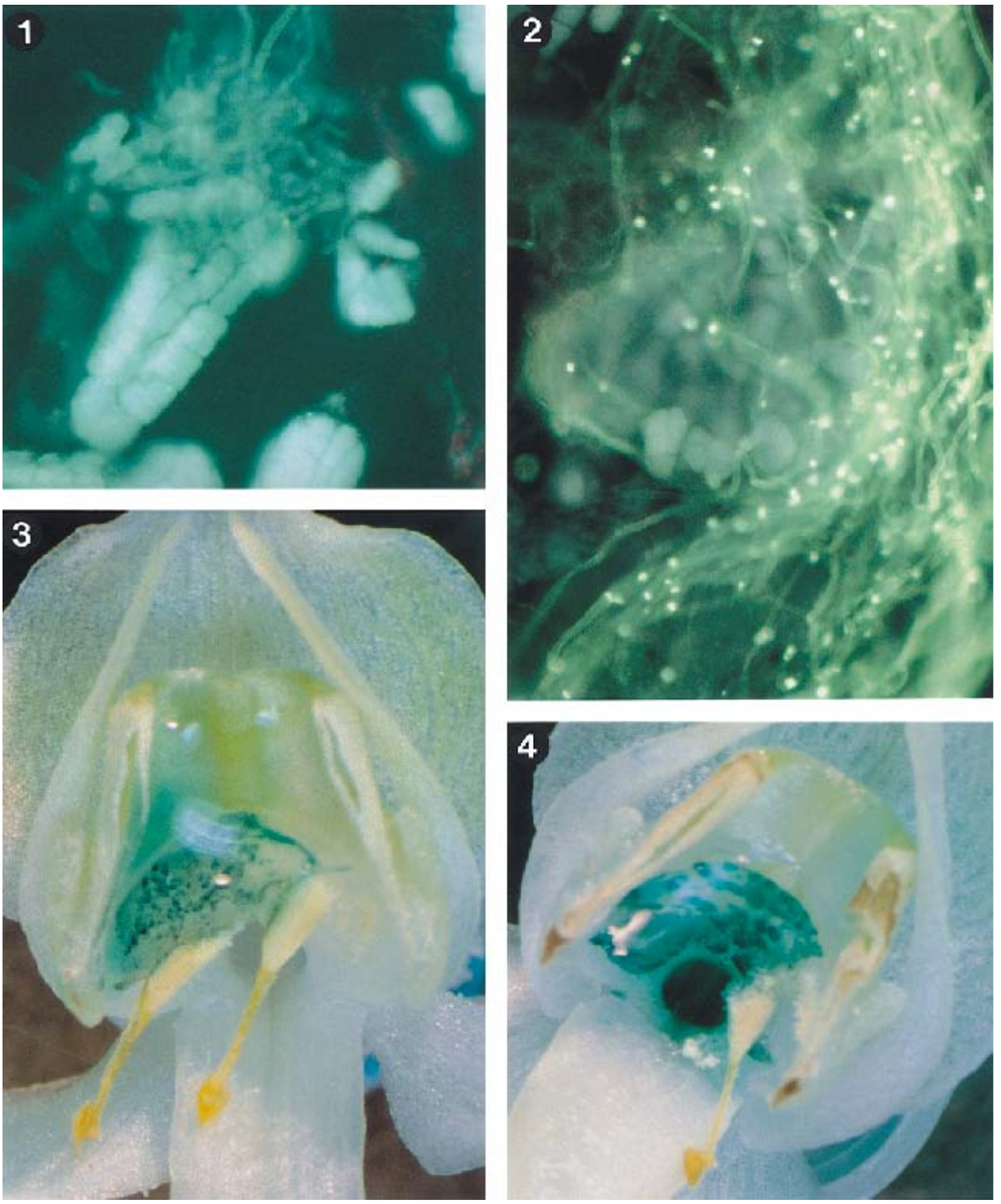

Fig. 1. Pollen tube germination 6 h after pollination. × 190. **Fig. 2.** Pollen tubes 24 h after pollination on stigma pollinated additionally, 6 days after the first pollination. × 190. **Fig. 3.** Stigma of flower pollinated on the 2nd day of anthesis and pollinated additionally on 8th day of anthesis. After 12 h the test is still positive (blue staining). × 19. **Fig. 4.** Positive Peroxtesmo Ko test 7 days after pollination. × 19.

in the Department of Botany of the Agricultural University in Lublin. In the examined inflorescences, 365 flowers (10–20 per inflorescence) were marked with a colored pencil on the day of full perianth opening. All visible changes connected with pollination and/or senescence were noted in each individual flower.

Five flowers were hand-pollinated at bud stage (1 day before anthesis) and 130 flowers were pollinated once with one pollinarium (xenogamy) at different intervals between the beginning and the 14th day of anthesis, in order to examine pollen tube growth in flowers of various ages.

To test whether pollination stops stigma receptivity and to see how long the stigma remains receptive after pollination, 40 flowers were pollinated initially on the 2nd day of anthesis and then at different intervals between the 1st and 6th days after the first pollination. Afterwards, for microscopic examination the stigmas were sampled one day after pollination and softened in 0.1 M NaOH at 60˚C for ~1 h. In bud-stage flowers the stigmas were sampled 6 h after pollination. Squashed stigmas were incubated with 0.1% aniline blue for 24–48 h. Slide preparations were viewed under a fluorescence microscope (Nikon Optiphot II) with blue (410 nm) excitation. In the case of the flowers with additional pollination, the stigma was divided into two parts, each with one pollinarium, and pollen tube growth on these two parts was examined separately.

Stigma receptivity was also tested by the presence of peroxidase using the Peroxtesmo Ko test, applied according to a modification of the method of Dafni and Maués (1998): one 15 × 15 mm Peroxtesmo Ko paper (MACHEREY-NAGEL D-52313 Dren, Germany) was soaked in 1 ml distilled water. A droplet of the fresh solution was applied directly onto the stigmas of untouched 30 flowers of varying ages, from buds (one day before anthesis) to 14-day-old flowers. In 25 flowers pollinated at the 2nd day of anthesis, the test was made from 12 h to 7 days after the pollinaria were deposited on the stigma.

The time necessary for pollen tube germination was tested 1, 3, 6, 12, and 24 h after pollination.

In 126 flowers hand-pollinated at different intervals between the 2nd and 19th days of anthesis, the efficiency of pollination was checked by capsule formation.

RESULTS

Platanthera chlorantha flowers are aggregated into spikes. The flowering period of an individual inflorescence lasted 2–3 weeks. Unpollinated flowers lived 15.5 days on average. After pollination, pollen tubes emerged from the massulae within 6 h (Fig. 1). The stigma was overgrown with pollen tubes 24 h after pollination (Fig. 2). At this time the first pollen tubes were observed in the ovary.

Receptivity was observed in buds one day before anthesis. At this stage the stigmas were able to sustain pollen tube germination and tested positive for peroxidase (Fig. 5), but did not have fluid on their surfaces and pollinaria did not adhere to them. Fluid appeared on the stigma surface concomitantly with anthesis or up to 24 h after it. Stigmatic receptivity lasted 15 days on average (range 7–19 days), to the end of the flower's life (Figs. 6–8). Pollen tubes did not germinate on stigmas with a dry surface in flowers with a dehiscent perianth. These flowers also showed negative results with the Peroxtesmo Ko test.

While pollination did not affect stigma receptivity, it did shorten flower longevity and the receptivity period. Stigmas retained receptivity one day after pollination and also 6 days after it. They were still wet and capable of pollen germination (Fig. 2) and turned blue in the Peroxtesmo Ko test (Figs. 3, 4).

On average, 89.68% of the hand-pollinated flowers set capsules (range 83.33–100%). Pollination was unsuccessful in flowers with a drying perianth. Pollination did not result in fruit set in flowers pollinated at maximum nectar secretion (when nectar escaped from the spur and the stigmatic cavity was covered with nectar).

DISCUSSION

The results demonstrate that stigma receptivity lasts 15 days on average in *Platanthera chlorantha* flowers. The examined flowers showed receptivity between the bud stage and the end of anthesis. As in *Platanthera*, Shivanna and Sastri (1981) detected stigma surface esterase and receptivity in early-stage pistil development, irrespective of the presence or absence of stigma exudates. Orchid species showed stigmas receptive throughout anthesis (Clifford and Owens, 1998). In numerous plant species stigmatic receptivity decreases as the flower ages. At senescence, the stigmatic papillae in *Actinidia* lost their integrity, cellular content was released into the stigmatic fluid, and the secretion contained phenolic compounds which may regulate whether pollen germination occurs (González et al., 1994; 1995).

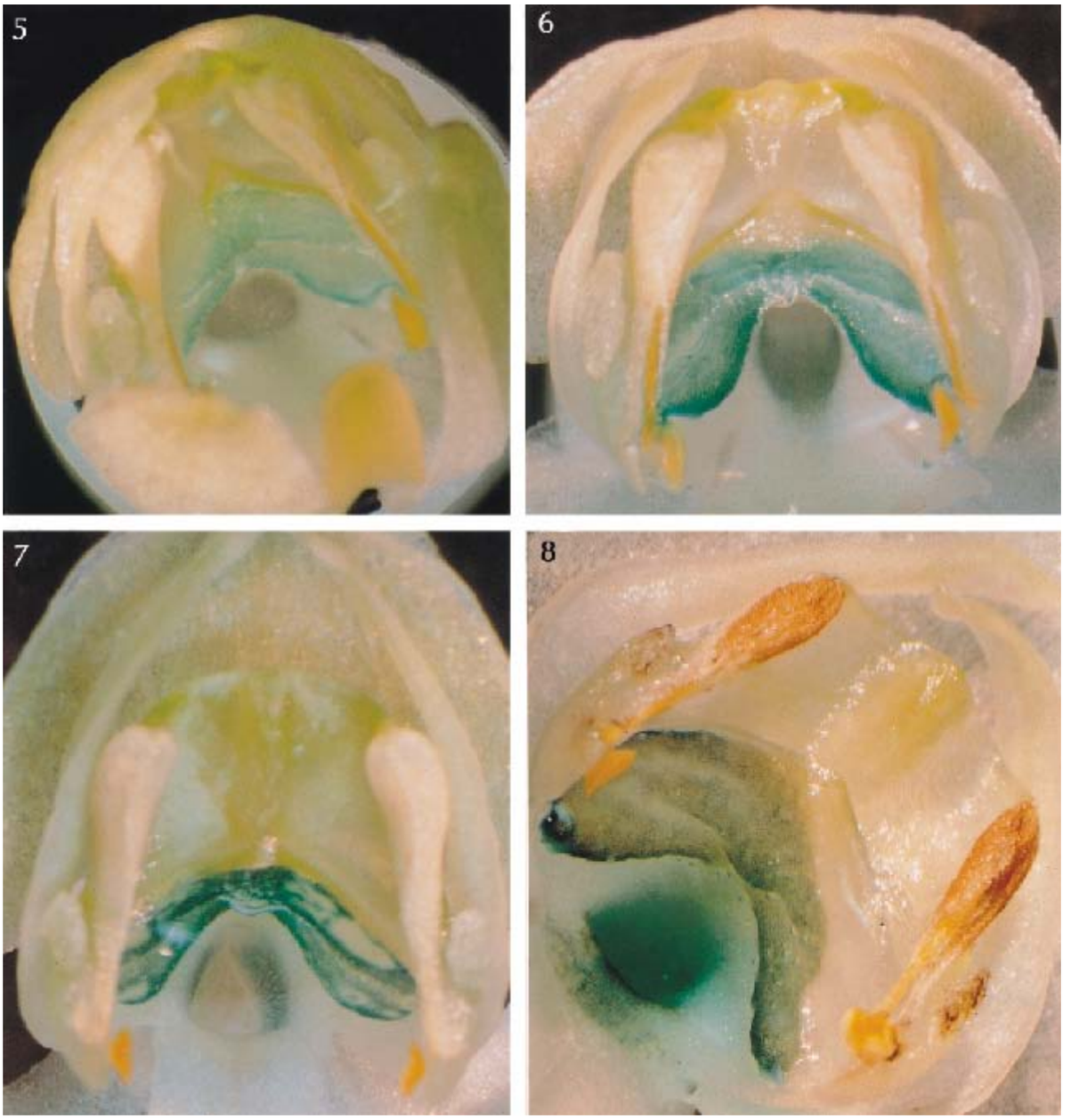

Fig. 5. Positive Peroxtesmo Ko test at bud stage. × 20. **Fig. 6.** Positive Peroxtesmo Ko test in freshly opened flower. × 20. **Fig. 7.** Positive Peroxtesmo Ko test in 2-day-old flower. × 20. **Fig. 8.** Positive Peroxtesmo Ko test in 15-day-old flower. × 20.

Platanthera chlorantha flowers with a drying perianth did not form capsules after pollination. Pollination was also unsuccessful in flowers with a fresh perianth when the stigmatic cavity was filled with nectar. The situation is the reverse in *Asclepias syriaca*, where nectar in the stigmatic chamber is needed as a germination medium for pollen (Kevan et al., 1989).

Pollination did not affect the duration of stigma receptivity in *P. chlorantha* flowers. The stigmas of flowers tested 6–7 days post-pollination continued to show a positive reaction for the presence of peroxidase, and were capable of pollen germination. Neiland and Wilcock (1995) reported similar findings: pollinated orchid flowers remained receptive for 8

days. Tropical orchids are characterized by more rapid post-pollination changes (Arditti, 1976). In Oncidiinae, stigma closure in pollinated flowers was observed within 1.76 days (Clifford and Owens, 1998). Sedgley (1979) found that pollination in avocado resulted in degeneration of the cytoplasm of stigmatic papillae, and suggested that it is of some importance in pollen tube nutrition. In some other plants, such as oil palm (Tandon et al., 2001), *Acacia* (Kenrick and Knox, 1981; Marginson et al., 1985) or *Citrullus* (Sedgley and Scholefield, 1980), pollination induced the release of stigmatic fluids as a post-pollination response. In these species, post-pollination secretions also provided additional nutrients for pollen tube growth. A different reaction was observed in blueberry, where fluid production stopped when the stigmatic surface was saturated with pollen tetrads (Parrie and Lang, 1992).

Stigma receptivity is a very important factor influencing effective pollination (Sanzol and Herrero, 2001). When it is restricted to a short period, it may limit fruit set as in apricot (Egea et al., 1991) or kiwifruit (González et al., 1995). Thus the need for research on easy and reliable methods of estimating stigma receptivity. Dafni and Maués (1998) compared the effectiveness of four tests for stigma receptivity, defined by the presence of different enzymes or peroxide. In their experiment the Peroxtesmo Ko test seemed the most reliable and advantageous one, and was recommended as a fast indicator of stigma receptivity. In *P. chlorantha* the results of the Peroxtesmo Ko test for the presence of peroxidase were fully in line with pollen germination in vivo, so the test is very useful for estimating stigma receptivity in this species.

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