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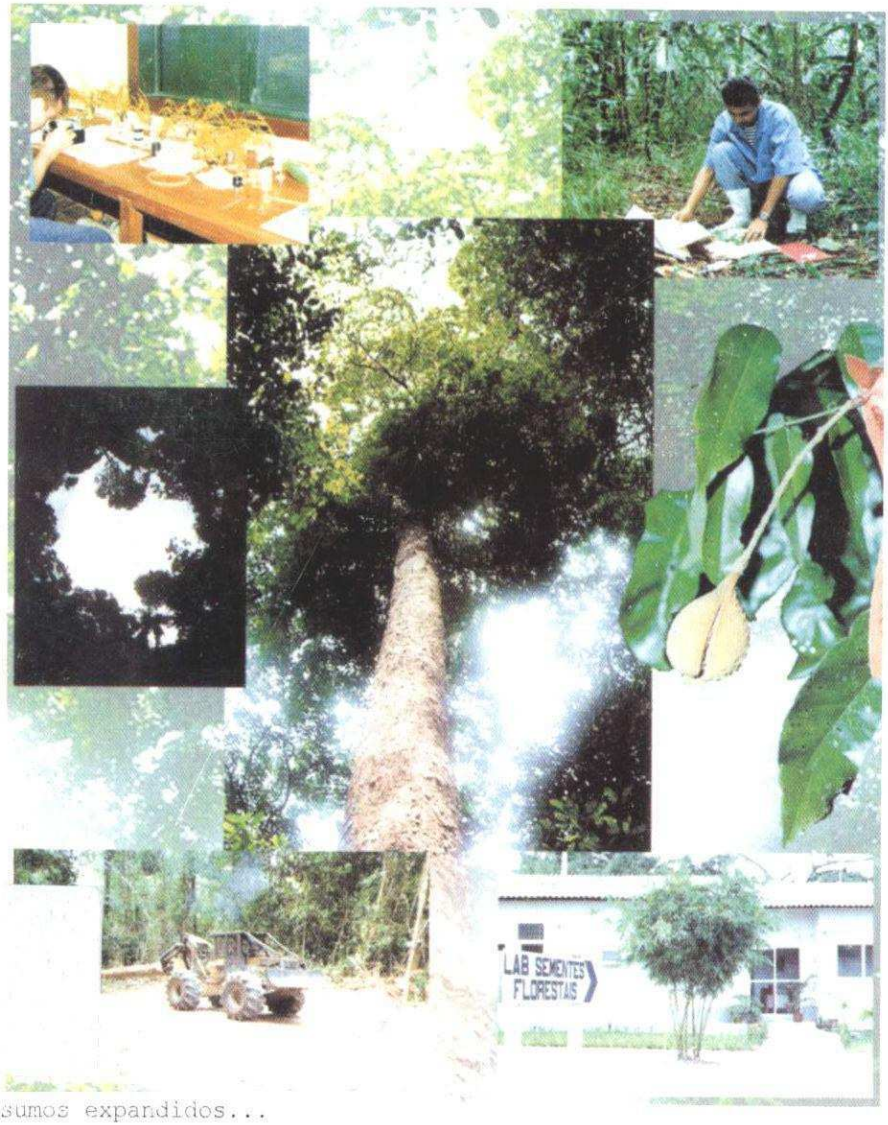
Simpósio

SILVICULTURA NA AMAZÔNIA ORIENTAL: CONTRIBUIÇÕES DOPROJETO EMBRAPA/DFID



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A RAPID AND SIMPLE PROCEDURE TO DETERMINE STIGMA RECEPTIVITY[1]

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Stigma receptivity is a crucial stage in the maturation of a flower which may greatly influence the rate of self-pollination , pollination success at different stages in the flower cycle, the relative importance of various pollinators, the interference between male and female functions, the rate of competition via improper pollen transfer, and the chances of gametophytic selection (Galen et al. 1987).

Any success in breeding experiments or artificial pollination procedures should be accompanied by tests on timing and duration of the stigma's receptivity (Stone et al. 1995). Receptive stigmas are characterised by high enzymatic activity. The presence of several enzymes is found to coincide with this developmental stage (Knox 1984; Shivana and Rangaswamy 1992) and consequently most of the methods to determine stigma receptivity in vitro are based on the identification of enzymatic activity (see Knox et al. 1986; Dafni 1992; Kearns and Inouye 1993 for reviews).

In practice, each method must be calibrated for each plan species (Firmage and Dafni 1997) and, if possible, by comparison to in vivo pollen germination on the stigma (Stone et al. 1995). This paper presents a simple method – the identification of esterase presence using a (Peroxtesmo Ko) paper indicator by converting it into a solution. The efficiency of this procedure is compared with three other methods for 14 plan species from Brazil.

Freshly cut stigmas of 14 species were collected in a secondary vegetation and primary forest in the National Forest of Tapajós, at Belterra (100km S of Santarém) or in experimental plots in Belém, at the Brazilian Agricultural Research Organisation – EMBRAPA, Centre for Agroforestry

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Research of the Eastern Amazon (Embrapa Amazônia Oriental) – in Brazil.

All stigmas examined were checked under a magnifier (x30) for presence of pollen and for any damage to the surface, either of which may cause enzymatic activity regardless of stigma receptivity (Dafni 1992; Kearns and Inouye 1993). From each species we used 30 to 50 stigmas at different stages of flower development and the results reflect the findings at the peak of receptivity.

Four tests were used to compare their effectiveness. Three methods test for the presence of different enzymes and one for peroxide.

1. Baker's procedure (Dafni 1992; Firmage and Dafni, unpublished): This test detects the presence of alcohol dehydrogenase. The fresh stigmas were cut and removed in the field directly into a large droplet of this test solution on a slide and incubated at room temperature in a closed Petri dish containing a moist filter paper in the bottom. The stigmas were inspected after 20-40 minutes under a magnifier (x20) or a microscope (x200) to locate the stained areas.

2. Perex Test (Firmage and Dafni 1997): This solution (Merck chemical 16206) tests for the presence of hydrogen peroxide. A droplet of solution was placed directly on the stigma and inspected after several minutes for yellow to orange coloration.

3. Hydrogen peroxide: A 6% solution was placed on the stigma and the appearance of bubbles was observed (Zeisler 1933).

4. Macherey-Nagel Peroxtesmo Ko peroxidase test paper (Motten 1982; Sullivan 1984): The normal use is to apply the paper directly on the stigma, and the appearance of a blue colour indicates the presence of peroxidases; however, the paper does not work on dry stigmas. Dafni (1992), Kearns and Inouye (1993) and Firmage and Dafni, unpublished found that if the paper was first briefly dipped in a drop of distilled water it was more effective (in general) and was also usable on some dry stigmas.

We went one step further and soaked one paper (15x15mm) in 1ml of distilled water and applied a droplet of the solution directly onto the stigma. If the stigma was very dark, and the blue colour was not noticeable, we left the Peroxtesmo solution droplet for three min and then collected the droplet with a small wedge of Whatman Number 1 paper to see if it had turned blue (1-3µl of the solution is adequate).

The results are presented in the Table 1. It is apparent that, except for *Carapa guianensis* (which shows no response to any of the chemicals) the

Peroxtesmo test was the only procedure that showed correspondence with all of the other tests when they indicated receptivity. There was not a single case where the other tests showed a positive response and the Peroxtesmo Ko solution a negative response.

Thirteen species reacted positively with the Peroxtesmo test, as well as with hydrogen peroxidase, 12 with Perex and 10 with Baker's test. There was full correspondence in the stained areas with Baker's and Peroxtesmo's tests, while in the Perex test it was hard to locate the coloured area.

It was found that the Peroxtesmo solution remains active under tropical conditions of 25-32°C for at least 4-5 days. One ml of the solution is sufficient for 50-100 tests.

We compared the four methods used to determine stigma receptivity. It is noted that the hydrogen peroxide may also react with old, non-receptive stigmas (Dafni unpublished), it is not quantitative and does not locate the receptive area. It is not advisible to use the hydrogen peroxide as the only indicator. The Perex test has the advantage of quantification and simplicity of application, but it has to be handled with care (it contains sulphuric acid) and is hard to locate the exact receptive areas. Although Baker's test gave good discrimination in most of the species and results were fully in accordance with Peroxtesmo test results, this method needs about 30 min before results can be read and some pre-test preparations. The Peroxtesmo Ko solution test indicates the presence of peroxidase – a reliable indication for stigma receptivity (Kandaswamy and Vivekanadan 1985; Schou and Mattson 1985; Galen aand Plowright 1987; Dupius and Dumas 1990; but see Ziestman and Botha 1995). The Peroxtesmo test has several advantages. The procedure for its preparation and application is simple, it has a long shelf life and is widely available. It also gives instant and accurate results, the exact location of the receptive areas and it can be used for dark stigmas when Baker's test and Perex solution are not applicable. It is efficient for dry as well as wet stigmas.

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SPECIES	BAKER'S	PEROXTESMO	H2O2	PEREX
Schizolobium amazonicum (Leguminosae)	Only the tip of the stigma stained dark brown-purple	Only the tip of the stigma turned blue	+	The tip of the stigma stained yellow (50-200)
Carapa guianensis (Meliaceae)	No response	No response	No response	No response
Jacaranda copaia (Bignoniaceae)	Only the 3-5mm inner surface of the forked stigma's tip stained purple-brown	Only the 3-5 mm inner surface of the forked stigma's tip stained blue	++	Only the 3-5 mm inner surface of the forked stigma's tip stained orange
Quassia amara (Simaroubaceae)	The stigma tip stained blue	The stigma tip stained blue	++	The stigma tip stained orange
Cedrela odorata (Meliaceae)	The stigma centre stained dark purple-brown	The stigma centre stained blue	+	No response
Bellucia sp. (Melastomataceae)	The whole multi-lobed stigma was stained brown	The whole multi-lobed stigma was stained blue	++	The whole multi-lobed stigma was stained orange
Passiflora foetida (Passifloraceae)	The wole surface stained dark blue	The wole surface stained blue		The whole surface stained light yellow (100)
Swietenia macrophylla (Meliaceae)	Only the centre of the stigma tained purple-brown	Only the centre of the stigma was stained blue	++	Only the centre of the stigma stained light orange
Marmaroxylon racemosum (Legominosae)	The stigma was stained purple around the depression (tip)	Only the 1mm tip and depression of the stigma was stained blue	+ Only in the groove	medium weak (100)
Cordia goeldiana (Boraginaceae)	No response on the stigma, but the pollen gains stained blues	Positive and immediate reaction (blue) on the whole stigma surface	++	Positive strong reaction with orange colour on the whole of the stigma surface (500)
Solanum juripeba (Solanaceae)	No response	Stigma stained blue, strong and queak reaction!	++	Slight yellow colour on the stigma surface. Immediate reaction (100)
Solanum crinitum (Solanaceae)	No response. The pollen on the stigma stained blue	Strong reaction, deep blue!	++ Strong reaction	Immediate orange (200)
Tabebuia serratifolia (Bignoniaceae)	Only tips of the papillae turned dark blue	Only the tips of the papillae	++	Weak reaction (100)
Hymenaea parvifolia (Leguminosae)	Only the base of the papillae stained in deep blue	Only the base of the papillae stained blue	+++ Over all the stigma, specially in the tip	Strong reaction (500) only in the tip