

INFLUENCE OF ATMOSPHERIC CO₂ ENRICHMENT, SOIL N, AND WATER STRESS ON NEEDLE SURFACE WAX FORMATION IN *PINUS PALUSTRIS* (PINACEAE)¹

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Interactive effects of increasing atmospheric CO₂ with resource limitations on production of surface wax in plants have not been studied. *Pinus palustris* seedlings were grown for 1 yr at two levels of soil N (40 or 400 kg N·ha⁻¹·yr⁻¹) and water stress (-0.5 or -1.5 MPa xylem pressure potential) in open-top field chambers under two levels of CO₂ (365 or 720 μmol/mol). Needle surface wax content was determined at 8 mo (fall) and 12 mo (spring) and epicuticular wax morphology was examined using scanning electron microscopy (SEM) at 12 mo. Wax content expressed on both a leaf area and dry mass basis was increased due to main effects of low N and water stress. No main effects of CO₂ were observed; however, a CO₂ × N interaction at 12 mo indicated that under low soil N the elevated CO₂ treatment had less wax (surface area or dry mass basis) compared to its ambient counterpart. Morphologically, low N needle surfaces appeared rougher compared to those of high N needles due to more extensive wax ridges. Although the main effect of water treatment on wax density was not reflected by changes in wax morphology, the CO₂ × N interaction was paralleled by alterations in wax appearance. Decreases in density and less prominent epicuticular wax ridges resulting from growth under elevated CO₂ and limiting N suggest that dynamics of plant/atmosphere and plant/pathogen interactions may be altered.

Key words: carbon dioxide; longleaf pine; needle wax; Pinaceae; *Pinus palustris*; resource limitations.

Anthropogenic emissions resulting from the burning of fossil fuels and deforestation are driving the atmospheric CO₂ concentration upward at rates without geologic precedence (Keeling et al., 1989; Sundquist, 1993). The direct effects of elevated CO₂ on plants have been recently reviewed; generally, elevated CO₂ increases biomass accumulation, water use efficiency, and photosynthetic rates (Rogers and Dahlman, 1993; Allen and Amthor, 1995; Amthor, 1995; Wittwer, 1995). Most CO₂ response research has been on crops grown with optimal inputs rather than on native plants, which often grow under sub-optimal conditions. Effects of soil resource limitations, interacting with elevated CO₂, on growth responses can be more variable than at ambient CO₂ levels (Ceulemans and Mousseau, 1994; Amthor, 1995). Native plants grown under CO₂ enrichment have shown increased growth under adequate fertility only (Bazzaz and Miao, 1993; Griffin, Thomas, and Strain, 1993), at both high and low fertility (Norby and O'Neill, 1991; Conroy et al., 1992; El-Kohen, Rouhier, and Mousseau, 1992), or at neither level of fertility (Oberbauer et al., 1986). Growth enhancement by elevated CO₂ has sometimes

been greater under water stress (Conroy and Barlow, 1986; Miao, Wayne, and Bazzaz, 1992). However, other studies have shown consistent effects of elevated CO₂ under several soil moisture levels (Samuelson and Seiler, 1993). Differential plant responses to CO₂, mediated by gradients in soil resources, could alter competitive relationships leading to changes in natural ecosystem structure and function (Bazzaz, 1990; Mooney et al., 1991). To predict the direction and magnitude of such change, it is essential to develop a more thorough understanding of how elevated CO₂ affects partitioning of assimilates throughout the plant for species exhibiting a range of adaptive suites.

Assimilate partitioning to cuticular wax under CO₂ enrichment, while simultaneously challenged with multiple soil resource limitations, has not been examined. Studies have, however, examined the effects of elevated CO₂ independent of other environmental factors. Thomas and Harvey (1983) suggested, based on observations of SEM micrographs, that soybean (*Glycine max*) leaves grown under high CO₂ appeared to have greater epicuticular wax deposition, and in the only known study that quantified the effects of CO₂ enrichment on leaf epicuticular wax deposition, *Agave deserti* (a common desert Crassulacean acid metabolism [CAM] species) was observed to have measurably less wax under CO₂ enrichment, which decreased the reflectance of photosynthetic photon flux (Graham and Nobel, 1996). In addition to the paucity of information on CO₂ effects, data on the effects of soil fertility on leaf epicuticular wax production are also rare. In one of the few investigations, Chiu et al. (1992) showed that, although wax density was unchanged, nee-

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TABLE 1. Main treatment effects for longleaf pine needle variables for seedlings harvested at two periods following initiation of CO₂ exposure.

Variable Sample date ^a	CO ₂ concentration (μmol/mol)			N fertility regime (kg·ha ⁻¹ ·yr ⁻¹)			H ₂ O stress regime (MPa)		
	365	720	Pr > F ^b	40	400	Pr > F	-1.5	-0.5	Pr > F
Needle area/fascicle (m ²)									
Nov 1993 (8)	19.40	20.62	NS	15.72	24.30	***	18.69	21.34	**
Mar 1994 (12)	18.21	17.67	NS	12.89	22.99	***	16.40	19.47	***
Needle dry mass/fascicle (g)									
Nov 1993 (8)	0.20	0.22	**	0.17	0.25	***	0.19	0.22	**
Mar 1994 (12)	0.21	0.22	NS	0.16	0.27	***	0.20	0.24	***
Specific needle mass (g/m ²)									
Nov 1993 (8)	101.65	106.94	*	106.96	101.63	***	104.42	104.17	NS
Mar 1994 (12)	118.44	124.50	NS	123.75	119.19	**	119.99	122.95	NS

^a Values in parentheses, following sample date, are months of exposure to CO₂ and N treatments. Water stress treatments were initiated after seedling establishment (19 wk).

^b *, **, *** significant at or below the 0.05, 0.01, and 0.001 probability level, respectively; NS = nonsignificant.

dles from Douglas-fir (*Pseudotsuga menziesii*) grown under high soil fertility were more "glaucous," which they attributed to altered wax morphology.

Epicuticular waxes, the interface between the atmosphere and the interior of the leaf, serve a variety of vital functions, including prevention of desiccation, insect and pathogen attack (Martin and Juniper, 1970), and damage by atmospheric pollutants (Turunen and Huttunen, 1990). Changes in wax quantity, composition, or morphology can alter many plant and ecosystem processes. For example, changes in surface features can alter fluid contact angles and thus retention time. Such changes in repellency can impact plant resistance to pathogen entry and insect attack, nutrient leaching due to ambient moisture (i.e., rainfall, mist, and dew), and permeability to chemicals including herbicides and pesticides (Martin and Juniper, 1970; Von Wetstein-Knowles, 1993). In addition to its influence on fluid repellency, surface wax may impact the reflection of light, boundary layer resistance, and moisture lost directly through epidermal cells (Jeffree, 1986). Several studies have shown that increased deposits of epicuticular wax resulting from water stress conditions (Bondada et al., 1996) confer an advantage by reducing water loss (Clarke and Richards, 1988; Jefferson, Johnson, and Asay, 1989; Jefferson et al., 1989; Premachandra et al., 1992).

Although effects of water stress on surface wax production are well established, few studies have examined independent effects of soil nitrogen and elevated CO₂, and no study has examined interactive effects of multiple resources on epicuticular wax production and morphology. In light of the importance of epicuticular wax to plant function, and the inevitability of global climate change, the objective of this study was to evaluate the effects of increased aerial CO₂ concentration, and its interactions with resource limitations (soil N and water), on epicuticular wax formation in longleaf pine, the keystone species in a dwindling but significant southeastern United States ecosystem (Peet and Allard, 1993).

MATERIALS AND METHODS

Three longleaf pine (*Pinus palustris* Mill.) seedlings were planted into each of 96 plastic containers (45 L) filled with a coarse sandy medium (pH 5.1) of very low fertility (P, K, Mg, and Ca = 0.9, 5.6, 6.9, and 26.6 mg/kg, respectively). Seedlings from a wild seed source were lifted from a Florida nursery (February 1993), stored (2°C, < 1 wk), and graded (root collar diameter mean = 13 mm; standard deviation = 2 mm) prior to planting.

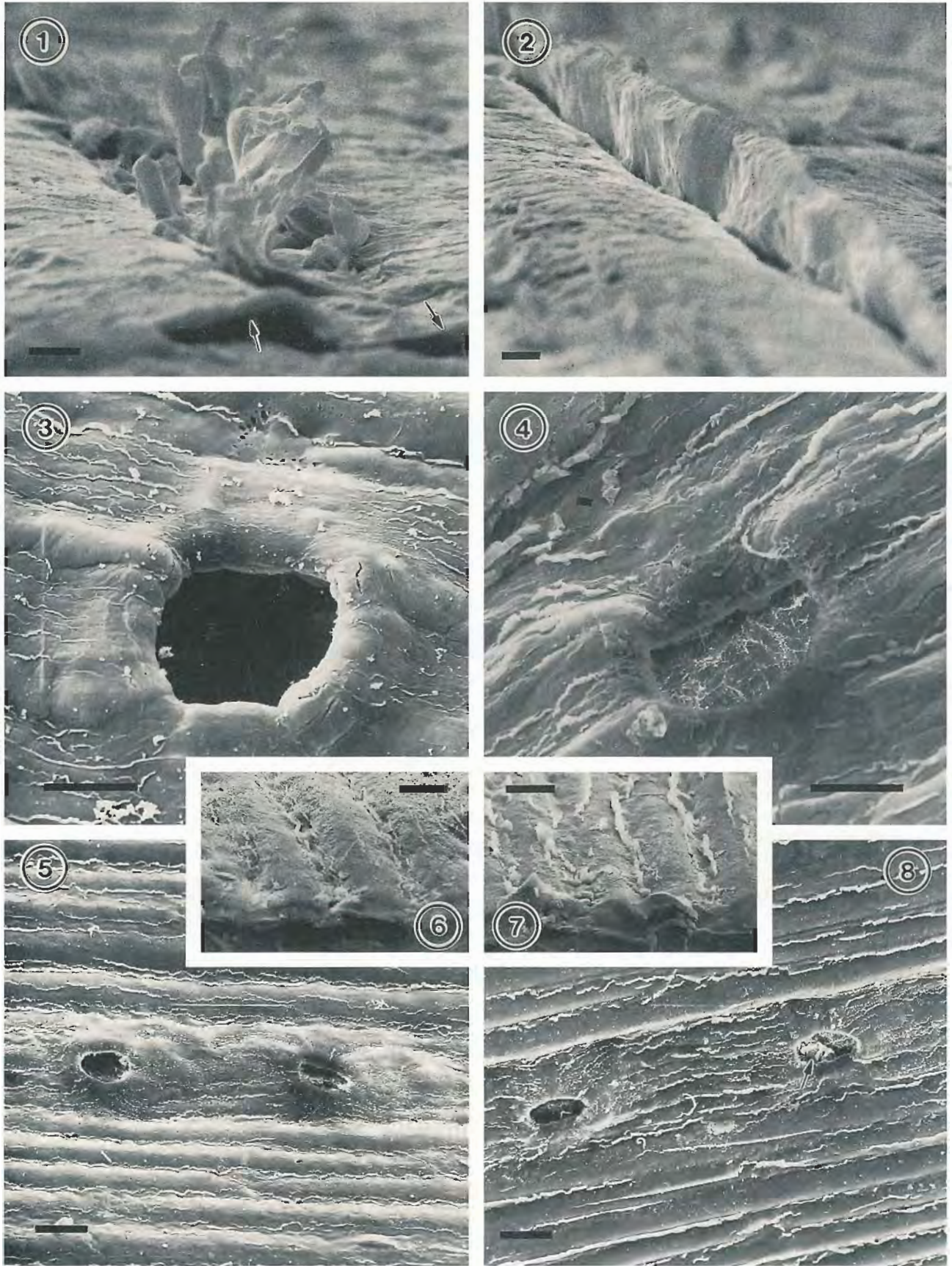
An open-top chamber system (Rogers, Heck, and Heagle, 1983) was used to expose seedlings to ambient (~365 μmol/mol) or elevated (~720 μmol/mol) atmospheric CO₂ conditions starting on 30 March

TABLE 2. Main treatment effects for longleaf pine needle variables for seedlings harvested at two periods following initiation of CO₂ exposure.

Variable Sample date ^a	CO ₂ concentration (μmol/mol)			N fertility regime (kg·ha ⁻¹ ·yr ⁻¹)			H ₂ O stress regime (MPa)		
	365	720	Pr > F ^b	40	400	Pr > F	-1.5	-0.5	Pr > F
Wax/fascicle (mg)									
Nov 1993 (8)	1.29	1.46	NS	1.16	1.58	***	1.39	1.36	NS
Mar 1994 (12)	1.92	1.84	NS	1.57	2.19	***	1.88	1.88	NS
Wax/needle area (mg/m ²)									
Nov 1993 (8)	676.04	721.15	NS	745.69	651.50	**	744.87	652.32	**
Mar 1994 (12)	1123.62	1083.39	NS	1236.47	970.54	***	1187.83	1019.19	***
Wax/needle dry mass (mg/g)									
Nov 1993 (8)	6.65	6.74	NS	6.98	6.42	NS	7.14	6.26	**
Mar 1994 (12)	9.47	8.75	NS	10.07	8.15	***	9.94	8.28	***

^a Values in parentheses, following sample date, are months of exposure to CO₂ and N treatments. Water stress treatments were initiated after seedling establishment (19 wk).

^b *, **, *** significant at or below the 0.05, 0.01, and 0.001 probability level, respectively; NS = nonsignificant.



1993. A detailed description of the chambers, CO₂ supply, and monitoring and dispensing systems has been described (Mitchell et al., 1995).

Nitrogen treatments initiated at planting were either 0.20 or 0.02 mg N·g soil⁻¹·yr⁻¹ applied at 3-mo intervals (sulfur-coated urea; 38-0-0). Optimum levels of other nutrients were established by applying sulfur-coated potassium (0-0-47=0.04 mg K·g soil⁻¹·yr⁻¹) and Micro-Max™ Plus (0-4-0; P=0.14, Ca=0.57, Mg=0.28, and S=0.05 mg·g soil⁻¹·yr⁻¹, plus a complete complement of micronutrients) at the time containers were filled. Iron chelate (0.007 mg Fe/g soil) was applied once (April 1993).

After seedling establishment (19 wk after planting), two water stress treatments (-0.5 or -1.5 MPa xylem pressure potential) were imposed. Rain exclusion covers (Teflon; 1.27 × 10⁻³ cm FEP film) were installed at the top of the chambers. Needle xylem pressure potentials (measured with a pressure bomb; Scholander et al., 1965) were correlated with gravimetric determinations using a weighing device (an electronic cable scale with pneumatic lifter) of our own design. Containers in the adequately watered treatment were irrigated every 3–4 d and containers in the water stress treatment every 12–20 d; seasonal evaporative demand determined frequency of watering. Fertility treatments were ensured by using deionized water at all irrigation events.

A split-plot design with six replications was used. The main plots were CO₂ levels, which were randomly assigned to chambers within replicates. The subplots consisted of N and water stress treatments, which were randomly assigned, in a 2 × 2 factorial design, to a total of eight containers within each chamber. To minimize position effect, container locations within each chamber were rerandomized monthly.

One container from each treatment in each chamber was sampled in November 1993 and March 1994, corresponding to 8 and 12 mo after initiation of the study. A total of 50 fascicles per container were collected from a cohort of fully expanded fascicles located near the top of the canopy for each treatment. Needle dry mass (oven dried at 55°C), needle area (measured photometrically), and needle epicuticular wax content were determined at both harvests. To extract the epicuticular wax, needles were immersed in chloroform; chloroform was transferred in batches to preweighed test tubes, evaporated using a nitrogen evaporator, and allowed to dry in a desiccator until mass no longer decreased. Wax content was determined as the difference between initial and final test tube masses. Wax data were expressed on both a needle surface area and dry mass basis.

Surface features of wax morphology were investigated using a scanning electron microscope (SEM; DSM 940 Zeiss, Germany). Needle segments were fixed with formalin-acetic acid-alcohol (FAA). After 24 h, tissue was dehydrated in an ethanol series, critical point dried, mounted on aluminum stubs, and coated with a sputter coater (SC-7, Ted Pella, Inc., Redding, California).

The GLM procedure of the Statistical Analysis System (SAS, 1985) was used for data analysis. Tests for significance of main effects variables and their interactions were conducted using error terms appropriate to the split-plot design. Differences were considered significant at the $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Needle growth, including area and dry mass per fascicle, decreased under low soil N at both harvests (Table

1). Increases in these variables due to CO₂ were not of large magnitude and were only significant at 8 mo under conditions of high soil N. Similar inconsistencies in total needle area and biomass per seedling due to interactive effects of N and CO₂ treatment were related to shifts in C allocation within seedlings (Prior et al., 1997). Plants grown under elevated CO₂ were not C limited and thus photosynthate was allocated belowground in order to acquire N, the most limiting resource. Greater C allocation belowground under high CO₂ and to aboveground tissue under ambient CO₂ (Prior et al., 1997) further supports the hypothesis that plant carbon budgets adapt to acquire the most limiting resource (Chapin et al., 1987). Water stress reduced needle area and dry mass per fascicle. However, a N × water interaction revealed that under high N, adequately watered seedlings had more needle area and biomass compared with water-stressed seedlings, whereas under low N fertility no difference between water treatments was noted (data not shown). The interaction of N with water at both harvests demonstrates the importance of N as a controlling resource for growth of longleaf pine seedlings (Prior et al., 1997). No CO₂ × water interactions were observed with respect to needle growth variables at either harvest.

In general, needle epicuticular wax per fascicle, per unit needle area, and per unit of dry mass was less at the fall vs. the spring sampling (Table 2). Seasonal differences in leaf epicuticular wax deposition have been reported previously (Leece, 1978). Furthermore, Baker (1974) found that decreases in temperature induced large deposits of wax. In the current study, winter occurred between harvests and thus the higher wax content found at the spring sampling may reflect the influence of low temperature on leaf surface wax production.

Variables describing needle epicuticular wax deposition were altered by main effects of N fertility (Table 2). Total wax masses were greater for the high soil N treatment (8 and 12 mo; Table 2) due to larger needles found under high N (Table 1). Although high N caused greater total wax per fascicle, density expressed as wax per unit needle area (significant at 8 and 12 mo) and per unit dry mass (significant at 12 mo, trend at 8 mo, $P=0.06$) was greater under conditions of limiting N (Table 2). Increased wax density under low soil N may protect foliage from insect/pathogen attack and may reduce nutrient leaching from leaf tissue (Martin and Juniper, 1970; Von Wetstein-Knowles, 1993). In comparison, high-N grown seedlings may be more susceptible to damage due to lower wax density, but since canopy size is more extensive, it could better tolerate tissue loss. These observations are especially relevant to conditions found in nature since longleaf pine forests often occur on low N soils. In comparison to these findings, Chiu et al. (1992) observed no

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Figs. 1–8. Scanning electron micrographs of surface features of *Pinus palustris* needles. **1, 2.** Wax ridges erupting from the juncture of epidermal cells. Ambient CO₂ (365 μmol/mol), low N (40 kg·N ha⁻¹·yr⁻¹) and water stress (-1.5 MPa). Arrows indicate wax layer that is apparently continuous over entire needle surface. Bars = 2 μm. **3, 4.** Florin rings (opening to epistomatal chamber) showing effect of N treatment on epidermal wax associated with stomatal areas. Bars = 10 μm. **3.** Ambient CO₂ (365 μmol/mol), high N (400 kg·N ha⁻¹·yr⁻¹), water stress (-1.5 MPa). **4.** Ambient CO₂, low N (40 kg·N ha⁻¹·yr⁻¹), and water stress. **5, 6.** Low (bar = 20 μm), and high (bar = 10 μm) magnifications of treatment consisting of ambient CO₂, high N and well watered (-0.5 MPa) conditions. **7, 8.** High (bar = 10 μm), and low (bar = 20 μm) magnifications of treatment consisting of ambient CO₂, low N, and well-watered conditions. Arrow on Fig. 8 indicates epistomatal wax.

effects of soil fertility on wax density in Douglas-fir, but did observe changes in epicuticular wax morphology.

Although there were no main effects of CO_2 , there was a significant $\text{CO}_2 \times \text{N}$ interaction for both wax per unit needle area and per unit dry mass during the spring harvest (12-mo exposure). Both wax density variables were decreased under elevated compared to ambient CO_2 only when N was low. Under conditions of low N fertility, mean values for wax per unit leaf area under elevated CO_2 were 1176 compared to 1297 mg/m^2 under ambient conditions. Expressed on a dry mass basis, respective mean values were 9.2 and 10.3 mg/g . Other studies have reported an increase (*Glycine max*; Thomas and Harvey, 1983) and a decrease (*Agave deserti*; Graham and Nobel, 1996) in epicuticular wax due to growth at elevated CO_2 . However, neither of these CO_2 studies examined the influence of N fertility.

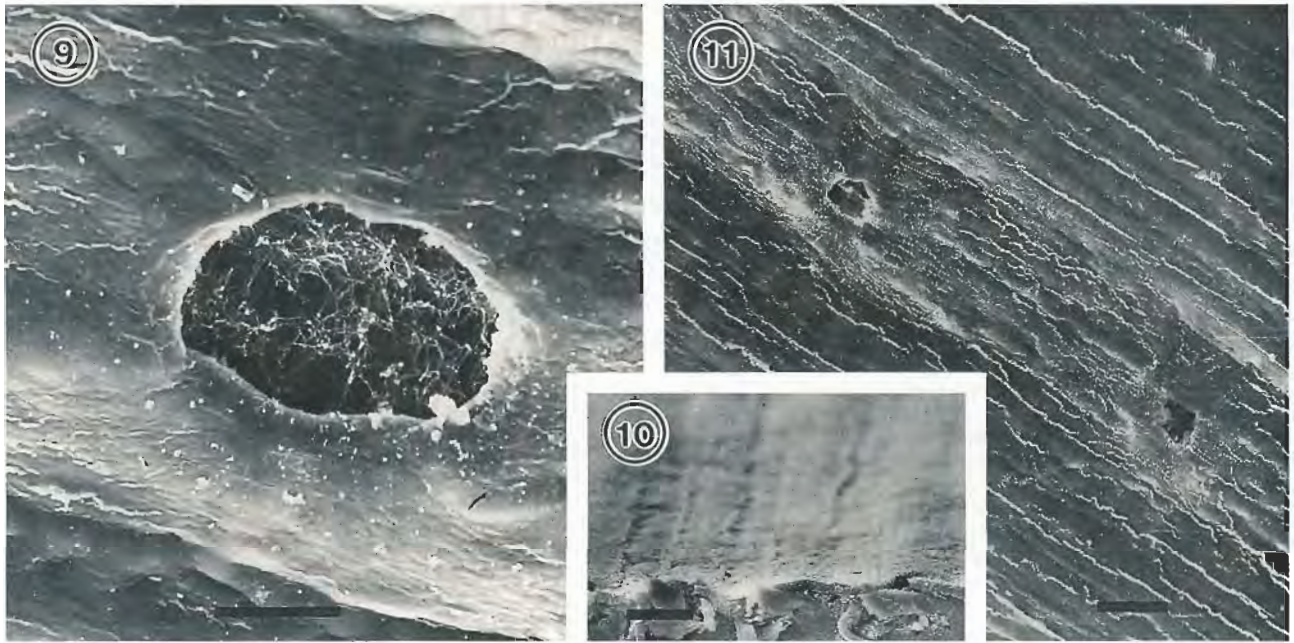
The sequence of metabolic events leading up to deposition of waxes on the leaf surface has not been fully elucidated, making it difficult to assess how changes in atmospheric CO_2 and soil N alter wax metabolism. Biosynthesis of wax precursors starts with fatty acid synthesis in plastids of epidermal cells, which in general contain no chloroplasts with the exception of guard cells (Kolattukudy, Croteau, and Buckner, 1976). Subsequent modification of fatty acids results in the diversity of compounds found in waxes (e.g., alcohols, aldehydes, ketones, etc.), but it is still not clear where these modifications occur (Kolattukudy, Croteau, and Buckner, 1976). Furthermore, the manner in which waxes are ultimately deposited on the leaf surface has been widely debated (see Ewers, Hammerschmidt, and Klomparens, 1994).

In the present study, specific needle mass, which often reflects patterns of nonstructural carbohydrate storage, was increased by main effects of low soil N and elevated CO_2 at the fall harvest (8-mo exposure). A $\text{CO}_2 \times \text{N}$ interaction was detected at 12 mo; CO_2 -enriched seedlings grown under low soil N had higher specific needle mass compared to ambient trees grown under low soil N (data not shown). Corresponding measures of total nonstructural carbohydrates are reflected by the trends observed in specific leaf mass. However, increases in carbohydrates in CO_2 -enriched tissue were mainly the result of increases in starch, whereas changes in low N tissue were attributed to increases in sugars (Mitchell et al., unpublished data). This leads to speculation on the effects of CO_2 and soil N conditions on sink-source relationships, and the subsequent effect of carbohydrate balance on wax production. The presence of starch instead of soluble sugar accumulation due to enhanced growth under elevated CO_2 may suggest that transport of triose phosphates out of chloroplasts was impeded. The inhibition in function of triose phosphate carriers in the chloroplast outer membrane brought about by a shortage of inorganic phosphates (see Stitt, 1991) may lead to starch accumulation, altered chloroplast structure (Cave, Tolley, and Strain, 1981; Pritchard et al., 1997), and thus photosynthetic acclimation (Wulff and Strain, 1981; Yelle et al., 1989). Others have suggested that the inability of sinks to metabolize sugars at the rate they are produced under CO_2 enrichment leads to starch buildup (Madson, 1968; Cave, Tolley, and Strain, 1981; Clough, Peet, and Kramer, 1981; Vu, Allen, and Bowes, 1989). It has been

suggested that the excessive accumulation of chloroplast starch could impede intracellular transport of CO_2 and possibly other metabolic products (Nafziger and Koller, 1976). Pritchard et al. (1997) reported that the growth of longleaf pine under conditions of elevated CO_2 , limiting N availability, and water stress resulted in excessive chloroplast starch inclusions that appeared to disrupt chloroplast integrity. Interestingly, this corresponds with the reduction in wax observed in the current study under elevated CO_2 and low N. Regardless of the mechanism, it appears that growth under elevated CO_2 either (1) reduced substrate at the site of wax synthesis, or (2) impeded the biosynthetic machinery responsible for either wax synthesis/modification, or extrusion to the needle surface. On the other hand, increased wax and sugars in needle tissue from seedlings grown under low soil N may indicate that sufficient substrates were available for wax synthesis and/or the rate of wax metabolism may have been sufficient to alleviate sink imbalances and thus starch buildup. However, these possibilities do not preclude other source-sink explanations and clearly more work is needed.

There are several reports on the relationship of leaf epicuticular wax and plant water deficit, predominantly of field crops. Jordan et al. (1984) reported that sorghum leaf wax densities $> 0.07 \text{ g/m}^2$ were effective in reducing cuticular water loss under most conditions. Water-stressed cotton leaves were found to have altered wax composition, greater cuticle thickness, and increased wax content, which collectively decreased penetration of chemical leaf defoliant (Oosterhuis, Hampton, and Wulschleger, 1991). Bondada et al. (1996) found that water-stressed cotton leaves had altered wax composition and increased wax content (leaves, bolls, and bract), but no discernible changes in wax morphology were observed. Several other investigations have found increases in leaf wax density due to water stress in other plant species (Clarke and Richards, 1988; Jefferson, Johnson, and Asay, 1989; Jefferson et al., 1989; Saneoka and Ogata, 1987; Premachandra et al., 1992). Fatty acid synthesis may be stimulated in drought-stressed tissue of cotton and the accumulation of substrates during water stress may result in an overproduction of epicuticular wax upon rehydration (Weete et al., 1978). In the present study, trees were subjected to repeated cycles of water stress, thereby being predisposed to produce more wax as suggested by Weete et al. (1978). The amount of needle epicuticular wax on both a surface area and dry mass basis was increased under water stress (Table 2). Increased wax density is important for reducing water loss, especially since longleaf pine forests occupy more xeric sites. No significant interactions between water level and other treatment variables were detected.

Longleaf pine has amorphous (Figs. 1, 2, 7) epicuticular wax morphology, with wax protrusions erupting from an apparently continuous sheet of cuticular wax (Fig. 1, arrows). Wax ridges arise predominantly from depressions between adjacent epidermal cells (Fig. 7). Similar amorphous wax protuberances were noted in stomatal regions (for example, Fig. 4). Occlusion of epistomatal openings (Fig. 8, arrow) by wax was common, but did not appear to be differentially affected by experimental treatments. Similar occlusions have been reported



Figs. 9–11. Scanning electron micrographs of surface features of *Pinus palustris* needles. **9.** Florin ring (opening into epistomatal chamber). Treatment consisted of elevated CO₂ (720 $\mu\text{mol/mol}$), low N (40 kg N·ha⁻¹·yr⁻¹), and water stress (-1.5 MPa) conditions. Bar = 10 μm . **10, 11.** High (bar = 10 μm), and low (bar = 20 μm) magnifications of treatment consisting of elevated CO₂, low N, and well watered (-0.5 MPa) conditions.

for pine species (Yoshie and Sakai, 1985) and other conifers (Hanover and Reicosky, 1971). It has been suggested that epistomatal wax originates from cells associated with the stomatal complex (i.e., subsidiary and guard cells), whereas epidermal wax arises from nonspecialized epidermal cells (Johnson and Riding, 1981). Thus, because these waxes arise from distinct physiological processes, one may expect that they would be regulated independently. From this study, it appears that production of epistomatal wax exhibits less plasticity in response to changes in environmental conditions than does epicuticular wax proper. Stomatal complexes must be adapted to maintain a sophisticated homeostasis in order to function, and thus maximize water use efficiency. Therefore, one may expect narrower plasticity in form due to the need to maintain cellular conditions within the range defining the physiological optimum for guard cell/stomatal function. Although the ontogeny of stomatal complexes may be altered by environmental factors, including CO₂ levels (Boetsch et al., 1996), the detection of, and reaction to immediate, short-term ambient CO₂, light, and water levels subsequent to stomatal development may partially override further plasticity to longer term conditions. Chiu et al. (1992) found that although epicuticular wax characteristics of nonstomatal areas of *P. menziesii* were significantly altered by soil fertility, wax in the epistomatal chambers was unaffected. Clearly epistomatal wax is crucial to maintaining plant water balance. For example, in sitka spruce (*Picea sitchensis*) such deposits have been estimated to decrease transpiration by two-thirds while decreasing photosynthesis by only one-third (Jeffrey, Johnson, and Jarvis, 1971).

Differences in wax density due to N limitation were consistently reflected by apparent wax on needle surfaces. Limiting N appeared to cause an increase in wax under

both water-stressed (Fig. 4 vs. Fig. 3) and well-watered (Fig. 7 and 8 vs. Figs. 5 and 6) conditions. The increase in surface wax caused the needles from the low N treatments to appear rougher in both the stomatal (Fig. 4 vs. Fig. 3) and nonstomatal (Fig. 8 vs. Fig. 5) regions. This rougher appearance was the result of more prominent wax ridges (Fig. 7 vs. Fig. 6). The CO₂ × N interaction for wax density variables was also apparently the result of differences in wax ridges in both stomatal regions and nonstomatal regions. Under limiting N, needles grown under elevated CO₂ appeared to be smoother (Fig. 9 vs. Fig. 4 and Fig. 11 vs. Fig. 8) and exhibited smaller epicuticular wax ridges than those grown under ambient CO₂ (Fig. 10 vs. Fig. 7). The increase in wax density variables due to the main effects of water stress was not obvious from electron micrographs of needle surface features (not shown). This suggests that the amorphous sheet of wax that covers the needles was thicker and that there was no appreciable alteration in morphology of the epicuticular wax.

Increases in leaf surface wax density (under N limitations and water stress) and altered epicuticular wax morphology (under low N) are of significant adaptive value, considering the well-drained, nutrient-poor habitats that are home to most longleaf pine ecosystems. Clearly, greater production of needle surface wax contributes to the classic longleaf pines xeromorphy, and represents a mechanism by which it can survive severe moisture stress by decreasing water lost via cuticular transpiration. Furthermore, such leaf surface wax production surely plays a role in decreasing needle wettability, thereby minimizing nutrient leaching and losses due to infection by fungal and bacterial pathogens (Martin and Juniper, 1970; Von Wetsein-Knowles, 1993). The decrease in wax density and altered morphology occurring under simultaneous N

stress and CO₂ enrichment suggest that, in nature, plant and ecological processes subject to influence by leaf surface wax characteristics may be altered if atmospheric CO₂ concentration rises. These changes may reduce adaptive advantages possessed by longleaf pine for existence on depauperate sites. Given that this research was conducted on pine seedlings, results may or may not extend to mature forests; however, these results should be directly relevant to questions of tree establishment.

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