Bioelectrical Phenomena in Relation to Pollination in Pinus

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The pollen grains of *Pinus* are transported by air currents from the microsporangium to the "stigmatic" surfaces of the micropyle. Because the dispersion of pollen in any large coniferous forest is essentially at random, it has been assumed that the arrival of the pollen at the micropyle is purely a chance phenomenon. In support of this view, it has been shown that the effectiveness of pollination in terms of the number of sound seeds per cone, is related to the size of the pollen crop in the previous year (Florence and McWilliam, 1956). Examination of freshly pollinated ovules reveals a greater concentration of pollen around the rim and arms of the micropyle than on any other surface of the strobilus. A similar condition has been reported in *Picea* by Sarvas (1955). This could be explained by the fact that pollen adheres to the slightly sticky surfaces of the micropyle, rather than to the smooth sloping surfaces of the ovuliferous scales. It also raises the quetion of possible electrical phenomena being involved in this non-random distribution of pollen within the strobilus.

The first of these two possibilities has been investigated (McWilliam, 1958), and it is considered that the micropylar surfaces do have a stigmatic function. The second possibility has not been investigated, although previous work has demonstrated the existence of electrical potentials in trees. Lund (1929 a, b; 1931 a, b) dernonstrated an electromotive force of from 30—200 mv. in the main stem of young Douglas fir trees. He also showed that the apical growing point, and the tips of the lateral branches, were electropositive in relation to the more basal parts of the stem. Burr (1945, 1947, 1956) also demonstrated measurable potential gradients in living trees. With relation to other electrical phenomena in nature, it is a well known fact that small particles, or droplets, suspended in air, often carry an electrostatic charge, the nature and magnitude of which depends on how the charge originated. There has been no work done in this regard with pine pollen, but Gregory (1957), in working with several *Basidiomycetes,* has demonstrated that the spores carry either a positive or negative electrostatic charge, depending on the species.

The purpose of this study was to investigate the existence of a potential gradient between the pollen and the receptive strobilus, in *Pinus.* An understanding of such a potential might help to explain further the mechanism whereby pollen collects on the micropyle during pollination.

Material and Methods

Receptive female strobili on cut branches and on grafted scions of Austrian pine, *Pinus nigra* Arnold, were used. The strobili were protected from stray pollen by enclosing them in transparent pollination bags immediately after they emerged from the terminal bud. Because it was not possible to make readings on individual micropyles, on account of their small size and inaccessibility, readings were taken on entire strobili.

The development of a reliable millivoltmeter, and the technique for its use, makes it possible to measure accurately the potential difference between any two points in a living system (Burr and Mauro, 1949). In this study the electrical polarity of the potted grafts and cut branches was measured, and also an attempt was made to record possible potential gradients existing between the pollen and the receptive strobilus.

Electrical contact was made through reversible silver-silver chloride electrodes, which were prepared after the method described by Burr and Mauro (1949). The electrodes were connected by shielded leads to a D. C. vacuum tube millivoltmeter, and the nature and magnitude of the potential difference (millivolts) was read from a standard galvanometer. The instrument was designed so that the potential gradients can be determined independently of changes in resistance, and under conditions in which practically no current is drawn from the system which is measured (Burr, *et al.,* 1936).

The electrical polarity of the grafts and cut branches was measured by placing electrodes in contact with the tissue at the base and at the terminal bud. These electrodes operated in tap water, and were mounted in small glass pipettes. Before measuring the potential difference between the pollen and the receptive strobilus, the tip of the shoot bearing the strobilus was enclosed in a transparent plastic tube which was open at the upper end, and sealed to the branch below the bud *(Figure 1A).* The tube was designed, so that when filled with the conducting solution, the upper level of the solution stood one inch above the apex of the strobilus. To measure the standing potential difference between the electrodes, readings were first taken with only tap water in the system. Following this, readings were taken with the strobilus inserted in the tube, and the pollen applied to the upper surface of the liquid. To obtain an estimate of the contribution of the pollen alone, similar readings were taken with the strobilus removed from the system. The pollen was applied from an atomizer-type syringe to simulate field conditions as closely as possible. However, it was found during the course of the experiment that the manner in which the pollen was applied made no difference to the measured potential. To gain a further measure of the

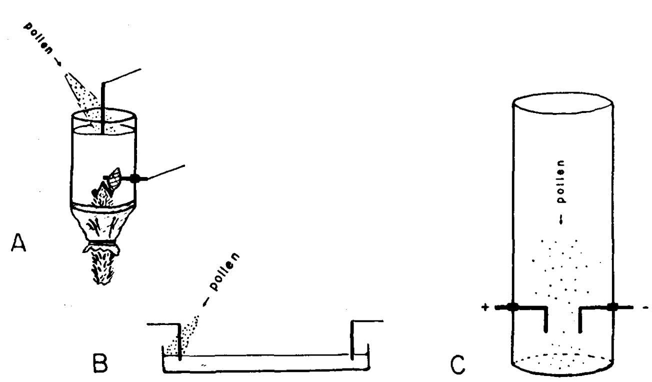

Figure 1. — Techniques *for* measuring bioelectrical phenomenn. — (A) Measurement of potential difference between pollen and strobilus. — (B) Agar plate for measuring the charge on the pollen. — (C) Apparatus for determining the nature of the charge carried by the pollen.

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potential which was supplied by the pollen, readings were taken across an agar plate, with and without pollen applied at one electrode *(Figure 1B)*. The values for the potential differences obtained were based on the average of three readings, all taken after the galvanometer had given a steady reading for several minutes.

The nature of the charge carried by the pollen was investigated by permitting pollen grains to fall in still air down a transparent plexiglass cylinder. At the base of the cylinder were two insulated copper electrodes, spaced two cm. apart with small blocks (1 cm^2.) of 1% agar on the surface of each. The electrodes were charged to + 135 and −135 v., respectively, by connecting them in series with two dry cell batteries *(Figure 1C)*. The pollen was released above the open end of the tube in a dark room, and its movement was observed as it passed through a narrow beam of light between the electrodes. After the pollen had been allowed to fall for two minutes, the agar blocks were removed, and a count made of the number of pollen grains adhering to the exposed vertical surfaces. The experiment was repeated six times reversing the charges on the terminals after each count to eliminate any bias in the design of the equipment. An similar number of (control) exposures were also made with no voltage differential across the electrodes.

Results and Discussion

The measurements of the potential differences are listed in *Table 1*. Readings taken over the entire branches and potted grafts indicated that, in every case, the bud carrying the female strobilus was positive with respect to the base. In the same way the charge on the pollen was determined to be negative with respect to the strobilus. The magnitude of the potential difference induced between the electrodes after the application of pollen, was dependent to a considerable extent on the quantity of pollen applied to the surface of the conducting medium. From the evidence of the controls, where pollen was applied to the system in the absence of the strobilus, it is clear that the pollen alone was carrying the charge responsible for the induced potential gradient. There is nothing to suggest that the strobilus was contributing to this measured potential difference, and the fact that a similar potential was measured across an agar plate, after the pollen was applied, is further evidence to support this view. Heat-killed pollen also gave a comparable potential difference when measured on an agar plate, which indicates that the charge carried by the pollen is not necessarily a property of the living cell.

Table 1. — Average potential differences (e. m. f.) recorded between pollen and receptive strobili in Austrian pine

Material	Electrode e. m. f. (mv.)	Total[1]) e. m. f. (mv.)	Induced e. m. f. (mv.)
Flowering scions	5	17	12
Cut Branches	10	19	9
Pollen applied with no strobilus	4	18	14
Pollen applied agar plate	7	19	12

[1]) Includes standing e. m. f. of electrodes plus the induced e. m. f.

The nature of the charge carried by the pollen was verified, both by visual observations, and by counts of the pollen grains adhering to the agar blocks on the electrodes. The deflection of the pollen grains towards the positive electrode was clearly visible, and the average distribution on both electrodes was as follows: positively charges, 264; negatively charged, 15. With no charge, the electrodes averaged 13 and 12 pollen grains respectively. This shows that many of the pollen grains carry a negative electrostatic charge. All pollen, however, does not appear to be carrying a charge, as some passed across the face of the positive electrode without any deflection. The pollen grains recorded on the negative electrode were probably of this type, their arrival being a matter of chance. The figures for the pollen counts on the uncharged electrodes (control) would also tend to support this view. Varying the voltage from 135 v. to 67.5 v., made no difference to the pattern of distribution on the electrodes.

Conclusions

No evidence could be obtained from this experiment which would support the theory that an electro-potential gradient is present which directs the pollen into the strobilus and on to the receptive surfaces of the micropyle. If such a gradient does exist, it is small in magnitude, and was not measurable with the instruments used. The measured potential between the receptive strobilus and the pollen has been attributed to the negative static charge carried by a high percentage of the pollen grains. This was readily demonstrated by passing the pollen between charged electrodes. With no real evidence to support any form of electrical attraction, an alternative mechanism is necessary to explain the arrival of the pollen at the micropyle. The most likely one is, that the windborne pollen passes between the ovuliferous scales and alights directly on the micropyle, or it alights on the smooth and inclined surface of the ovuliferous scale, and slides down to contact the micropyle at its base. The pollen adheres to the neck and arms of the micropyle until it is drawn into the ovule by the absorption of the micropylar fluid. Even under these chance conditions the process is quite effective on account of the high density of pollen present in the atmosphere during a normal pollination season.

Acknowledgements

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Summary

The possibility of bioelectrical forces being involved in the pollination of *Pinus* was investigated on strobili of Austrian pine. The terminal bud and the strobilus were found to be electro-positive with relation to more basal regions of the stem. A high percentage of the pollen was shown to be carrying a negative electrostatic charge. There was no evidence, however, to support the view that electro-potential gradients set up between the pollen and the receptive strobilus, are responsible for directing the pollen to the micropyle

Zusammenfassung

Titel der Arbeit: *Bioelektrische Erscheinungen im Zusammenhang mit der Bestäubung bei Pinus.*

Die Möglichkeit des Einwirkens bioelektrischer Kräfte bei der Bestäubung von Kiefern wurde an den Blüten von *Pinus nigra* var. *austriaca* untersucht. Endknospen und Blüten erwiesen sich im Vergleich zu den basalen Stammregionen als elektro-positiv. Ein großer Teil des Pollens zeigte negative Aufladung. Es waren jedoch keine Hinweise zu erkennen, daß zwischen Pollen und weiblichen Blüten verschiedene elektrostatische Aufladungen bestehen, die für das gerichtete Wachstum des Pollens zur Mikropyle verantwortlich sind.

Résumé

Titre de l'article: *Phénomènes bioélectriques dans la pollinisation des pins.*

On a recherché sur des fleurs femelles de pin noir d'Autriche l'intervention possible de phénomènes bioélectriques dans la pollinisation des pins. Le bourgeon terminal et la fleur femelle sont chargés positivement par rapport aux régions basales du rameau. Une forte proportion des grains de pollen sont chargés négativement. Il n'a cependant pas été possible de vérifier l'hypothèse que des gradients électriques existent entre les fleurs femelles et le pollen, et dirigent ce dernier vers le micropyle.

Literature Cited

Burr, H. S.: Diurnal potentials in the maple tree. Yale Jour. Biol. and Med. 17: 727—734 (1945). — Burr, H. S.: Tree Potentials. Yale Jour. Biol. and Med. 19: 311—318 (1947). — Burr, H. S.: Effect of a severe storm on electrical properies of a tree and the earth.. Science 124: 1204—1205 (1956). — Burr, H. S., Lane, C. T., and Nims, L. F.: A vacuum tube microvoltmeter for the measurement of bioelectrical phenomena. Yale Jour. Biol. and Med. 9: 65—76 (1936). — Burr, H. S., and Mauro, A.: Millivoltmeters. Yale Jour. Biol. and Med. 21: 249—253 (1949). — Florence, R. G., and McWilliam, J. R.: The influence of spacing on seed production. Z. Forstgenetik 5: 97—102 (1956). — Gregory, P. H.: Electrostatic charges on spores of fungi in air. Nature 180: 330 (1957). — Lund, E. J.: Electric polarity in Douglas fir. Publ. Puget Sd. Biol. Sta. 7: 1—28 (1929a). — Lund, E. J.: The relative electrical dominance of growing points in the Douglas fir. Publ. Puget Sd. Biol. Sta. 7: 29—37 (1929b). — Lund, E. J.: External polarity potentials in the apex of the Douglas fir before and after mechanical stimulation. Plant Physiol. 6: 507—517 (1931a). — Lund, E. J.: Electric correlation between living cells in cortex and wood in the Douglas fir. Plant Physiol. 6: 631—652 (1931b). — McWilliam, J. R.: The role of the micropyle in the pollination of *Pinus*. Bot. Gaz. (In press). — Sarvas, R.: Investigations into the flowering and seed quality of forest trees. Comm. Inst. For. Fenn. 45 (7): 1—37 (1955).

Buchbesprechungen

The genetic basis of selection. By I. M. Lerner. John Wiley & sons, New York, 1958. 298 Seiten. Preis 8,25 Dollar.

Das neue Werk Lerners ist ausschließlich den Problemen der natürlichen und artifiziellen Selektion bzw. deren genetischen Grundlagen gewidmet. Es ist gleichzeitig das einzige neuere Buch, in dem versucht wird, eine solche integrierende Zusammenfassung des in der Literatur verstreuten, umfangreichen Wissens um diese Dinge zu geben. Deshalb wird es sicherlich einen großen Leserkreis unter all denen finden, die sich in ihrer experimentellen Arbeit oder auch in theoretischen Studien mit Selektionsproblemen auseinanderzusetzen haben. Besonders auch für Forstgenetik und Forstpflanzenzüchtung, deren schwerfällige und langfristige Versuche eingehende und zweckentsprechende Planung zur Voraussetzung haben, und in denen Selektionsfragen auch in Zukunft eine hervorragende Rolle spielen werden, wird das Buch eine fühlbare Lücke zu schließen helfen. Es ist dabei kein Nachteil, daß Beispiele und Verfahren, die in das Buch aufgenommen wurden, meist, jedoch nicht ausschließlich, aus der Literatur der Haustierzüchtung stammen.

Die Lösung der schwierigen Aufgabe, ein Wissensgebiet für Biologen darzustellen, in dem komplizierte biologische Fragen und mathematisch-statistische Beweisführungen und Ableitungen eng nebeneinanderstehen und die biologischen Schlußfolgerungen oft nur unter den Voraussetzungen der mathematischen Modelle verständlich werden und verstanden werden können, ist dem Verfasser gut gelungen. Das notwendige mathematische Rüstzeug ist in 30 „Boxes" augenfällig gegen den Text abgesetzt. Einen breiten Raum nehmen praktische oder praxisnahe Beispiele ein, mit denen die theoretischen Erörterungen oder die Anwendbarkeit der Methoden belegt werden.

Besonders interessant für den Forstpflanzenzüchter dürfte die Darstellung der Planung von Selektionsversuchen sein, sowie der Schätzung der Varianzkomponenten genotypischer und phänotypischer Variation, die Voraussetzung aller Versuchsplanungen auf diesem Sektor der Züchtung ist. Ebenso dürften gerade den Forstpflanzenzüchter die Erörterungen über die Erhaltung der genotypischen Variation, der Inzuchtfragen u. a. interessieren, die in enger Beziehung zu seiner Arbeit stehen und deren Vernachlässigung zu praktisch irreversiblen Schäden an seinem Versuchsmaterial führen kann.

Das Inhaltsverzeichnis mag einen Überblick der behandelten Probleme geben: (1) Die Mendelpopulation. — (2) Polygenvererbung. — (3) Aufrechterhaltung der genetischen Variation. — (4) Reaktion auf Selektionsdruck. — (5) Selektion bei additiver Genwirkung. — (6) Selektion bei nichtadditiver Varianz. — (7) Züchtung auf bestimmte Merkmale. — (8) Ausblick. Stern

Conifere a rapido accrescimento. (Raschwüchsige Nadelhölzer.) Monti e Boschi IX, Sondernummer 7/8, 1958.

In der Einführung werden von Piccarolo die geeigneten Gebiete, die Kulturmethoden und die in Frage kommenden Holzarten gekennzeichnet.

Die „beschleunigte Holzzucht" findet ihre Rechtfertigung 1. in dem dauernden Holzmangel und der daraus folgenden Preiserhöhung und 2. in dem Vorhandensein weiter Gebiete, die von der Landwirtschaft nicht oder nicht mehr gewinnbringend genutzt werden können. Solche Gebiete umfassen besonders das Heideland, die weniger fruchtbaren Böden im Hügelland und in den Voralpen und manche seit kurzem entwässerte Böden.

Die Anbaumethode strebt danach, den Zuwachs zu steigern und die Umtriebszeit zu verkürzen. Dafür werden grundsätzlich folgende Richtlinien angenommen: (a) stärkeres Pflanzgut, als in der Forstwirtschaft üblich ist; — (b) gründliche Bodenbearbeitung und Düngung; — (c) landwirtschaftliche Zwischenkulturen; — (d) Pflegemaßnahmen, wie Aufastung, Bodenpflege, Schädlingsbekämpfung.

Piccarolo unterscheidet zwischen „intensiver Forstwirtschaft" und „Holzzucht für Industriezwecke". Für erstere kommen manche einheimischen Arten, besonders die Fichte, und einige fremden Hölzer, wie Douglasie, Weymouthskiefer, *Pinus insignis*, *P. rigida* und japanische Lärche in Betracht. — Für die „Holzzucht" bieten die raschwüchsigen fremdländischen Arten bessere Aussichten. Als besonders wichtig werden folgende Eigenschaften betrachtet: (1) stark entwickeltes Wurzelwerk: — (2) gute ökologische Anpassungsfähigkeit; — (3) Raschwüchsigkeit; — (4) Vereinbarkeit mit dem Unterbau; — (5) gute Auswertung der Pflegemaßnahmen; — (6) Krankheits- und Schädlingsresistenz; — (7) Anpassung an die Forderungen des Marktes und der Industrie.

In den nachfolgenden Artikeln werden die genannten Holzarten beschrieben.

In der *Douglasie* unterscheidet Pavari die drei Arten *Pseudotsuga Douglasii, Ps. glauca* und *Ps. macrocarpa*, sowie zahlreiche Zwischenformen. Nur die erste Art wird von ihm für den Anbau in Italien empfohlen, da *Ps. glauca* zwar frosthärter ist, dafür aber nicht nur langsamer wächst, sondern auch eine extreme Empfindlichkeit für *Rhabdocline Pseudotsugae* aufweist. *Ps. macrocarpa* ist besonders langsamwüchsig und ihre gerühmte Trockenresistenz scheint diejenige von *Ps. Douglasii* nicht wesentlich zu übertreffen. Für letztere Art wird eingehend das ökologische Verhalten in ihrer Heimat beschrieben. Morandini schildert ihren Anbau in verschiedenen europäischen Ländern, während ihr Verhalten in Italien wiederum von Pavari erörtert wird. Piccarolo beschreibt die *Weymouthskiefer* in ihrer Heimat, ihre Ansied-