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Application of Ovary and Ovule Culture in *Populus alba* L. x *P. euphratica* OLIV. Hybridization

By A. JAFARI MOFIDABADI, A. R. MODIR-RAHMATI and A. TAVESOLI

Research Institute of Forests and Rangelands, Jaber ibn Hayyan Plant Sciences Labs., P.O. Box 13185-116, Tehran, Iran

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Summary

An *in vitro* method was used to develop *Populus alba* x *P. euphratica* hybrid plants. Developed ovaries and ovules were isolated from *P. alba* female branches which were pollinated with *P. euphratica* pollen grains by using twig and pot breeding technique. Isolated ovaries and ovules were then transferred to growth regulator free half concentrated MS agar medium for embryo germination. Maximum three plantlets were observed per ovary and more than 90% of cultured ovules successfully produced plantlets, whereas the efficiency of ovary culture to produce plantlets was 70%. Plantlets were cultured in the same medium within jars, before being transferred to potting soil. Seventy five interspecific *P. alba* L. x *P. euphratica* OLIV. hybrid plants were successfully acclimatized in greenhouse.

Key words: Ovule culture, embryos, ovary, *Populus euphratica*, *Populus alba*, interspecific hybridization.

FDC: 165.442; 165.72; 163; 176.1 *Populus alba*; 176.1 *Populus euphratica*.

Introduction

There has been a long sustained interest in the hybridization of poplars largely by the benefits derived from capturing heterosis and combining desirable traits ultimately expressed in the amount and quality of wood production (STETTNER, 1980). Owing to the resistance to drought and salinity, *Populus*

euphratica OLIV. was chosen as parental species in *Populus* breeding, but the incompatibility was observed between this species with some of other poplar species (WILLING and PRYOR, 1976). During last decade, a number of observations have been made on pollen-stigma interactions in interspecific crosses or *Populus*, with emphasis on the sections Aigeiros, Leuce and Tacamahaca (GAGET *et al.*, 1984; GURIES and STETTNER, 1976; HESLOP-HARRISON, 1975; KNOX *et al.*, 1972). Except for interspecific combination involving Leuce poplars and Aigeiros-Tacamahaca poplars, most hybridization experiments were reported to be successful with methods of bottle grafting and the technique of twig and pot (technique described in KOUIDER *et al.*, 1984; see also RAJORA and ZSUFFA, 1984). The reasons for incompatibility in trees can be typically due to premature abscission of flowers, early dehiscence of the capsules, pollen mortality, grafting failure and some unknown physiological disorders occurring within the flowering branches (reviewed in RAMMING, 1990 for fruit trees). In addition, with interspecific crosses, intergeneric and crosses between diploids and tetraploids, the endosperm often develops poorly or not at all. By aseptically culturing the embryo in a nutrient medium, this problem may be overcome. This technique was used in many different crosses and seems to be amenable to large-scale application in poplar hybridization (LI *et al.*, 1983; KOUIDER *et al.*, 1984; LI and LI, 1985; NOH *et al.*, 1986 and SAVKA *et al.*, 1987).

In order to produce *P. alba* x *P. euphratica* plantlets, we report here the development of an easier *in vitro* system for embryo maturation and germination based on ovary and ovule culture.

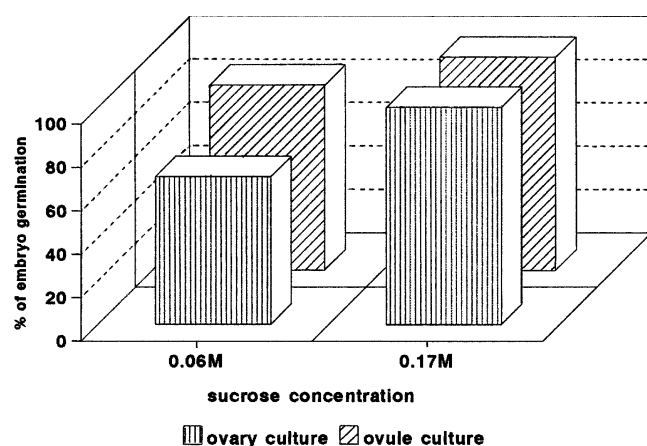
Materials and Methods

Artificial pollination was made between *P. alba* L. and *P. euphratica* OLIV. (in one direction). Branches of *P. euphratica* were collected from Research Center of Alborz - Karaj - Iran. In order to provide pollen grains, the male flowers of *P. euphratica* were forced to anthesis in greenhouse. Twig and pot technique (tap water culture) were used for artificial hybridization. The female inflorescence buds were covered with transparent papers before anthesis to avoid contamination. Artificial pollination was then carried out on covered female inflorescence by high dusting of *P. alba* stigma with *P. euphratica* pollen grain. The control pollination was made with *P. alba* x *P. alba*. Catkins were collected from branches 10, 14 and 21 days after pollination. Closed capsules, still attached to the axis of the catkin, were then surface disinfected for 12 min in a solution of calcium hypochlorite followed by three 5 min rinses in sterile deionized water. To rescue fertilized embryos, isolated ovaries and ovules were transferred to half concentrated MS agar medium in 100 mm x 15 mm petri dishes. Cultures were incubated at 24°C under 16 h photoperiod with light provided by 40 watt cool white fluorescent lamps (4000 lux to 5000 lux). Plantlets 1 cm to 2 cm in height were transferred to jars containing the same medium (half-MS) and kept for two months before acclimatized.

The tested medium for the embryo germination and plantlet development contained half concentration of MURASHIGE and SKOOG inorganic salts (MURASHIGE and SKOOG, 1962) with FeEDTA 10⁻⁴ M and pH adjusted to 5.8. Two concentrations of sucrose (0.06 M and 0.17 M) were used. No growth regulators were added to the medium. The medium was autoclaved for 20 min at 120°C and then dispensed in sterile petri dishes and jars with 10 ml and 20 ml respectively.

Results and Discussion

Embryos were germinated when the half-capsules of ovaries and isolated ovules were placed on the surface of agar medium. Highest embryo germination was observed on half-MS medium containing 0.17 M sucrose in both ovary and ovule cultures (Fig. 1). Germination of ovule embryos was started within one week after culture initiation. Comparing to ovary culture



% of embryo germination was calculated on total number of explants producing plantlets obtained from initially cultured explants

Figure 1. – Effect of sucrose concentration on embryo germination in ovary and ovule cultures.

ovules produced only one tiny shoot (Table 1 and 2). In ovary culture average 70% of cultured capsules produced plantlets, while this value was 90% for ovule culture. The mean effect of explants (ovary and ovule) and age of embryo in the time of isolation on plantlet production were compared (Table 3). There was significant difference between ovary and ovule as explants on mean effect of embryo germination. Due to multiple plantlets induction (induction of 2 to 3 plantlets in each cultured ovary), the high number of induced plantlets (yield)

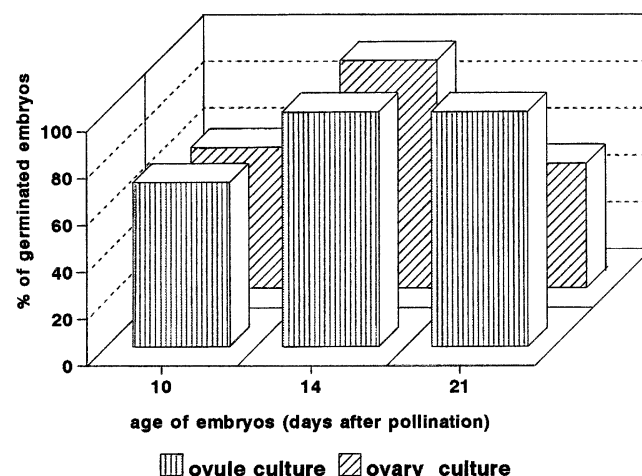


Figure 2. – Influence of the age of embryos on seedling production of *Populus alba* L. x *P. euphratica* OLIV. ovary and ovule cultures.



Figure 3. – A: *Populus alba* L., root stock maternal plant. B, C, D, E, and F: *Populus alba* L. x *P. euphratica* OLIV. hybrid plants.

Table 1. – Influence of the age of embryos on seedling production of *Populus alba* L. x *P. euphratica* OLIV. in ovary cultures on nutrient medium with 0.17 M sucrose.

Crosses	No. of days after pollination	No. of capsules isolated	Seedling			
			No. of capsules producing plantlets	% of capsules producing plantlets	Total No. of plantlets	Yield *
<i>P. alba</i> x <i>P. euphra.</i>	10	62	37	59.7	76	1.2
<i>P. alba</i> x <i>P. euphra.</i>	14	130	126	96.9	168	1.3
<i>P. alba</i> x <i>P. euphra.</i>	21	78	41	52.5	81	1.1
<i>P. alba</i> x <i>P. alba</i> (contr.)	14	30	28	93.3	30	1

*) Yield = Total number of plantlets ÷ total number of isolated ovaries

Table 2. – Influence of the age of embryos on seedling production of *Populus alba* L. x *P. euphratica* OLIV. in ovule cultures on nutrient medium with 0.17 M sucrose.

Crosses	No. of days after pollination	No. of ovules isolated	Seedling			
			No. of ovules producing plantlets	% of ovules producing plantlets	Total No. of plantlets	yield *
<i>P. alba</i> x <i>P. euphra.</i>	10	20	14	70	14	0.7
<i>P. alba</i> x <i>P. euphra.</i>	14	30	30	100	30	1
<i>P. alba</i> x <i>P. euphra.</i>	21	25	25	100	25	1
<i>P. alba</i> x <i>P. alba</i> (contr.)	14	25	25	92	25	1

*) Yield = Total number of plantlets ÷ total number of isolated ovules

were observed in ovary culture. In spite of non-significant effect of embryo age on embryo germination, results in figure 2 demonstrated that an increase in yield can be obtained by culturing 14-day-old embryos. Two week old embryos appeared to be best for embryo germination (yield and percentage of explants producing plantlets) in both ovary and ovule cultures. In contrast to RAQUIN and TROUSSARD (1993), we were able to isolate 21-day-old embryo ovaries and ovules from ovaries and ovules by keeping twigs and pots in a cool greenhouse (25°C). A total of 127 *P. alba* x *P. euphratica* plantlets was successfully obtained by the technique of ovary and ovule embryo culture. Average 5.7% malformed plantlets and 23% necrosed ovaries were found in ovary cultures. 8.2% of cultured ovules became necrotic. Only 9.8% malformed plantlets were observed and there were no malformed ovules in this culture. Malformed ovules have been described in other tree species, such as: Sourcherry (FUROKAWA and BUKOVAC, 1989); fertilized and unfertilized apricot ovules (EATON and JAMONT, 1992). The fastest germination was observed with the combination of *P. alba* x *P. alba* (control pollination) after four to five days with 10-day-old ovaries (10 days after pollination). The time necessary for germination of *P. alba* x *P. alba* (control) ovary was the same as for *P. alba* x *P. alba* ovary cultures reported by RAQUIN and TROUSSARD (1993). Seventy five interspecific *P. alba* x *P. euphratica* hybrid plants were successfully acclimatized in greenhouse and planted in the field (Fig. 3).

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The authors are grateful to KALAGARY, M., ASADI, F. and Miss KAZAMI F. for their scientific cooperation.

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Buchbesprechungen

Trees – Contributions to Modern Tree Physiology. By H. RENNINGBERG, W. ESCHRICH and H. ZIEGLER (Eds.). 1997. Backhuys Publishers, Leiden. ISBN 90-73348-67-g. 565 pages with 161 figures, 19 photographs, 20 plates and 37 tables. Hardbound NLG 235,-, US\$ 130,-.

The present book contains 32 articles in 7 sections and represents the scientific outcome of a program named 'Physiology of trees' sponsored by the German Science Foundation (Deutsche Forschungsgemeinschaft). This program was initiated in 1985 by HUBERT ZIEGLER to bring together numerous German plant physiologists working on different fields of tree physiology. The aim was to increase their basic knowledge in tree physiology in the background of forest decline phenomena. Within the 8 years duration of the program progress has been published in numerous reports which is the scientific basis of the book. In addition, it contains contributions of guests that presented their views on particular topics at the annual meetings of the special program. The whole field of tree physiology is covered by the book. It starts from structure and function of tree surfaces, moves to biomass production, water relations and tree nutrition and ends with mykorrhizae, tree development and wood formation. Interestingly, the last section handles with the important question of differences between trees and herbs and discusses peculiarities in tree physiology. One minor limitation of the book is that genetic relationships within species are poorly considered when comparing physiological values between different species. Nevertheless, this book contains important papers on tree physiology which might not only be interesting to scientists working in this field.

M. FLADUNG (Grosshansdorf)

Zukunft der Gentechnik. Originalausgabe. Von P. BRANDT (Herausgeber). 1997. Birkhäuser Verlag, Basel, Berlin und Boston. ISBN 3-7643-5662-6. 294 Seiten mit ca. 50 Abbildungen und 26 Tabellen. Broschiert DM 68,-/öS 497,-/sFr 58,50.

Nach dem Erfolgsbuch von Autor PETER BRANDT 'Transgene Pflanzen' aus dem gleichen Verlag, das eine Übersicht über den derzeitigen Stand gentechnischer Arbeiten an Pflanzen gibt, hat PETER BRANDT in dem vorliegenden Buch kompetente Autoren zusammengebracht, die alle Spezialisten auf einem Gebiet der Gentechnik sind. Unzweifelhaft nimmt in den neunziger Jahren das Thema Gentechnik eine Stellung ein, wie sie in den sechziger und siebziger Jahren die Atomtechnik innehatte. Daher ist es nicht verwunderlich, dass besonders auch die Auswirkungen der Gentechnik auf die menschliche Gesundheit und die Umwelt heute und in der Zukunft die Menschen berührt. Es ist den Autoren gelungen, die verschiedenen Teilbereiche der Gentechnik verständlich, aber fachlich fundiert darzulegen, dass auch der naturwissenschaftliche Laie sich in der komplizierten Materie nicht verloren vorkommt. So können die Visionen der Befürworter der Gentechnik sowie Befürchtungen der Kritiker für jeden verständlich nachvollzogen werden. Aufbauend auf einem einführenden Kapitel zum derzeitigen Entwicklungsstand der Gentechnik werden in den folgenden Kapiteln die zukünftigen Chancen für die menschliche Ernährung und die medizinische Versorgung beim Einsatz dieser Technik aufgezeigt. Darüber hinaus finden Beiträge zu weltanschaulichen oder soziologischen Belangen in der Zukunft Beachtung. Zusammengefasst ist das Buch jedem Interessierten zu empfehlen, der fachlich fundierte und ideologisch neutrale Informationen über die Zukunft der Gentechnik sucht.

M. FLADUNG (Grosshansdorf)

Farbatlas der Basidiomyceten. Colour Atlas of Basidiomycetes. Lieferung 15. Von M. MOSER und W. JÜLICH, unter Mitarbeit von C. FURRER-ZIOGAS. 1997. Verlag G. Fischer, Stuttgart, Jena, New York. ISBN 3-437-25506-1. 200 Seiten mit 150 Abbildungen. DM 98,-.

Der im Jahre 1985 im Loseblattformat begonnene Farbatlas der Basidiomyceten wird nunmehr in der 15. Lieferung mit weiteren 134 abgebildeten Pilzarten aus 24 Gattungen und 3 Ordnungen (*Polyporales*, *Boletales*, *Agaricales*) fortgesetzt. Zusätzlich zu den Bildtafeln enthält die Lieferung 7 Gattungsdiagnosen in deutscher, englischer, französischer und italienischer Sprache. In ihnen werden die für eine eindeutige Identifizierung notwendigen makroskopischen und mikroskopischen Merkmale beschrieben, ökologische Eigenschaften und Besonderheiten genannt und Merkmale diskutiert, durch die eine Abgrenzung zu verwandten Pilzgattungen möglich ist. Zudem sind wichtige Literaturzitate aufgeführt. Die Farabbildungen sind wie in den bisherigen Lieferungen Studioaufnahmen, die überwiegend von sehr guter Qualität sind. Die Pilze werden in unterschiedlichen Altersstufen und unter Berücksichtigung typischer Bestimmungsmerkmale dargestellt.

B. R. STEPHAN (Grosshansdorf)

Grundpraktikum zur Pflanzenphysiologie. UTB 2026. Von U. KUTSCHERA. 1998. Quelle & Meyer Verlag, Wiesbaden. ISBN 3-8252-2026-5. 160 Seiten mit 125 Abbildungen und 7 Tabellen. Kartoniert DM 36,80.

In Anlehnung an das vom selben Autor verfaßte „Kurze Lehrbuch der Pflanzenphysiologie“ stellt dieses Buch eine gelungene Auswahl an einfach und realistisch durchführbaren Experimenten vor, um alle Grundlagen der Pflanzenphysiologie anschaulich zu vermitteln. Die dargestellten Versuche sind exakt und ausführlich beschrieben. Ein „Labor-Anfänger“ müßte in der Lage sein, die Experimente mit Hilfe der enthaltenen Erläuterungen erfolgreich und sinnvoll durchzuführen.

Unter Verwendung einer Vielzahl von aktuellen Modellorganismen ist es ULRICH KUTSCHERA gelungen, aus einer Fülle von Fremdwörtern und Spezialbegriffen einen gut lesbaren, leicht verständlichen Text zu formulieren. Das Ziel heißt offenbar: „Gib den Studenten Freude am Lesen, dann haben sie auch Freude am Forschen“.

Vor allem die klare Gliederung innerhalb einer Versuchsbeschreibung, die prägnanten theoretischen Hintergrund-erklärungen und die sehr einfach gehaltenen Zeichnungen erhöhen die Verständlichkeit des teilweise schwer zu vermittelnden, partiell physikalischen und mathematischen Stoffes. Des weiteren wird die klare Strukturierung eines wissenschaftlichen Versuchs für spätere eigene Experimente ersichtlich, was für Diplomstudenten meist erst während der Diplomarbeit ein akutes Thema darstellt.

Die Auswahl an Versuchen aus bestimmten Themenbereichen (z.B. Phytohormone) ist bemerkenswert groß, so daß je nach Ausstattung das ein oder andere Experiment tatsächlich zur Durchführung gelangen kann und kein Teilaspekt des umfassenden Lehrstoffes mangels entsprechender Materialien oder Apparaturen entfällt. Für Betreuer und Gymnasiallehrer wird eine Zusammenstellung von inhaltlich verschiedenen Praktika ermöglicht.

Die durchgehende Einführung in die internationale Fachterminologie ist als sehr positiv zu bewerten. Allerdings wurde in einigen Fällen wohl über das Ziel hinaus geschossen. So sollte beispielsweise eine Chloroplasten-Isolierung nicht mit dem deutschen Wort „Isolation“ beschrieben werden.

Ein durchaus wünschenswerter Anhang für dieses Praxisbezogene Buch wäre außerdem eine Auflistung der zu verwendenden Lösungen und Reagenzien mit der Einteilung in Gefahrstoffklassen gewesen, was sicherlich ein Manko darstellt.

Allgemein ist das Buch vor allem solchen Praktikanten/-innen zu empfehlen, deren Verständnis komplexer theoretischer Zusammenhänge durch praktische Versuche um ein Vielfaches erleichtert ist.

M. FASKE (Grosshansdorf)

Biodiversität und nachhaltige Forstwissenschaft.

Herausgegeben von G. MÜLLER-STARCK. 1996. Ecomed Verlagsgesellschaft, Landsberg. ISBN 3-609-69360-6. 340 Seiten mit zahlreichen Abbildungen und Tabellen. Paperback DM 48,-/öS 350,-/sFr 46,-.

Im Oktober 1995 wurde vom Lehrbereich Forstgenetik der Forstwissenschaftlichen Fakultät der Universität München eine Tagung des Forum's Genetik – Wald – Forstwirtschaft ausgerichtet. Der Fachverlag Ecomed konnte gewonnen werden, die 22 Vorträge aus dem deutschsprachigen Raum zum Thema 'Biodiversität und nachhaltige Forstwissenschaft' in Buchform zu veröffentlichen. Das Buch gliedert sich in 4 Kapitel: Im Kapitel 'Biodiversität, Genetik und Forstpflanzenzüchtung' geben 7 Beiträge eine Übersicht über aktuelle Tendenzen im Bereich der Forstgenetik und Forstpflanzenzüchtung. 5 Beiträge gehen im Kapitel 'Beeinflussung der Biodiversität durch den Waldbau' auf waldbauliche Möglichkeiten der Erhöhung der Biodiversität sowie potentielle Risiken und ertragskundliche Aspekte ein. Probleme und Verbesserungsmöglichkeiten im Zusammenhang mit der Beschaffung, dem Vertrieb und der Charakterisierung von forstlichem Vermehrungsgut haben 5 Beiträge im Kapitel 'Forstliches Vermehrungsgut (Baum- und Straucharten)' zum Inhalt. Die 5 Beiträge des Kapitels 'Genetisch nachhaltige Forstwirtschaft' befassen sich mit Aspekten einer sich wandelnden Forstwirtschaft und dem Schutz genetischer Ressourcen. Den einzelnen Beiträgen, bestehend aus Einleitung, Methodendarstellung, Ergebnissen, Folgerungen, Zusammenfassung und Literatur, ist jeweils ein Summary in englischer Sprache vorangestellt.

Insgesamt bietet das Buch eine interessante Sammlung aktueller Beiträge über Biodiversität und nachhaltige Forstwirtschaft, die in diesem Umfang bisher nicht zur Verfügung stand. Wissenschaftliche Institutionen, Waldbesitzer, Erzeuger von forstlichem Saat- und Pflanzgut und Verwaltungen, die sich mit naturnahem Waldbau befassen, werden dieses Buch schätzen.

M. LIESEBACH (Grosshansdorf)

Beech Forests. Geobotany, vol. 24. By R. PETERS. 1997. Kluwer Academic Publishers, Dordrecht, Boston, London. ISBN 0-7923-4485-5. XII and 169 pages, illus. Hardbound US\$ 141.-/SFr 198.-/GBP 86.-/NLG 220.-.

Beech forests are important in Europe, eastern Asia and North America. The author has done research on beech forests

in each of these three regions for about 10 years, and his large body of data is the starting point for comparisons.

The book includes 8 chapters. The first four chapters present in-depth literature reviews of the historical background of the present beech forests, spread (including a short passage to the genetic diversity of European *Fagus* stands) and specification of *Fagus* during the Tertiary and Quaternary periods, and intercontinental comparisons of the climates and soils associated with beech forests. These chapters provide a wealth of information on the evolutionary and developmental underpinnings of beech forests. The following three chapters compare the trees and forests themselves, on growth and form of beech, on the existence of geographic trends in species composition, on tree population structure, on spatial aspects of beech forests, on tree ring chronologies and climatic patterns, as they all relate to beech forest dynamics. The discussion of vertical structure is illustrated with clearly arranged profile diagrams. The book's last chapter on synthesis and conclusion features speculations on the future development of beech forests. Sustainable use of beech forest resources is an underlying theme. At all over 100 original figures and tables are presented, and dozens of original black and white photographs enrich the text. The book includes 10 pages references with some misspellings, nevertheless this is not a weak point of the book.

In this book the author gives a global but broad description of what 'has happened' to beech forests, in order to give a better prediction to how they will develop in future. No other book covers beech on this scale.

M. LIESEBACH (Grosshansdorf)

Impacts of Global Change on Tree Physiology and Forest Ecosystems.

Forestry Sciences, vol. 52. By G. M. J. MOHREN, K. KRAMER and S. SABATÉ (eds). 1997. Kluwer Academic Publishers, Dordrecht, Boston, London. ISBN 0-7923-4921-0. XI and 372 pages, illus. Hardbound US\$ 169.-/NLG 295.-/GPB 99.-.

At Wageningen, The Netherlands, in November 1996 an International Conference on impacts of global change on tree physiology and forest ecosystems was held. As outcome of this conference 46 papers are presented in these proceedings. The book contains 46 papers. They are grouped in 3 sections: I. Impacts of CO₂, air pollution and temperature on tree physiology (19 papers); II. Impacts of global change on forest ecosystems (13 papers) and III. Modelling and integrated assessments (14 papers). The papers, each between 5 and 13 pages, present an overview of the current knowledge of effects of air pollution and climate change, at the biophysical, biochemical and physiological level of trees, against the background of climatic conditions and natural stresses. The authors give an overview of their recent work and provide an entrance to their particular field of research rather than presenting unpublished material. It would be too much for this review to discuss the papers in particular. In summary, the present proceedings form an invaluable reference to all scientists working on impacts of air pollution or global change on forest ecosystems.

M. LIESEBACH (Grosshansdorf)

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