Ann Sci For (1997) 54, 301-310

Original article

Electrostatic dusting: an efficient technique of pollination in larch

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(Received 5 December 1995; accepted 29 March 1996)

Summary – Electrostatic pollination has been developed for mass production of seeds of hybrid larch (*Larix* × *eurolepis* Henry). Pollination occurs within a tank carried at the front of a tractor (fig 1). A high voltage is maintained between the pollen dispenser and the tree to be pollinated. Pollen charged with static electricity is blown on the tree by an electrostatic gun, fastened at the top of the tank. This technique favors pollen deposition on the flowers (five and 15 pollen grains per bract on the average for 0 and 40 kV, respectively) (fig 2, table II). Compared to conventional pollen blowing, electrostatic dusting enhanced full seed percentage (32 vs 23%) without reduction in seed viability although the amount of pollen used was much smaller. Moreover, this technique involves less time than the conventional method. However, seed set decreased from the top to the bottom of the crown (39 and 26% full seeds, respectively) (table III). Manual utilization of an electrostatic gun should result in good pollination, judging by the excellent seed set obtained in the branches situated near the gun (table IV).

pollination / pollen / electrostatic dusting / *Larix eurolepis* / *Larix decidua*

Résumé – Le poudrage électrostatique : une technique de pollinisation efficace chez le mélèze. La pollinisation par poudrage électrostatique a été évaluée dans un verger d'hybridation de mélèze (*Larix* × *eurolepis* Henry). Cette technique consiste à instaurer une forte tension entre le pistolet électrostatique et l'arbre à polliniser et à souffler du pollen chargé en électricité statique. La pollinisation a lieu dans une cuve portée sur tracteur pour s'affranchir du vent. Ce procédé favorise le dépôt du pollen sur les fleurs (13 et 15 grains de pollen / bractée pour 15 et 40 kV, contre cinq grains / bractée sans électrisation). La supériorité du poudrage électrostatique diminue du haut vers le bas de l'arbre, à mesure que l'on s'éloigne de la source de pollen. Dans le bas, seule la tension de 40 kV diffère significativement de 0 kV. La pollinisation électrostatique aboutit à de meilleurs rendements en graines que la technique de pollinisation classique (32% de graines pleines contre 23%) sans perte de pouvoir germinatif tout en permettant une économie importante de pollen. Cependant, le pourcent-

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age de graines pleines décroît du sommet vers le bas de l'arbre (de 39 à 26 %). Sur les branches situées à proximité du pistolet, le rendement en graines est très satisfaisant (48 % de graines pleines) et les variations entre branches et entre cônes sont plus faibles que dans le cas de la pollinisation classique. Ces résultats rendent optimiste quant à l'utilisation manuelle du pistolet électrostatique en pollinisation dirigée.

pollinisation / pollen / poudrage électrostatique / *Larix eurolepis* / *Larix decidua*

INTRODUCTION

Supplemental mass pollination (SMP) is the broadcast application of pollen to female flowers that are not isolated from airborne pollen (Wakeley et al, 1966). It enables the seed orchard manager to increase seed yield and to improve panmixis and genetic gains, especially by reducing pollen contamination, self-fertilization or by making crossings between the best genotypes (El-Kassaby et al, 1990).

SMP is absolutely necessary in French hybrid larch (*Larix* x *eurolepis* Henry) seed orchards where a lag between flowering of European larch (*Larix decidua* Mill) and Japanese larch (*Larix kaempferi* Carr) clones prevents natural hybridization. In a first step, a mechanized pollen harvester has been built to collect quickly large amounts of pollen (Philippe and Baldet, 1992). In a second step, improvement of the pollination method is needed in order to increase seed yield and to save pollen.

The most common technique of SMP consists of blowing pollen, sometimes mixed with talc, in an air flow toward the female inflorescences. Many devices can be used: mechanical insecticide dusters, compressed-air paint sprayers for the pollination of individual or clustered flowers, motorized dusters operated from the ground or from a platform for larger scale treatments (Bridgwater and Trew, 1981). More sophisticated ways of distributing pollen have also been attempted but without success, for example, via helicopter in Douglas-fir (Webber, personal communication) and radio-controlled model helicopter in Scots pine (Hadders, 1984).

A new technique, electrostatic dusting, has been developed in Cemagref since 1988. This method is used in industry to coat objects of unusual form, such as frames. Paint particles are charged with static electricity and blown by an electrostatic gun toward a grounded object to be painted. It results in a strong attraction, the object being painted uniformly with minimal paint loss owing to the 'electrostatic wrapping effect'. In addition, the particles of paint are guided toward the most conductive parts of the object when it is made of several materials. As resistivity measurements proved that larch flowers were more conductive than the old branches or the trunk (Philippe and Valadon, 1992), it was to be expected that pollen would be attracted specifically by the flowers. Therefore, this industrial process has been transposed to pollination with the coated object being the flower and the coating particles being the pollen grains.

Feasibility tests demonstrated that larch pollen characteristics were compatible with electrostatic coating technology. Preliminary trials indicated that this technique did not affect pollen quality and that a fair number of pollen grains were visible on the flower bracts and on the stigmatic flaps (Philippe and Valadon, 1992). The purpose of the present work was i) to quantify the effect of voltage intensity on pollen deposition on flowers situated at different places in the crown; ii) to ascertain whether electrostatic dusting was detrimental to ovules and finally iii) to compare seed yields obtained by electrostatic dusting with those obtained by the conventional pollination technique.

MATERIALS AND METHODS

Pollinator

The pollinator was carried by a four-wheel drive tractor and composed of three main components: a front pollination tank designed to encompass the tree, the electrostatic dusting device and an energy unit (fig 1).

The pollination tank was made of the same materials and had the same shape as the pollen harvester tank (Baldet and Philippe, 1993). Nylon nuts and screws were added in order to prevent loss of current. After the tree was enclosed within the tank, a sharp metallic tip grounded to the tank frame and to the tractor was pushed into the base of the trunk to ensure electrical conductivity between the tree and the positive pole of the electrostatic device. The tractor itself was grounded by a heavy steel chain.

The electrostatic dusting device was composed of three components: i) a control module in the tractor cab, which regulated the voltage and which also adjusted pressure and flow rates of the air stream that blew the pollen; ii) a pollen conveyer, fixed to the tank, which delivered pollen to the gun; iii) an electrostatic gun (Ransburg-Gema, model AP 761) fastened on the top of the tank and driven by a rotating arm. At each rotation (20 s duration), it blew 1.25 g of pollen in a dry air flow (1 bar pressure). An integrated cascade power supply maintained the high voltage, adjustable from 15 to 80 kV. A nozzle situated at the tip of the gun contained four electrodes which electrostatically charged pollen grains.

The energy unit, located at the rear, drew electricity and generated compressed dry air. It combined an air compressor with an air drier, both electrically powered from a generator driven by the tractor's power train.

Experiment 1

Treatments

Experiment 1 compared three voltages: 0 kV (uncharged pollen), 15 kV and 40 kV. Pollen of Japanese larch was blown on a single target tree (2.5 g per application), a 3 m tall hybrid larch. Determination of pollination efficiency was based on the average number of pollen grains per bract (per flower) on the same set of 20 flowers distributed over the whole tree (situated at 40–210 cm from the electrostatic gun). Only those pollen grains located on the visible part of the upper side of the bracts were counted. Thus, pollen deposited on the lower side of the bracts and pollen that may have landed directly on the

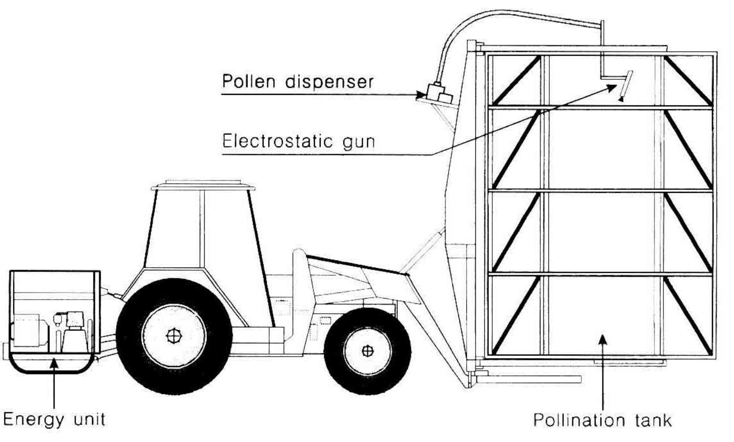

Fig 1. Pollinator.

stigmatic hairs were not taken into account. Between applications, pollen present on the sampled flowers was carefully removed by blowing gently compressed air. Absence of remaining pollen was checked. Each treatment was replicated once.

Statistical analysis

Data were analyzed by the Statgraphics procedure. The sampled flowers were grouped into three crown sections including eight flowers at the top (situated at a distance of 40–95 cm from the pollen source), seven flowers in the middle (110–130 cm) and five flowers at the bottom (160–210 cm).

Mean numbers of pollen grains per bract (per flower) were subjected to the analysis of variance according to the following linear model:

$$Y_{ijkl} = \mu + V_i + C_j + R_k + V{*}C_{ij} + V{*}R_{ik} + C{*}R_{jk} + V{*}C{*}R_{ijk} + e_{ijkl}$$

where Y_{ijkl} = the response variable, ie, the mean number of pollen grains/bract of flower l in replication k in crown section j in treatment i; μ = the grand mean; V_i = the effect of voltage for i = 1, 2, 3; C_j = the effect of crown section for j = 1, 2, 3; R_k = the effect of replication for k = 1, 2; $V{*}C_{ij}$, $V{*}R_{ik}$, $C{*}R_{jk}$, $V{*}C{*}R_{ijk}$ = the main effect interactions and e_{ijkl} = the experimental error.

Prior to analysis, mean number of pollen grains was transformed by the cube root to conform to assumptions of ANOVA. Means were compared with the Tukey test.

Experiment 2

Seed orchard

The pollinator was tested in a 0.7 ha seed orchard located in 'Les Barres', in the center of France. It included 114 grafts of a self-incompatible clone of European larch (coded V44), used as maternal parent, and 150 grafts of clones of Japanese larch, used as pollen producers. Grafts were planted in 1976–1977, at 5 x 5 m spacings and they have been pruned regularly. In 1992, the year of the experiment, the average tree was 3 m high and 2 m wide. Cone production was excellent, thanks to girdling + gibberellin ($GA_{4/7}$) treatments in 1991.

Treatments

Three treatments were compared on the V44 clone: i) manual pollination of 13 grafts by the conventional method, using compressed air; ii) electrostatic pollination of ten grafts (2.5 g per application, 40 kV); iii) a control treatment intended to ascertain whether the high tension inherent in electrostatic dusting did not disturb normal reproduction events. Four grafts were pollinated as in treatment 1, then were submitted to a 40 kV tension in the pollinator (without additional blowing of pollen).

Pollen was a polymix of 56 clones of Japanese larch (6–7% moisture content). In treatments 1 and 3, it was diluted with cedar pollen (1:1 volume), a usual practice in Cemagref, in order to save larch pollen. Cedar pollen, which is easy to collect in great quantities, was preferred to talc which sometimes damaged flowers. As electrostatic dusting makes pollination possible even with small amounts of pollen, the pollen used in treatment 2 was pure.

Grafts included in treatments 1 and 3 were pollinated on 17, 20 and 25 March 1992. Daily counts of flowers (860 flowers distributed on 36 trees) indicated that 72, 87 and 96% of the flowers had emerged at these respective dates. Grafts included in treatment 2 were pollinated twice only, on 17 and 20 March. This period was characterized by favorable climatic conditions for pollination (sunny weather, low air moisture, limited wind). For each treatment, quantity of pollen used and working time were determined.

Cone collection

Cones were collected according to different procedures, depending on the objectives pursued (table I).

Determination of seed set and germination capacity for each tree; homogeneity of seed set within the tree

In treatments 2 and 3, cones were sampled in the upper, middle and lower crown of all the grafts in order i) to determine the percentage of full seeds and the germination percentage for each tree (taking into account the number of cones present in each crown section of each tree) and ii) to study variations of seed set among crown sections. In treatment 1, previous results had shown that seed set did not vary within the tree. There-

Table I. Summary of cone collection and seed analysis.

<table>
<tr><th></th><th colspan="3">Variables studied</th></tr>
<tr><th></th><th>% full seeds/tree
% germination/tree</th><th>Variations in seed set within tree</th><th>Variations in seed set among branches and cones</th></tr>
<tr><td>Type of treatment and collection</td><td>treatment 1: type I
type II</td><td>treatment 1: type II</td><td>treatment 1: type III
treatment 2: type III</td></tr>
<tr><td></td><td colspan="2">treatment 2: type II (10)
treatment 3: type II (4)</td><td></td></tr>
</table>

Types of cone collection: type I: 100 cones collected over entire tree (ten trees); type II: 33 cones collected from each of three different crown sections (three trees unless otherwise indicated); type III: cones collected from uppermost branches (three trees, three branches/tree, 20 cones/branch + ten cones collected separately from one branch per tree).

fore, only three grafts were harvested on crown section basis as a control and the ten remaining grafts were harvested in bulk.

Assessment of electrostatic dusting efficiency in the case of manual utilization of the electrostatic gun

If the electrostatic gun was disconnected from the machine and used manually, the operator would direct alternatively the pollen flow toward groups of flowers or flowering branches as in treatment 1. In order to predict the efficiency of this method, we compared the effect of treatments 1 and 2 on flowers situated near the pollen source. In both treatments, cones were collected from the top branches, located in the upper part of the upper crown section. Mean percentage of full seeds as well as variations of seed set between branches and between cones of the same branch were studied.

Seed analysis

The percentage of full seeds was determined by cutting 200 seeds per sample (or the totality of the seeds when cones were collected individually). Germination percentage was estimated by germinating 300 to 700 seeds per tree (full and empty mixed), without prechilling, on filter paper, in an incubator set at alternating temperatures of 30 °C (8 h) and 20 °C (16 h). Light (25 $\mu mol \cdot cm^{-2} \cdot s^{-1}$) was provided during the high temperature period. A seed was considered germinated when the root length reached at least three times the seed length. Germinated seeds were counted and removed on days 6, 10, 17 and 19. At this point, all the seeds that failed to germinate were cut in order to determine, for each tree, the number of full seeds put in the incubator. Germination percentage was based on the number of full seeds (number of germinated seeds x 100/number of full seeds).

Statistical analysis

In a first step, mean percentage of full seeds per tree and mean germination rate per tree of the 27 trees included in the experiment were analyzed. For trees harvested on a crown section basis, mean percentage of full seeds was calculated by taking into account the number of cones produced in each crown section of each tree. Data were subjected to ANOVA according to the following model:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where Y_{ij} = the mean percentage of full seeds or the mean germination rate of tree j in treatment i; μ = the grand mean; P_i = the effect of pollination for i = 1, 2, 3 and e_{ij} = the experimental error.

In a second step, homogeneity of seed set within the tree was determined by comparing, in each treatment, the percentage of full seeds observed in the three crown sections. ANOVA was performed according to the following model:

$$Y_{ij} = \mu + C_i + e_{ij}$$

where Y_{ij} = percentage of full seeds of tree j in crown section i; μ = the grand mean; C_i = the effect of crown section for i = 1, 2, 3; e_{ij} = the experimental error.

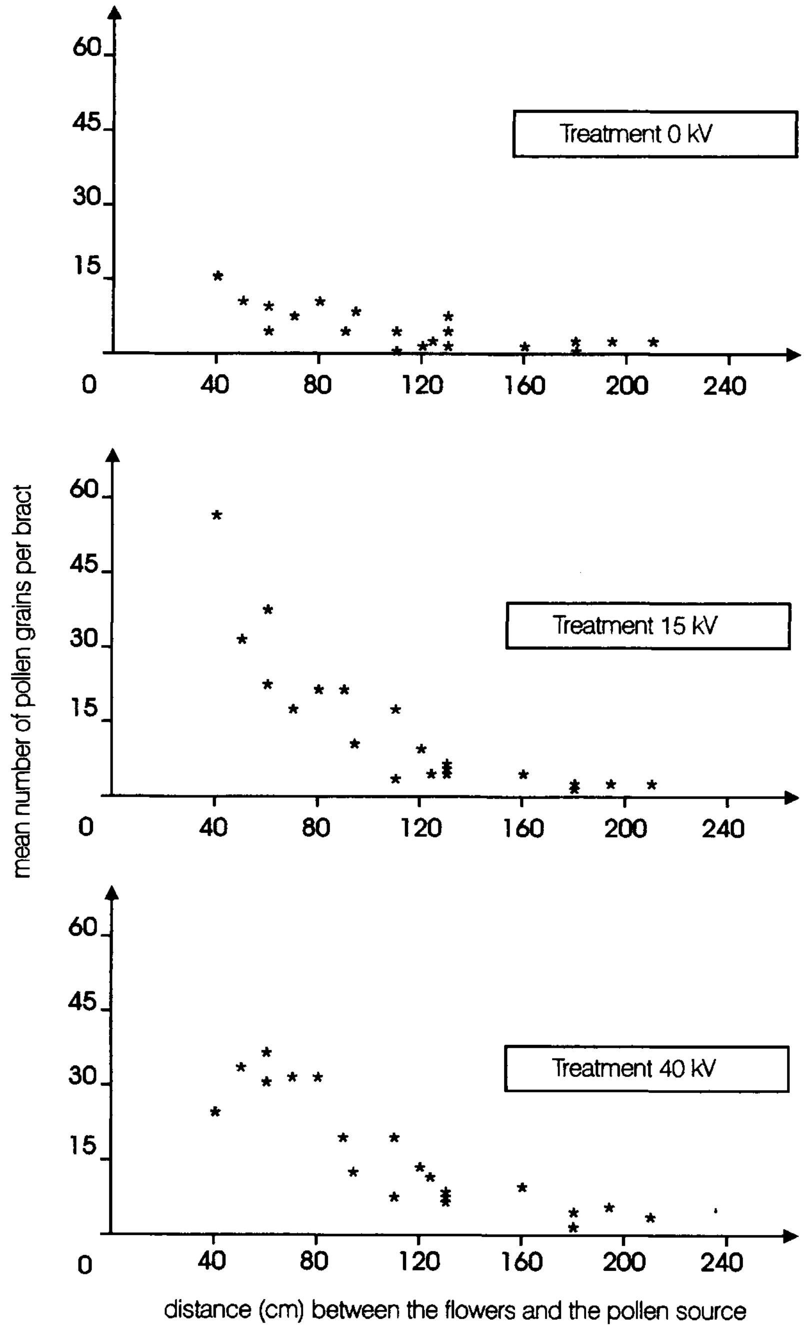

Fig 2. Number of pollen grains per bract as a function of the distance between the flowers and the pollen source.

Prior to analysis, the residuals were checked to conform to assumptions of ANOVA. Means were compared with the Scheffe test.

RESULTS

Experiment 1

Irrespective of voltage, a gradient of pollination from the top to the bottom of the crown was observed (fig 2). There was a strong negative correlation between the mean number of pollen grains per bract (after logarithmic transformation) and the distance from the flowers to the pollen dispenser: $r = -0.81$, -0.90 and -0.90 for 0, 15 and 40 kV, respectively ($P < 0.001$ in all cases).

Flowers pollinated with charged pollen received three times more pollen (13 and 15 grains per bract for 15 and 40 kV, respectively) than those pollinated with uncharged pollen (five grains per bract). This effect was highly significant ($P < 0.001$). In addition, many pollen grains (not taken into consideration in pollen counts) could be seen on the lower side of the bracts after electrical pollination, in the top and, to a smaller extent, in the middle part of the tree. In each crown section, we observed a significant effect of electrostatic dusting except in the lower crown where 15 kV did not differ from 0 kV (table II). Replication effect as well as interactions between replication and voltage and replication and crown section were not significant.

Experiment 2

Quantity of pollen used and working time

Manual pollination (treatments 1 and 3) demanded 16 g of larch pollen per tree (7, 7, and 2 g on 17, 20 and 25 March, respectively). Trees included in treatment 2 received 5 g of pollen (2.5 g on 17 and 20 March).

Manual pollination took 5 min per graft on the average. Electrostatic dusting required only 2 min, including tank positioning, pollination and removal of the tank.

Cone production

In treatments 1, 2 and 3, cone production averaged 1 400, 1 100 and 1 500 cones per graft, respectively. The differences were not significant. On average, 20% of the cones were situated in the upper part of the crown, 45% in the middle and 35% in the lower part.

Percentage of full seeds

Mean percentage of full seeds per tree averaged 23, 32 and 24% in treatments 1, 2 and 3, respectively. The superiority of electrostatic dusting over the conventional tech-

Table II. Effect of voltage on pollen deposition.

Voltage (kV)	*No of pollen grains per bract*		
	Upper crown	*Middle crown*	*Lower crown*
0	9^{a}	4^{a}	2^{a}
15	27^{b}	8^{b}	3^{ab}
40	28^{b}	11^{b}	6^{b}

[ab] In each column, values followed by the same letter do not differ significantly at the 5% level.

Table III. Variations of full seed percentage among crown sections.

Crown section	*Treatment 1*	*Treatment 2*	*Treatment 3*
Top	20[a]	39[b]	24[a]
Middle	20[a]	32[ab]	27[a]
Bottom	16[a]	26[a]	23[a]

[ab] In each column, values followed by the same letter do not differ significantly at the 5% level.

nique was significant ($P = 0.01$). In particular, seven of ten trees had more than 30% full seeds whereas, among the trees pollinated manually, only four of 13 reached this threshold. Treatments 1 and 3 did not differ significantly.

As shown in table III, no difference was found among crown sections for treatment 1 ($P = 0.63$) and treatment 3 ($P = 0.88$). In contrast, electrostatic dusting did not result in homogeneous seed yield since there was a significant decrease from the top to the bottom of the crown ($P < 0.001$).

Data from three trees pollinated by electrostatic dusting indicated that seed yield was even higher in the top branches than in the upper third of the crown (48% full seeds vs 41%). However, the difference was clear for only one tree where percentage of full seeds increased from 32 to 49%. This increase was hardly perceptible for the two other trees (43 vs 39% and 53 vs 52%). Electrostatic dusting resulted in more homogeneous seed set in the top branches than manual pollination, interbranch and intercone variations being lower (table IV).

Seed germination

Mean germination percentages per tree were 79, 78 and 82% for treatments 1, 2 and 3, respectively. Differences were not significant ($P = 0.63$). Intermediate germination percentages, obtained on days 6, 10 and 17, did not differ significantly, indicating that germination speed was similar in the three treatments.

DISCUSSION

Experiment 1 clearly showed that electrostatic effect favored pollen deposition on flowers. Electrostatic dusting resulted in three times more pollen on the flowers than uncharged pollen blowing (15 and five pollen grains per bract, respectively). However, the quantity of pollen that settled on the bracts varied strongly according to the posi-

Table IV. Variations in seed set in the top branches, between branches and between cones.

	Between branches		*Between cones*	
	Treatment 1	*Treatment 2*	*Treatment 1*	*Treatment 2*
Mean % full seeds (min/max)	23 (8/40)	48 (39/58)	23 (2/45)	44 (19/79)
Coef. of variation	39%	15%	53%	31%

tion of the flowers within the crown, the nearest from the pollen source being pollinated more efficiently.

Although 15 and 40 kV gave similar results (13 and 15 grains per bract, respectively), 40 kV led to more homogeneous distribution of pollen than 15 kV. In particular, only 40 kV differed significantly from 0 kV in the lower crown. On the other hand, a previous experiment demonstrated that 70 kV resulted in more heterogeneous pollination than lower voltages (Philippe and Valadon, 1992). Therefore, 40 kV proved to be the most suitable voltage.

Although lack of pollination is a major cause of empty seeds in larch (Kaji, 1974; Hall and Brown, 1977; Owens and Molder, 1979; Kosinski, 1985, 1986), good pollination is only the first link of the chain that leads to seed production. In particular, it was important to ensure that electrostatic dusting did not disrupt normal reproduction (meiosis, female gametophyte development, fertilization, embryo development). Experiment 2 indicated that the high tension inherent in this technique did not prevent viable seed formation. Trees included in treatments 1 and 3 were characterized by similar seed yields (23 and 24% full seeds, respectively) and similar germination percentages (79 and 82%, repectively). In addition, variations in seed set among crown sections were not significant in both treatments, indicating that the upper flowers, potentially in danger owing to their proximity to the electrostatic gun, were not affected by electrostatic effects.

Electrostatic dusting resulted in improved seed yield. Percentage of full seeds was, on the average, 40% higher than that obtained by manual pollination (32 vs 23%) although it used three times less pollen per tree. Contrary to manual pollination, a significant decrease in seed set from the top to the bottom of the crown was observed with electrostatic dusting (39, 32 and 26% full seeds in the upper, middle and lower crown, respectively). It is consistent with the gradient of pollination observed in experiment 1. Nevertheless, whatever the crown section, seed set was greater than or equal to that obtained by the manual dusting. Ultimately, the full seeds produced by both techniques had satisfactory germination percentages (78 and 79% for electrical and manual pollination, respectively).

The study of seed set in the branches situated near the electrostatic gun gave a good idea of electrostatic dusting efficiency if the gun was to be operated by hand. Compared to the conventional technique, it resulted in more homogeneous seed set among branches and cones (table IV). Moreover, percentage of full seeds as high as 48% can be regarded as excellent in larch (Hall and Brown, 1977; Kosinski, 1982; Hall, 1985; Kosinski, 1985).

For branches located near the electrostatic gun, it seems that only limited gain in seed yield can be obtained by further improvement in pollination, at least for the clone used in the present seed orchard. Firstly, the increase in seed set from the upper third of the crown to the top branches was not clear for the two trees characterized by high seed set in the upper crown section (39 and 52% full seeds), whereas pollen deposition should have been better in the top branches. Secondly, if one ignores one exceptional cone which contained 79% full seeds, the percentage of full seeds per cone in the top branches never rose above 60% although huge amounts of pollen could be seen on most of the flowers. This indicates that the quantity of pollen is no longer a limiting factor when it exceeds a certain threshold. Formation of empty seeds must be connected with factors other than pollination, such as female gametophyte degeneration, failure of fertilization or embryo abortion (Hall and Brown, 1977; Owens and Molder, 1979; Kosinski, 1985, 1986; Owens, 1995). A better understanding of prezygotic and postzygotic events and development of better tools to evaluate pollen viability would help to reduce the high percentage of empty seeds that is not due to pollination failure.

At pollination time in 1992, air humidity was low, which was favorable to electrical pollination. Further work is needed to ascertain whether efficiency of electrostatic dusting is affected by climatic conditions, and air hygrometry in particular.

ACKNOWLEDGMENTS

The authors wish to thank the Cemagref staff in Montoldre (France), who actively participated in the construction of the pollinator and Ransburg-Gema Company for technical advice. They express also their gratitude to JD Boyé for field work and to M Bonnet-Masimbert and P Von Aderkas for their advice and for reviewing the manuscript.

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