

Title: Some genetic effects of agrochemicals on nitrogen fixing bacteria

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Ambush (Cypermethrin), Delan (Dithianon) and Dithane M-45 (Nabam) are widely used agrochemicals (pesticides). Their lethal doses (LD50) are well known. The three induce C-metaphases, micronuclei, anaphase bridges, condensation and mitostasis in *V. faba* but information is lacking on their effects on nitrogen fixing bacteria. This study aimed at assessing the genetic effects of the three agrochemicals on nitrogen fixing bacteria. This was done by investigating the induction of forward and reverse mutations in the laboratory and field strains of *Rhizobium phaseoli* strain 445, 446 & CC-511, *Derxia gummosa* and *Azotobacter chroococcum*. The effect on nodulation and dry weight was also investigated with *Phaseolus vulgaris*. The spot and direct tests were conducted. In the spot test sterile filter discs, 6 mm in diameter, soaked in solutions of each agrochemical at concentrations 10^{-6} to 10^{-4} ppm were placed at 9 separate points on media on which separate strains had previously been plated. Control samples of discs soaked in sterile water were used. Genetic activity was assessed in terms of proportion of discs around which growth occurred out of the total number used. Toxicity was assessed by scoring zones of inhibition of growth. In the direct test, killing effect (toxic) was assessed by plating approximately 400 cells suspended in the respective agrochemical on complete medium and assessing the emergent colonies out of the total number expected. In an attempt to map the genome of *R. phaseoli* 445, 446 and CC-511, the bacteria were grown minimal media supplemented with amino acids eliminated one at a time. Antibiotic resistance/sensitivity was assessed by introducing discs of antibiotics to bacterial

Abstract: cultures inoculated on complete media. The effects of Ambush, Delan and Dithane M-45 on nodulation by *P. vulgaris* were investigated by applying the agrochemicals at 103 ppm (the recommended rate), 105 ppm and 101 ppm every 10 days for 56 days. Every week for four consecutive week two plants per replicate were selected and the number of nodules was scored and the dry weight was determined. No agrochemical was applied in the controls. Ambush, Delan and Dithane M-45 reduced colony emergence and inhibited growth of *R. phaseoli*, *D. gummosa* and *A. chroococcum* at high agrochemical concentration (104 ppm). At low concentration (10^{-6} to 100 ppm) the 3 agrochemicals induced selective growth of *R. phaseoli* around filter discs but had no observable effect on the growth of *A. chroococcum* and *D. gummosa*. Experiments with amino acids did not elucidate the nature of growth probably due to auxotrophic pre-emption. Dithane M-45 induced antibiotic resistance and/or sensitivity in *R. phaseoli* 445, *R. phaseoli* CC-511, and in *A. chroococcum* in the antibiotic resistance test. High concentration (105 ppm) of Ambush, Delan and Dithane M-45 significantly ($P < 0.05$) reduced the dry weight and number of nodules of *P. vulgaris* especially during the sixth to eighth week of growth. Overall the results indicate that Ambush, Selan and Dithane M-45 at high concentration are toxic to nitrogen fixing bacteria. Selective growth of *R. phaseoli* suggests genetic activity of the three agrochemicals. Dithane M-45 at low concentration induces antibiotic resistance and/or sensitivity in *R. phaseoli* 445, *R. phaseoli* CC-511, and in *A. chroococcum*. There is also evidence for reduction of nodulation and dry weight of *P. vulgaris* by Ambush, Delan and Dithane M-45.

