Morphological Variation in Epicuticular Wax of SO_2-Sensitive and -Tolerant Eastern White Pine Clones

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ABSTRACT

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Needle surfaces of sulfur dioxide-tolerant and -sensitive clones of *Pinus strobus* were examined by scanning electron microscopy to determine if variability related to sensitivity class correlated with differences in epicuticular wax morphology. Epistomatal wax formed a continuous covering over the stomata of tolerant clones, but was split longitudinally over the stomata of sensitive clones.

Additional key words: cold-stage microscopy, osmium-vapor fixation, *Pinus strobus*, scanning electron microscopy, split epistomatal wax.

Intraspecific variation in sensitivity of *Pinus* species to pollutants has been reported (1,2,3,9,10), and several studies have investigated the relationship of needle anatomy to sensitivity, as well as histochemical and histological responses to pollutant exposure (4,11,13,14).

Epicuticular waxes on conifer needles have also been studied. Hanover and Reicosky (5) described epicuticular wax morphology in *Pinus strobus*, noting that stomatal chambers were not occluded by surface waxes. On *P. strobus* needles subjected to ambient air pollution, however, stomatal chambers appeared to be completely occluded by cuticular waxes (12). Trimble et al (15) concluded that cuticular wax structure was not correlated with ozone sensitivity, since wax structure was similar on needles sampled from one ozone-sensitive and one ozone-tolerant clone of *P. strobus*.

The purpose of this study was to determine if unique morphological features exist in the foliar surface anatomy of eastern white pine clones previously known to be tolerant or sensitive to sulfur dioxide.

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MATERIALS AND METHODS

Initial selections of pollution-sensitive or -tolerant eastern white-pine (*Pinus strobus* L.) ortets were made in three Christmas tree plantations in central Ohio by Eckert and Houston (2). All three of the plantations were located within the airsheds of large industrialized, urban centers. Sensitive and tolerant ortets were selected in pairs on the basis of current-year foliage characteristics. An ortet was considered sensitive if mottling could be found on needles (6), while tolerant ortets were characterized by a complete lack of mottling. Members of each pair were located within 7 m of each other to minimize site differences. Sensitivity to SO_2 was subsequently verified during controlled fumigations of SO_2 at 5.0 ($\pm$0.5) pphm for 2 hr by Eckert and Houston (2).

Preparation of clonal material. Scions collected from ortets were side-grafted to potted nursery-run 2-yr-old seedlings. Ramets were maintained in a greenhouse at 25–30 C under mist until growth was

apparent on the scion (20–30 days). After bud-break occurred, grafts were moved to a lath house for the remainder of the growing season and fertilized monthly with Ingestad's solution (7).

Experimental treatments. Needles of a tolerant clone and sensitive clone were grown in a charcoal-filtered greenhouse and sampled for scanning electron microscopy (SEM) at early elongation, mid-elongation and complete elongation stages. In addition, completely elongated needles of 10 tolerant clones and 12 sensitive clones were grown in ambient air and sampled for SEM. To investigate intraclonal variability, samples were also collected from six ramets each of a tolerant clone and a sensitive clone grown in ambient air at Delaware, OH. Ambient air was monitored for SO_2 and ozone that occurred at background levels.

Scanning electron microscopy. In order to fix and kill guard cells

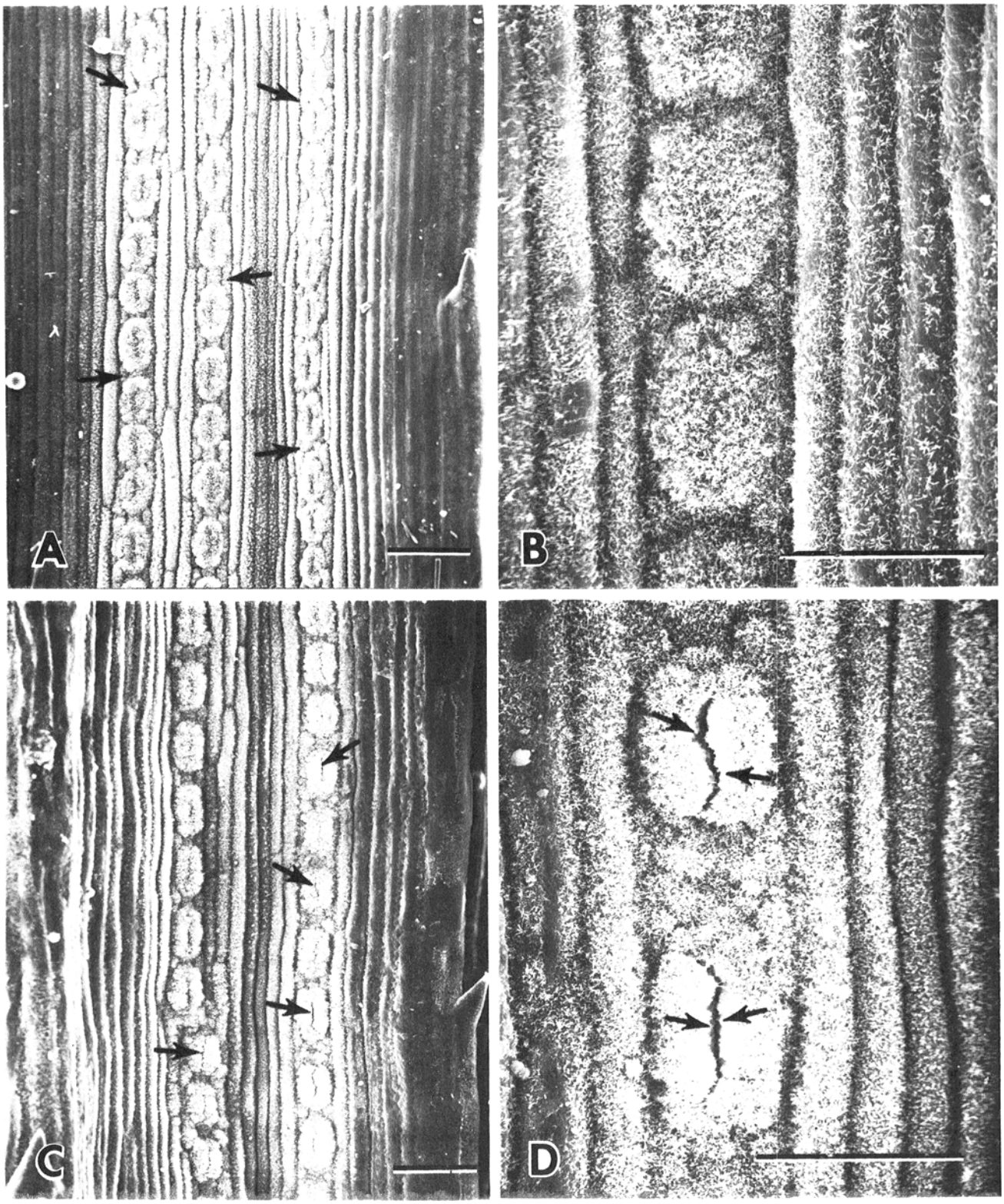

Fig. 1. (A and B) Needles of an SO_2-tolerant clone of *Pinus strobus* grown in charcoal-filtered air (bars = 10 μm). **A,** Needle surface covered by downy epicuticular wax with turgid subsidiary cells (arrows) in channeled rows. **B,** Note continuous epistomatal wax over stomatal apertures. **(C and D)** Needles of an SO_2-sensitive clone of *P. strobus* grown in charcoal-filtered air (bars = 10 μm). **C,** Needle surface covered with downy wax, turgid subsidiary cells in channels but **D,** exhibiting discontinuous split epistomatal wax over stomatal appertures, arrows.

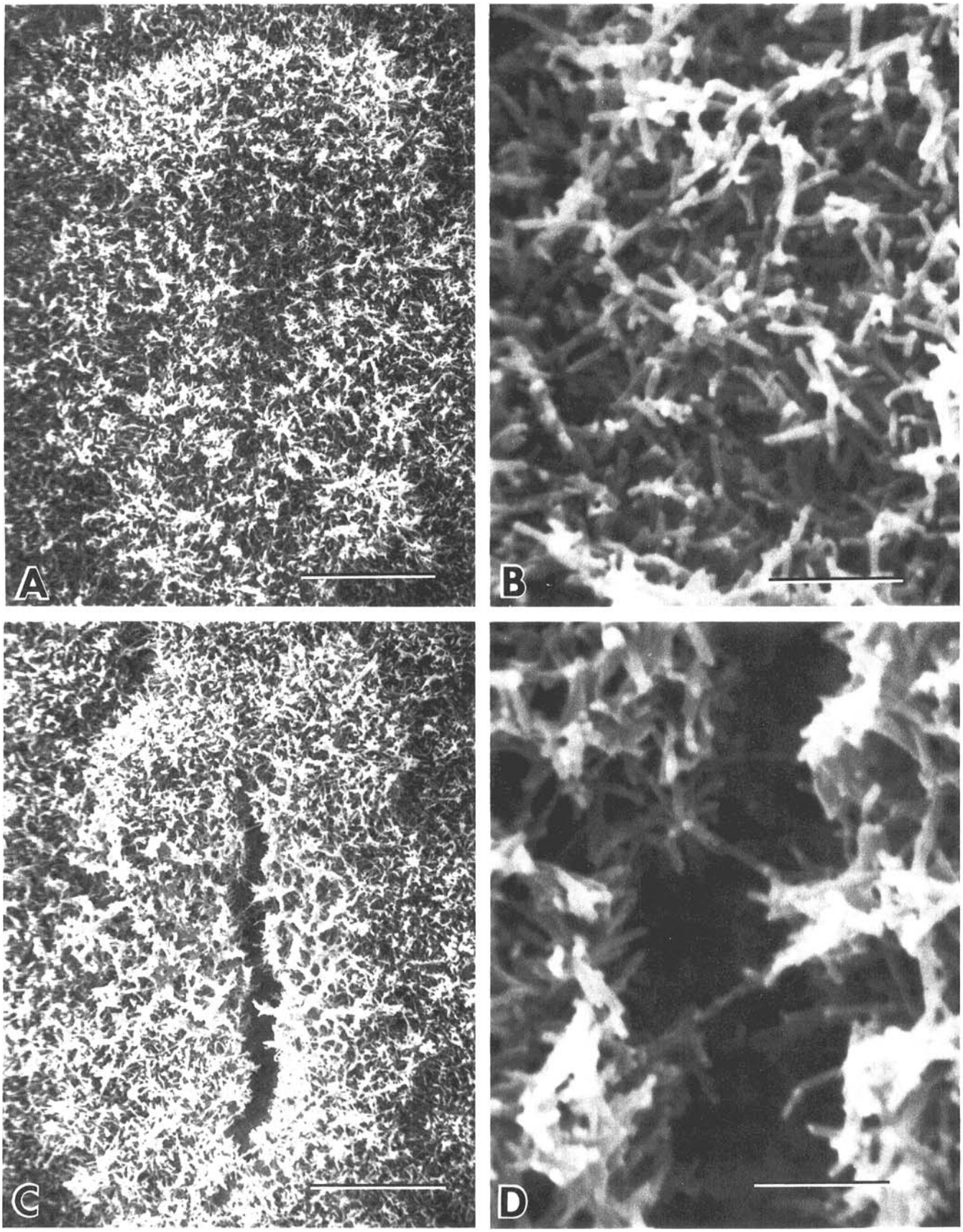

Fig. 2. (A and B) Needles of an SO_2-tolerant clone of *Pinus strobus* grown in charcoal-filtered air. **A,** Individual stoma with continuous epistomatal wax (bar = 2 μm). **B,** Continuous wax fibrils over stomatal aperture (bar = 0.5 μm). **(C and D)** Needles of an SO_2-sensitive clone of *P. strobus* grown in charcoal-filtered air. **C,** Individual stoma with discontinuous epistomatal wax (bar = 2 μm). **D,** Split epistomatal wax fibrils over stomatal aperture (bar = 0.5 μm).

and immobilize any movement of the epistomatal apparatus, the middle one-third of ten needles from each treatment was fixed by using an OsO_4-vapor technique as follows. Fresh needles were immediately suspended on nylon netting that had been placed on an uncovered preparation dish (38 mm) filled with 2% OsO_4 in a pH 7.2 phosphate buffer. The preparation dish was placed into a larger preparation dish (100 mm), covered to create a saturated OsO_4 atmosphere and maintained at 22 C. Osmium-vapor fixation was used in lieu of standard SEM preparative procedures. Krause and Townsend (10) found that standard SEM solvents could remove or alter epicuticular wax of *Pinus* spp. Samples from each treatment were mounted with conductive cement on specimen stubs and sputter-coated with gold (Hummer V; Technics, Springfield, VA 22153). Tissue was examined with a Hitachi S-500 SEM set at 20 kV and 5 mm working distance with 40-degree tilt. A cold stage adjusted to −80 C was used during examination to reduce heat-induced artifacts.

RESULTS AND DISCUSSION

The appearance of the epicuticular waxes observed with SEM was similar to that described by Hanover and Reicosky (5) as "structural," ie, of a "fibrillar, netted, or thread-like consistency." The "amorphous" wax type described in previous reports (5,12,15) was not observed in the 22 clones examined in this study, even in those grown for a full season in ambient air.

SEM-examined needles of all tolerant clones, grown in either charcoal-filtered or ambient air, appeared to be covered by downy, or fibrillar, epicuticular wax (Fig. 1A). Stomata were flanked by turgid, subsidiary cells in channeled rows (Fig. 1A). Wax fibrils formed a continuous covering over stomatal apertures (Figs. 1B, 2A and B). All 10 SO_2-tolerant clones had identical epistomatal wax configurations, regardless of the stage of needle development.

Needles of SO_2-sensitive clones were also covered by fibrillar epicuticular wax (Fig. 1C), identical in appearance to that observed on tolerant needles. Epistomatal wax, however, appeared to be discontinuous and was split, or cracked, longitudinally across the stomates (Figs. 1D, 2C and D). The appearance of these stomates was very similar to those pictured in a report by Trimble et al (15). They studied epicuticular waxes of one ozone-tolerant and one ozone-sensitive clone of *P. strobus*, but did not identify the sensitivity class of the tissue in their photographs. Split epistomatal wax was evident in all 12 sensitive clones in our study, regardless of whether they were grown in charcoal-filtered or ambient air, and regardless of stage of needle development.

These results suggest that epistomatal wax characteristics may serve as a marker to screen for SO_2 tolerance in eastern white pine selection and breeding programs. The presence of cracks across stomata may also enhance gas exchange (8), resulting in increased SO_2 absorption by sensitive individuals.

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