# THE WAX STRUCTURE OF THE DEVELOPING NEEDLES OF PINUS SYLVESTRIS PROGENIES INFECTED BY LOPHODER-MELLA SULCIGENA

RISTO JALKANEN, SATU HUTTUNEN and SEIJA VÄISÄNEN

#### SELOSTE:

NUORTEN NEULASTEN VAHARAKENNE HARMAAKARISTEISISSA MÄNTYJÄLKELÄISTÖISSÄ

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The development of the stomatal area wax structure of pine needles was studied in flushing needles with a scanning electron microscope. The needles were obtained from eleven Finnish plus pine (Pinus sylvestris L.) progenies. The needles were taken from nearly uninfected and heavily infected trees. No differences in the early developmental stages of stomatal wax structure were observed between the southern Finnish, central Finnish and northern Finnish progenies. The general structure differed in the stomatal cavity chamber size. The stomatal openings were larger in heavily infected trees than in healthy trees. This might have an influence on the mechanical penetration of the fungal hyphae.

## INTRODUCTION

In the late 1970's, an epidemic of gray and ARMITAGE 1968, HAWTHORN and needle cast (Lophodermella sulcigena (Rostr.) v. Höhn. was observed in Finland (JALKANEN 1979). The disease is common in cultivated pine stands, including progeny trials, in which distinct genetic differences can be found between the progenies as to their resistance to the disease (JALKANEN 1980). A preliminary study was made in order to determine the significance of surface structure for the resistance and avoidance of penetration.

Cuticular wax is generally a mixture of relatively simple hydrocarbons, wax esters, and fatty alcohols, ketones and acids. The morphology of the wax projections is greatly influenced by environmental factors, such as humidity and temperature (BAKER 1974), genetic variability, and variability of environmental factors (DALY 1964, LEYTON

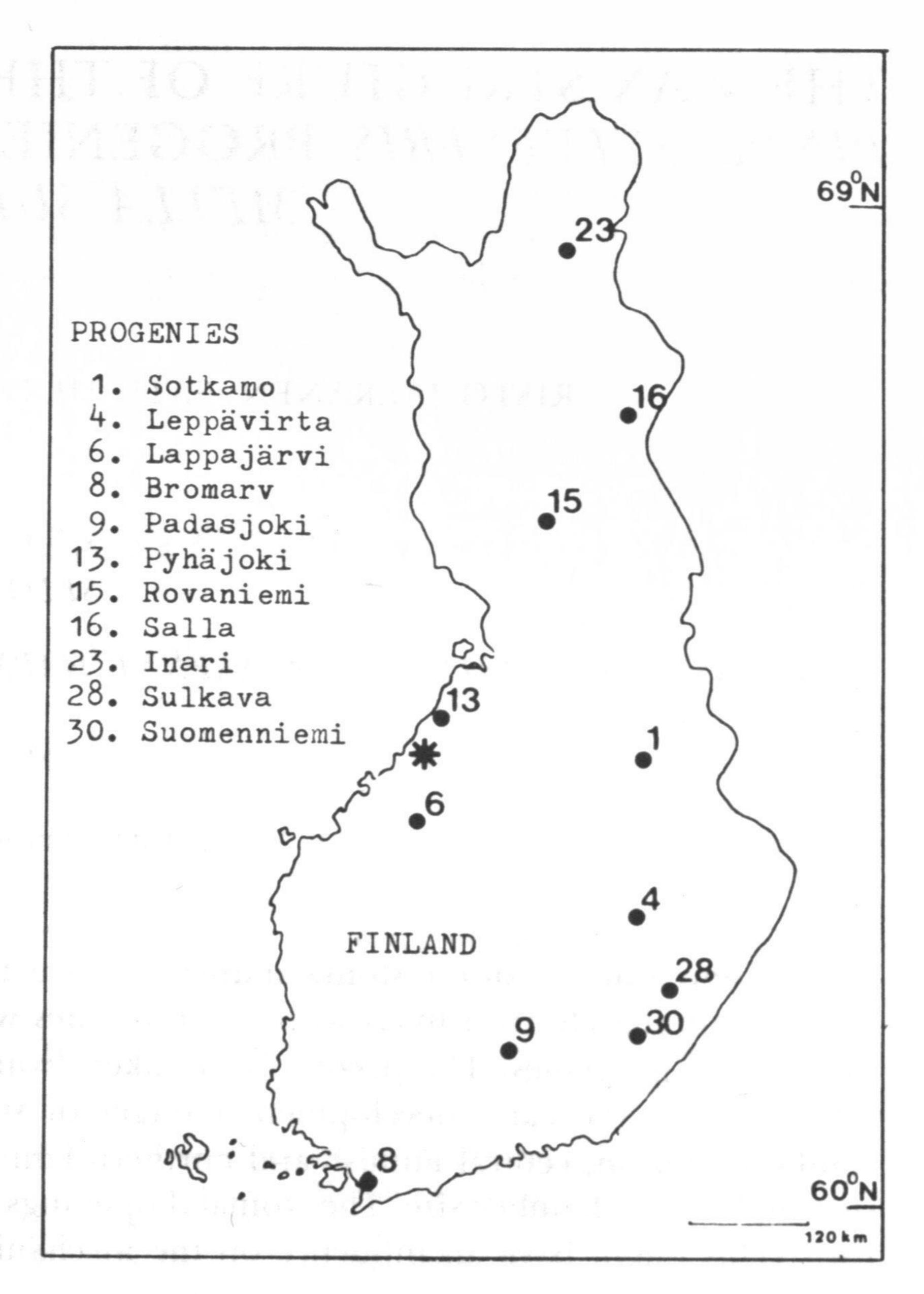
STEWART 1970, MARTIN and JUNIPER 1970, ROOK et al. 1971). The amount and quality of the waxes vary seasonally (SCHUCK 1972, 1976, SCHÜTT and SCHUCK 1972, 1973, REICOSKY and HANOVER 1976).

The main part of Pinus sylvestris L. needle surface possesses an amorphous surface wax, but the stomatal area consists of a hairylooking crystallous wax with fibrous wax structures (HANOVER and REICOSKY 1971, HUTTUNEN and LAINE 1979). The function of the stomatal cavity occlusions of conifers is not yet fully known. Several functions have been described, e.g. that of an antitranspirant (JEFFREE et al. 1975) and a mechanical barrier to the penetration of fungal hyphae, which normally enter the needle through stomata, etc.

Needle samples were obtained on the 4th

# MATERIAL AND METHODS

and 17th of June at Himanka, Kekolahti, from a fourteen-year-old progeny trial. The progeny trial is composed of eleven subtrials, all of which have been established by the Department of Forest Genetics of the Finnish Forest Research Institute. The subtrial was infected by L. sulcigena in summer 1979. The degree of infection in 1979 and 1980 was inventoried as described by Jalkanen in 1980. Eleven progenies from 29 were taken on the basis of latitude and the degree of infection for collection of needle samples (fig. 1). One almost healthy and one heavily infected tree were then marked for sampling purposes in every progeny. The needle samples were taken into the laboratory in cool containers. The samples were allowed to dry for twenty minutes at room temperature. Small needle cuttings were covered by a thin 20-30 nm gold film with Polaron E5100 sputter equipment. The samples were scanned under a Jeol JSM-35 scanning electron microscope at the Institute of Electron Optics, University of Oulu. Micrographs (with magnification of 1000x, 2000x and 10000x) were used for purposes of inspection. The micrographs were taken from the basal parts of young developing needles. During the sampling Fig. 1. The Pinus sylvestris progenies which were used for



period the needles were about 0-5 mm long. study in the progeny trial at Himanka (asterisk).

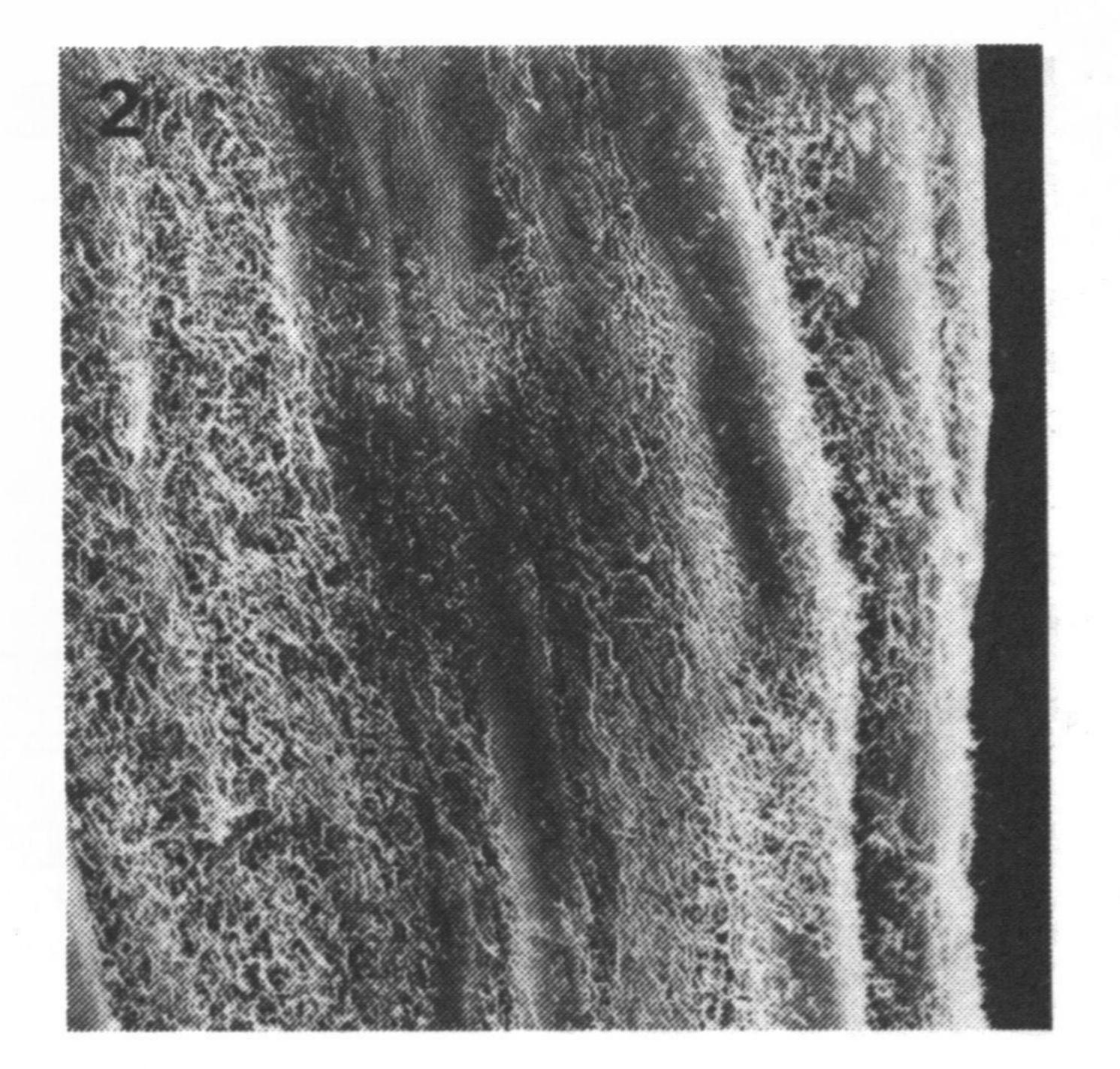
# RESULTS AND DISCUSSION

The wax structure develops along with needle elongation. The stomatal surfaces of displayed retarded needle development needles are covered by hairy-looking crystallous waxes soon after the flushing of the needle from the fascicular sheaths. The early development of needles inside the fascicular sheaths was monitored in the samples obtained on June 4th, 1980. A gradual development of fibrous wax structure could be seen (fig. 2 and 3). The areas free from fascicular sheaths had a typical hairy-looking wax structure, but the needle areas just beneath the fascicular sheaths continued to have an amorphous epicuticular wax structure (fig. 4 and 5).

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Later in June the progenies which (shorter needles) showed a premature structure of waxes in the stomatal areas. The fibrous hairy-looking wax structure was still under development.

In other progenies no significant differences in the epicuticular wax development were observed. The fibrous structure was developed in stomatal areas. The amount of fibrous structures just around the stomata was slightly smaller in some progenies and the stomatal cavity was larger. But the fibrous structure seemed mature even at a mangification of 10 000x (fig. 6). The



needles of the Pyhäjoki progeny (1000x), June 4th, 1980 waxes under the fascicular sheaths. Needles of the Pyhä-(more infected tree).

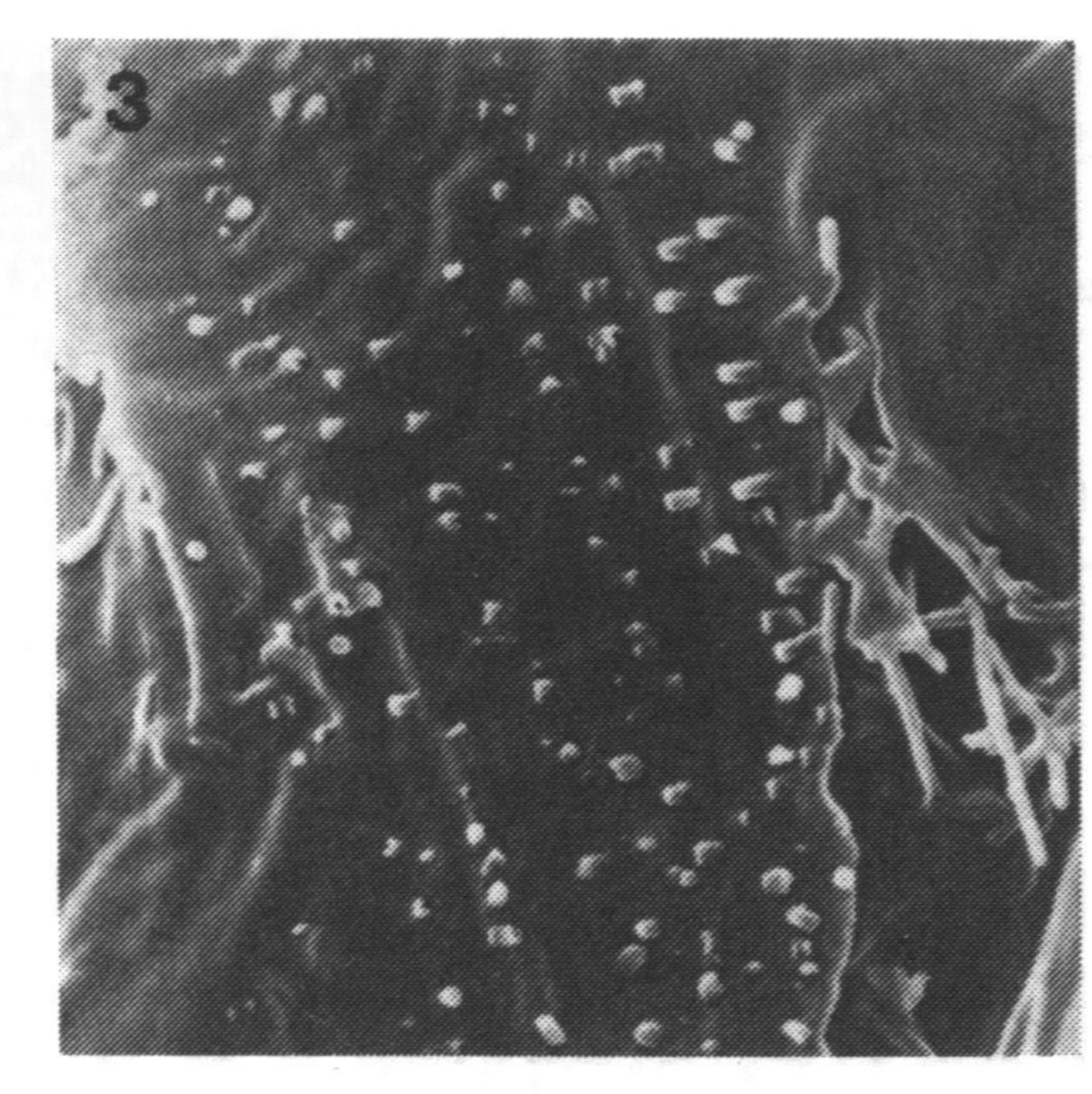
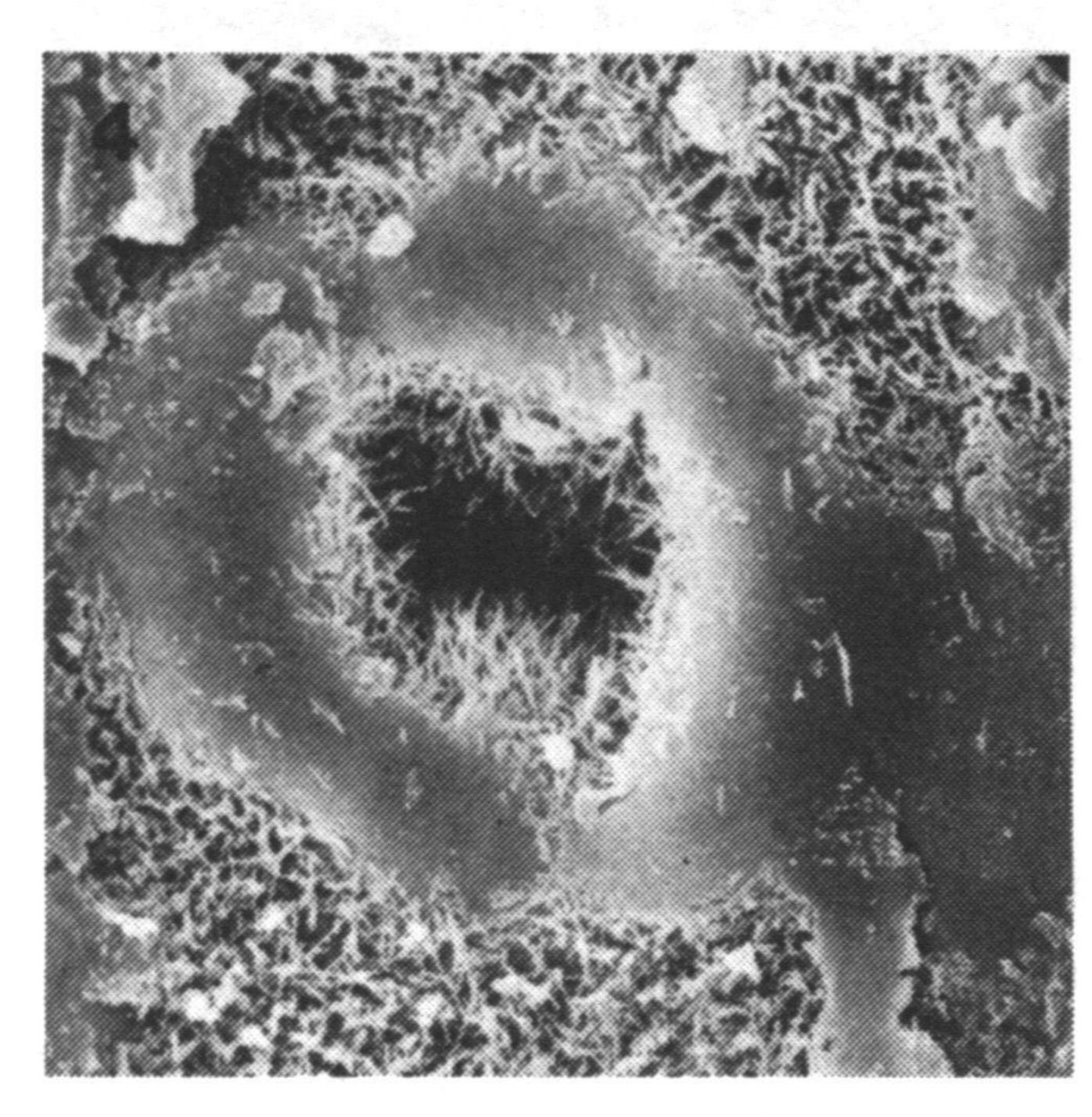


Fig. 2. The structure under the fascicular sheaths of the Fig. 3. The early development of fibrous epicuticular joki progeny (1000x), June 4th, 1980 (more infected



needles of the Sulkava progeny. The fibrous wax around structure in the needles of the Inari progeny (2000x) (mothe stomatal cavity is still developing (2000x), June 17th, re infected tree). 1980 (less infected tree).

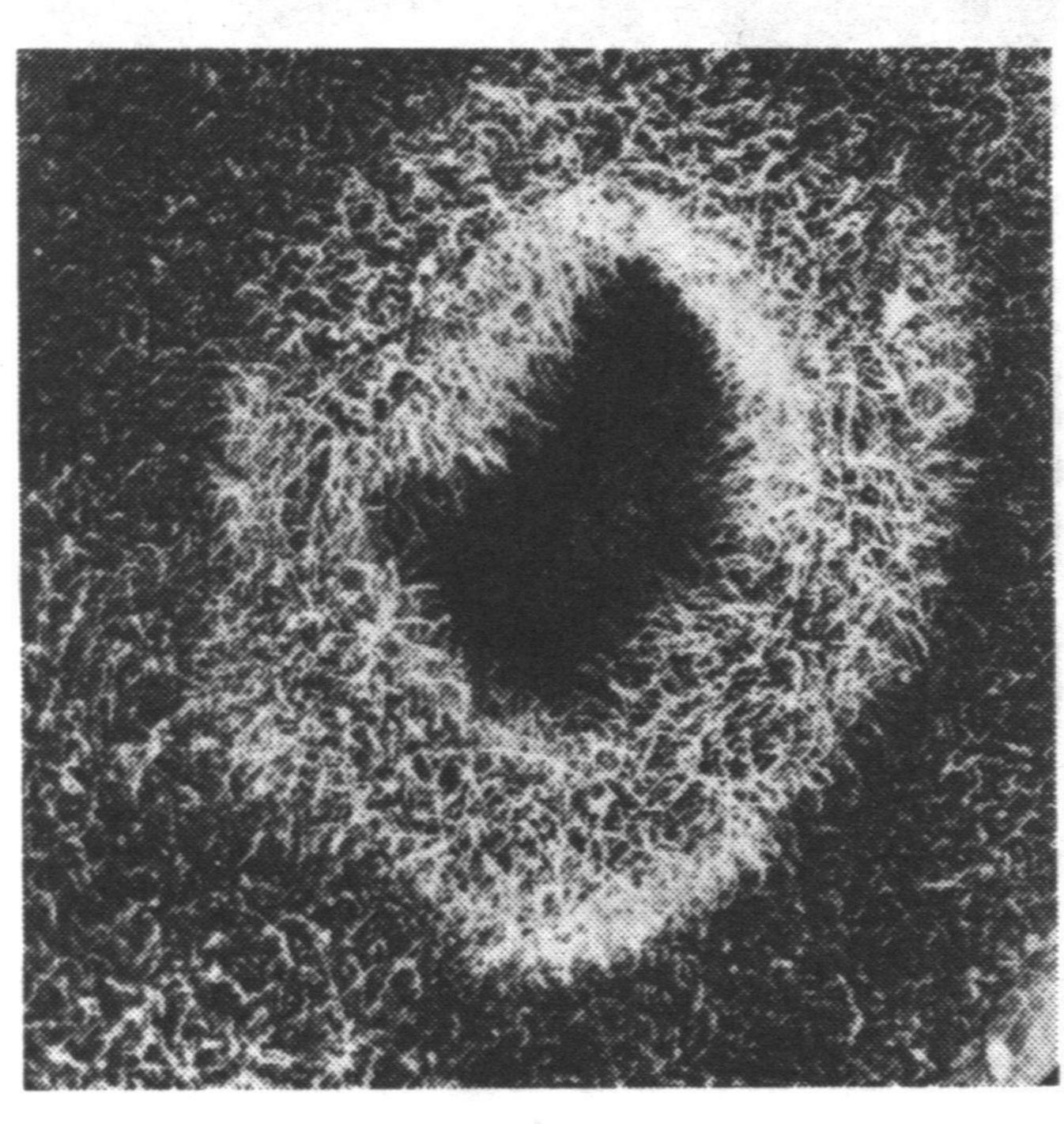


Fig. 4. The epicuticular wax surface of the stomata of the Fig. 5. Almost fully developed stomatal area fibrous wax

stomatal area waxes in the different progenies the tree. The mean area of stomatal cavity were highly similar in a micrograph series of openings was 214.5  $\mu$ m<sup>2</sup> (n=11) in the more June 17th (fig. 7, 8 and 9). The needles were infected trees and only 169 µm² (n=11) in the still under elongation. There were no visible almost healthy trees. The significance of this differences between the southern and more observation is not straight forward, but rather northern progenies, as seen in the figures 7, 8 indirect, suggesting a favourable structure and 9. The stomatal cavity size or pore size of and microenvironment for fungal penetramore infected trees was greater than that of less infected trees. This cavity size has nothing to do with the actual morphology of the stomatal cells, being the size of the opening in the wax architecture. The size of the opening seemed to vary with the degree of infection of

tion. Later on, observations were made on L. sulgicena in the stomatal cavities.

The development of wax structure had no clear connection with the susceptibility of a progeny to L. sulcigena. In infected and almost healthy trees the wax was synthetized

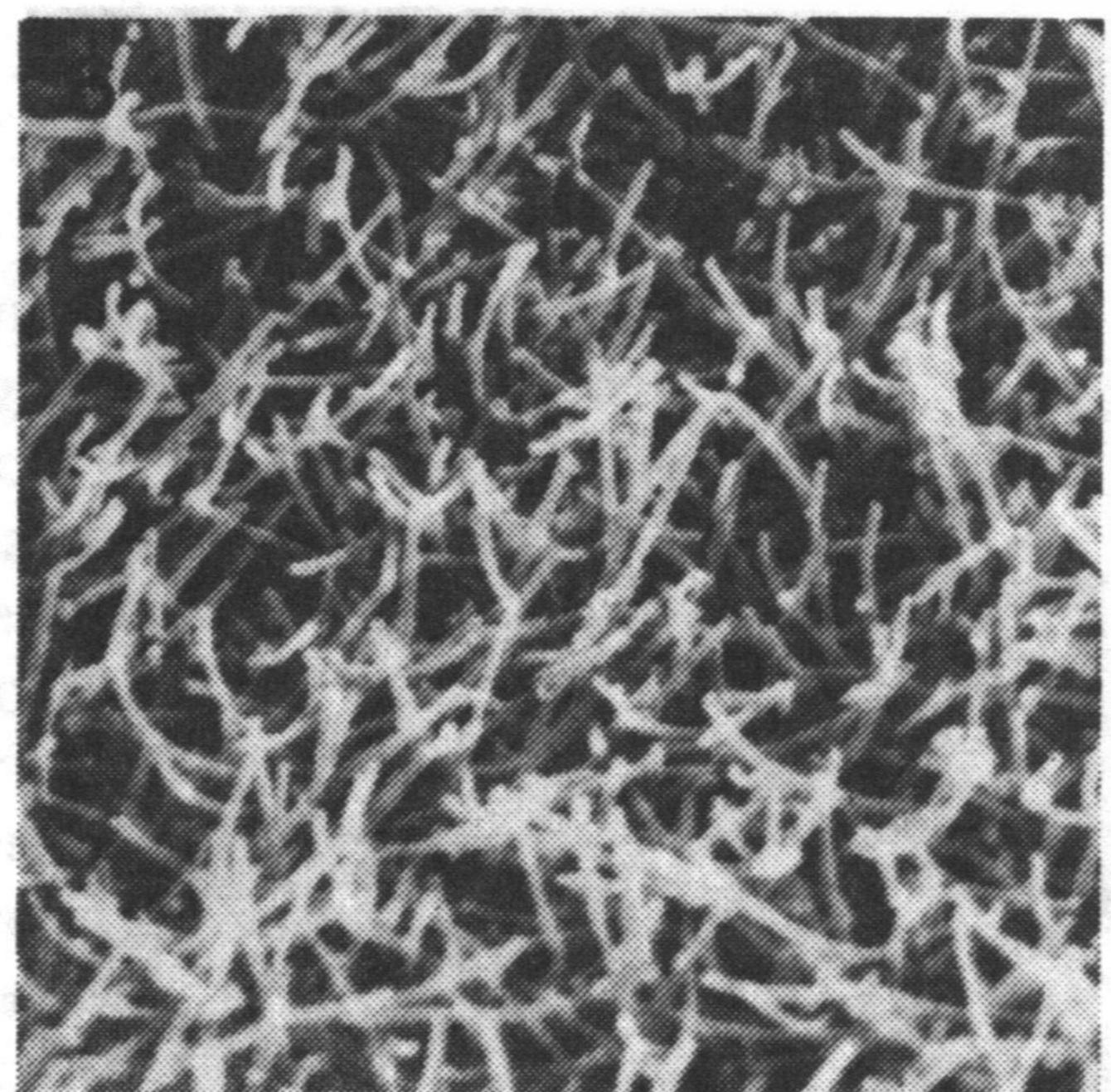
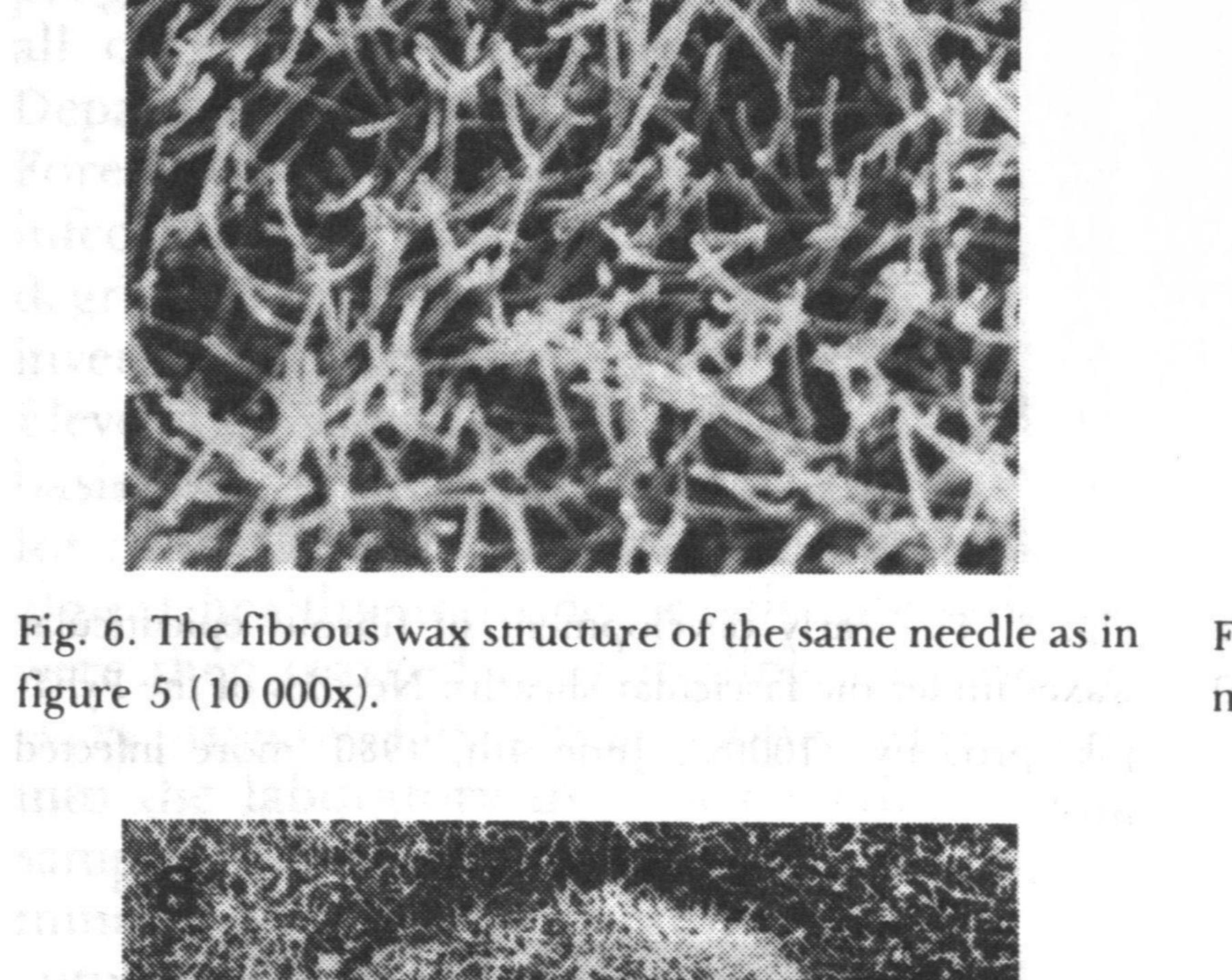


Fig. 6. The fibrous wax structure of the same needle as in figure 5 (10 000x).



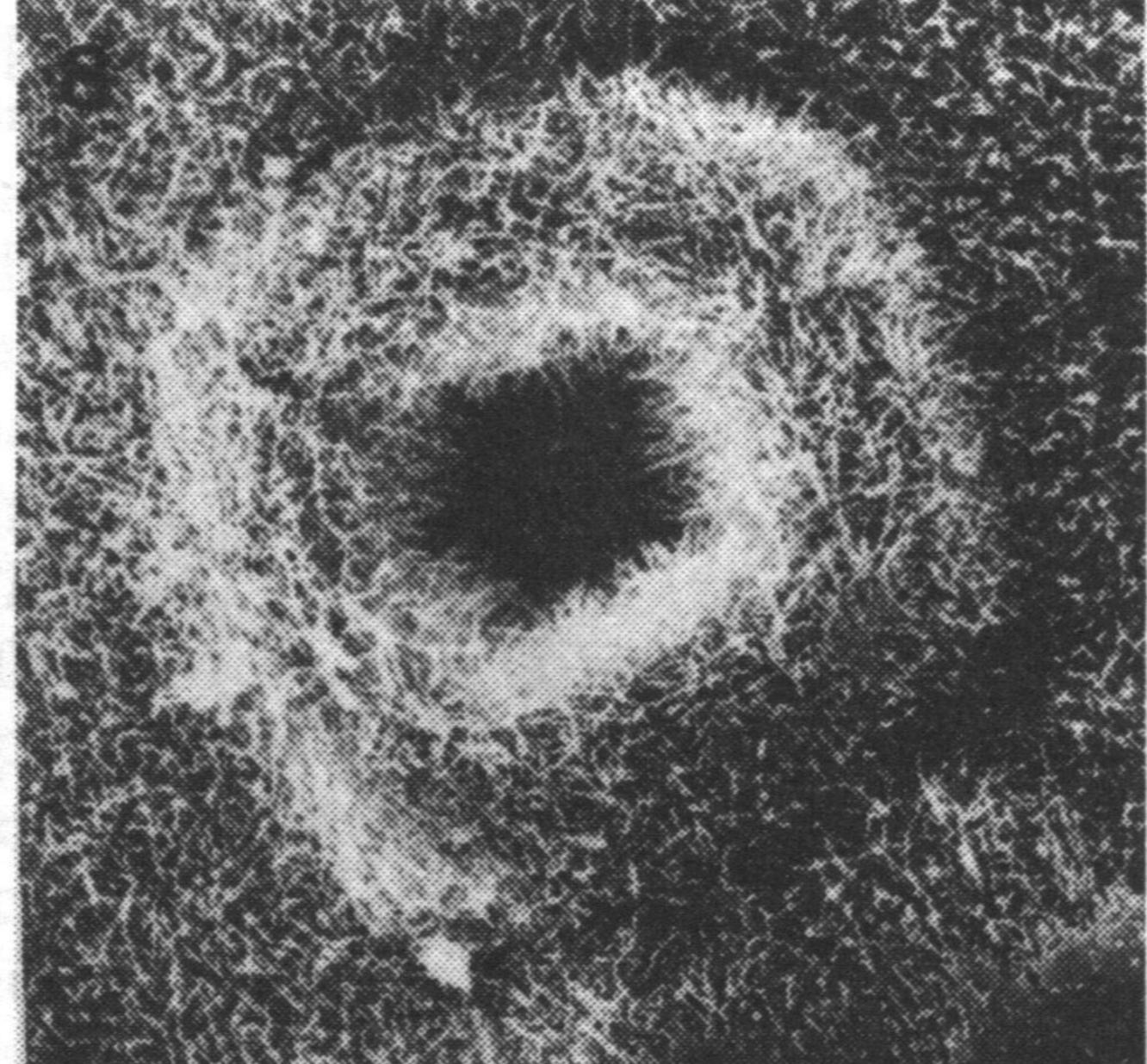


Fig. 8. The stomatal wax surface of the progeny of Suomenniemi (2000x) (less infected tree).

fascicular sheaths. According to other observations, too, the wax structure is not directly connected with the susceptibility to Lophodermium pinastri (Schrad.) Chev. (SCHUCK 1972). It has also been indicated that the amount of wax in the resistant older needles is the same as in younger needles, which are susceptible to Diplodia pinea (Desm.) Kick (WALLA and PETERSON 1976). In amount of waxes vary with needle age 1973, MITCHELL et al. 1976). (SCHUTT, and SCHUCK 1973, FRANICH

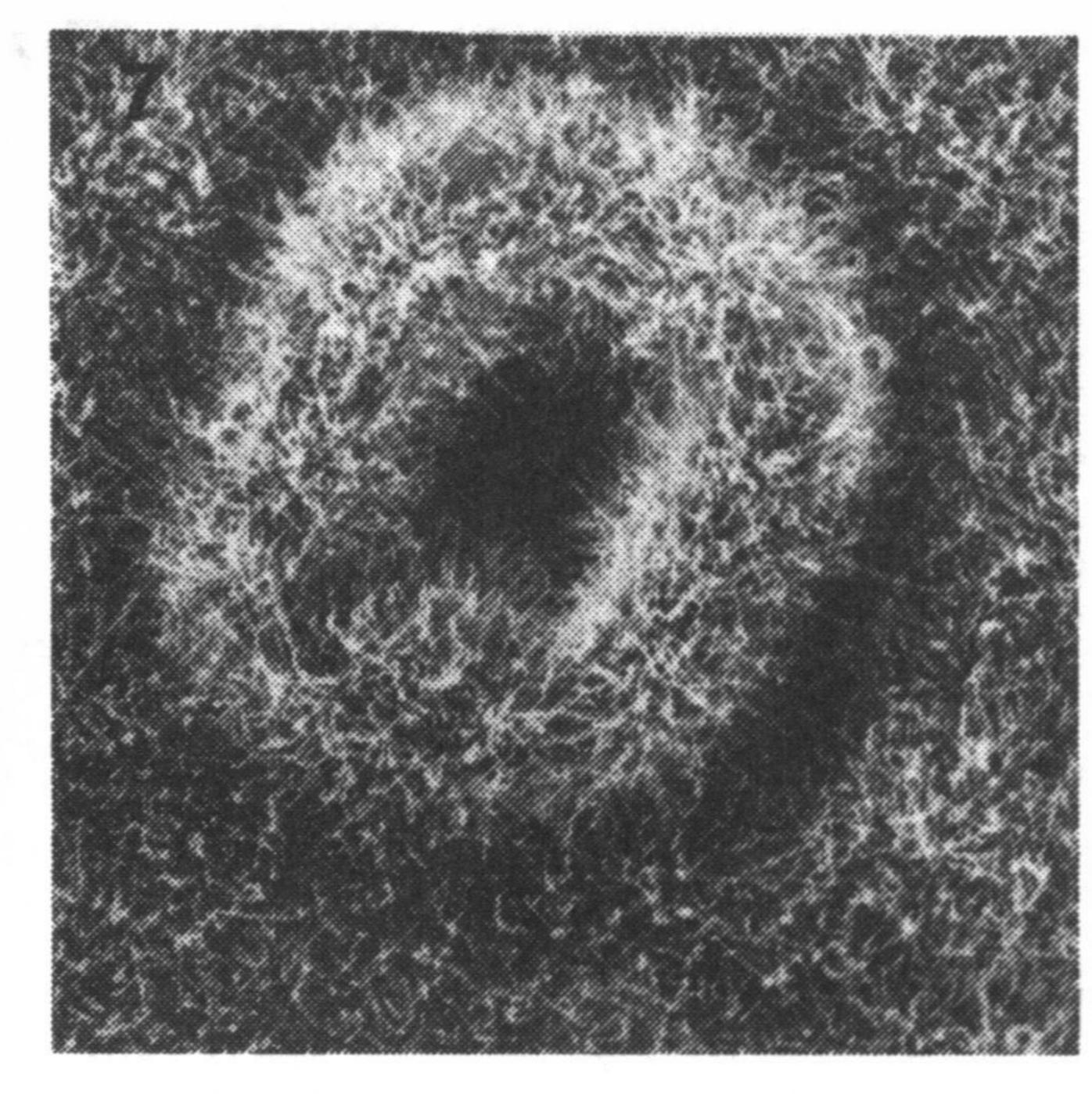


Fig. 7. The stomatal wax surface of the progeny of Bromary (2000x) (less infected tree).

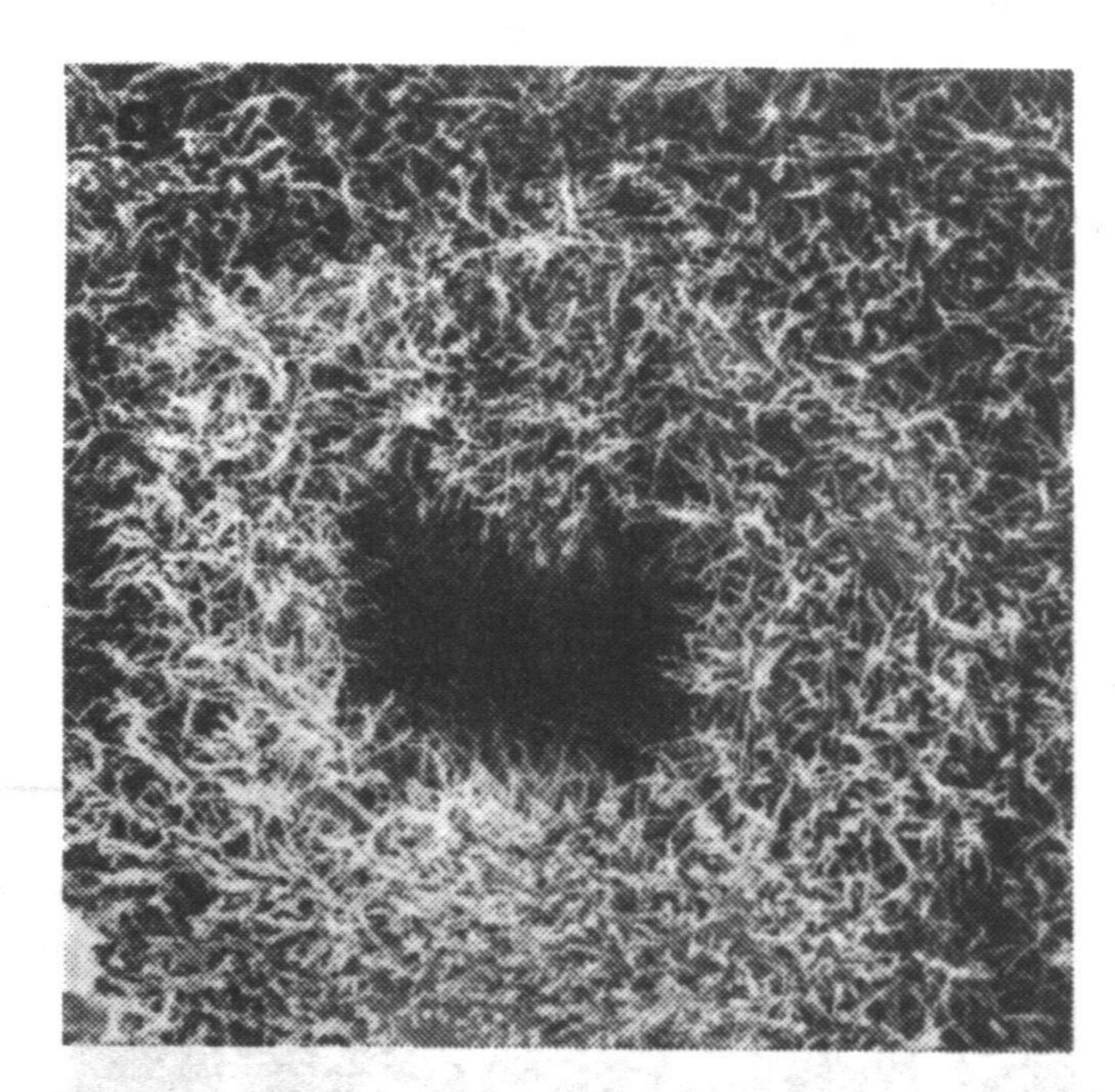


Fig. 9. Stomatal area wax surface in the Lappajärvi progeny (2000x) (more infected tree). A sparsely fibrous wax surface.

simultaneously with the release from SCHUCK 1972, HUTTUNEN and LAINE 1979). In this case the stomatal cavity size was found to be different in different trees. These are of course normal differences due to the progeny of trees, but it should be pointed out that the largest cavities were seen in the more infected trees without a definite connection to the progeny. This suggests that L. sulcigena enters the needle through the stomata. The microclimate of the stomatal some other studies, however, it has been cavities of different trees might have some observed that the wax structure and even the influence on the penetration (CAMPBELL

It has been proposed that the zone which is et al. 1977. The air pollution induced susceptible to gray needle cast is about 5 mm wax erosion has been observed to alter the from the basal area of the needle surface surface structure of needles (SCHÜTT and (MILLAR 1970). If the ascospore is farther

away from the base of the needle, it does not 1972, SCHÜTT and SCHUCK 1973). The germinate at all (CAMPBELL 1972, 1973). highest quotients were observed at the early This might have something to do with cavity stage of needle development. Later on the size and cavity microenvironment in pine quotient decreases. The highest percentage of needles. The chemical composition of waxes the ester phase is observed in July and the vary with needle age.

chemical composition of the cuticular waxes case of L. sulcigena infection. Further studies in of P. sylvestris, a high quotient of some ester this respect are needed. phase substances has been found (SCHUCK

increase is very rapid. These changes in wax In studies of seasonal changes in the composition might be of importance in the

## LITERATURE

BAKER, E. A. 1974. The influence of environment on leaf wax development in Brassica oleracea var. gemnifera. New. Phytol. 73: 955-966.

CAMPBELL, R. 1972. Electron microscopy of the epidermis and cuticle of the needles of Pinus nigra var. maritima in relation to infection by Lophodermella sulcigena. Ann. Bot. (London) 36: 307-314.

- "- 1973. Ultrastructure of asci, ascospores and spore release in Lophodermella sulcigena (Rostr.) v. Höhn. Protoplasma 78: 69-80.

DALY, G. T. 1964. Leaf surface wax in Poa colensoi. J. Exp. Bot. 15: 160-165.

FRANICH, R. A., WELLS, L. G. & BARNETT, J. R. 1977. Variation with tree age of needle cuticle to-Don. Ann. Bot. (London) 41: 621-626.

HANOVER, J. W. & REICOSKY, D. A. 1971. Surface wax deposits on foliage on Picea pungens and other conifers. Am. J. Bot. 58: 681-687.

HAWTHORN, W. R. & STEWART, J. W. 1970. Epicuticular wax forms on leaf surfaces of Zizania aquatica. Can. J. Bot. 48: 201-205.

HUTTUNEN, S. & LAINE, K. 1979. The structure of pine needle surface (Pinus sylvestris L.) and the deposition of air-borne pollutants. Archives of Env. Protec. In print.

JALKANEN, R. 1979. Harmaakariste vaivaa männyntaimistoja. Metsä ja Puu 6-7: 34-35.

- " - 1980. Occurrence of Lophodermella sulcigena (Rostr.) v. Höhn. in clones and progenies of Scots pine. 3rd International IUFRO S 2.05 Workshop on the Genetics of Host-Parasite Interactions in Forestry, Wageningen, the Netherlands, September 14-21,

JEFFREE, C. E., BAKER E. A. & HOLLOWAY, P. J. 1975. Ultrastructure and recrystallization of plant epiculticular waxes. New Phytol. 75: 539-549.

LEYTON, L. & ARMITAGE, I. P. 1968. Cuticle structure

and water relations of the needles of Pinus radiata Don. New Phytol. 67: 31-38.

MARTIN, J. T. & JUNIPER, B. E. 1970. The cuticles of plants. Edward Arnold, London. 347pp.

MILLAR, C. 1970. Role of Lophodermella species in premature death of pine needles. Rep Forest Res., London 1970: 176-179.

MITCHELL, C., WILLIAMSON, B. & MILLAR, C. 1976. Hendersonia acicola on pine needles infected by Lophodermella sulcigena. Eur. J. For. Path. 6: 92-

REICOSKY, D. A. & HANOVER, J. W. 1976. Seasonal changes in leaf surface waxes of Picea pungens. Am. Bot. 63: 449-456.

pography and stomatal structure of Pinus radiata ROOK, D. A., HELLMERS, H. & HESKETH, J. D. 1971. Stomata and cuticular surfaces of Pinus radiata needles as seen with a scanning electron microscope. J. Ar. Academ; of Science 6: 222-225.

SCHUCK, H. J. 1972. Die Zusammensetzung der Nadelwachse von Pinus sylvestris in Abhängigkeit von Herkunft und Nadelalter sowie ihre Bedeutung für die Anfälligkeit gegenüber Lophodermium pinastri (Schrad.) Chev. Flora 161: 604-622.

-"- 1976. Quantitative Variation der Wachsauflange und der cuticulären Kallenwasserstoffe bei Picea abies Nadeln. Flora 165: 303-314.

SCHÜTT, P. & SCHUCK, H. J. 1972. Zusammenhänge zwischen Rauchhärte und Cuticularwachsen bei Koniferen – in Wirkungen von Luftverunreinigungen auf Waldbäume. Mitt. Forst. Versuchs. Anst. Wien 97(11): 399-417.

- "- 1973. Jahreszeitliche Schwankungen in der Zusammensetzung der Cuticularwachse von Pinus sylvestris. Flora 162: 206-214.

WALLA, J. & PETERSON, G. 1976. Dothistroma pini and Diplodia pinea not affected by surface wax of pine needles. Plant Dis. Rep. 60 (12): 1042-1046.

#### SELOSTE:

### NUORTEN NEULASTEN VAHARAKENNE HARMAAKARISTEISISSA MÄNTYJÄLKELÄISTÖISSÄ

koopilla 11 eri mäntyjälkeläistön neulasten vaharaken- Tutkimuksessa havaittiin kuitenkin eroja vaharakenteen teen kehitystä nuorissa kasvavissa neulasissa. Tutkimuk- suojaavissa ominaisuuksissa. Neulasten ilmarakokuoppa sessa vertailtiin vaharakennetta sairaissa ja hyvä- oli huonommin vahanukalla suojattu ja suurempi sairailkuntoisissa puissa. Ilmarakoalueen kiteisten vahojen la puilla kuin terveillä. Tällä on ilmeisesti merkitystä kehityksessä ei ollut havaittavissa merkittäviä ajallisia sieni-infektiossa. eroja sairaiden ja terveiden puiden välillä. Eroja ei

Tutkimuksessa tarkasteltiin pyyhkäisyelektronimikros- myöskään ollut havaittavissa eri jälkeläistöjen välillä.