The structure and interrelation of stomata, cuticle, and wax were studied without subjecting the plant material to drastic prestudy treatment. The scanning electron microscope, which has a greater depth of field than either the high magnification of the light microscope or the transmission electron microscope, was used. We basically followed the technique described by Mozingo et al. (1970). Needles of Monterey pine (Pinus radiata D. Don) have been studied in detail with the light microscope (Fonde, 1969) and with the transmission electron microscope, Leyton and Armitage (1968). The present study was of anatomical details while the latter one was of the form of the surface wax. Both studies used impressions or fixed plant material.

EXPERIMENTAL PROCEDURE. — In the present study the surface wax formation and stomatal form in P. radiata seedlings grown under a range of temperature and drought conditions were investigated. All the seedlings were grown in the Phytotron at Duke University from seed obtained from the New Zealand Forest Service tree breeding program, breeding trees 55 x 121, and from the California State Division of Forestry from a native stand of P. radiata. Seedlings from the New Zealand seed were grown in a temperature-controlled glasshouse at day/night temperatures of 26°/23°C, respectively, under two different degrees of water stress. One set of seedlings was watered daily; the other was watered once every two weeks. Seedlings from the California seed were grown under one of the following temperature regimes: 32°/29°C, 27°/23° or 17°/11°C (day/night temperatures, respectively) in controlled-temperature glasshouses. The lowest and the highest of these temperature regimes are near the high and low temperature limits for growth of P. radiata seedlings (Rook, 1969). The secondary needles used in this study were taken from approximately mid-height of the seedlings which were 7 months old. The primary needles were removed from near the base of 2-month-old seedlings. Three needles, each from a separate plant, were taken from each treatment. P. radiata, being a 3-needled pine, has leaves triangular in cross-section. The midportion of the needle was scanned in each case, and all three surfaces were examined.

Fresh plant material was examined as soon after detachment as possible. All photographs were taken on material which after mounting on a block were shadowed with gold in a vacuum of $2 \times 10^{-4}$ torr. This treatment took 10 to 15 minutes. The leaves were then examined with the scanning electron microscope.

RESULTS AND DISCUSSION. — The surface of a secondary needle consists of a series of ridges with sunken cuticular ledges beneath holes in a thick, overarching waxy layer (Fig. 1). The surfaces of the secondary needles of the seedlings watered daily and those watered once every two weeks did not differ in appearance (Figs. 1 and 2). Both consist of a smooth layer of wax which covers ridges and valleys (Fig. 3). The wax layer forms a raised border around each stoma (Figs. 1-3).

Leyton and Armitage (1968) observed that both the abaxial exposed surfaces of P. radiata needles have basically a similar structure with numerous tubular outgrowths of wax arising from a relatively smooth layer. These outgrowths were scattered i-
regularly over the needle surface and were thought to make up only a very small proportion of the surface. The surface structure of current needles from a mature tree and fully grown needles from young transplants were similar. None of our photographs of secondary needles showed any evidence of tubular outgrowths which should have been observable at a magnification of X3000. Kolattukudy (1970) notes that considerable genetic variation in quality and quantity of cuticle can be expected.

The stoma shown in Figure 3 includes a view looking through the hole in the smooth, overarching wax layer into the pore of the stoma. The edges of two subsidiary cells, one on each side of the stomatal pore, are visible clearly. The guard cells are less clearly visible below these cells. In one instance while a stoma was being examined with the scanning electron microscope, the subsidiary cells were seen to come together. Both cells moved slowly, at a regular rate, going from a fully opened to a completely closed stoma in about 30 seconds.

To determine the relative position of the cells in the stoma, needles were cut at right angles with a sharp razor blade slicing through a stoma (Fig. 4). The needle segment, approximately 2 mm in length, was mounted with the cut end pointing vertically upwards. The mesophyll collapsed to some extent, but this collapse was limited by the support from the more rigid epidermal and vascular tissues. One stoma was cut almost through the middle (Fig. 4). The subsidiary cells with a thick coat overlie the guard cells. The guard cells have thick walls, especially on those walls facing the pore and stomatal cavity. A small cell, triangular in cross-section with thin walls, lies immediately adjacent to each of the guard cells and in close contact with the mesophyll layer. These photographs were taken of secondary needles of seedlings watered once every two weeks.

Whereas the wax surface of secondary needles appears smooth, the surface of the primary needles is covered with waxy flakes (Fig. 5). Primary needles from seedlings grown from both the New Zealand
and California seed showed this feature. No obvious differences in this surface structure could be detected with growing temperature, but cuticle thickness was not examined. The needle shown in Figure 5 was from seedlings grown from both the New Zealand regime of 32°/29°C, while Figure 6 shows the surface of a primary needle grown at 17°/11°C. Another set of primary needles from the 26°/23°C was similar in appearance. The surfaces of the cotyledons, likewise, are slightly ridged and pubescent.

Differences in morphology of the surface waxes could have been expected with differences in growing temperature (Kolattukudy, 1970), but no obvious differences could be found.

To check on the morphological similarity of the cuticle surface of *P. radiata* with another conifer, the surfaces of needles of *Picea engelmannii* (Parry) were examined (Fig. 7). The wax surface of the spruce is considerably rougher, with chunks of wax lying on the ridged surface which also bears a thick flaky covering. On surface view the stomata of both species appear similar.

**SUMMARY** — The cuticular surfaces of the foliage of *P. radiata* (Monterey pine) are ridged. The primary needles and cotyledons are covered with flakes of wax, while the secondary needles are smooth. No differences in surface topography between drought conditions or growing temperatures were observed.
LITERATURE CITED


