The effects of colchicine on number of giant cell nuclei and nematode development in *Pisum sativum* infected by *Meloidogyne incognita*

Robert J. WIGGERS 1,*, Nathan T. THORNTON 1 and James L. STARR 2

Giant cells induced by *Meloidogyne* sp. are characteristically enlarged and multinucleate. Each nucleus has more chromosomes than unaffected root tip cells. In *Vicia faba*, only euploid nuclei were detected (Huang & Maggenti, 1969) whereas in *Pisum sativum* euploid and aneuploid nuclei were reported (Wiggers et al., 1990). The origin of the increased chromosome numbers is not known. The presence of strictly euploid nuclei in *V. faba* suggests endomitosis and possibly nuclear fusion which was observed by Huang and Maggenti (1969). Evidence of mitotic abnormalities, such as lagging chromosomes and nuclear fusion, which could generate aneuploid nuclei, has been observed in *P. sativum* (Wiggers et al., 1990). Individual nuclei within a single giant cell also have greatly increased DNA contents, ranging up to 16 times that of unaffected plant cells (Wiggers et al., 1990). In *P. sativum*, mitotic divisions were absent in giant cells at 12 days after inoculation (DAI), yet DNA contents continued to increase until 21 DAI, suggesting that endoreduplication had occurred (Wiggers et al., 1990).

Despite these well-known nuclear abnormalities of giant cells, little is known of the relationship between the multinucleate condition of the giant cells and development of the nematode parasite. Bird and McGuire (1966) reported that 6-azauridine and 5-bromo-2'-deoxycytidine, inhibitors of DNA and RNA synthesis, inhibited *M. javanica* development in tomato roots. However, they did not relate effects on nematode development to giant cell development or nuclear condition of the giant cells. Additionally, 6-azauridine was phytotoxic and the observed effects on nematode development may have been an indirect consequence of suppressed host growth.

A more recent study (Engler et al., 1999) examined the effects of the cell cycle blockers hydroxyurea and oryzalin on giant cells induced by *Meloidogyne incognita* in *Arabidopsis*. It was found that a single exposure to either agent at 3 DAI reduced the number of nuclei per giant cell and inhibited nematode maturation. No statistical treatments of nuclear counts in exposed giant cells vs controls were made nor were the effects on nematode development quantified by determining the proportions of nematodes in various stages of maturation. Additionally, the effects of exposure to either hydroxyurea or oryzalin at times other than 3 DAI were not determined.

The purpose of this study was to expand upon the work reported by Engler et al. (1999) by quantifying the effects of the microtubule poison colchicines on nuclear numbers in giant cells when applied at 3 and 7 DAI. We also determined the effects of treating the plants with colchicine on the development of the infecting nematodes.

**Materials and methods**

An isolate of *Meloidogyne incognita* from cotton was established on tomato plants (*Lycopersicon esculentum* Mill. cv. Rutgers). Eggs were obtained by agitation of infected roots containing mature nematodes and egg masses in 0.6% NaOCl solution for 4 min and sequential filtration through 75 and 20 μm aperture filters. Isolated eggs were incubated at room temperature for hatch.

Seeds of *Pisum sativum* cv. Little Marvel were established in rag-dolls, i.e., seeds between two layers of seed cloth wrapped in wax paper (Carter et al., 1977), and allowed to germinate and develop until the emerging root was ca 5 cm long, when each seedling was inoculated with 20 to 50 second-stage juveniles (J2) of *M. incognita*. Inoculated seedlings were maintained at 25°C with a 12 h day/night cycle.

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Seedlings were incubated in aqueous colchicine (0.1% w/v) for 4 h at room temperature or in distilled water (controls), at either 3 or 7 DAI. Following the incubation period, all seedlings were rinsed thoroughly in distilled water and re-rolled in the rag-dolls. Feeding sites from all plants were collected at 10, 21 and 30 DAI, fixed in 3:1 (v/v) ethanol: glacial acetic acid for 24 h, then transferred to 70% ethanol for storage. Additionally, five colchicine exposed and five control seedlings from each sample date were stained with acid fuchsin according to the protocol of Byrd et al. (1983) and examined microscopically to determine stage of nematode development.

For nuclear counts, giant cells were stained with Feulgen stain following established protocols (Wiggers et al., 1990). Once stained, giant cell complexes could be readily observed under a dissecting microscope and surgical forceps were used to remove individual giant cells from these feeding sites. Upon removal, giant cells were placed in a droplet of 40% acetic acid on a glass microscope slide. Pressure was applied to a cover slip to spread the giant cells. To make the slides permanent, they were placed at −80°C for 30 min. Immediately upon removal from the freezer, the glass cover slips were removed and the slides allowed to dry overnight in the dark. After 24 h, the glass slides were removed from the dark and one drop of Permount adhesive (Sigma Chemical Company, St. Louis, MO, USA) was placed directly over the tissue on each slide. New glass cover-slips were placed over the drop of Permount, and the slides allowed to dry for 24 h before examination. Giant cells were observed using bright-field microscopy at 400 to 1000× magnification. Nuclei were counted in at least ten giant cells from each treatment.

Results

Treatment with colchicine at 3 and 7 DAI resulted in reduced numbers of nuclei per giant cell at all sample dates when compared to control plants (Table 1). Plants treated with colchicine at 3 DAI contained 7.2 to 8.5 nuclei/giant cell and counts at 10, 21 and 30 DAI were not significantly different \( (P > 0.05) \). At the same sample dates, plants treated at 7 DAI contained 13.8 to 15.7 nuclei/giant cell, not significantly different among dates \( (P > 0.05) \). In contrast, in untreated controls numbers of nuclei/giant cell increased \( (P \leq 0.05) \) from 20.2 at 10 DAI to 51.8 at 30 DAI. At all sample dates, giant cells from colchicine incubated seedlings had fewer nuclei than their control counterparts \( (P \leq 0.05) \).

<table>
<thead>
<tr>
<th>Colchicine</th>
<th>Sample Date (DAI)</th>
<th>LSD ( P \leq 0.05 )</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>20.2 41.5 51.8</td>
<td>6.38</td>
</tr>
<tr>
<td>3 DAI</td>
<td>7.2  7.7  8.5</td>
<td>ns</td>
</tr>
<tr>
<td>7 DAI</td>
<td>14.7 13.8 15.7</td>
<td>ns</td>
</tr>
<tr>
<td>LSD ( P \leq 0.05 )</td>
<td>4.38 6.75 6.06</td>
<td></td>
</tr>
</tbody>
</table>

Numbers of nematodes in the roots were not affected by colchicine treatment (data not shown) but nematode development in colchicine treated plants was affected. At 10 DAI, all colchicine treated plants and the controls contained only J2. By 21 DAI, in plants treated with colchicine at 7 DAI and those not treated, 37.4 and 34.3% of the nematodes were adults. In plants treated with colchicine at 3 DAI, only 4.6% of the nematodes present were adults. At 30 DAI, in plants treated at 7 DAI or untreated, more than 80% of the nematodes were adults, but in plants treated at 3 DAI only 10% were adults and 70% were still J2. Chi-square analysis indicated that at 30 DAI the proportion of nematodes in each stage of development for plants treated at 3 DAI was different \( (P < 0.05) \) from those in plants treated at 7 DAI or not treated.

Discussion

These nuclear counts indicate that numbers of nuclei per giant cell in untreated giant cells increased from 14 to 21 DAI, differing from the report of Starr (1993). This apparent difference may be due in part to the large variation in numbers of nuclei per giant cell, making it difficult to precisely determine when mitosis ceases in giant cells. In giant cells treated with colchicine, a single application at either 3 or 7 DAI prevented further accumulation of nuclei. In this respect, our results agree with those of Engler et al. (1999) who reported that a single exposure to either hydroxyurea or oryzalin at 3 DAI stopped giant cell development.

Colchicine depolymerises already formed microtubules, thus affecting any cellular functions dependent on microtubules, including mitosis (Artvinli, 1987). That colchicine exposure at 3 DAI significantly reduced nematode development assessed at 21 and 30 DAI is in agreement with the report by Engler et al. (1999) that a single expo-