Identification of Salt Spray Injury to *Pinus* species with Scanning Electron Microscopy

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ABSTRACT

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Studies were conducted to determine whether surface changes induced by NaCl spray could be detected with scanning electron microscopy (SEM) and whether these changes varied between *Pinus aristata* and *P. parviflora*. SEM revealed that, in contrast to control plants, salt-sprayed needles were injured, showing coalesced wax and flaccid subsidiary cells. Apparently, salt injury to pines can be seen by SEM examination before macroscopic symptoms are expressed.

Additional key words: salt tolerance, bristlecone pine, Japanese white pine.

Pines (*Pinus* spp.) are frequently planted along streets or highways, where they are subject to injury by deicing salt spray (2,8,9). Assessment of relative tolerance to salt spray has been based on field observations rather than on environmentally controlled studies (6,7).

Scanning electron microscopy (SEM) was used to detect surface injury to Scots pine needles (*Pinus sylvestris*) damaged by windborne salt (5). Salt crystals, lacerations, and splits on needle surfaces were observed. Barrick et al (1) used SEM to observe unsprayed *P. nigra* and *P. strobus* and then related the amount of epicuticular wax to salt spray resistance. Logan (4) found wax platelets around stomatal openings of *P. strobus* viewed with SEM and suggested that they serve as a salt barrier. These structures, however, appeared to be coating artifacts induced by heat from vacuum evaporation. Differential foliar sensitivity to NaCl spray was found among *Pinus* spp. at this laboratory (A. M. Townsend, *unpublished*). Bristlecone pine (*P. aristata* Engelm.) was more susceptible than Japanese white pine (*P. parviflora* Sieb. and Zucc.). The purpose of the present study was to determine whether surface changes induced by NaCl spray could be detected with SEM and whether these changes varied between these two species.

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MATERIALS AND METHODS

Experimental treatments. Seedlings of bristlecone pine and Japanese white pine were potted and grown in a peat-perlite-soil mixture (2:2:1) within a lathhouse. After one season of growth (March to September), the pots were sealed in plastic bags. Two groups with 10 replications of each species were placed in a cold room at 2–4 C with a 12-hr photoperiod. One group of plants was sprayed twice daily for 10 days with distilled water and the other group with 2% aqueous NaCl solution to runoff. A pump sprayer set at 207 kPa (30 psi) was used to apply treatments. Plants were then placed in a greenhouse for development of symptom expression.

Temperatures varied from 7 to 13 C and relative humidity varied between 40 and 75% for the duration of the study. The percentage of foliar symptoms (ie, browning, banding, necrosis, and chlorosis) was estimated for each seedling immediately after spraying ended and 2 wk later. Survival was recorded 10 mo after treatments were completed.

Scanning electron microscopy. At the end of the spray period, 20 fresh foliar samples from each treatment were mounted on specimen stubs sputter-coated with gold to avoid heat artifacts. Tissue was examined with a Hitachi S-500 SEM set at 20kV and 5 mm working distance with a 40° tilt. Fresh specimens were examined because solvents used in standard SEM preparative procedures (3) could remove or alter epicuticular wax and salt crystal distribution.

RESULTS AND DISCUSSION

Macroscopic observations. Necrosis was not observed on either species sprayed with H_2O. Needle browning and banding were apparent at the end of the 2-wk treatment period on all *P. aristata* seedlings sprayed with NaCl, with an estimated average of 38% of the needles showing symptoms. Two weeks later, the symptomatic needles of *P. aristata* averaged 66%. Only 12% of the needles of *P. parviflora* exhibited symptoms at the end of the treatment period, but after 2 wk in the greenhouse an estimated 57% of the needles exhibited injury. Ten months after treatment, 30% of the treated *P. aristata* plants were still alive. This was significantly less than the 92% survival of *P. parviflora*.

Surface changes. When H_2O-sprayed *P. aristata* needle surfaces were examined with SEM, they appeared to be covered by downy epicuticular wax with turgid subsidiary cells (Fig. 1A) in channeled

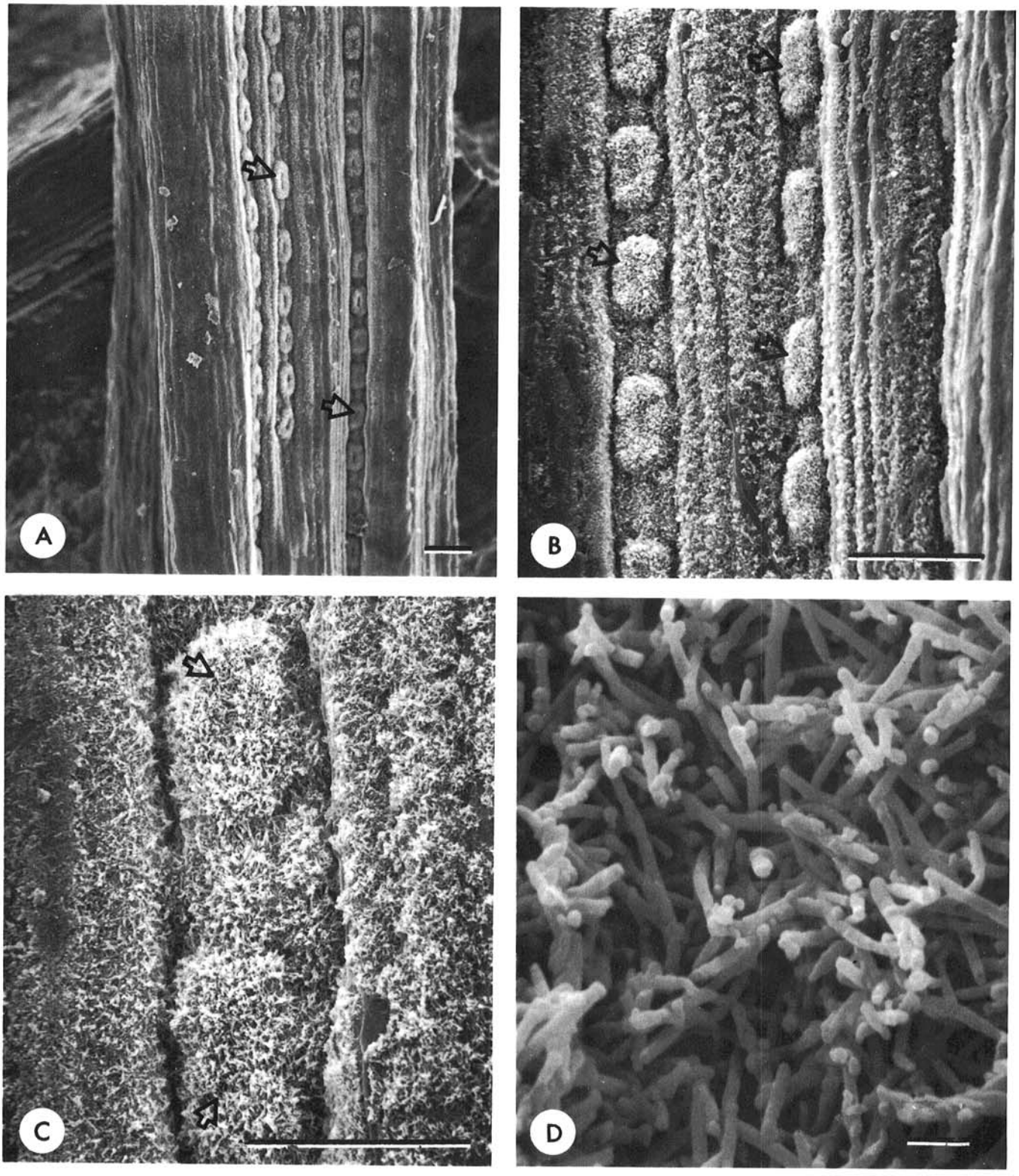

Fig. 1. Scanning electron micrographs of H_2O-sprayed *Pinus aristata* needles. **A,** Surfaces covered by downy epicuticular wax (arrows) (bar = 5 μm); **B,** turgid subsidiary cells (arrows) in channeled longitudinal rows (bar = 5 μm); **C,** wax loosely covering subsidiary cells (arrows) (bar = 5 μm); **D,** wax crystals appearing tubular in shape (bar = 0.5 μm).

longitudinal rows (Fig. 1B). Wax loosely covered the subsidiary cells (Fig. 1C). At higher magnification, wax crystals appeared tubular in shape (Fig. 1D). The needle surfaces of *P. parviflora* sprayed with H_2O appeared similar to those of *P. aristata* when examined with SEM (Fig. 2). Rows of turgid subsidiary cells (Fig. 2A and B), loosely arranged wax (arrows, Fig. 2C), and tubular-shaped crystals (arrows, Fig. 2D) could be seen. No microtopographical differences were found between H_2O-sprayed plant samples examined immediately after treatment or 2 wk after treatment.

SEM examination of NaCl-sprayed pines revealed changes in surface structure. *P. aristata* needles lacked downy epicuticular wax and had rows of flaccid subsidiary cells visible at lower magnifications (Fig. 3A and B). Flaccid subsidiary cells and coalesced wax were more apparent at higher magnifications (Fig. 3C and D). *P. parviflora* needles sprayed with NaCl exhibited similar microtopographical symptoms (Fig. 4A and B); flaccid subsidiary cells and coalesced epicuticular wax dominated the

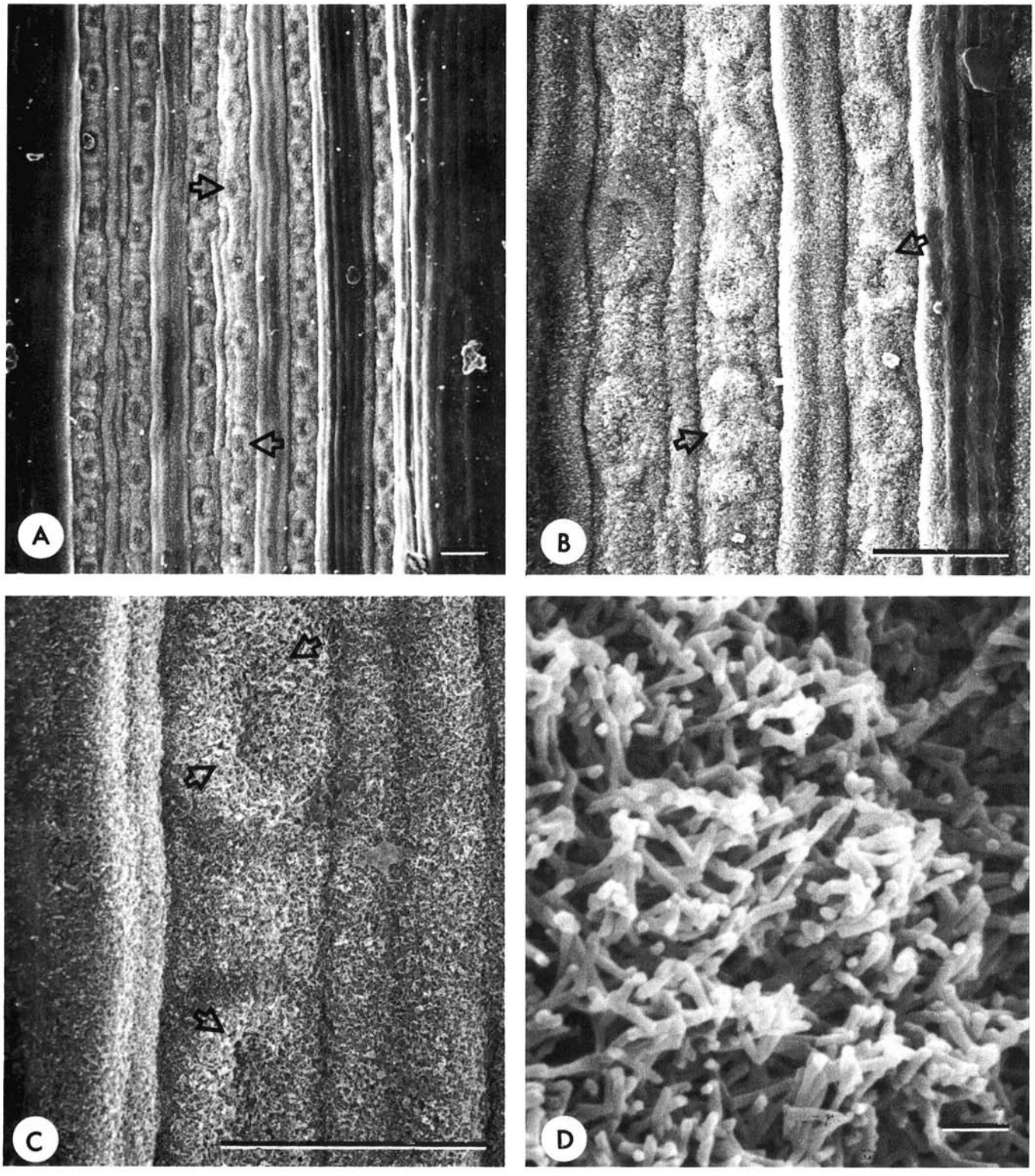

Fig. 2. Micrographs of H_2O-sprayed *Pinus parviflora* needles. **A,** Surfaces covered with downy epicuticular wax (arrows) (bar = 5μm); **B,** turgid subsidiary cells in rows (arrows) (bar = 5 μm); **C,** wax loosely covering subsidiary cells (arrows) (bar = 5 μm); **D,** wax cystals appearing tubular in shape (bar = 0.5 μm).

needle surfaces (Fig. 4C and D). Although both species sprayed with salt exhibited similar microtopographical symptoms (ie, flaccid subsidiary cells and coalesced wax) initially as well as 2 wk later, *P. parviflora* showed less macroscopic injury. Previously injured needles exhibited flaccid subsidiary cells and coalesced epicuticular wax when examined with SEM 10 mo after salt spray. No stomatal apertures of *P. aristata* or *P. parviflora* appeared to be plugged with wax following salt treatments. Epicuticular wax in stomata does not seem to be a barrier to salt as noted by Logan (4).

Salt spray affects epicuticular wax structures and subsidiary cell configurations on sensitive and tolerant *Pinus* spp. Salt-induced changes were detected with SEM before macroscopic symptoms were expressed on needles of *P. parviflora*. SEM can be used to identify salt injury to needles of *Pinus* spp. or to detect changes in the physical structure of epicuticular wax that might predispose affected pines to pathogens or to abiotic stress.

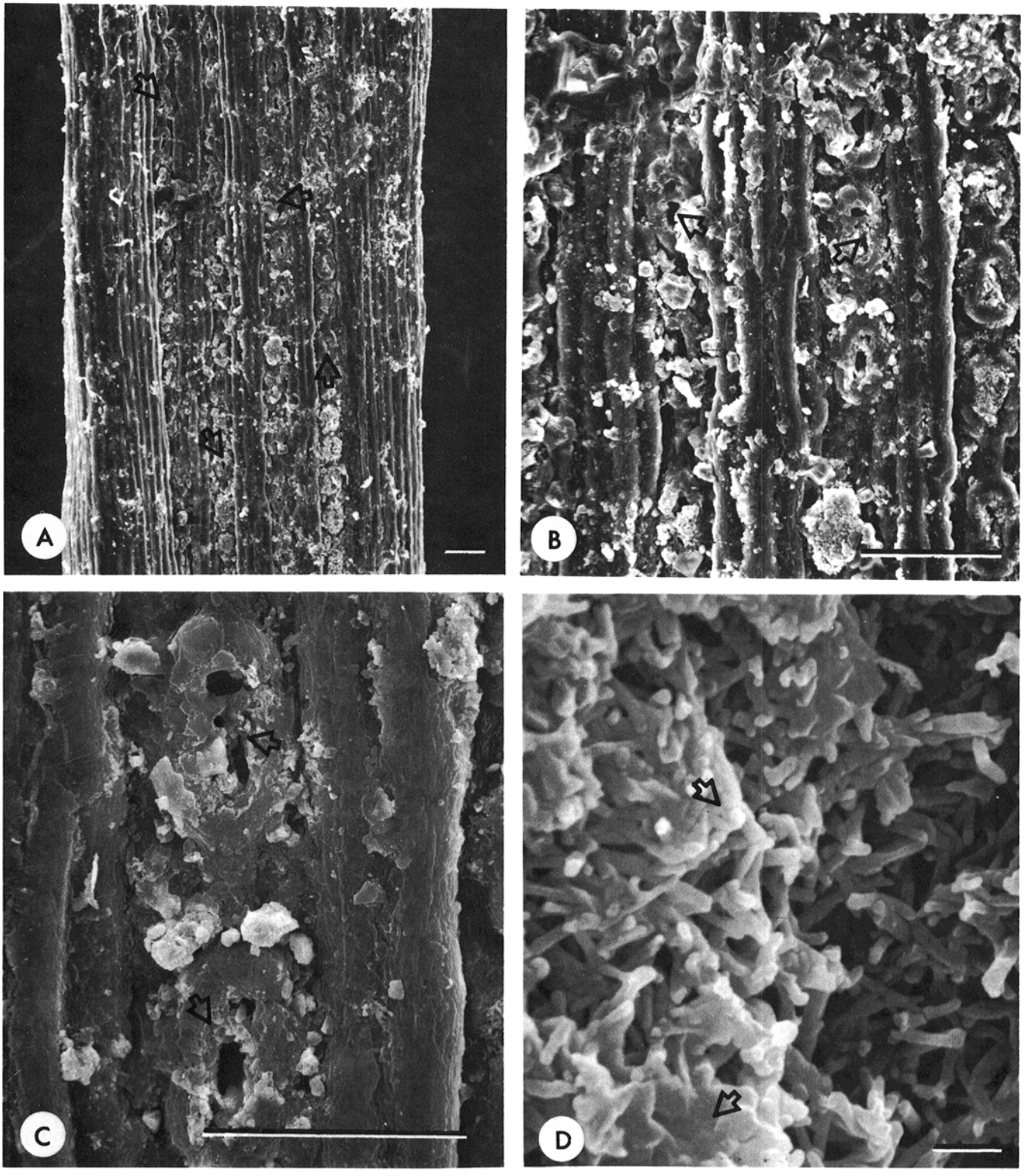

Fig. 3. Micrographs of *Pinus aristata* that were sprayed with NaCl. **A,** Lack of downy epicuticular wax (arrows) (bar = 5 μm); **B,** rows of flaccid subsidiary cells (bar = 5 μm); **C,** flaccid subsidiary cells covered by coalesced wax (arrows) (bar = 5 μm); **D,** epicuticular wax appearing coalesced (arrows) (bar = 0.5 μm).

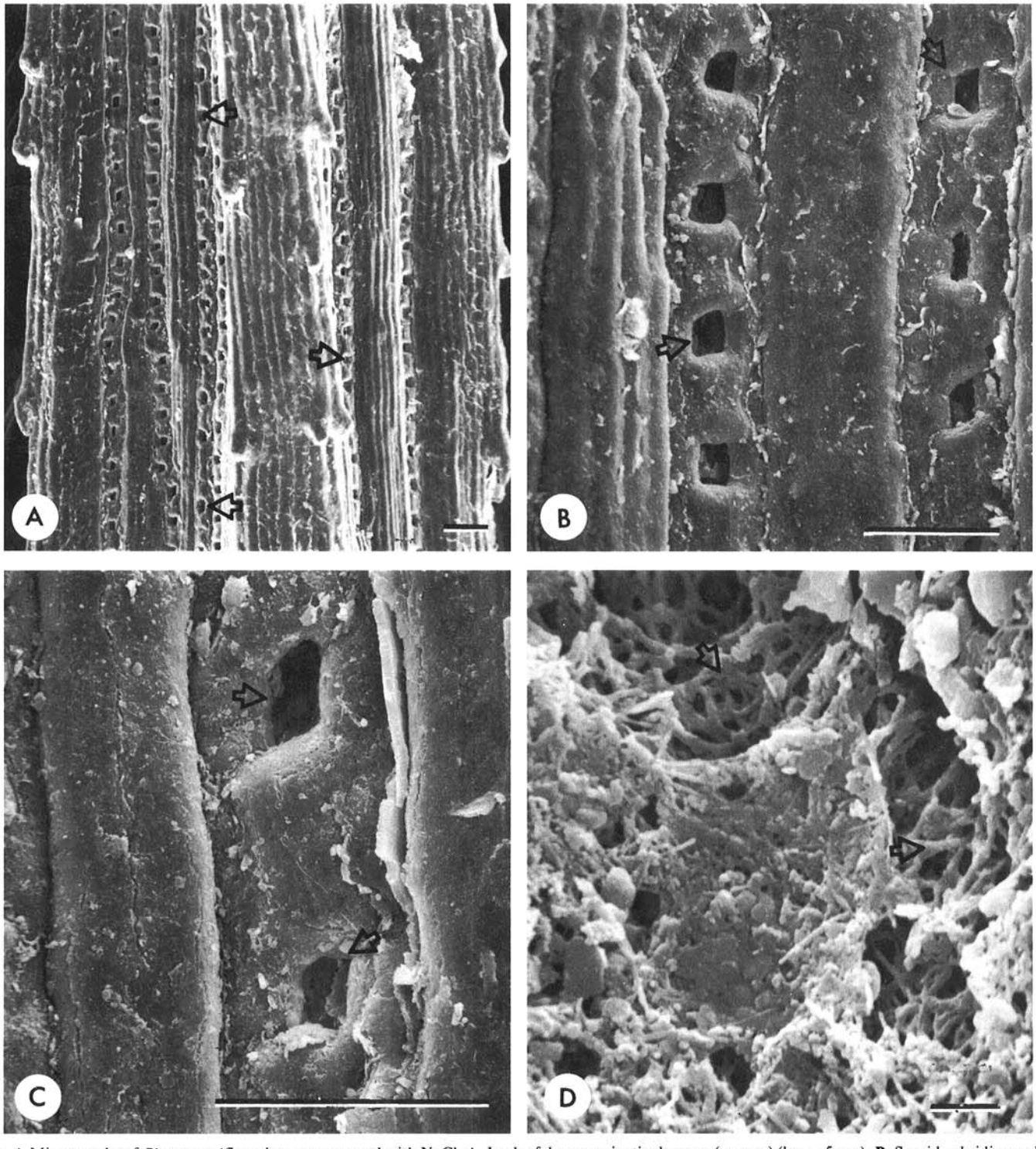

Fig. 4. Micrographs of *Pinus parviflora* that were sprayed with NaCl. **A,** Lack of downy epicuticular wax (arrows) (bar = 5 μm); **B,** flaccid subsidiary cells (arrows) with wax appearing scaly (bar = 5 μm); **C,** scaly epicuticular wax and flaccid subsidiary cells (arrows) (bar = 5 μm); **D,** coalesced wax (arrows) similar to that of NaCl-sprayed needles of *P. aristata* (bar = 0.5 μm).

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