THE INFLUENCE OF COLCHICIN ON ROOT NODULE FORMATION IN *TRIFOLIUM PRATENSE*

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INTRODUCTION

In 1938 Wipf and Cooper described the occurrence of polyploid cells in the root nodules of leguminous plants. This observation has been confirmed repeatedly. Very recently, Mitchell made microspectrophotometric determinations of the DNA content of the nuclei of cells in the root nodules of peas. Although the nuclei of the superficial cell-layers were of the 2c type, there was a continuous layer of cells in the cortex which had 4c type nuclei, and in the centre of the nodules cells having 8c or even 16c type nuclei were observed.

As a result of their detection of the spontaneous occurrence, in uninfected roots, of small groups of polyploid cells near the endodermis in the vicinity of secondary root meristems Wipf and Cooper put forward the hypothesis that the occurrence of polyploid cells in root nodules is due to the nodules having arisen from these small groups of polyploid cells.

Nutman suggested that successful infection is only possible at preformed foci which are in some way related to the secondary root primordia. It seems possible that these preformed foci and the small groups of polyploid cells are one and the same thing.

If the presence of polyploid cells is indeed a prerequisite for a successful nodulation, an increase in the number of such cells should lead to an increase in the number of nodules.

Bonnier was the first to observe that addition of colchicin, which is known to induce polyploidy, increased the number of
nODULES DEVELOPING ON THE ROOTS OF *Medicago sativa* AND *Trifolium pratense*. This effect was confirmed by Trolldenier and Weir for various races of *Trifolium pratense*, *T. hydridum*, *Medicago lupulina*, and *Ornithopus sativus*.

Although concentrations of colchicin above a certain level were definitely toxic, lower concentrations (e.g. for *Trifolium* about 10 mg/l) always increased the number of nodules developing after inoculation. This stimulation of nodule formation was observed even in polyploid races, which led Trolldenier to the conclusion that juxtaposition of cells of differing degree of polyploidy was essential for successful nodulation.

This conclusion will remain open to criticism as long as it is not known whether the effect of colchicin is due to its capacity to induce the production of an increased number of groups of cells of a higher degree of polyploidy in the root cortex or to secondary effects. The problem is the more important because the necessity for the prior formation of cells of a higher degree of polyploidy is made doubtful by other observations. (e.g. Bhaskaran and Swaminathan.) It therefore seemed desirable to reexamine the effects of colchicin, paying special attention to possible alternative explanations for its stimulation of nodulation.

**MATERIAL AND METHODS**

Seeds of *Trifolium pratense*, obtained from various sources, were used: a diploid strain (CB 2n) from the 'Vereenigingsbedrijf van het Centraal Bureau,' Hoofddorp; a tetraploid strain (CB 4n) selected by the same bureau; some commercial diploid strains (French red clover, Groninger red clover (blue cert.), English red clover, Violet red clover, Kuhn red clover) from D. J. van der Have of Kapelle-Biezelinge, and a tetraploid strain (HR 41) from the same source. The seeds were disinfected by first placing them in 96% alcohol for 15 seconds, then in 0.2% HgCl2 for 6 minutes followed by 8 washings in sterile water (Fred, Baldwyn, and McCoy). They were then placed on moist, sterilized filter paper at room temperature in the dark for 4 days. During the second half of the fourth day the plants were exposed to light to stimulate loosening of the seed coats.

After germination the seedlings were grown under various conditions:

1. The method used by Trolldenier, in which the seedlings are hung by a thin thread in glass tubes, 15 cm long and 1.3 cm wide, closed with a cap of transparent plastic. The tubes are filled 2/3rds full with nutrient solution and wrapped to that level with black paper to prevent algal growth in the nutrient solution. These cultures will be referred to as 'tube cultures.'