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The abnormal cell plates formed after microtubule disrupter herbicide treatment are enriched in callose

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Abstract

Mitotic disrupter herbicides are best known for their macroscopic effect on root tip swelling and their microscopic effect on the progression of chromosomes through mitosis. However, irregularities with the phragmoplast microtubules and cell plate formation occur at lower herbicide concentrations than these more familiar effects. Instead of the relatively straight cell plates found in control tissue, cell plates after mitotic disrupter treatment are often branched and grow irregularly throughout the cytoplasm. Sometimes these abnormal plates adhere to one wall and in most cases do not effectively divide the potential daughter cytoplasms. To determine the chemical composition of these abnormal cell plates, thin sections of treated onion root tips were probed with a battery of antibodies and cytochemical probes. Abnormal cell plates are greatly enriched in callose compared to control cell plates and accumulate very low levels of cellulose. The development of these wildly undulated and excessively branched or heavily thickened cell plates indicates the importance of microtubules in forming a proper cell plate and perhaps the necessity of stable microtubule arrays for the addition of cellulose to these structures. Because the abnormal plates occur at herbicide concentrations below that required for induction of mitotic arrest or root tip swelling, this effect may be the primary phytotoxic effect of these herbicides.

Keywords: Callose; Cell plate; Mitotic disrupter herbicides; Herbicide action

1. Introduction

Mitotic disrupter herbicides induce a wide variety of macroscopic and microscopic effects on plant cells [1,2]. These herbicides block the addition of tubulin dimers onto the "+" end of microtubules [3]. Microtubules are dynamic structures so that the gradual loss of tubulin dimers from the "−" end result in progressively shorter microtubules, and eventually no microtubules. This loss has dramatic effects on the ability of a plant to deal with functions required of microtubules, chiefly control of cell shape and mitosis. The loss of both of these microtubular functions results in the classic "clubroot" formation that is characteristic of this group

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of herbicide [1,2]. The club root is the result of isodiametric cell growth in the zone of root elongation, leading to a large increase in girth in this area that is normally elongate.

In a survey of mitotic disrupter herbicides on the very mitotic disrupter herbicide sensitive oat plant, Hoffman and Vaughn [4] found that all of the herbicides caused the same kind of structural changes to the microtubule array but varied in herbicide efficacy. In all cases, the most sensitive arrays were the phragmoplast array, an array that is involved in the organization and production of the cell plate [5], and the spindle apparatus that controls chromosome movement. Mitotic disrupter herbicide treatments resulted in the production of branched and abnormally oriented cell plates, either directly as an effect on the phragmoplast or as a consequence of a multipolar division. In some of the weakest of these herbicides, such as DCPA, the only effect observed was on the phragmoplast arrays and cell plate formation [4,6–8]. Numerous studies have reported the production of abnormal cell plates after microtubule disrupter herbicide treatment. To date, however, there has been no characterization of the plate itself to determine what differences are present in the branched and abnormal cell plates compared to those in untreated root tips. In this study, antibodies to polysaccharides and a cytochemical probe for cellulose are used to probe sections to determine the relative chemical composition of these abnormal cell plates on onion root tips treated with a variety of these herbicides. Because onion is less sensitive than oats to these herbicides, more of the primary effects might be captured.

2. Materials and methods

2.1. Plant material and treatment

Onion seeds (*Allium cepa* cv. 'Red Hamburger,' Park Seeds) were germinated in 9 cm petri dishes on Whatman's #1 dampened with distilled water at room temperature in the dark. After 4–5 days, the seedlings were checked for uniformity and were transferred to new petri dishes dampened with various concentrations of mitotic disrupter herbicides. Concentrations chosen were those that had been shown previously to cause the formation of abnormal cell plates and at higher concentrations where mitosis was abnormal. Herbicide treatments include terbutol, oryzalin, sindone B, CIPC, pronamide, and dithiopyr. All of these herbicides, with the exception of sindone B, were purchased from ChemService (Warrington, PA) and were essentially pure compounds. Sindone B was provided by Dr. Paul Bartels. All of the solutions were prepared from acetone stocks and the control experiments were prepared in 0.1%(v/v) acetone. Each herbicide was applied over a 1000-fold concentration range (see Table 1). The herbicides were chosen to include a representative of at least one chemical family of mitotic disrupter that affects onion. Lengths of roots from control and treated roots were measured for length before the treatment and after 24 h in the herbicide. Eighteen roots were sampled from each treatment.

2.2. Microscopic techniques

2.2.1. Light and transmission electron microscopy

Samples for TEM were prepared as described by Vaughn and Turley [9]. Sections from these same block faces were cut with a Delaware Diamond Histoknife, mounted on subbed slides, and stained with 1% toluidine blue in 1% sodium borate in water to determine the sort of mitotic irregularities present. At least six different block faces were examined for each herbicide concentration and the presence of abnormal cell plates scored as a % of the total cells in cytokinesis. Mitotic abnormalities including lagging chromosomes, C-mitosis, and multipolar mitosis were determined as the total % of cells in some stage of mitosis.

2.2.2. Immunogold electron microscopy

Thin sections from the blocks prepared for the electron microscopy were cut at 99 nm with a Reichert Ultracut ultramicrotome and mounted on uncoated 300 mesh gold grids. The grids were then floated on the series of solutions and times as described by Vaughn [10]. Four different antibodies/probes were used on these sections that work well on samples embedded in epoxy resin. To