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Some Phytotoxic Effects of Fenitrothion on the Germination and Early Seedling Growth of *Picea glauca* (MOENCH) Voss and *Betula alleghaniensis* Britton

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Seed germination is a crucial phase in the establishment and maintenance of natural populations of plants. The dry seed is characterized by a low state of metabolism capable of withstanding extreme variations in its environment. Hydration of the seed is often all that is required to initiate an intricate and interlocked sequence of anabolic and catabolic processes essential for germination and growth. At this time, the seed is extremely vulnerable to environmental stress. The addition of biocides to the environment of the germinating seed may constitute an additional stress factor and the induced biochemical and histochemical changes in the germination and seedling growth of a number of herbaceous seed species has recently been followed, (DHILLON & ANDERSON 1972. To date, the effect on forest seeds has largely been neglected.

The pesticide fenitrothion (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate) is annually sprayed on several million acres of Canadian forests to control forest insect defoliators, such as spruce budworm. It is generally applied at a dosage rate of 2-6 oz/acre over a period of a few weeks in mid to late spring. Although fenitrothion is generally not considered to be persistent, one year following spraying it was present at a level of 10% of the original deposit on some conifer foliage (YULE & DUFFY 1972), and red maple (an angiosperm) retained as much as 3 times more fenitrothion on the foliage than similarly treated conifer species. Recent investigations have indicated that following normal spraying local deposits of fenitrothion may differ by a factor of ten or more (CARROW 1974). The spruce budworm larvae are not all killed by a single spraying of fenitrothion (FETTES 1975) and it has often been necessary to repeat applications on an annual basis. Flight patterns also cause some areas to be oversprayed many times in one season. Thus, there is the potential for ecological damage from successive fenitrothion spraying. BUCKNER (1975) observed significant damage and subsequent fall of up to 70% of the total foliage of sugar maple, trembling aspen and white birch, following a 1.4 kg/ha (20 onz/ha) spray of fenitrothion however, the conifer species examined here, significant effects were not show. Our earlier studies confirmed the apparent lack of significant toxicity of the pesticide on selected conifer species while indicating the sensitivity of yellow birch seeds (HALLETT *et al.* 1975).

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In the present study the seeds of white spruce (Picea glauca) (Moench/Voss) and yellow birch (Betula alleghaniensis Britt) were exposed to 10, 100 and 1000 ppm fenitrothion during the course of stratification. Ten ppm represents a normal field concentration, the two higher levels are environmentally relevant, as aerial spraying patterns sometimes lead to overspraying. Evidence(s) of toxicological change(s) was monitored by examining seeds and seedlings of untreated and fenitrothion treated seeds during germination and early seedling growth. The toxicological indicators were changes in total proteins, carbohydrates, succinic dehydrogenases and non-specific esterases.

MATERIALS AND METHODS

Seeds and Storage: The seeds of white spruce, Picea glauca Moench/Voss and yellow birch, Betula alleghaniensis Britt., were obtained from the Pettawawa Forest Experiment Station in the fall of 1972/73 and 1974/75. The seeds were collected from areas not previously sprayed with insecticide. All seeds were stored in tightly sealed glass containers at 10°C until required for experimental procedures (WANG 1973).

Conditions of Imbibition and Stratification: Seeds were routinely sterilized with a 2% hypochlorite solution and thoroughly washed for $\frac{1}{2}$ hr in running deionized distilled water prior to use. White spruce and yellow birch seeds required 14 and 21 days of stratification at 5°C, respectively, in order to germinate consistently (U.S.D.A., 1969). During the course of stratification the seeds (10 per glass petri plate), were exposed to aqueous solutions of final concentration of 10, 100 and 1000 ppm of fenitrothion.

Following stratification, seeds were transferred to clean petri plates (10 seeds per plate) and germinated (in the absence of fenitrothion) in darkened controlled environmental growth chambers. In the case of white spruce, a 12 hr temperature regime of 20°C and 30°C was followed (U.S.D.A. 1969). Yellow birch seeds were germinated with a 12 hr diurnal temperature of 15°C and 32°C.

Sampling Procedures: White spruce and yellow birch embryos were dissected from surrounding seed tissue at three morphologically comparable periods during germination, namely, 3, 6, and 10 days for white spruce and 4, 6, 10 and 13 days for yellow birch.

To prevent tissue dessication small amounts of phosphate buffer were added during the dissection. A random sampling of at least three embryos from each of the four treatments (control, 10, 100- and 1000 ppm) was obtained at each sampling date for each procedure. Further, some seedlings were harvested after 3 and 15 weeks and their gross morphology was examined. For all tests, apart from the enzyme studies, the embryos or seeds of all series at each sampling period were fixed in a 10% neutral formalin solution for three hours and then washed in running water overnight. Samples were dehydrated in a graded tertiary-butyl alcohol-

ethanol series prior to infiltration and embedding in paraffin or paraplast. Ten micron sections were obtained on a conventional rotary microtone and adhered to clean glass slides with freshly prepared Haupt's adhesive (PURVIS et al. 1964). Following rehydration they were permanently mounted with Canada balsam.

For the enzyme localization studies (succinic dehydrogenases, and general esterases) intact embryos were excised and placed in the appropriate reaction mixture, then fixed in Carnoy's fluid (PURVIS et al. 1964) and photographed with a 35 mm Leicaflex camera assembly.

Localization Procedures: Total protein was obtained by the mercuric-bromophenol blue method (WEST 1968). Rehydrated sections were placed in a solution consisting of 0.05% bromophenol blue and 0.1% $HgCl_2$ in 2% acetic acid, for exactly 2 hours. Sections were then rinsed in 0.5% acetic acid for 5 min then immersed in water and transfered through absolute tertiary butyl alcohol (TBA) (2 hours), a 1:1 mixture of xylol: TBA, and finally absolute xylol.

Total carbohydrates (insoluble polysaccharides) were localized by the periodic acid-Schiff's (PAS) reaction (HOTCHKISS 1948). The sections were rehydrated and placed in 1% periodic acid for precisely 10 min., washed in water and stained in freshly prepared Schiff's reagent (JENSEN 1962) for 35 min. Following a brief water rinse, sections were placed in 2% sodium bisulfite for 2 min, rehydrated, then mounted.

Esterases and succinic dehydrogenases were localized in intact white spruce and yellow birch embryos by the method of HOLT (1958) and AVERS (1958). In both procedures, incubation in the reaction mixtures was carried out at 37°C for precisely 2 hr. Controls were heat killed and placed in a complete reaction mixture. Distribution and intensity of staining was monitored in embryos from each treatment group.

RESULTS

In white spruce there was no evidence that even the highest level of fenitrothion permanently affected any of our toxicological indicators. General esterase activity appeared somewhat affected, but this was not significant at the 5% level.

The response of yellow birch seeds and seedlings to fenitrothion treatment was different. The two higher levels of fenitrothion resulted in unusual seedling development, poor seedling vigor and death after 2-3 wk. While seedlings from the 10 ppm group appeared to grow normally, intracellular protein changes were evidenced by the presence of large anomalous bodies "protysomes" (readily digested with the enzyme protease). These were different from protein bodies in other seeds (ADAMS & NOVELLIE 1975). The "protysomes" were only present in treated tissues and were present through most of the root and hypocotyl tissues of the seedlings. The higher the fenitrothion concentration, the greater

the number of "protysomes" evidenced, Plate 1.

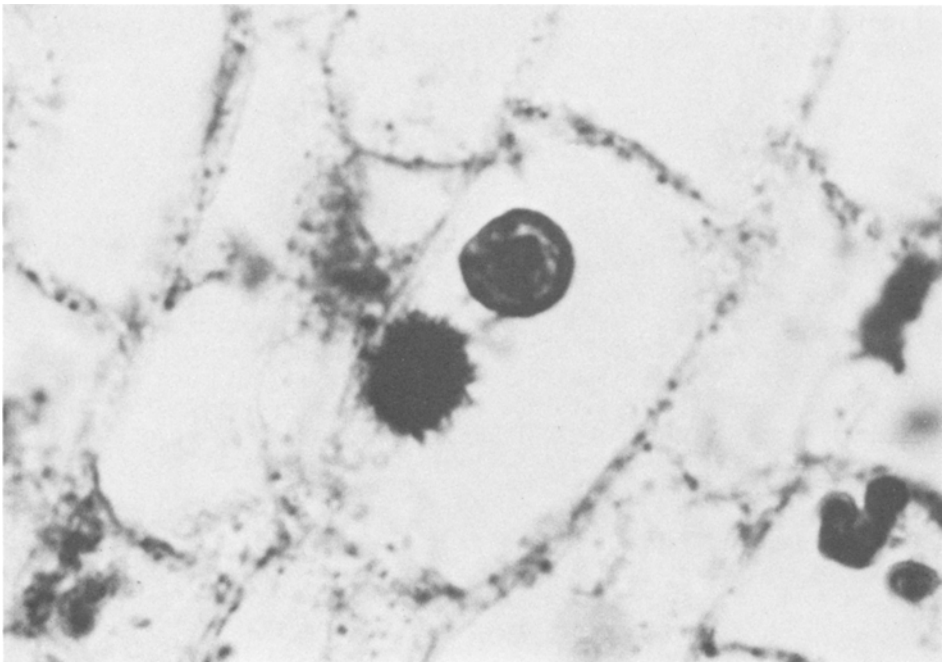

Plate 1. "Anomalous protein bodies present in yellow birch root and hypocotyl tissues following fenitrothion treatment (10 to 1000 ppm).

By contrast, in seedlings from the 10 ppm group, the succinic dehydrogenases and esterases were unaffected; localization in treated tissues was not notably different from comparable controls. However, fenitrothion treatment did drastically affect the way in which succinic dehydrogenases were distributed in the 100 ppm and 1000 ppm fenitrothion treated embryos. Following 6 days germination, embryos from these groups showed more intense localization in the radicle, whereas in the controls the cotyledons were the main site of localization. Ten and thirteen days after germination, succinic dehydrogenases were restricted to the root apex in the controls whereas the enzyme was apparent throughout the seedlings derived from the 1000 ppm treatment. The distribution and activity of non-specific esterases was also affected following exposure to the two higher levels of fenitrothion. These changes were most evident following 10-13 dy of growth. In the control seedlings and those derived from 10 ppm fenitrothion treated seeds, esterase activity was widely distributed in all the tissues and

was especially intense in the cotyledons whereas in the 100- and 1000 ppm fenitrothion treatment groups esterase activity was insignificant in the cotyledons and mainly confined to the procambial tissues and the root tips of the seedlings.

DISCUSSION

Field concentrations of fenitrothion (10 ppm) do not seem to have any long lasting toxicological effects on white spruce or yellow birch seeds. The embryos gave rise to normal seedlings and apart from some minor, short term effects, the seeds withstood being bathed in a 10 ppm fenitrothion water medium for 14 and 21 dy respectively. White spruce seeds were able to withstand exposure to 1000 ppm fenitrothion with no evidence of long term damage and little evidence of cytological change. This substantiates, at the cytological level, the earlier observations of undamaged stands of conifers following spraying with fenitrothion (BUCKNER 1975).

The sensitivity of yellow birch seeds to 100- and 1000 ppm fenitrothion indicate that these angiosperm seeds are less toxitolerant than those of the conifer, white spruce.

Our observations on yellow birch have shown that the bulk of storage reserve material consists of protein. Inhibition of its utilization and suppression of hydrolytic enzyme activities (POMBER et al. 1974) undoubtedly contributes to the morphogenetic anomalies we have observed in fenitrothion treated embryos. A number of germination inhibitor substances, including organophosphorous insecticides, have been shown to impair specific enzymatic activities and to disrupt protein metabolism in plants (DALVI et al. 1972, DALVI et al. 1974, ASHTON et al. 1968, DHILLON & ANDERSON 1972). It is therefore not surprising that the most no-table indications of toxicological sensitivity to both low and higher concentrations of the fenitrothion was evidenced in the apparent disfunction of protein metabolism. The appearance of the large "protysomes" present in the yellow birch seeds exposed to any of the three fenitrothion concentrations was unexpected especially as they are dissimilar from the typical protein bodies found in many seeds (ADAMS & NOVELLIE 1975). These gave an early indication of aberrance in protein metabolism. Alone, this change did not seem to be harmful as 10 ppm treated seeds gave rise to seedlings which appeared to have seedling vigor equal to that of non-treated controls. Exposure to the higher fenitrothion concentrations also affected seed and seedling succinic dehydrogenases and general esterases, particularly in the cotyledons. This was paralleled by gross changes in development and early death of the seedlings.

The difference in reactivity of the white spruce and yellow birch seeds may be due, in part, to the inherent differences in primary sites of metabolism in the early germination process.

The conifer seeds obtain their required nutrient from the surrounding gametophytic tissue, however, there is evidence that the cotyledons of yellow birch are designed for both storage and photosynthesis (MARSHALL & KOZLOWSKI 1974). Our enzyme localization studies have shown that the regions most affected by fenitrothion exposure in yellow birch are the cotyledons. The retardation of hydrolytic processes of reserve nutrient in cotyledons has been observed in mung bean seeds following exposure to a range of pesticides (DALVI et al. 1974, DHILLON & ANDERSON 1972). Thus, it it possible to speculate that the different responses of birch and the conifer seeds to the insecticide may be due in part to the extensive surrounding gametophytic tissue found in white spruce in contrast to the small amount of nutrient material in the birch cotyledons. Also, it is possible that there may be intrinsic differences in toxi-resistance of target enxymes in seeds and seedlings of the two species. (HALLETT et al. 1977).

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