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Cellular Characteristics of Dinitroaniline Herbicide-Resistant Goosegrass (*Eleusine indica*)[1]

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Abstract. **Primary root cells from five dinitroaniline-resistant (R) and three susceptible (S) goosegrass biotypes from North Carolina and South Carolina were observed by transmission electron microscopy to determine whether resistance was associated with changes in cell wall formation. Cell wall malformations were found in some cells from two of the R-biotypes and in one of the S-biotypes. Malformations consisted of partially deposited cell walls and the inclusion of cell wall material in the cytoplasm. Some of the affected cells also had abnormal, lobed nuclei and malformed mitochondria. There seems to be little or no correlation between dinitroaniline resistance and cell wall malformations. Nomenclature: Goosegrass, *Eleusine indica* (L.) Gaertn. #[3] ELEIN.**

Additional index words. **Ultrastructure, cell wall, ELEIN.**

INTRODUCTION

Dinitroaniline herbicides injure plants by interfering with the normal function of microtubules (MTs)[4] during cell division (1). MTs are organelles present in nearly all eukaryotic cells (4, 7, 8). They are long tubular structures composed of tubulin. Tubulin, a heterodimeric protein with a molecular weight of 110 kilodaltons (kD) is composed of alpha and beta subunits (approximately 55 kD each). These subunits are electrophoretically separable and exhibit different amino acid compositions (15, 20). MTs, in association with other proteins, form the mitotic spindle, determine the plane of cell division, and orient microfibril deposition in walls of growing plant cells. Thus, MTs are significant determinants of morphogenesis (8, 9, 19, 21).

During mitosis, four MT assemblies succeed one another (6, 10): the *cortical MTs* which are involved in cell elongation (16), the *preprophase band*, the *mitotic spindle*, and the *phragmoplast*. The phragmoplast, an MT assembly unique to higher plants, appears at telophase after the chromosomes have migrated to the cell poles. During cytokinesis, phragmoplast MTs help guide wall-forming vesicles to the site where they coalesce to form the cell plate, which separates the replicated cell contents. In the absence of MTs, the cell plate does not form (14).

MTs do not seem to move from one location to another in the cell, but rather depolymerize into their component tubulin. Early work by Hess and co-workers (12, 13, 22) and other researchers (3) provided evidence that dinitroaniline herbicides act on plant tissue by preventing tubulin from polymerizing into MTs (11, 14). Without the formation of MTs, normal separation of chromosomes during mitosis is prevented. The cell plate does not appear and cells fail to divide. Microfibrils in the cell walls become disoriented and cells expand isodiametrically. Meristematic cells of roots treated with dinitroaniline herbicides may contain unusually large, lobed, or multiple nuclei (23).

Morejohn et al. (18) found that the dinitroaniline herbicide oryzalin [4-(dipropylamino)-3,5-dinitrobenzenesulfonamide] at concentrations as low as 0.1 μM depolymerized plant MTs and prevented the polymerization of new ones. The chromosome condensation cycle, however, was unaffected by oryzalin. Oryzalin (0.1 μM and higher) stopped chromosome movement within 30 s after perfusion of *Haemanthus katherinae* endosperm cells (2). Morejohn et al. (17) also demonstrated binding of oryzalin to tubulin isolated from cultured rose cells. In a dinitroaniline-resistant (R)[4] biotype of goosegrass [*Eleusine indica* (L.) Gaertn.], MTs formed in the presence of the herbicides (26). The resistant biotype exhibits an altered beta tubulin (27), which is approximately 3 kD larger than that of the susceptible[5]. Vaughn (26) indicated that MTs in the R-biotype were less abundant and appeared to be altered in function. This observation suggests that the alteration in tubulin that apparently is responsible for the lack of activity of dinitroanilines in this biotype also causes aberrations in some of the MT functions (26). Vaughn (26) also found wall protuberances and abnormally oriented or incompletely formed walls in some cells from the untreated R-biotype. If these abnormalities are common to all R-biotypes and if other MT-related physiological processes are affected negatively because of the evolution of resistance, the reduced fitness observed in an R-biotype (24) could be explained by the physiological alterations resulting from a modified tubulin. Our objective was to examine the integrity of cell walls and ultrastructure of several biotypes to determine if cytological aberrations are common among R-biotypes of goosegrass.

MATERIALS AND METHODS

Response to trifluralin. Plant material included five biotypes from North Carolina (Anson, Lee, Scotland, Wake, and

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[3]Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

[4]Abbreviations: MT, microtubule; kD, kilodalton; R, dinitroaniline-resistant goosegrass biotype; S, dinitroaniline-susceptible goosegrass biotype; GR_{50}, herbicide rate required to reduce dry weight 50%; PIPES, [piperazine-*N*,*N*′-bis (2-ethanesulfonic acid)]; EGTA, [ethyleneglycol-bis (b-aminoethylether)-*N*,*NN*′,*N*′-tetracetic acid]; kV, kilovolt.

[5]Vaughn, K. C. 1986. Personal communication. South. Weed Sci. Lab., USDA-ARS, Stoneville, MS.

Robeson)[6] and three from South Carolina (Calhoun, Darlington, and Dillon). Seed were dehulled mechanically and caryopses were germinated in petri dishes containing Whatman No. 2 filter paper moistened with 20 mM KNO_3. Dishes were placed in a growth chamber at 35 C/30 C day/night temperatures. Light was about 100 μmol m^{-2} s^{-1} with a 14-h photoperiod. Seedlings (2 to 3 cm long) were transplanted into soil treated with trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine]. Trifluralin was incorporated into air-dried soil in a tumbler at rates of 0, 0.06, 0.18, 0.54, 1.62, and 4.86 ppm in enough water to bring soil moisture to 10% (wt/wt). Each biotype was tested by transplanting 15 seedlings per treatment in a randomized complete block design with three replications. Plants were harvested 3 to 4 weeks after transplanting and foliage dry weights were recorded. Dry weights were converted to percentage of the untreated control. Each experiment was conducted twice and the data were combined. GR_{50}[4] values (herbicide rate required to reduce dry weight by 50%) for each population were calculated from fitted regressions.

Electron microscopy study. Seed were germinated in a growth chamber as before. Untreated primary roots were fixed when they were 1 to 2 cm long (48 h after germination) following a procedure similar to that of Vaughn (26). Additionally, roots of a susceptible biotype (Wake) were incubated for 48 h in 0.1 μM trifluralin before tissue fixation. Initial fixation was in 5% glutaraldehyde containing 0.05 M PIPES[4] buffer [piperazine-*N*,*N*-bis(2-ethanesulfonic acid)] pH 7.4, and 1 mM EGTA[4] [(ethyleneglycol-bis(b-aminoethylether)-*N*,*NN*′,*N*′-tetracetic acid] for 2 h at room temperature. Roots were washed twice for 15 min in PIPES-EGTA and postfixed in 5% OsO_4 in 0.10 M cacodylate buffer pH 7.2 for 2 h at 4 C. After a brief buffer wash, roots were left overnight in 50% acetone. Dehydration was completed by washing the specimens with 50% acetone, staining in 70% acetone saturated with uranyl acetate for 1 h, and incubating three times for 15 min each in 100% acetone. The tissue was then embedded in 1:1 (v/v) Spurr's resin in 100% acetone for 1 h. The proportion of Spurr's resin was increased to 2:1 for an additional 2-h period, and samples were finally transferred to 100% Spurr's resin and placed in an oven at 60 C. Ultrathin sections (gold-silver reflectance) were cut with an ultramicrotome[7], placed on formvar-coated grids and stained with 75 mM lead citrate. When stability of the tissue was not sufficient, specimens were carbon coated. Samples were observed with an electron microscope[8] at 60 kilovolts (kV) (or 40 kV when contrast needed improvement).

RESULTS AND DISCUSSION

Response to trifluralin. The goosegrass populations examined could be grouped into three categories based on their sensitivity to trifluralin (Figure 1). Biotypes from Anson, Lee, and Wake Counties were susceptible to trifluralin with GR_{50} values of 0.44, 0.63, and 0.53 ppm, respectively. Resistant biotypes (Darlington, Dillon, Robeson, and Scotland) exhibited GR_{50} values beyond the highest herbicide rate used (4.86 ppm). The biotype from Calhoun County had a GR_{50} of 2.50 ppm, an intermediate resistance level, as previously observed (5). Resistant populations came from counties located near the border between North Carolina and South Carolina and were from fields with a history of dinitroaniline use (24).

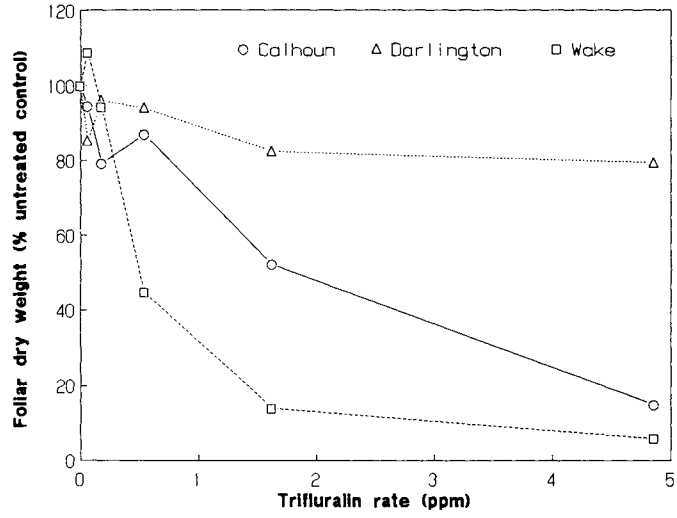

Figure 1. Typical dose-response curve of a dinitroaniline-resistant biotype (from Darlington County, SC), a susceptible biotype (from Wake County, NC), and the biotype with intermediate resistance (from Calhoun County, SC).

Electron microscopy study. Cell wall malformations were not observed in the dinitroaniline-susceptible (S)[4] biotypes (Figure 2), except the biotype from Anson County (Figure 3). Even in this biotype, most cells had normal ultrastructure and walls (Figure 3C). Occasionally, however, cells were found that exhibited remnants of cell wall material embedded in the cytoplasm (Figures 3B and 3C). The cell in Figure 3A apparently completed mitosis without cytokinesis and thus was binucleated, although roots had not been exposed to dinitroaniline herbicides. When present, these abnormalities were observed in no more than 5% of the cells of any of the biotypes examined.

Two R-biotypes, Dillon and Scotland, exhibited some ultrastructural abnormalities (Figure 4). Some cells in the biotype from Dillon County had abnormal cell walls and mitochondria, as well as lobed nuclei (Figures 4A and 4B). However, other cells were normal and showed long, clearly defined MTs in oblique section (Figure 4C). Cell walls in the biotype from Scotland County often were not completely formed in the plane of cell division, and endoplasmic reticulum was unusually concentrated in this area (Figure 4D). Normal cells were observed in cell preparations from Robeson (Figure 2), Darlington, and Calhoun (micrographs not shown). Vaughan et al. (25) also reported recently that a biotype from South Carolina with intermediate dinitroaniline resistance produced normal cell walls.

[6]Biotypes were named for the county in North Carolina or South Carolina where seed were collected.
[7]Porter-Blum MT-2.
[8]Philips EM 300.

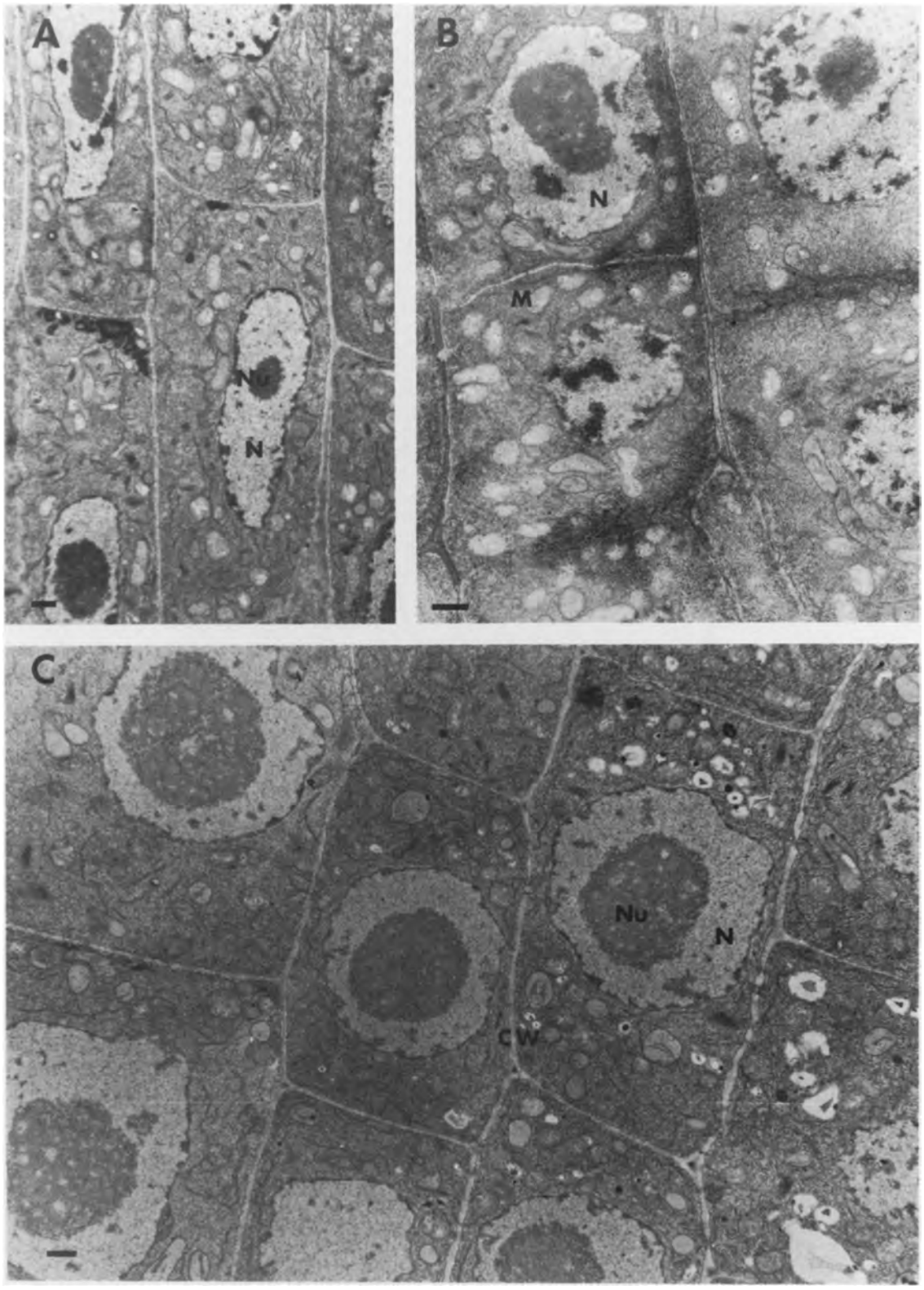

Figure 2. Root cells from an R-biotype (A, B) from Robeson County, NC and an S-biotype (C) from Lee County, NC with normal cell walls (CW) and ultrastructure. Nuclei (N) show prominent nucleoli (Nu) in most cells, M: mitochondrion. Bar = 1 μm.

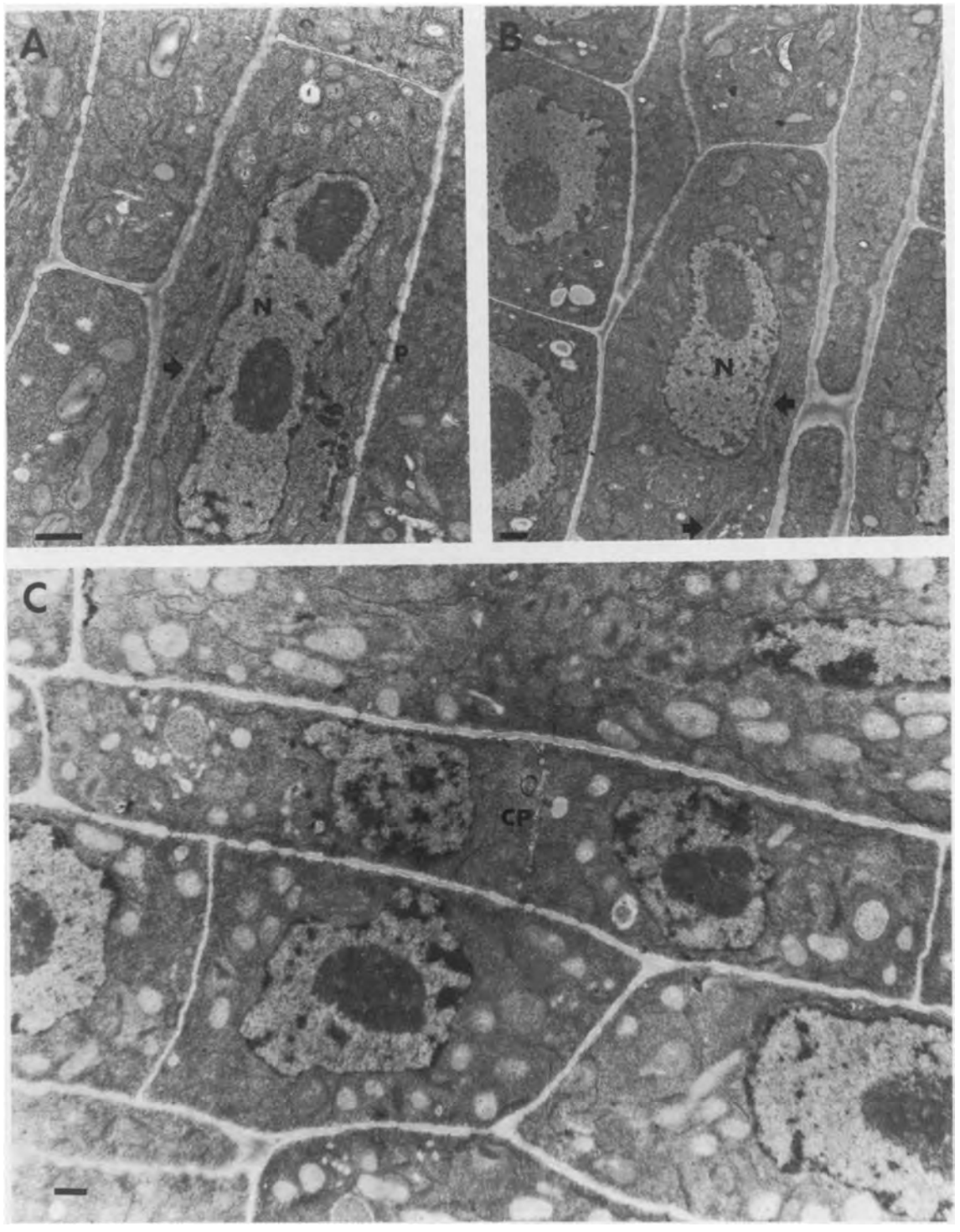

Figure 3. Ultrastructure of primary roots from an S-biotype from Anson County, NC. (A) Binucleated cell that completed mitosis without formation of a cell wall between the daughter cells. Remnants of what appeared to be cell wall material are embedded in the cytoplasm (arrow). (B) Another cell wall inclusion (arrow) in the cytoplasm of an otherwise normal cell. (C) Cell in telophase in which the cell plate (CP) is being deposited. Cells in this micrograph are normal. N: nucleus, P: plasmodesmata. Bar = 1 μm.

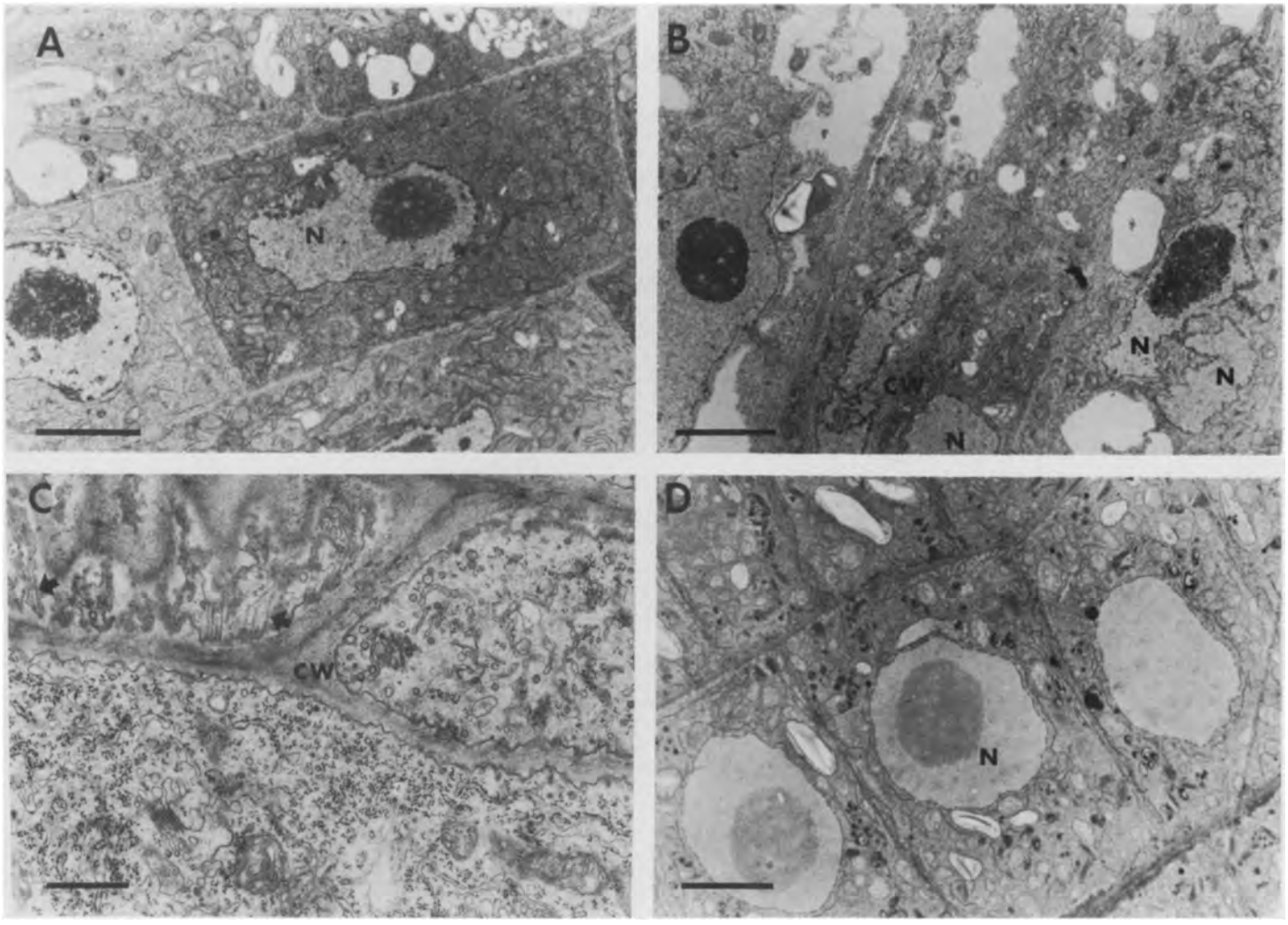

Figure 4. Electron micrographs of root cells from R-biotypes from Dillon County, SC (A,B,C) and Scotland County, NC (D) showing some ultrastructural abnormalities. (A) Cell with lobed nuclei (N). (B) Cells with lobed nuclei and abnormal walls (CW). (C) Oblique section through a normal cell wall with abundant microtubules (arrows) aligned near to the wall. (D) Cells with walls not completely formed and surrounded by abundant endoplasmic reticulum. Bar = 5 μm in all micrographs.

Cytological abnormalities observed in some of the R-biotypes resembled those that occur when cells are exposed to dinitroaniline herbicides or other mitotic disrupters. For example, lobed nuclei, binucleated cells, and cells with abnormally deposited cell walls were observed (Figure 5) as a result of incubating roots of an S-biotype (Wake) in 0.1 μM trifluralin before tissue fixation. These roots showed typical symptoms of dinitroaniline toxicity such as swelling of the tip and reduced growth. The R-biotypes that exhibited abnormalities may have MTs that are altered in function because of a modified tubulin molecule as suggested by Vaughn (26). If this is the case, the abnormal behavior of MTs from those biotypes could affect the cell cycle and produce the abnormalities described.

It would be enlightening to quantify the amounts of tubulin present in several R- and S-biotypes and to determine its electrophoretic or immunological properties or both. Plant tubulin isolation and characterization are difficult in the case of goosegrass. One approach would be to obtain large amounts of actively dividing cells from suspension cultures. However, goosegrass has not yet been successfully cultured as a cell suspension. Additionally, knowing the dinitroaniline-binding characteristics of tubulin and the behavior of MTs during the cell cycle in untreated cells from those sources could end much of the speculation concerning the cause of the cytological malformations observed. We have found that cell wall abnormalities are not widespread among all R-biotypes of goosegrass. In fact, they even occur in some S-

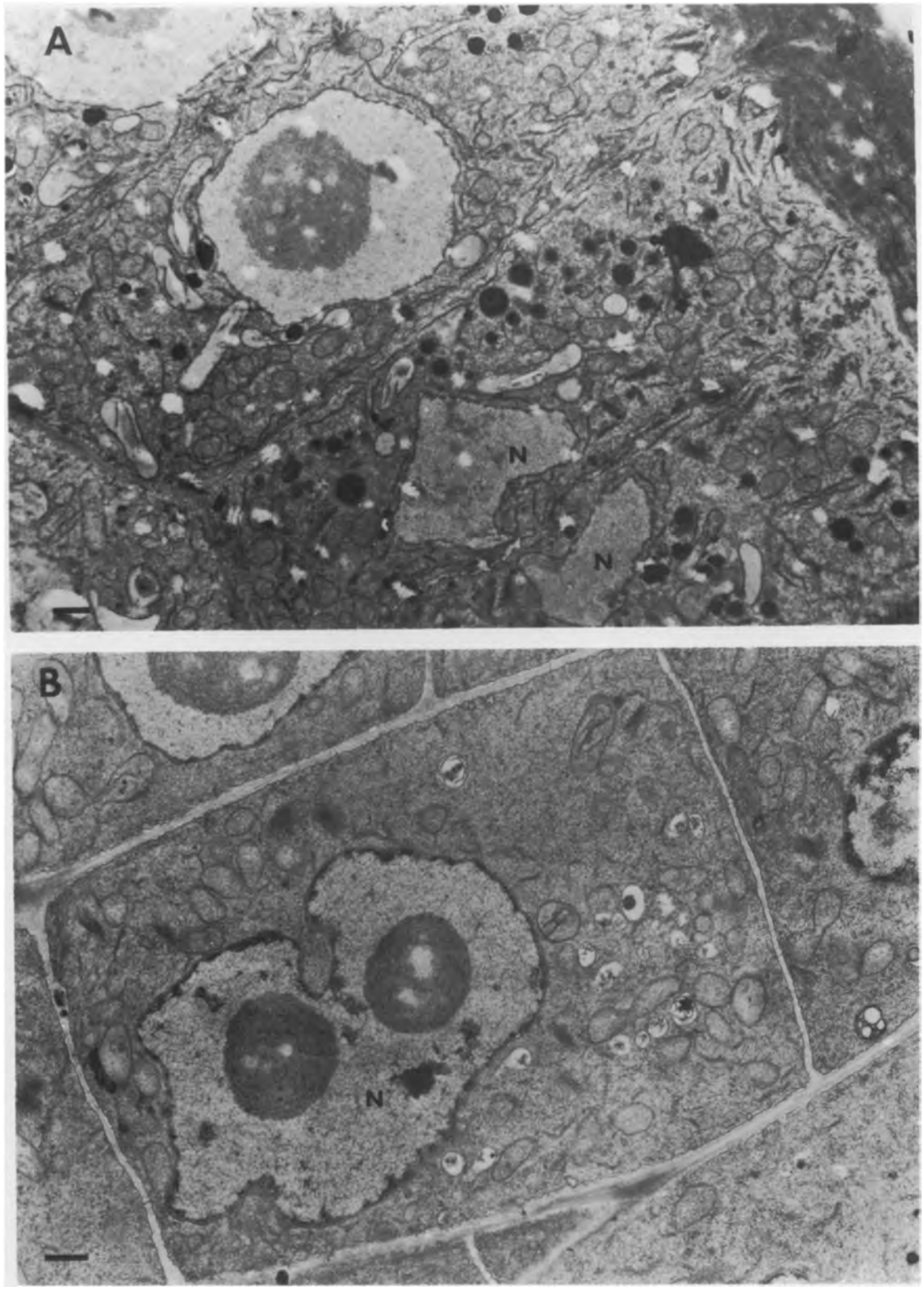

Figure 5. Cells from roots of an S-biotype from Wake County, NC treated with 0.1 µM trifluralin for 48 h before fixation. (A) Micrograph showing two daughter cells with lobed nuclei (N). (B) Binucleated cell. Bar = 1 µm.

biotypes not exposed to dinitroaniline herbicides and do not seem to be associated with herbicide resistance.

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