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A Dinitroaniline-Resistant Mutant of *Eleusine indica* Exhibits Cross-Resistance and Supersensitivity to Antimicrotubule Herbicides and Drugs[1]

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ABSTRACT

A dinitroaniline-resistant (R) biotype of *Eleusine indica* (L.) Gaertner. (goosegrass) is demonstrated to be cross-resistant to a structurally non-related herbicide, amiprophosmethyl, and supersensitive to two other classes of compounds which disrupt mitosis. These characteristics of the R biotype were discovered in a comparative test of the effects of 24 different antimitotic compounds on the R biotype and susceptible (S) wild-type *Eleusine*. The compounds tested could be classified into three groups based upon their effects on mitosis in root tips of the susceptible (S) biotype. Class I compounds induced effects like the well known mitotic disrupter colchicine: absence of cortical and spindle microtubules, mitosis arrested at prometaphase, and the formation of polymorphic nuclei after arrested mitosis. The R biotype was resistant to treatment with some class I inhibitors (all dinitroaniline herbicides and amiprophosmethyl) but not all (*e.g.* colchicine, podophyllotoxin, vinblastine, and pronamide). Roots of the R biotype, when treated with either dinitroaniline herbicides or amiprophosmethyl, exhibited no or only small increases in the mitotic index nor were the spindle and cortical microtubules affected. Compounds of class II (carbamate herbicides and griseofulvin) cause misorientation of microtubules which results in multinucleated cells. Compounds of class III (caffeine and structually related alkaloids) cause imcomplete cell walls to form at telophase. Each of these last two classes of compounds affected the R biotype more than the S biotype (supersensitivity). The cross-resistance and high levels of resistance of the R biotype of *Eleusine* to the dinitroaniline herbicides and the structurally distinct herbicide, amiprophosmethyl, indicate that a mechanism of resistance based upon metabolic modification, translocation, or compartmentation of the herbicides is probably not operative.

A dinitroaniline-resistant biotype of *Eleusine indica* (goosegrass) was discovered in agricultural fields in certain counties of South Carolina where dinitroaniline herbicides had been used continuously for many years (25). Presently, this is the only documented R[2] weed biotype. An initial study (35) of the R biotype showed that its growth and cellular ultrastructure are not affected by treatment with the dinitroaniline herbicide, trifluralin, at concentrations as high as 20,000 times that which has been shown to affect the susceptible (S) biotype.

The mode of action of dinitroaniline herbicides has generally been attributed to their ability to inhibit the polymerization of tubulin into microtubules (12, 14, 15, 22, 24, 33), although other, less likely, mechanisms have been proposed (13). Evidence supporting a direct interaction between dinitroaniline herbicides and tubulin are the loss of both spindle and cortical microtubules shortly after dinitroaniline treatment (15, 35), the inhibition of tubulin polymerization *in vitro* (22), and the binding of a ^{14}C-labeled dinitroaniline herbicide to tubulin subunits (16, 33). The macroscopic (14) and ultrastructural (15, 35) effects of these compounds are similar to the effects of the well studied microtubule disrupter colchicine (14), although there is evidence that colchicine and dinitroaniline herbicides do not bind at the same site on the tubulin molecule (2).

In *Eleusine*, concentrations of dinitroaniline herbicides 3 to 4 orders of magnitude greater than that causing a complete loss of microtubules in the S biotype have no effect on the microtubules of the R biotype (35). The microtubules of nontreated R biotype, however, appear to be altered so that microtubule-controlled processes such as the plane of cell division and cell wall formation are somewhat abnormal (35). These abnormalities in microtubule-directed processes indicated that the mutation in the R biotype might involve cellular components which contribute to the structure, stability, and function of microtubules, such as tubulin.

Tubulin mutants of fungi (26, 27, 32, 34) and other organisms (7, 8, 31) often exhibit cross-resistance to two or more mitotic disrupters but sensitivity, or even supersensitivity, to other antimicrotubule drugs. In this report, we compare the resistance of the R and S biotypes of *Eleusine* to various mitotic disrupters. Based on the response of these biotypes to the various mitotic disrupters, three classes of compounds are reported: (a) those compounds to which R and S biotypes are equally sensitive, (b) those compounds to which the R biotype is more sensitive than the S biotype, and (c) those compounds to which the S biotype is much more sensitive than the R biotype. Based on the high levels of resistance of the R biotype to both dinitroaniline herbicides and APM and the similarities between known tubulin mutants and the R biotype in their responses to antimicrotubule compounds, we discuss the possibility that either an alteration of tubulin, or proteins that interact with microtubules, are responsible for the resistance phenotype of the R biotype of *Eleusine*.

MATERIALS AND METHODS

Plant Growth and Treatment. For cytological examinations seeds were germinated and grown for 3 d in the dark in petri

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[2] Abbreviations: R, dinitroaniline-resistant; APM, amiprophosmethyl; CIPC, isopropyl *m*-chlorocarbanilate; DCPA, dimethyl tetracholoterephthalate; IPC, isopropyl carbanilate; S, dinitroaniline-sensitive.

dishes on filter paper saturated with distilled H_2O. Test compounds were then applied for 24 h in the dark. At the time of the treatment, most of the cells in the root tip were in interphase (35). Table I summarizes the chemical treatments used, the concentrations, and the sources of the reagents. All solutions contained acetone at a final concentration of 1.0% (v/v). Neither microtubule structure or plant growth is adversely affected by incubation of plants for 24 h in solutions containing 1.0% acetone (data not presented). Compounds with low water solubility were dissolved in 100% acetone and then diluted with water with constant stirring. The pH of all solutions was adjusted to 5.7 with either dilute NaOH or HCl to eliminate possible effects due to differential pH. Each of the treatments was performed at least twice and at least three roots from each treatment were observed by microscopy. Results were consistent in all cases.

For tests of the effects of the dinitroaniline herbicide, oryzalin, and APM on germination and early seedling growth, surface sterilized seeds of R and S biotypes were planted in separate rows in petri dishes containing 1% (w/v) agar, Murashige and Skoog salts (Gibco)[3], 2% (w/v) sucrose, and various concentrations of the two herbicides. Dilutions of oryzalin and APM were made from 10 mM stock solutions in DMSO. At no concentration of herbicide did the DMSO concentration exceed 0.3% (v/v). This concentration of DMSO caused no significant growth reduction in our system. Petri dishes were sealed with parafilm and incubated for 2 d at 37°C in the dark and then transferred to the light at 27°C for 5 d.

Microscopy. A number of fixation regimes were tested to obtain optimal fixation conditions for microtubules in *Eleusine* root tips. For example, glutaraldehyde fixation in the presence of 2% (w/v) tannic acid allowed good preservation and clarity of microtubule structures, but poorly preserved the remainder of the cytoplasm. Tannic acid was therefore deleted from the fixation procedure. The best fixation regime for microtubules, that also successfully preserved cell structure, was a 2 h fixation at room temperature in 6% (v/v) glutaraldehyde, 0.05 M PIPES-NaOH buffer (pH 7.4), and 1 mM EGTA. After the initial fixation, roots were washed with two changes, 15 min each, of 0.10 M sodium cacodylate buffer (pH 7.2) at 0 to 4°C. They were then fixed with 1% (w/v) OsO_4 in 0.10 M sodium cacodylate buffer (pH 7.2) for 2 h at 0 to 4°C. After a cold water wash, the sections were stained *en bloc* with 1% (w/v) uranyl acetate for 2 h at 0 to 4°C. Dehydration, embedding, sectioning, and observation were as described earlier (35).

Mitotic indices of samples from each treatment were measured using the procedures of Holmsen and Hess (18). More than four thousand cells were scored for each concentration of herbicide used. The data are expressed in terms of cells in division/thousand (35).

RESULTS AND DISCUSSION

Effects of Antimicrotubule Compounds. The ultrastructural effects of 24 different compounds which disrupt mitosis were tested on the sensitive biotype of *Eleusine indica*. Based on these studies, we classified the effects of each compound on cellular ultrastructure into one of three groups (Table I).

Many of the antimicrotubule compounds cause a loss of spindle microtubules so that, although the chromosomes condense, they do not proceed past prometaphase (Fig. 1A). This condition is often termed *C*-metaphase (colchicine metaphase) (14). After the futile attempt at mitosis, the nuclear membranes reform around the chromosomes, resulting in a deeply lobed, polymorphic nucleus (Fig. 1B). Cortical microtubules are absent or sparse after treatment, so that cells in the zone of elongation become isodiametric rather than elongated. Macroscopic evidence for the loss of cortical microtubules is the swelling of the root tip due to aberrant cell enlargement in the zone of elongation (14). Nearly all of the well-characterized drugs which disrupt mitosis (*e.g.* colchicine, vinblastine, podophyllotoxin) as well as several herbicides (*e.g.* pronamide, amiprophosmethyl, the dinitroaniline herbicides) are in this group. Similar effects have been observed by other workers who used these compounds on other plant species (3, 14, 15, 17).

A second group of compounds does not cause a loss of microtubules, but rather affects the orientation of the microtubules in the cell (3, 4, 11). Compounds in this group include the classic mitotic disrupter drug, griseofulvin, and the carbamate herbicides, IPC and CIPC. Cells treated with these compounds have abundant spindle microtubules at prometaphase (*cf.* Fig. 1A with Fig. 2A), but the microtubules appear to be oriented towards many poles. The multipolar mitosis which follows causes formation of cells with multiple nuclei and walls that are laid down at all angles instead of along a single plane of division (Fig. 2B). It is difficult to ascertain directional misorientations in nonspindle microtubules from thin section analysis; however, the abnormal orientation formation of cell walls (Fig. 2B) after treatment with these compounds suggest that phragmoplast microtubules may also be affected. Two of the compounds tested, terbutol and DCPA, induced these wall abnormalities and multipolar divisions as well as arrested prometaphases and polymorphic nuclei; these later effects were observed primarily after treatment with the highest herbicide concentration. Holmsen and Hess (18) recently reported similar effects of CIPC and DCPA on the structure of treated *Avena sativa* roots.

Table I. *Antimicrotubule Compounds, Concentrations Used, Source, and Effects*

Compound	Concentrations	Sources	Effect[a]
Colchicine	0.1, 1.0 mM	Sigma	1
Podophyllotoxin	0.1, 1.0 mM	Sigma	1
Vinblastine	0.1, 1.0, 10 mM	Sigma	1
Pronamide	1, 10, 100 μM	Chemical Services	1
8-OH-quinoline	1, 10 mM	Sigma	—
Oncodazole	0.1, 1 mM	Sigma	—
Griseofulvin	0.1, 1 mM	Sigma	2
CIPC	0.01, 0.1, 1 mM	Polysciences	2
IPC	0.01, 0.1, 1 mM	Sigma	2
Terbutol	1, 10, 100 μM	Chemical Services	1, 2
DCPA	1, 10, 100 μM	Chemical Services	1, 2
Caffeine	0.1, 1, 10 mM	Sigma	3
Theophylline	0.1, 1, 10 mM	Sigma	3
Aminophylline	0.1, 1, 10 mM	Sigma	3
Caffeic acid	0.1, 1, 10 mM	Sigma	3
Trifluralin	0.01, 0.1, 1, 10 μM	Polysciences	1
Oryzalin	0.01, 0.1, 1, 10, 100 μM	Chemical Services	1
Profluralin	0.1, 1, 10 μM	Chemical Services	1
Ethafluralin	0.1, 1, 10 μM	Chemical Services	1
Benefin	0.1, 1, 10 μM	Chemical Services	1
Isofluralin	0.1, 1, 10 μM	Chemical Services	1
Amiprophosmethyl	0.01, 0.1, 1, 10, 100, 1000 μM	Mobay	1

[a] 1, Loss of spindle and cortical microtubules; cells arrested at prometaphase and reformed, lobed nuclei; 2, microtubules present but cell division is multipolar; 3, phragmoplast formation abnormal, vesicles do not fuse to form the cell wall; —, no effect.

[3] Mention of a trademark, proprietary produce or vendor does not constitute a guarantee or endorsement of this item by the USDA and does not imply its approval to the exclusion of vendors that may also be suitable.

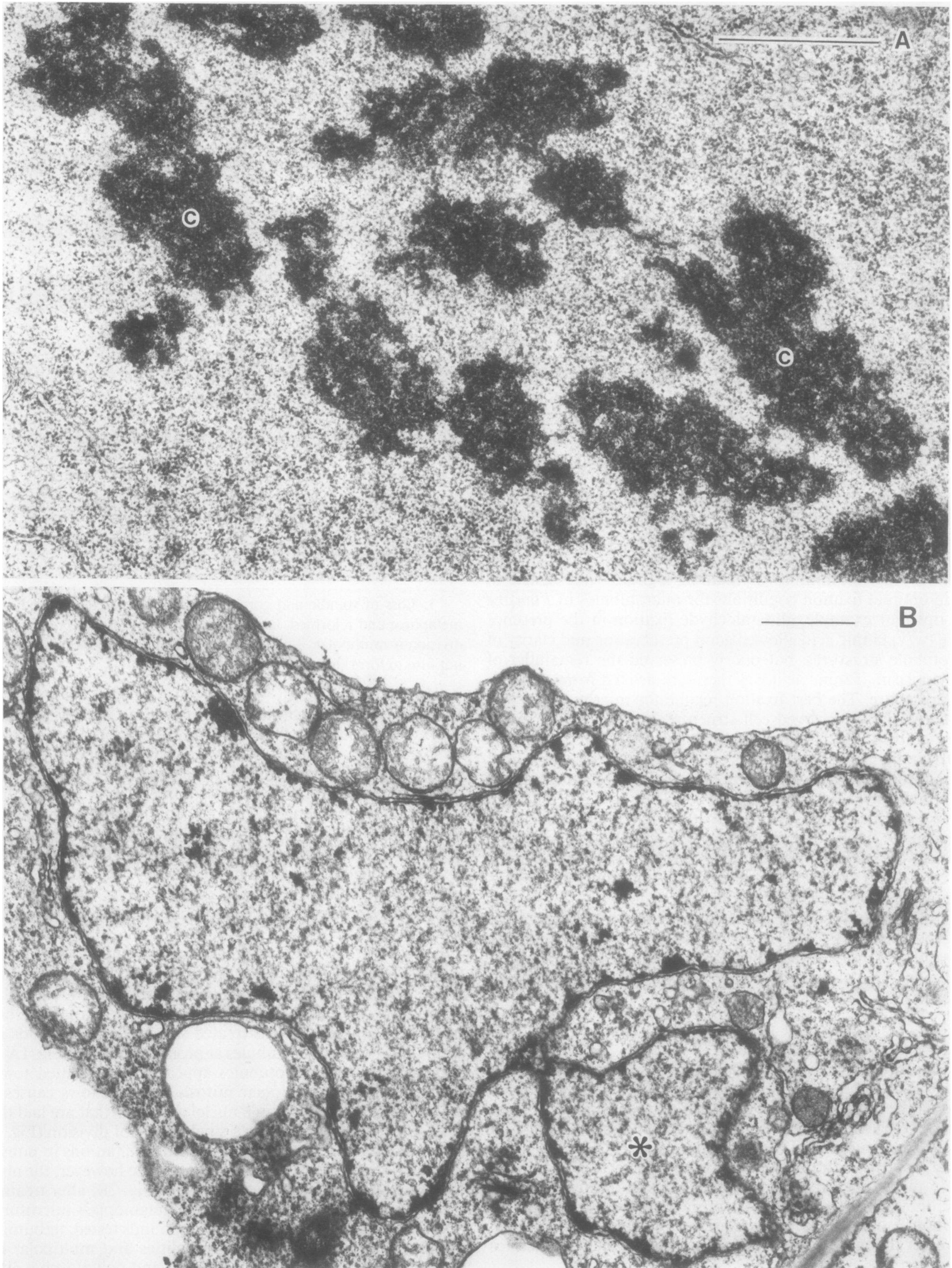

FIG. 1. A, S biotype after treatment with 1.0 μM APM for 24 h. Chromosomes (c) are heavily condensed but no spindle microtubules are noted. B, Heavily lobed nucleus (* = lobes) after treatment of S biotype with 1.0 μM oryzalin. Bar, 1.0 μm.

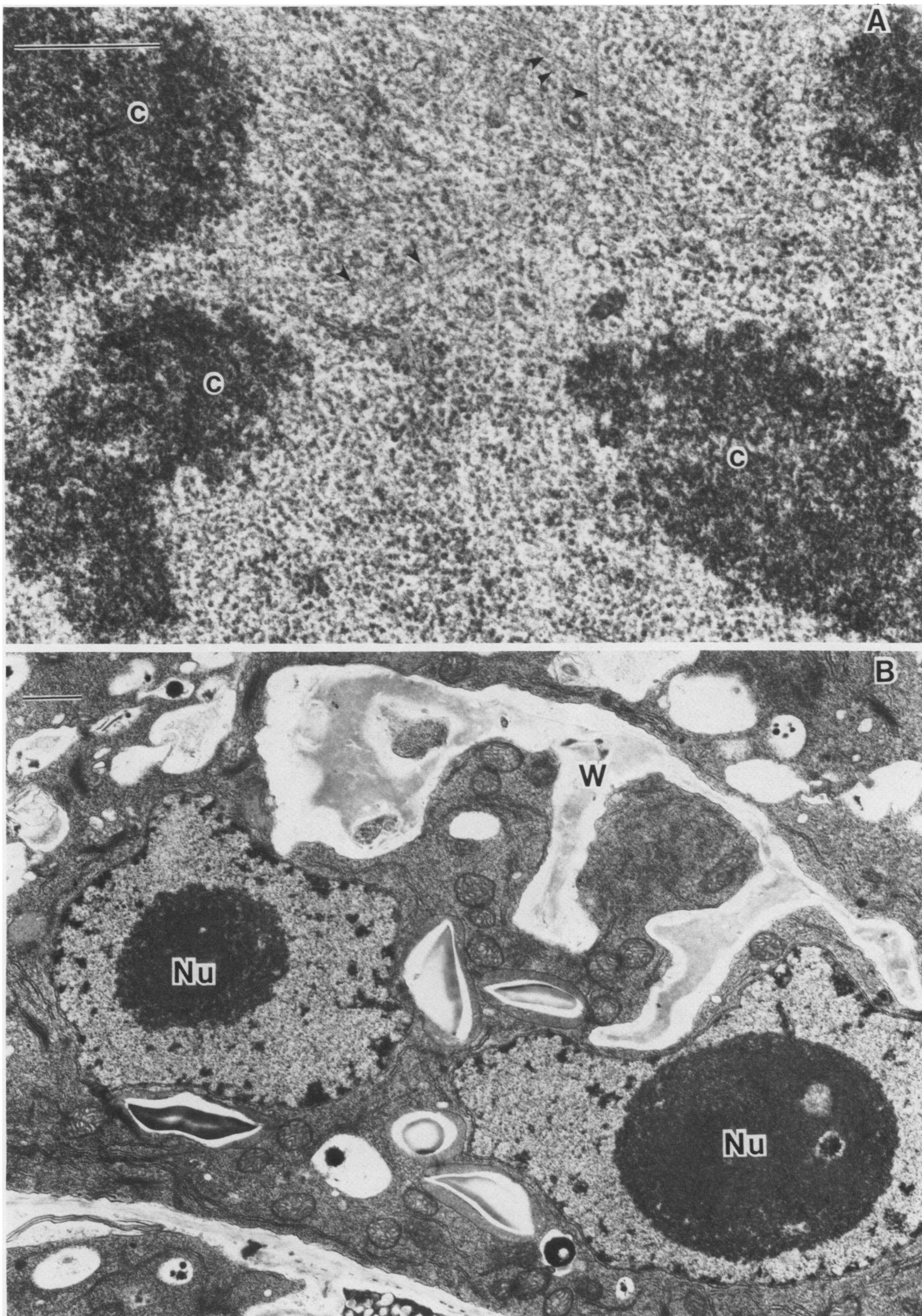

FIG. 2. A, Prometaphase after treatment with 1.0 mM CIPC: many spindles microtubules (arrows) are present but they are oriented toward several poles. c, chromosome; bar, 0.5 μm. B, Nuclei (Nu) are found in several compartments after treatment with 1 mM CIPC and walls (W) are arranged in an abnormal fashion. m, mitochondrion; bar, 1.0 μm.

A third group of compounds, caffeine and related alkaloids, cause a failure of vesicle fusion during the formation of cell walls (28). Fully or partially vesiculate cell walls are formed in the presence of these compounds (Fig. 3A). At high concentrations, especially in the R biotype, no recognizable cell wall is formed and binucleate cells result (Fig. 3B).

Two compounds, oncodazole and 8-hydroxyquinoline, did not induce any mitotic irregularities at the concentrations examined even though these compounds induce mitotic irregularities in other species (KC Vaughn, MA Vaughan, unpublished data).

Comparison of Structural Effects on the R and S Biotypes. Many of the compounds tested had similar effects on both R and S biotypes (Table II). These include most of the well-known antimicrotubule drugs (*e.g.* colchicine, podophyllotoxin, vinblastine) as well as several herbicides (*e.g.* pronamide, terbutol, DCPA). In addition to the ultrastructural data, root tip squashes

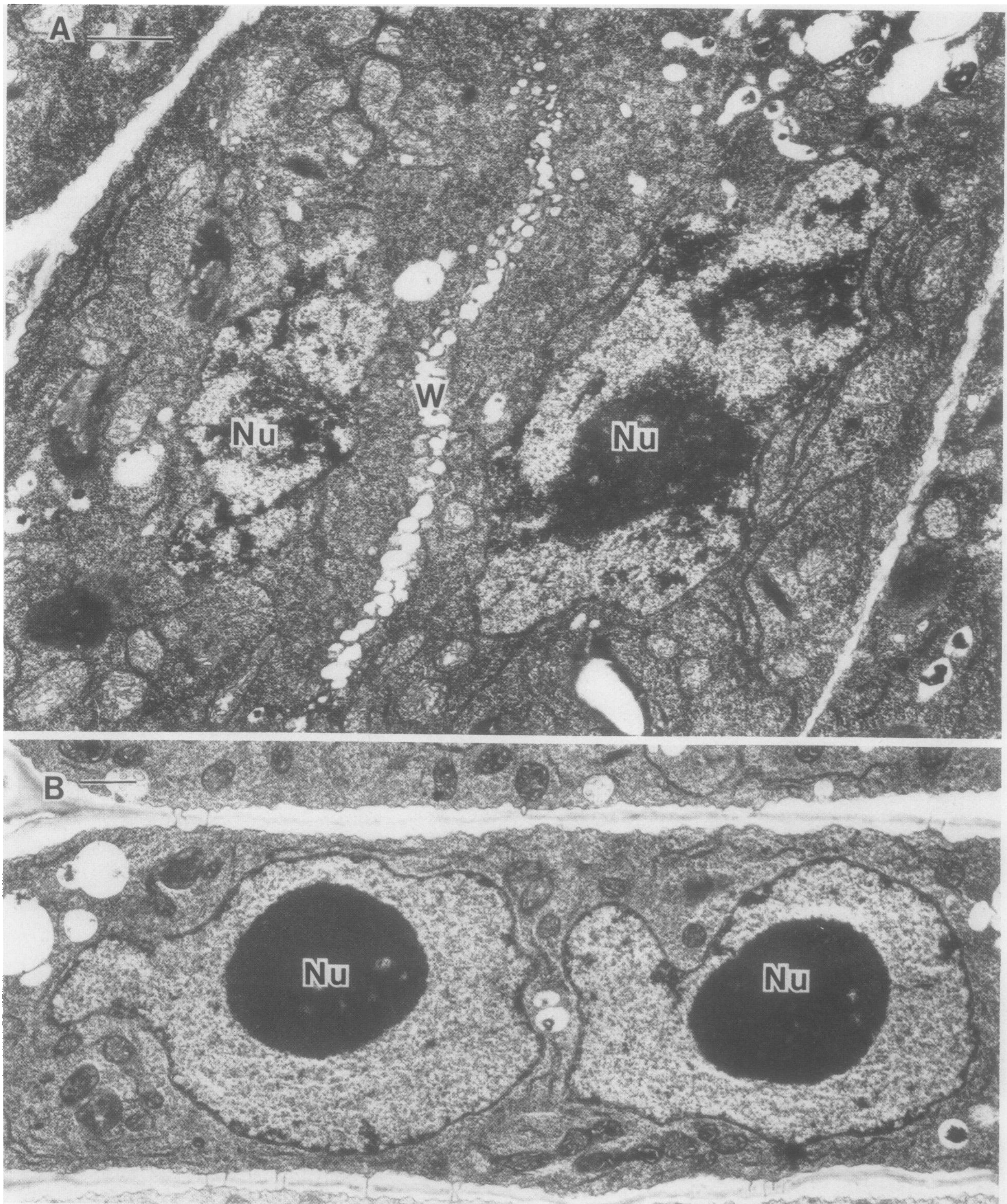

FIG. 3. A, Vesiculate cell wall (W) formation after treatment of the S biotype with 10 mM caffeine. Nu, nucleus; bar, 1.0 μm. B, Binucleate cell formed after treatment of the R biotype with 1 mM caffeine. Nu, nucleus; bar, 5.0 μm.

Table II. *Response of R and S Biotypes of Eleusine to Various Mitotic Inhibitors*

The groupings were determined by ultrastructural effects on mitosis, by increases in the mitotic indices, and the severity of the effect.

Group 1. (R and S biotypes equaliy affected)
Colchicine, podophyllotoxin, vinblastine, pronamide, terbutol, DCPA, 8-hydroxyquinoline, oncodazole.
Group 2. (R more affected than S)
griseofulvin, CIPC, IPC, caffeine, theophylline, aminophylline, caffeic acid.
Group 3. (S more affected than R)
trifluralin, oryzalin, profluralin, ethafluralin, isopropalin, benefin, amiprophosmethyl.

reveal a similar level of increase in the mitotic index in both R and S biotypes after treatment with many of the antimicrotubule drugs (*e.g.* Fig. 4A).

Nearly all of those compounds that caused wall abnormalities (Tables I and II) affected the R biotype more than the S biotype. Typical examples of the increased sensitivity of the R biotype to compounds that cause wall abnormalities are the data from CIPC and caffeine treatments described here. Other compounds in these groups gave similar results. Mitotic indices were virtually the same in each biotype (151/1000 cells in the R biotype and 158/1000 cells in the S biotype) after treatment with 100 μM CIPC. The effects observed were more pronounced in the R biotype, however. At 100 μM CIPC, 37% of the cell profiles observed by transmission EM had multinucleate cells whereas only 8% of the cells in the S biotype were multinucleate. Similarly, at 1 mM caffeine 57% of the cells of the R biotype contained binucleate cells or cells with a small amount of wall material whereas only 11% of the cells of the S biotype had vesiculate walls at this same concentration. An example of this difference in sensitivity to caffeine is illustrated in Figure 3. Effects of caffeine on the S biotype similar to those in the R biotype similarly required a 10-fold higher concentration of mitotic disrupters. The only exceptional cases are the responses of the R biotype to terbutol and DCPA, in which the two biotypes appear equally sensitive despite the wall abnormalities which these herbicides induce (Table II). The enhanced sensitivity to those mitotic disrupters that induce wall abnormalities is only one order of magnitude, as compared to the 1000-fold or greater resistance reported previously for trifluralin (35) and for other dinitroaniline herbicides and APM, described below. In a previous report (35), the ultrastructure of the R and S biotypes of *Eleusine* was described. Although no abnormal cell walls were formed in the untreated S biotype, evidence of incompletely formed or abnormally oriented cell walls were frequently noted in the untreated R biotype. Because of this abnormality in the R biotype, it is not surprising that mitotic disrupters which affect cell wall orientation or biogenesis would be more effective at disrupting the R biotype than the S. From this, two explanations for the supersensitivity of the R biotype to caffeine, CIPC, and other compounds that affect wall orientation are likely: (a) that the already disturbed wall structure is especially sensitive to agents that further perturb wall structure (*i.e.* a pleiotrophic effect of the mutation) or (b) that the alteration in the R biotype conferring dinitroaniline-resistance allows enhanced effect (binding?) of these compounds. Interestingly, several colchicine-resistant lines of *Chlamydomonas* are also caffeine sensitive (38), which might indicate that enhanced caffeine sensitivity is a consequence of resistance to compounds that arrest mitosis like trifluralin or colchicine. Other possible reasons for the enhanced sensitivity are discussed below.

Mudge *et al.* (25) and Chernicky (9) found that the R biotype, although originally selected as a trifluralin-resistant strain, exhibited cross-resistance to all of the dinitroaniline herbicides examined as determined by growth comparisons of the R and S biotype in the presence of herbicide. Likewise, we found that the R biotype was resistant to six different dinitroaniline herbicides as determined from mitotic indices and the lack of ultrastructural changes that are characteristic of the S biotype when treated with dinitroaniline herbicides (Fig. 4B). Oryzalin is one of the most water soluble commerically available dinitroaniline herbicides. This fact allowed us to determine the level of resistance of the R biotype to supersaturated concentrations (*i.e.* a theoretical 100 μM in 1% [v/v] acetone) of this class of herbicides. Root tip squashes of the S biotype treated with oryzalin, even at low concentrations, reveal a large increase in the mitotic indices due to arrested prometaphases. In contrast, the mitotic index of the R biotype is little affected even at high herbicide concentrations (Fig. 4B). This magnitude of resistance to herbicides has only been reported for site-of-action mutations (29). Much lower levels of tolerance/resistance are noted in mutants which alter the levels of target protein production (1), increase rates of herbicide decomposition (19), or decreased effective herbicide

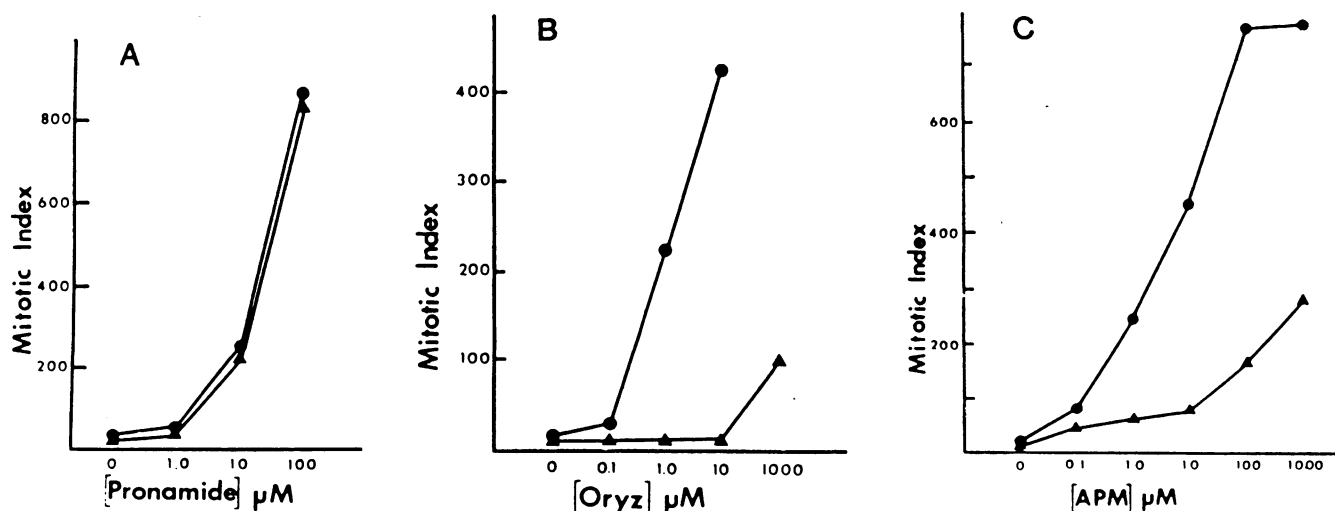

FIG. 4. Mitotic indices (cells in division/1000 cells) in plant root tips treated with various microtubule disrupter herbicides. Note the similarity of effects on the R and S biotypes with mitotic disrupters such as pronamide (A) and the dissimilarity between the biotypes when treated with oryzalin (B) or amiprophosmethyl (C) ● R biotype; ▲, S biotype.

concentrations by altering intracellular compartmentation (37). The high level of resistance observed therefore indicates that the R biotype of *Eleusine* is a site-of-action mutant.

The R biotype is also resistant to the structurally unrelated herbicide APM. This herbicide is much more water soluble than the dinitroanilines and thus more concentrated solutions (*i.e.* 0.1–1.0 mM) of the herbicide could be tested. The microtubules of the R biotype are not affected by herbicide concentrations as high as 0.1 mM (not shown) and the nuclear abnormalities that result from loss of spindle microtubules (*e.g.* Fig. 1, A and B) are not seen in the R biotype after treatment (Fig. 5). The mitotic index is increased only at the two highest concentrations (0.1–1.0 mM) tested (Fig. 4C). It is possible that the increase in the mitotic indices in the R biotype observed at these very high levels may not be due to microtubule interactions. Rather, the increase may be due to interaction of APM with other cellular processes which may slow the time for mitosis sufficiently so that more cells are observed in mitosis. For example, it is well known that oryzalin and APM both cause Ca^{2+} release from the mitochondria at high (10–100 μM) concentrations (13) and indeed, mitochondria from both R and S biotypes are affected at these high herbicide concentrations (KC Vaughn, unpublished data). Based upon mitotic indices, the resistance of the R biotype to APM is only slightly less than that observed for dinitroaniline herbicides. Nevertheless, the R biotype is still clearly resistant to APM.

Comparison of Whole Plant and Ultrastructural Data on Resistance. When seeds of the R and S biotypes are imbibed and germinated in direct contact with oryzalin or APM, the R biotype exhibits an approximately 100-fold greater resistance to the two herbicides. Figure 6 illustrates that R biotype seedlings grow well in oryzalin at 1.0 μM and APM at 3.0 μM. Growth of seedlings of the R biotype is markedly inhibited by concentrations of 3.0 μM oryzalin and 6.0 μM APM. Seedlings of the S biotype survive only on concentrations of <0.05 μM of either herbicide (not shown). At high concentrations (6 μM), APM causes an overall decrease in seedling vigor (Fig. 6F) whereas at high concentrations (3 μM) of oryzalin (Fig. 6C), root growth is comparatively more inhibited than shoot growth. Nevertheless, it is evident that the R biotype is strongly resistant to both herbicides and that the degree of resistance to both herbicides in this germination and growth test is approximately the same as that noted by mitotic indices measurements. It is possible that the inhibition of the seedling growth of the R biotype at high herbicide concentrations may be due to secondary inhibitory effects of the compounds and not to effects on microtubules *per se*. In fact, no arrested mitoses are noted in the ultrastructural studies of the R biotype treated with APM (*e.g.* Fig. 5), indicating that the decrease in seedling vigor is due to a nontubulin-related effect of the herbicide.

Because of the structural dissimilarity between APM and dinitroaniline herbicides, it appears unlikely that the cross-resistances of the R biotype to these compounds can be explained by a mechanism of resistance based upon metabolism, uptake, translocation, or compartmentation of the herbicides. Indeed, Chernicky (9) found no consistent difference in dinitroaniline herbicide uptake or translocation between the S and R biotypes. There are several mechanisms by which co-resistance to dinitroaniline and phosphoric amide herbicides might be conferred. These mechanisms include: (a) a mutation in a site on a tubulin subunit to which both herbicides bind; (b) a mutation that alters the structure of a tubulin subunit in such a way that two independent herbicide binding sites are affected; (c) a mutation that alters tubulin subunit structure in such a way that one or both herbicides can still bind, but no longer interfere with microtubule assembly (see Refs. 8 and 26 for discussions of such types of mutants); (d) a mutation in a protein that interacts with tubulin or microtubules (*e.g.* a microtubule-associated protein) and blocks or nullifies the binding of the two herbicides; or (e) a

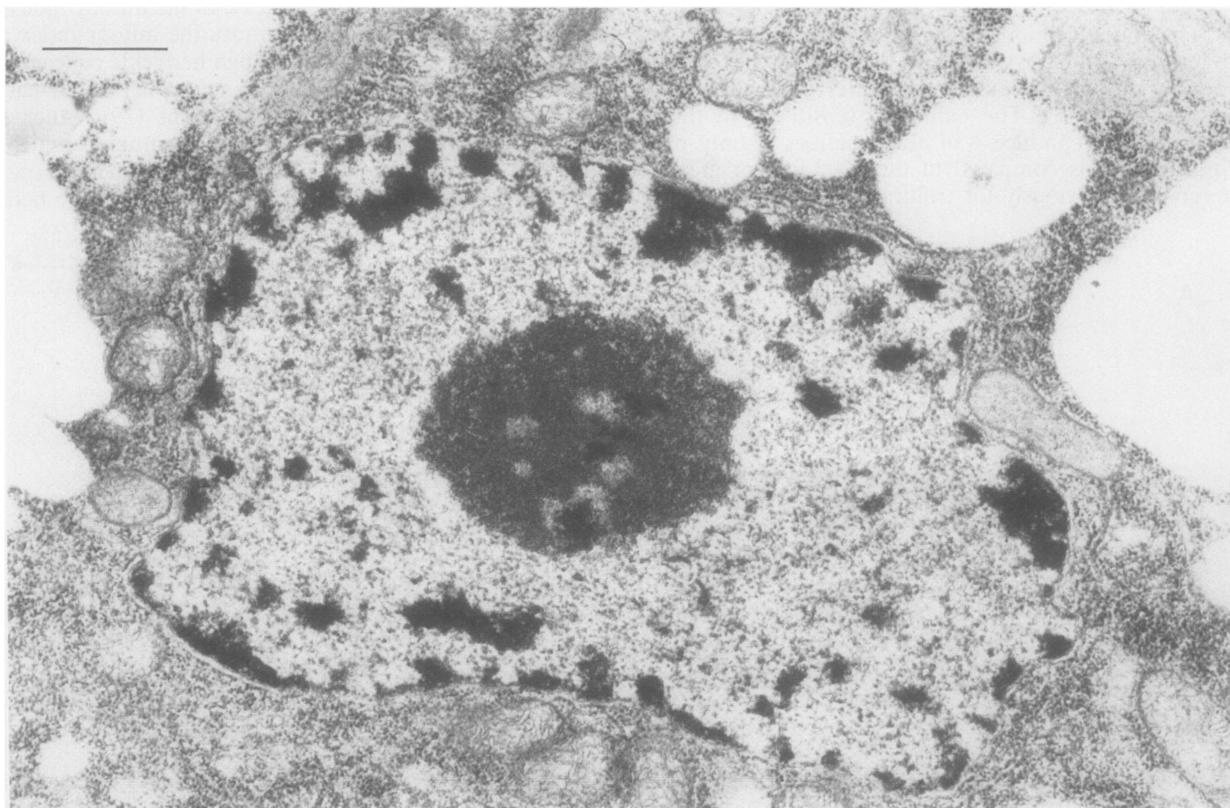

FIG. 5. No apparent effect on nuclear structure of the R biotype after treatment with 10 μM APM. Bar, 1.0 μm.

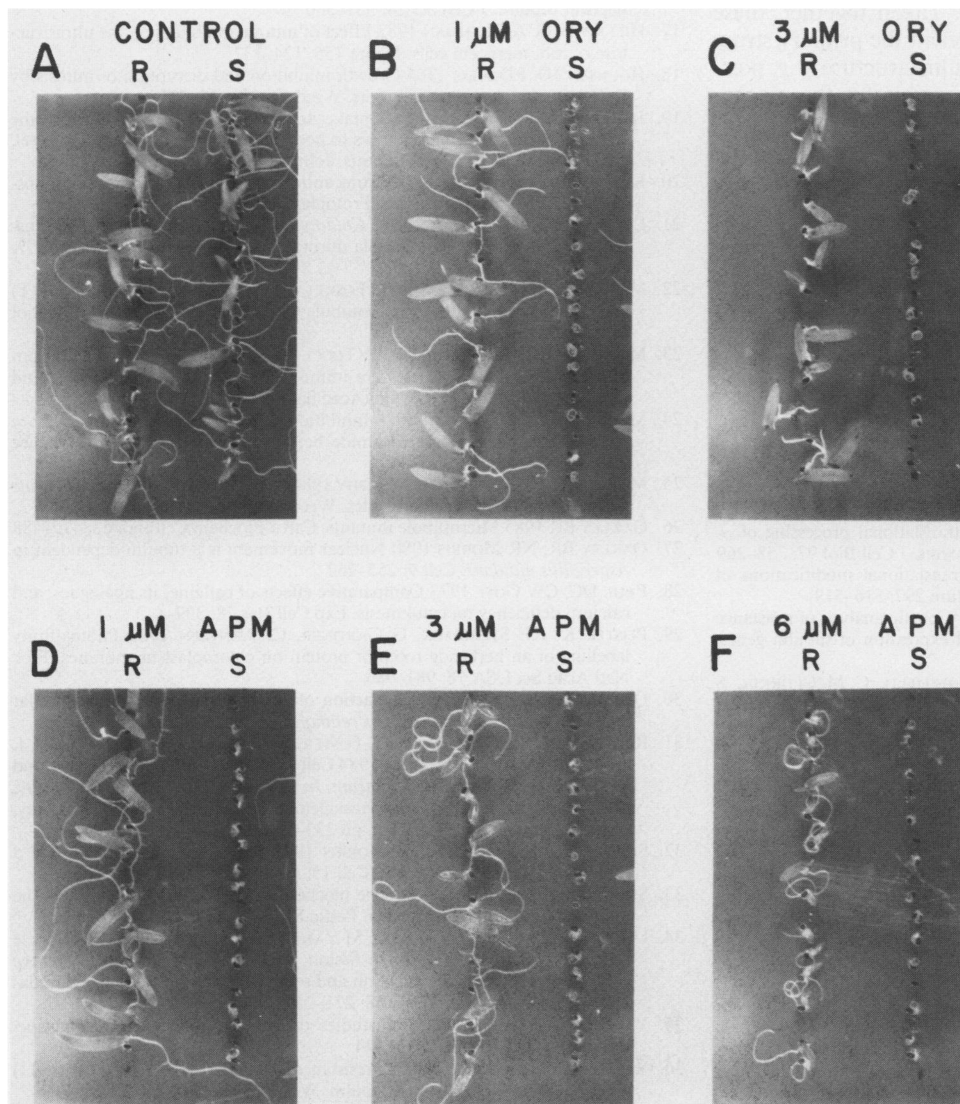

FIG. 6. Response of germinating resistant (R) and susceptible (S) biotypes of *Eleusine indica* to various concentrations of oryzalin (ORY) and amiprophosmethyl (APM). Seeds of both biotypes were planted in separate rows on a nutrient medium (see "Materials and Methods") containing 1% (w/v) agar and allowed to germinate for 2 d in the dark at 37°C followed by 5 d in the light at 27°C.

mutation that promotes a post-translational modification of tubulin (*e.g.* Refs. 5, 6, 21) which, in turn, prevents or nullifies the binding of dinitroaniline herbicides and APM.

Although the R biotype of *Eleusine* appears to be the first case of a microtubule mutant of higher plants, several microtubule mutants of fungi, slime molds, algae, and cultured animal cells have been described previously (for reviews see Refs. 8, 26, 31). Most fungal and mammalian mutants were initially selected for resistance in media containing microtubule-disrupting agents. Many such mutants selected on one antimicrotubule drug exhibit cross-resistance to compounds of the same chemical class. Such is the case for the R biotype of *Eleusine* (Table II). Supersensitivity has also been documented in many of the microtubule mutants studied (see below). Changes in microtubule stability are thought to be responsible for enhanced sensitivity in some mutants (*e.g.* 26, 27) although, for most, the basis of the altered sensitivity is unknown. In the case of the *Eleusine* biotype, the enhanced sensitivity could be due to (a) a change at the dinitroaniline binding site that confers a greater access for other compounds to their binding sites or (b) further exacerbation of microtubule-related processes (*e.g.* cell wall formation) already impaired in the mutant.

Is the R biotype of *Eleusine indica* a tubulin mutant? While definitive experiments remain to be performed, a number of observations point to a strong possibility that the R biotype may contain a mutation which affects tubulin. First, the R biotype is resistant to at least two structurally distinct classes of herbicides which are known to bind to tubulin (10, 22, 30) and whose primary modes of action are known to be as inhibitors of microtubule formation in higher plants (14, 24, 33). Second, there are marked similarities between the types of resistances and sensitivities exhibited by the R biotype and those exhibited by mutants of fungi, slime molds, and mammalian cells that can grow in the presence of antimicrotubule drugs as a result of alterations in the structure of their tubulin subunits (*e.g.* 7, 8, 26, 34). These similarities include: (a) cross-resistance in many mutants to antimicrotubule agents of the same structural class as the compound used in the original screening process (dinitroaniline in the case of *Eleusine*); (b) cross-resistance to some of the agents with similar mode or site of action, but which have distinctly different chemical structures (amiprophosmethyl in the case of *Eleusine*); and (c) supersensitivity to yet other classes of microtubule disrupters (for *Eleusine*, these compounds include griseofulvin, carbamates herbicides, and compounds structurally related to caffeine). Third, while only minor cytological differences can be detected between R and S biotypes without herbicide treatment (35), those differences that are observed are closely tied to processes mediated by microtubules (*e.g.* cell plate formation). Finally, biochemical results indicate that the R biotype has a beta-tubulin subunit that is distinctly different in apparent

mol wt from that of the S biotype (36). Taken together, these results suggest that direct (genetic) changes in the primary structure of tubulin or indirect changes in tubulin structure (*e.g.* post-translational modification) may be responsible for the mechanism of resistance in the R biotype of *Eleusine*.

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