Ovule Receptivity and Pollen Viability in Japanese larch (Larix Leptolepis Gord.)

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Summary

Ovule receptivity and pollen viability were studied in order to determine the cause of low seed production in the hybridization of *Larix*. Ovule receptivity may be less than 48 hours for samples kept in a temperate greenhouse. Some external morphological characters of the female conelet may be used as indicators of ovule receptivity; specifically, cone curvature upwards at a 90° angle with respect to the short shoot and full opening of the bracts. Pollen stored at 4° C may remain viable for at least 3 to 12 months as estimated by fluorochromatic reaction.

Key words: gymnosperm, larch, receptivity, pollen, viability.

Résumé

Dans le but de déterminer les causes du faible rendement en graines lors d'hybridations dans le genre *Larix*, les deux paramètres principaux conditionnant la réussite de la pollinisation ont été étudiés. Il s'agit d'une part de la réceptivité ovulaire, d'autre part de la viabilité pollinique.

La réceptivité ovulaire est brève. Elle peut être estimée à moins de 48 heures chez des individus élevés en serre. Elle est repérable grâce à des caractères morphologiques du cône femelle: courbure à 90° de l'axe du cône sur le pédoncule et écartement des écailles. La viabilité du pollen appréciée par le test fluorochromatique peut se prolonger de 3 à 12 mois au moins en stockage à 4° C.

Introduction

Larix decidua Mill., the European larch, is a highly-prized wood. Unfortunately, the species has a limited distribution in France at high elevations, which prevents its use in reforestation. The hybrid *Larix eurolepis* Henry, which results from a cross between *L. decidua* and *L. leptolepis* (the Japanese larch) can grow successfully both at low and moderate elevations. This hybrid shows a distinct growth superiority compared to the pure species. Its vigor has been frequently noted in Europe (Bastien and Keller, 1980; Schober, 1981; Paques, 1989). However, increased use of the hybrid in active reforestation is hindered by low seed production. In fact, *L. eurolepis*, like its parents, is characterized by a low seed yield. Several factors have been reported to explain this low seed production: influence of external factors, especially temperature, on microsporogenesis (Ekberg and Eriksson, 1967) and megasporogenesis (Hall and Brown, 1977; Kosinski, 1986), lack of pollination (Hall and Brown, 1977; Kosinski, 1986) and early embryo degeneration as a result of self-pollination (Kosinski, 1982, 1986).

In order to increase the yield of hybrid seeds using controlled pollination, the pollen viability and the ovular receptivity of each parent must be known. Since the beginning of the century many aspects of sexual reproduction have been described in *Larix* (review in Owens and Molder, 1979; review in Chesnoy, 1987; Korinekova et al., 1988): however, never have pollen viability and ovular receptivity been studied simultaneously. Therefore, this paper is a report on a study of the structure and the viability of the pollen grain as well as the receptivity of the female cone and of the ovule of *Larix leptolepis*.

Material and Methods

Plant Materials

Specimens of *Larix leptolepis* (Sieb. and Zucc.) Gord. synonymous with *Larix kaempferi* (Lamb.) Carr. grown in containers in the National Institute of Agriculture Research (INRA) Station of Orleans (France) were conveyed to our laboratory (University of Lyon, France) in late January and were kept in a temperate greenhouse (15° C to 18° C) during the flowering time.

Pollen viability

Fresh pollen grains were collected from pollen cones at the time of pollen shedding (early March in Lyon). They were divided in two lots: the first was kept at 20° C in open air whilst the second was stored at 4° C in sealed glass vials.

Pollen water content was determined after drying for 5 min. at 85° C with an infrared dessicator (1265 MP Sartorius Göttingen, FRG). It was expressed as a percentage of the fresh weight. The determination was made immediately after collection of fresh pollen, or after one year at 4° C for stored pollen.

Pollen germination in vitro was tested at 28° C in the dark, on solid medium (agar 1%) with Brewbaker and Kwack (1963) mineral salts, saccharose (20%) and ovule homogenates. The homogenates were made at 4° C, either with ovules isolated from cones collected at pollination (P) or with ovules dissected from cones collected at fertilization (F), i.e. six weeks after pollination. Fresh pollen was sown on the P-homogenate medium whereas the pollen kept six weeks at 4° C was sown on the F-homogenate medium. In order to visualize pollen development during germination tests, the pollen grain was stained with Auramine O and Calcofluor White (Heslop-Harrison, 1979) and the pollen nuclei were stained by the acetocarmin method (Johansen, 1940).

The fluorochromatic reaction test (FCR test, Heslop-Harrison and Heslop-Harrison, 1970) was performed in order to estimate pollen viability. The test is based on the penetration of fluorescein diacetate (nonfluorescent and apolar) into the pollen grain and its hydrolysis by an esterase to yield fluorescein (fluorescent and polar). Viable pollen shows a bright yellow fluorescence and is, therefore, FCR-positive in contrast to FCR-negative which does not fluoresce. For each sample, around 400

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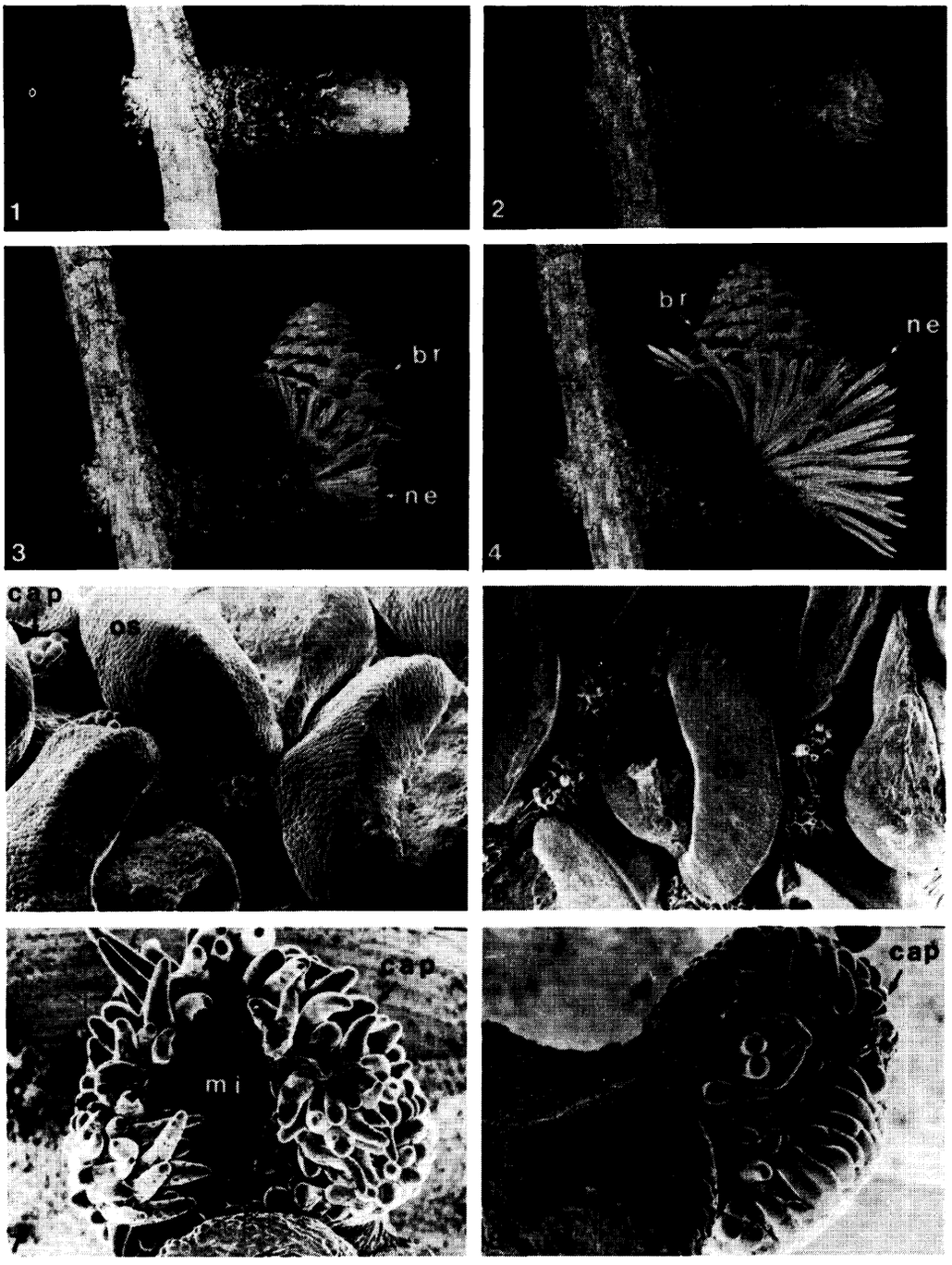
grains were counted using a Leitz (Wetzlar, FRG) microscope with UV light. The test was performed daily over a 3 months period for fresh pollen. It was assessed monthly over a one year period for stored pollen. The reaction was allowed to proceed for 10 minutes at room temperature before scoring.

1H-NMR measurements

Parallel to the FCR tests, the water content of fresh pollen during aging was estimated by ^{1}H-NMR measurements in a GWP 80 Brucker spectrometer. The amount of liquid water was proportional to the area under the NMR line (DUMAS et al., 1983).

Cone and ovule receptivity

Developing cones were monitored periodically. They were photographed weekly during January and early February (resting period prior to pollination), daily from



Figs 1 to 4: — Female cone photographs. br: bracts, ne: needles, s: short shoot. x 3. *Fig. 1.* — Elogating early cone bud. *Fig. 2.* — Beginning of the axis curving: the origin stage. *Fig. 3.* — 3 days after the origin stage. *Fig. 4.* — 6 days after the origin stage: receptive stage. *Figs 5 to 8.* — Scanning electron micrographs of ovuliferous scales and pollen collecting apparatus after liquid N_2 freezing. *Fig. 5.* — Same stage as *figure 1* after removal of bracts. The papillae of the pollen collecting apparatus (cap) are elongating. os: ovuliferous scale; ov: ovule x 40. *Fig. 6.* — Same stage as *figure 3.* The papillae of the pollen collecting apparatus (cap) are fully developed and erect. The micropyle has not opened. os: ovuliferous scale. x 40. *Fig. 7.* — Same stage as *figure 4:* the micropyle (mi) is fully open. x 140. *Fig. 8.* — 7 days after the origin stage: collapse of the pollen collecting apparatus (cap). x 140.

late February to mid-March (pollination period) and weekly from March to September (fertilization and embryogenesis period).

Simultaneously, cone buds and developing cones were collected. Cone buds, ovuliferous scales, or isolated ovules were stuck to metal specimen stubs with silver laquer, frozen in liquid N_2 and observed at 1500 volts using a S-600 scanning electron microscope.

Young seed cones showing several stages of development during and after pollination were fixed in Boin-

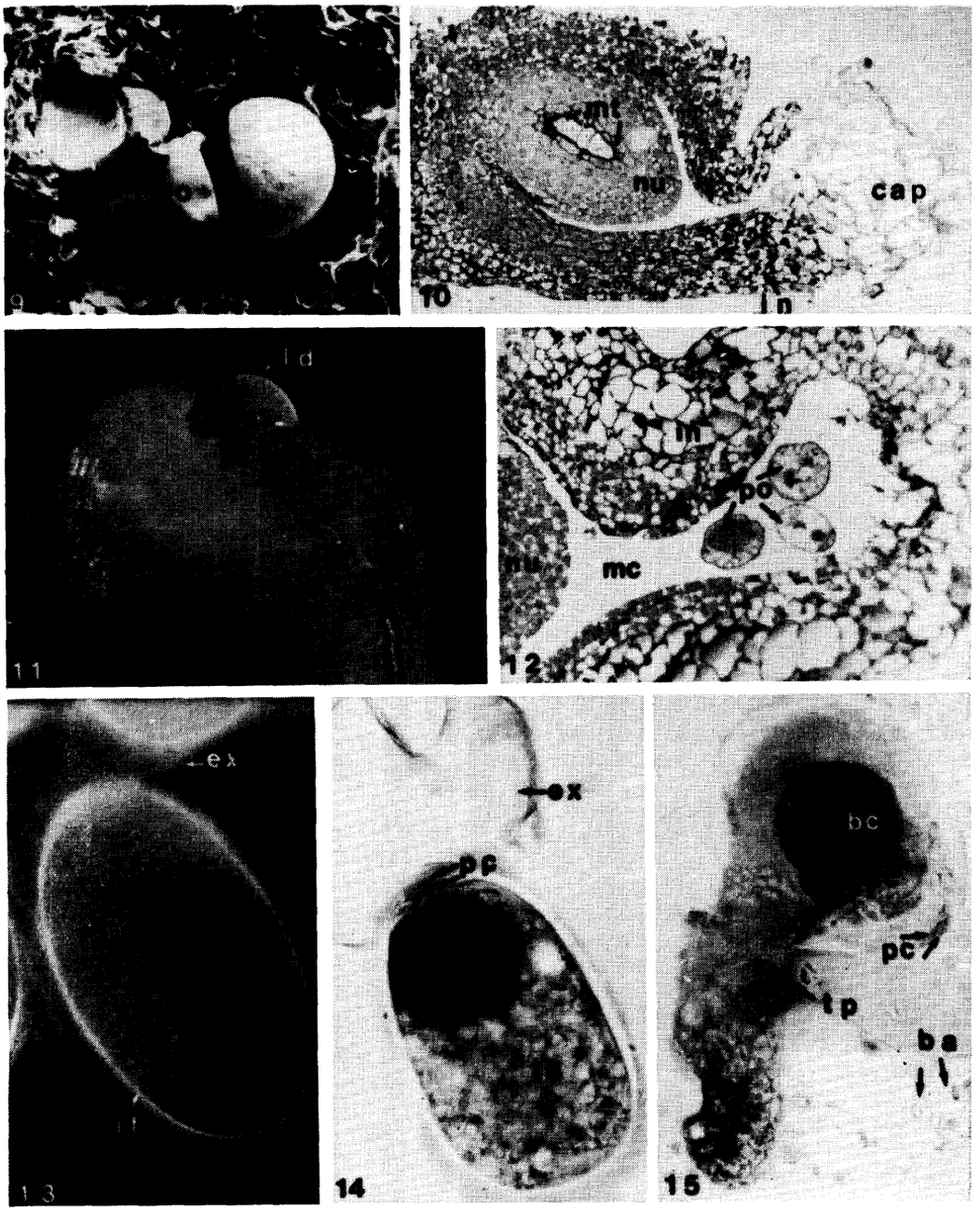


Fig. 9. — Scanning electron micrograph of an ovule (after liquid N_2 freezing) at pollination time (the ovule tip has been sectioned): three dry cup-shaped pollen grains (po) may be observed, engulfed in the micropylar canal. x 300. *Fig. 10.* — Semi-thin section of an ovule at pollination time, six days after the origin stage. Meiosis has just occured and the linear tetrad of megaspores (mt) may be observed. cap: pollen collecting apparatus, in: integument, nu: nucellus. PAS and toluidine blue staining. x 115. *Fig. 11.* — Apical view of an ovule showing liquid drop (ld) six weeks after pollination; ot: ovule tip. x 115. *Fig. 12.* — Semi-thin section of the ovule six weeks after pollination; three hydrated pollen grains (po) may be observed in the micropylar canal (mc). in: integument; nu nucellus. PAS and toluidine blue staining. x 600. *Fig. 13.* — On artifical medium, most of pollen grains, elongated towards the distal pole, showed broken exine (ex) and appeared to be surrounded only by intine (it) fluorescing with Calcofluor White. x 800. *Fig 14.* — Pollen grain on artifical medium. Its broken exine (ex) remains as a cap. The five cells of the grain may be observed. pc: prothalial cells; sc: stalk cell; bc: body call; tc: tube cell. Acetocarmin staining. x 800. *Fig. 15.* — When homogenate of fertilizable ovules was added to artificial medium, some pollen grains produced a short pollen tube (tp). Bacteria (ba) contamined the culture pc: prothalial cells, sc: stalk cell; bc: body cell; Acetocarmin staining. x 600.

Hollande liquid fixative, dehydrated with graded solutions of ethanol and butyl-alcohol, embedded in Paraplast and then sectioned to 6 μm. The sections were stained by PAS reaction (JENSEN, W. A., 1962) followed by ferric hematoxylin staining.

For purposes of comparison, specimens collected from open-grown trees were fixed and treated the same way.

Pollen and ovule structure

Mature pollen grains and ovules at pollination were fixed 3 hours by 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), washed several times in 0.1 M cacodylate buffer, and stored overnight in the same buffer. They were then postfixed for 1 hour in 0.1 M, cacodylate buffered 1% OsO4 and washed 3 times for 20 minutes in the same buffer before dehydration. Dehydration was routinely performed with graded solutions of ethanol. After dehydration, the samples were embedded in Epon. Semi-thin sections of 1 μm were stained by PAS reaction after removal of epoxy resin.

Results

Seed-cone, ovule, and female gametophyte development

Morphological development

In late February, a few days before pollination occurred, seed-cones were about 0.6 cm long and had burst through the bud scales. They were borne on short shoots of vigours branches in the upper regions of the crown. First, seed cones were aligned with the axis of the short shoot (*Fig. 1*). Then, they began to curve upwards (stage 0; *Fig.* 2) and they progressively arrived at a 90^0 angle to the short shoot axis (*Fig. 3*). Six days after the curvature began (stage 0 + 6 days; *Fig. 4*), the bracts fully opened on the female cone, leaving between them large spaces through which pollen could sift down to the ovuliferous scales. Bracts remained fully open, allowing pollen access to the ovules for 24 to 48 hours. Then, the bracts elongated and thickened causing the cone to close. It should be observed that on the same tree, all the seed-cones were not at the same stage: some were aligned with the axis of the short shoots whilst others were perpendicular to the axis. The paired-ovules at the base of each bract were simultaneously developing. A few hours before stage 0, the papillae of the pollen-collecting apparatus were elongating (*Fig. 5*). From days 0 to 5, the fully developed papillae of the collecting apparatus covered the micropyle, leaving no orifice (*Fig. 6*). By day 6, the papillae opened outward, revealing the micropyle (*Fig. 7*). At this period, the papillae exhibited liquid exudate and acted as pollen receptors. By day 7, the active growth inward of the papillae closed the micropyle (*Fig. 8*) and engulfed the pollen grains in the micropylar canal (*Fig. 9*).

Structure

By day 6, meiosis had just occurred in the nucellus, and a linear tetrad of megaspores had formed (*Fig. 10*). Female gametophyte development continued over several weeks and during this time, pollen grains remained at the top of the micropylar canal. In samples grown in open air and observed for comparison, nearly all of the ovules of the mid-portion of the seed cone contained two to three pollen grains.

Under greenhouse conditions, the female gametophyte was mature and fertilizable six weeks after pollination. At this time, we observed the presence of a liquid in the micropylar canal. Sometimes, this liquid formed a drop at the tip of the ovule (*Fig. 11*). This liquid seemed to be exuded by nucellar cells that showed glandular aspects in sections. In the micropylar canal, the pollen grains appeared turgid, with the exine broken or entirely without exine (Fig. 12). Some grains were germinating on the nucellar surface.

The male gametophyte

Morphology and structure

Dry *Larix leptolepis* pollen grains were bowl-shaped (*Fig. 9*), inaperturate and about 60 μm in diameter. In sections, they appeared five-celled, and completely enclosed by the pollen grain wall. This wall had two divisions, a thin outer exine and a thick inner in tine.

In vitro germination tests

The pollen grains sown on germinating medium became spheroid. After 2 days of incubation, some grains remained spheroid and enclosed in the intact exine. Other grains became elliptic; then their exine broke in two halves or split open widely, remaining as a cap on the pollen grain (*Fig. 13, Fig. 14*). These grains, then enclosed only by the intine fluorescing with Calcofluor White (*Fig. 13*), elongated up to 80 μm (*Fig. 14*). The elongation of the pollen always occurred towards the distal pole for consistancy and there was no apparent increase in the width of the pollen. This elongation was observed both for fresh pollen incubated on P-homogenate medium (medium with homogenate of ovules collected at pollination) and F-homogenate medium (medium with homogenate of ovules collected at fertilization). A few pollen grains produced a short pollen tube on F-homogenate medium (*Fig. 15*).

In any case, *in vitro* pollen germination (i. e., production on artifical substrate of a short pollen tube) was difficult in larch and could not be used as a pollen viability test. Thus, the fluorochromatic reaction (FCR test) was used to estimate pollen quality.

Fluorochromatic reaction test

The FCR score was about 62% at anthesis (*fig. 16*). When pollen grains were kept at room temperature, the FCR score decreased quickly to 50 percent and then remained stable at about 50% for 3 months.

For pollen stored at 4^0 C, pollen viability according to the FCR results was about 45 percent after one year of storage.

^{1}H-NMR measures

The loss of water from fresh pollen is expressed directly on *figure 17* by the proportionality between the areas under the NMR line and the water content obtained during aging of the pollen grain. About 25% of the water content was lost during the first day and then water content remained quite constant until the 14th day of

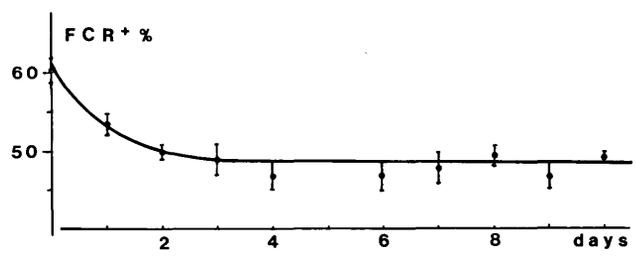


Fig. 16. — Percentage of FCR$^+$ (FCR positive) grains during pollen aging.

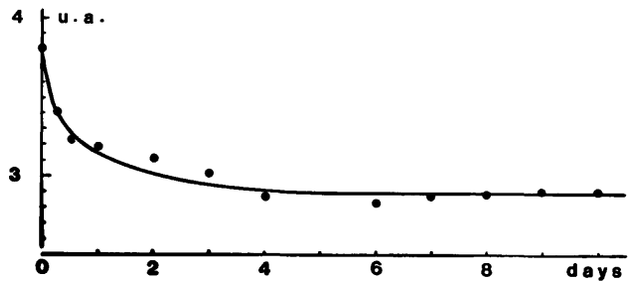


Fig. 17. — Loss of water content during pollen aging (^{1}H NMR measurements; a.u.: arbitrary units).

measurement. The general features of curves of *figures 16* and *17* are quite similar.

Water content

Fresh larch pollen appeared to have a low level of hydration; the moisture content was about 16% at pollination time and decreased to 8% after storage for one year at 4° C.

Discussion

Pollen viability

The methods used to estimate pollen viability may be criticized. Pollen viability is usually based on germinability tests; *i. e.,* on pollen ability to produce pollen tubes in *in vitro* growing conditions. In *Larix leptolepis,* we encountered several difficulties in obtaining successful pollen germination *in vitro*: a few pollen grains produced pollen tubes, most of them did not. Similar observations are reported in the literature. Barner and Christiansen (1960). Christiansen (1970), Owens and Blake (1985) found pollen germination *in vitro* impossible to observe in Larix; in *L. sibirica,* Ho and Rouse (1970) described germination as limited to a splitting of the exine and an elongation of the intine without any suggestion of the formation of a pollen tube; in *L. leptolepis,* Kaji (1974) observed some rare pollen tubes. Therefore, other techniques than a germination test are needed for assessing larch pollen quality, and we chose the fluorochromatic reaction (Heslop-Harrison and Heslop-Harrison, 1970). The relationship between fluorochromasia and pollen germinability has been established in angiosperm pollen grains (Shivanna and Heslop-Harrison, 1981) and this proposed correlation may not be applicable to *Larix leptolepis* pollen grains. Nevertheless in angiosperms, the fluorochromatic procedure verifies the integrity of the plasmalemma of the vegetative cell. Tests of electrical conductance, based on the premise that, as pollen ages, its membranes change and deteriorate, have been correlated to germinability in some gymnosperm pollen (Ching and Ching, 1976; Goddard and Matthews, 1981). Analogies between angiosperm and *L. leptolepis* vegetative cell plasmalemmas have been reported (Said, 1989).

Our FCR results are correlated with changes of pollen water content during pollen aging. Similar correlations have already been reported in angiosperm pollen grains and a parallel decrease in FCR score and in pollen water content would indicate loss of pollen viability (Dumas et al., 1983; Kerhoas et al., 1987).

Pollen germination tests, in species with pollen that is easier to germinate *in vitro* than that of larch, has allowed some researchers (Jensen, 1964; Cram and Lindquist, 1984) to report high pollen viability in conifers, even after storage. Our results suggest that 50 percent of the pollen grains kept in open air or stored at 4° C are still alive at least 3 months after pollination; i. e., for a time longer than the six weeks needed for maturation of the female gametophyte. Therefore, we conclude with Hall and Brown (1976) that pollen viability has a small effect on the empty seed yield of larch.

Ovule receptivity

Our observations on seed cone and ovules have established that ovular receptivity is short in *Larix leptolepis,* lasting 24 hours to 48 hours in our experimental conditions. Obviously, the environmental conditions, especially temperature, in natural stands are not constant and ovular receptivity is, perhaps, slightly different. The duration of maximal receptivity in *L. leptolepis* appears to be shorter than in other conifers, perhaps because of its different pollination mechanism. In *Tsuga heterophylla,* (Colangeli and Owens, 1989), where the pollen grains are not taken into the micropyle, the seed cones are receptive for 8 to 9 days. In *Chamaecyparis* (Owens et al., 1980), *Picea* (Owens and Blake, 1984), and *Pinus* (Owens et al., 1981 a; Greenwood, 1986; Brown and Bridgwater, 1987), which all produce pollination drops, a cone is receptive for about 1 week and individual ovules are receptive for about 2 to 5 days because not all drops are exuded at the same time. In *Pseudotsuga menziesii* (Owens et al., 1981b), which has a stigmatic tip, each cone is receptive for 4 to 6 days. In *Larix decidua,* which has also a stigmatic tip, Korinekova et al. (1988) report that the effective period of receptivity is short but they do not define it in days. In *Larix leptolepis* (Villar et al., 1983; and this work), cone and ovule receptivity define a brief, effective pollination period that could be a limiting factor for pollination success.

However, in spite of the brevity of the effective pollination period, we have observed that open pollinated ovules actually contain two or three pollen grains. Moreover, other genera of Pinaceae with similar (e.g. *Pseudotsuga)* or different (e. g. *Pinus)* pollination mechanisms do not have such a low level of seed production (Owens et al., 1981b; Owens et al., 1982). Other mechanisms may explain poor seed yield in *Larix.* Recognition barriers known in angiosperms have been considered (Said et al., 1985; Said, 1988a and b) but the existence of recognition barriers remains to be proved.

The occurrence of a liquid in the micropylar canal, 6 weeks after pollination, seems important for pollen transport and germination. This liquid, which seems to be exuded by the nucellus, can play two roles: firstly, it would allow pollen grains to reach the nucellus; secondly, it would hydrate pollen and allow germination. Indeed, pollen grains are turgid and elongate when secretion occurs, and in addition, the introduction of exuding ovule homogenate to artificial germination medium allowed us (also Kaji, 1974) to obtain *in vitro* germination of larch pollen. The occurrence of this liquid in *L. decidua* has been previously noticed by Barner and Christiansen (1960) and Villar et al. (1984) but was not observed by Owens and Molder (1979) in *L. occidentalis.* Our observations confirm its existence in *L. leptolepis.*

Conclusion

Based on the above observations and discussion, improving controlled pollination techniques could increase hybrid seed production. Pollen quality should be tested by the FCR method, and the time of pollination carefully

determined. Larch conelets should be pollinated as soon as possible after they have curved upwards at a 90° angle in respect to the short shoot axis. An increase of seed yield (up to 50 percent in the mid-portion of the seed cone has been observed after artificial pollination during the days defined as "receptive" (SAID, unpublished data). Nevertheless, this method requires daily monitoring of the trees. Moreover, repeated pollination is necessary because the stage of female cone development is variable between clones and even between ramets of the same clones.

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References

BARNER, H. and CHRISTIANSEN, H.: The formation of pollen, the pollination mechanism and the determination of most favorable time for controlled pollination in *Larix*. Silvae Genet. **9**, 1—11 (1960). —— BASTIEN, J. CH. and KELLER, R.: Intérêts comparés du Mélèze hybride (*Larix x eurolepis* HENRY) avec les deux espèces parentes. Rev. For. Fr. **32**, 521—530 (1980). —— BREWBAKER, J. L. and KWACK, B. H.: The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. **50**, 859—865 (1963). —— BROWN, S. D. and BRIDGWATER, F. E.: Observations on pollination in loblolly pine. Can. J. For. Res. **17**, 299—303 (1987). —— CHESNOY, L.: La reproduction sexuée des Gymnospermes. Bull. Soc. bot. Fr. Actualités bot. **134** (1) 63—85 (1987). —— CHING, T. M. and CHING, K. K.: Rapid viability tests and aging study of some coniferous pollen. Can. J. Forest Resj 6, 516—522 (1976). —— CHRISTIANSEN, H.: On the germination of pollen of *Larix* and *Pseudotsuga* on artificial substrate and on viability tests of pollen of coniferous forest treeds. Silvae Genet. **18**, 104—107 (1970). —— COLANGELI, A. M. and OWENS, J. N.: Postdormancy seed-cone development and the pollination mechanism in western hemlock (*Tsuga heterophylla*). Can. J. For. Res. **19**, 44—53 (1989). —— CRAM, W. H. and LINDQUIST, C. H.: Pollen viability studies for *Picea pungens* For Chron. **60**, 93—95 (1984) —— DUMAS, C., DUPLAN, J. C., SAID, C. and SOULIER, J. P.: 1H Nuclear Magnetic Resonance to correlate water content and pollen viability. pp. 15—20. In: Pollen Biology and Implication for Plant Breeding. MULCAHY, D. L. and OTTAVIANO, E. (ed.). Elsevier Biomedical, New York (1983). —— EKBERG, I. and ERIKSSON, G.: Development and fertility of pollen in three species of *Larix*. Hereditas **57**, 303—311 (1967). —— GODDARD, R. E. and MATTHEWS, F. R.: Pollen testing. In: Pollen Management handbook. Franklin, E. C. (ed.). USDA Forest Serv. Agric. Handbook **587**, 40—43 (1981) —— GREENWOOD, M. S.: Gene exchange in loblolly pine: the relation between pollination mechanism, female receptivity and pollen availability. Am. J. Bot. **73**, 1443—1451 (1986). —— HALL, J. P. and BROWN, I. R.: Microsporogensis, pollination and potential yield of seed of *Larix* in NE Scotland. Silvae Genet. **25**, 132—137 (1976). —— HALL, J. P., and BROWN, I. R.: Embryo development and yield of seed in *Larix*. Silvae Genet. **26**, 77—84 (1977). —— HESLOP-HARRISON, J.: Aspects of the structure, cytochemistry and germination of the pollen of Rye (*Secale cereale* L.). Ann. Bot. **44**, Suppl 1, 1—47 (1979). —— HESLOP-HARRISON, J. and HESLOP-HARRISON, Y.: Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technol. **45**, 115—120 (1970). —— HO, R. H. and ROUSE, G. E.: Pollen germination of *Larix sibirica* (Siberian larch) *in vitro*. Can. J. Bot. *48*, 213—215 (1970). —— JENSEN, C. J.: Pollen storage under vacuum. Danish Atomic Energy Com. Ann. Kongelige Veterinarerog Landbohjskole Arsskrift. pp. 133—146 (1964). —— JENSEN, W. A.: Botanical histochemistry. 408 pp. ed. WHITAKER, D. M. Freeman, San Francisco, London (1962). —— JOHANSEN, D. A.: Plant microtechnique. Mc Graw-Hill Book Co. Inc., New York and London (1940). —— KAJI, K.: On the pollination and development of ovules and on the sterility of seeds in Japanese larch (*Larix leptolepis* GORD.). Bull Hokkaido For. Exp. Station. **12**, 1—12 (1974). —— KERHOAS, C., GAY, G. and DUMAS, C.: A multidisciplinary approach to the study of the plasma membrane of *Zea* mays pollen during controlled dehydration. Planta **171**, 1—10 (1987). —— KORINEKOVA, M., KRIZO, M., and BIES, R.: The development of integument and pollen-collecting apparatus of european larch (*Larix decidua* MILL.). Biologia **43** (5), :93—399 (1988). —— KOSINSKI, G.: Genetic load in empty seeds of European larch (*Larix decidua* MILL.) Arboretum. Kornickie **26**, 231—236 (1982). —— KOSINSKI, G.: Megagametogenesis, fertilization and embryo development in *Larix decidua*.. Can. J. For. Res. **16**, 1301—1309 (1986). —— OWENS, J. N. and BLAKE, M. D.: The pollination mechanism of Sitka spruce (*Picea sitchensis*). Can. J. Bot. **62**, 1136—1148 (1984) —— OWENS, J. N. and BLAKE, M. D.: Forest tree seed production. Petawawa National Forestry Institute (Canadian Forestry Service information report). 161 pp. (1985). —— OWENS, J. N. and MOLDER, M.: Sexual reproduction of *Larix occidentails*. Can. J. Bot. **57**, 2673—2690 (1979). —— OWENS, J. N., SIMPSON, S. J. and MOLDER, M.: The pollination mechanism in yellow cypress (*Chamaecyparis nootkasensis*). Can. J. For. Res. **10**, 564—572 (1980). —— OWENS, J. N., SIMPSON, S. J. and MOLDER, M.: Sexual Reproduction in *Pinus contorta*. I. Pollen development, the pollination mechanism, and early ovule development. Can J. Bot. **59**, 1828—1843 (1981a). —— OWENS, J. N., SIMPSON, S. J. and MOLDER, M.: The pollination mechanism and the optimal time of pollination in Douglas-fir (*Pseudotsuga menziesii*). Can. J. Forest Res. **11**, 36—50 (1981b) —— OWENS, J. N., SIMPSON, S. J. and MOLDER, M.: Sexual reproduction of *Pinus contorta*.. II. Postdormancy ovule, embryo and seed development. Can. J. Bot. **60**, 2071—2083 (1982). —— PAQUES, L. E.: A critical review of larch hybridization and its incidence on breeding strategies. Ann. Sci. For. **46**, 141—153 (1989) —— SAID, C.: La microsporogénèse chez *Larix leptolepis:* étude ultrastructurale. In: Neuvième colloque international de Cytobiologie de la Reproduction Sexuée des Plantes Supèrieures. Annales scientifiques de l'Université de Reims **23**, 54—57 (1988a). —— SAID, C.: Différenciation et reconnaissance des gamétophytes mâle et femelle chez *Larix leptolepis*: étude structurale. Thesis Université Claude Bernard Lyon I (1988b). —— SAID, C.: Some characteristics of pollen wall cytochemistry and ultrastructure in Japanese larch (*Larix leptolepis* GORD). Sex. Plant Reprod. **2**, 77—84 (1989). —— SAID, C., ZANDONELLA, P. and VILLAR, M.: Pollen ovule interaction in *Larix leptolepis*. New data and hypothesis. pp 507—509 In: Biotechnology and Ecology of Pollen. Ed. MULCAHY, D., MULCAHY, G. B. and OTTAVIANO, E. Springer, Berlin, Heidelberg, New York (1986). —— SCHOBER, R.: A. crossing of *Larix europaea* D. C. of Schlitz provenance with *Larix leptolepis* GORD. Allg. Forst. u. J. Ztg. **151**, 101—112 (1981). —— SHIVANNA, K. R. and HESLOP-HARRISON, J.: Membrane state and pollen viability. Ann. Bot. **47**, 759—770 (1981). —— VILLAR, M., KNOX, R. B. and DUMAS, C.: Effective pollination period and nature of pollen-collecting apparatus in the Gymnosperm *Larix leptolepis*. Ann. Bot. **53**, 279—284 (1984).

Variation in Susceptibility to Dothistroma Needle Blight among Provenances of Pinus radiata var. radiata

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Summary

Considerable variation in susceptibility to *Dothistroma* needle blight was found among provenances of *Pinus radiata* var. *radiata*. Cambria was the most susceptible. Monterey and Ano Nuevo were very similar; both were