SOME PHYSIOLOGICAL EFFECTS OF FENITROTHION ON THE GERMINATION AND SEEDLING GROWTH OF *Betula alleghaniensis* Britt. AND *Picea glauca* (Moench) Voss

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RÉSUMÉ

On a utilisé des techniques de localisation histochimiques pour déterminer les effets du fénitrothion (10, 1000 p.p.m.) sur les succinodéshydrogénases, sur les estérases non spécifiques, sur les nucléoprotéines basiques, sur la mitose et l'ADN des graines de merisier (Betula alleghaniensis) et d'épinette blanche (Picea glauca) au cours de la stratification. Dans les embryons d'épinette blanche, seules les estérases ont été touchées; leur teneur, d'abord faible, est redevenue normale au début de la germination. Des effets similaires ont été confirmés dans le cas des embryons de merisier après emploi de 10 p.p.m. de fénitrothion. Toutefois, au taux de 1000 p.p.m., ce produit a entraîné une diminution marquée des teneurs en ADN, en estérases non spécifiques et en succinodéshydrogénases, lesquelles sont demeurées faibles au cours de la germination et au début de la croissance.

INTRODUCTION

Seed germination is a crucial phase in the establishment and maintenance of natural populations. The dry seed is characterized by a low state of metabolism capable of withstanding extreme variations in the environment. Jack pine seeds, for example, may experience temperatures as low as -60°C during cold boreal winters; yet seeds are only released from the serotinous cones following exposure to excessive heat due to forest fires (Mirov, 1967). 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. However, the metabolism of germinating seeds is extremely vulnerable to environmental stress. For example, Mukherjee et al (1974) have found that temperatures beyond the optimum levels, usually decrease the vigor of rice seedlings and this corresponds to decreased amylase, ATPase, and phytase activities. The addition of various biocides in the environment thus constitutes an additional parameter affecting the germination and seed metabolism.

Information correlating biochemical and histochemical changes in germinating seeds treated with biocides has only recently been examined. Dhillon and Anderson (1972) have shown that propachlor inhibits root and shoot growth by preventing normal autolysis of protein and lipid reserves. Similar impairment responses of mung bean seed hydrolytic metabolism has been shown following exposure to the organophosphorous aphicide, menazon (Dalvi, et al, 1972; Dalvi, et al, 1974).

Previous studies carried out by the authors have shown that the organophosphorous insecticide, fenitrothion, is quite effective during seed germination and subsequent growth of yellow birch (Betula alleghaniensis) (Pomber et al, 1974). Using cytological and histochemical techniques, we extended the earlier study to determine whether there were any coincident metabolic changes during the germination of yellow birch and white spruce (Picea glauca) seeds following exposure to low and high levels of fenitrothion.

MATERIALS AND METHODS

(i) Seed Materials:

Seeds of white spruce and yellow birch were obtained from the Petawawa Forest Experiment Station, Chalk River, Ontario from areas not previously sprayed with fenitrothion. They were stored at 20°C in tightly sealed glass containers until required for experimental use.

White spruce and yellow birch seeds require 14 and 21 days of stratification at 5°C, respectively, in order to germinate consistently (U.S.D.A., 1949; Crossley and Skov, 1951). During the course of stratification the seeds were exposed to aqueous solutions of 10 ppm and 1000 ppm of fenitrothion.

Following the stratification period, seeds were placed in petri plates (10 seeds per plate) and germinated in darkened controlled environmental growth chambers. In the case of white spruce, a twelve hour temperature regime of 68° to 86° F. was followed (U.S.D.A., 1949). Yellow birch seeds were germinated with a twelve hour diurnal temperature regime of 59° to 90° F. (U.S.D.A., 1949).

(ii) Sampling Procedure:

White spruce and yellow birch embryos were dissected from surrounding seed tissue at three morphologically comparable periods during germination. These time periods were 3, 6, and 10 days in the case of white spruce and 4, 6, 10 and 13 days for yellow birch.

Small amounts of phosphate buffer were added during the dissection in order to prevent tissue dessication. A random sampling of at

least three embryos from each of the three treatments (control, 10 ppm, and 1000 ppm) was obtained at each sampling date for each histochemical localization procedure.

(iii) Tissue Preparation:

Identical procedures were used with both white spruce and yellow birch embryos. Embryos to be examined by light microscopy (Feulgen, PAS (periodic acid - Schiff) and basic nucleoprotein localizations) were initially fixed in a 10 percent 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. Ten micron sections were obtained on a conventional rotary microtome and adhered to clean glass slides with freshly prepared Haupt's adhesive (Purvis, 1964). Following rehydration and cytochemical localization, sections were dehydrated and permanently mounted with Canada balsam.

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

(iv) Localization Procedures:

Basic nucleoproteins, DNA, succinic dehydrogenases and non-specific esterases were examined in white spruce and yellow birch embryos.

(a) Basic nucleoproteins (histones)

Histones were localized in tissue sections by the method of Prent and Lyon (1973). Nucleic acids were first extracted

from the sections in 5 percent trichloroacetic acid at 60°C for precisely 80 minutes. Sections were then rinsed for 15 minutes in running tap water and placed in a 1 percent Fast Green FCF solution adjusted to pH 8.1 with 0.1M HCl-borate buffer for 30 minutes. Timing is critical and complete extraction of the nucleic acids was confirmed by a negative Feulgen reaction.

(b) DNA

Sections were stained for DNA by the conventional Feulgen method (Jensen, 1962). Prior to staining, sections were hydrolized in 1N HCl at 60°C for 30 minutes. From each treatment, embryos were then examined for intensity of staining in the cotyledons, shoot and root apices. Mitotic figures were then examined in the cotyledons of white spruce embroys from each treatment following 3, 6, and 10 days of germination in order to obtain an indication of cell division rates.

(c) Esterases and succinic dehydrogenases

Both enzymes were localized in intact white spruce and yellow birch embryos by the methods of Holt (1958) and Avers (1958) as detailed in an earlier report (Pomber et al, 1974). In both procedures, incubation in the reaction mixtures was carried out at 37°C for precisely 2 hours. Controls were heat killed and placed in a complete reaction mixture. Distribution and intensity of staining was monitored in embroys from each treatment group.

RESULTS

A. Effects of Fenitrothion on White Spruce:

Succinic dehydrogenases were localized in the white spruce treatment groups following the 14 day stratification-exposure period and 3 and 8 days of subsequent germination. The enzyme appeared to be unaffected by the fenitrothion treatments. Embryos from the control, 10 ppm and 1000 ppm fenitrothion groups showed similar intensities and distribution of succinic dehydrogenases at each sampling time.

Following stratification, non-specific esterases were distributed throughout the embryos of all treatment groups. Intensity of localization was as control > 10 ppm > 1000 ppm. Similar relationships between treatments were apparent following 4 days germination. Following 10 days of germination, however, intensity of non-specific esterase localization and distribution of the enzyme did not differ significantly between treatments.

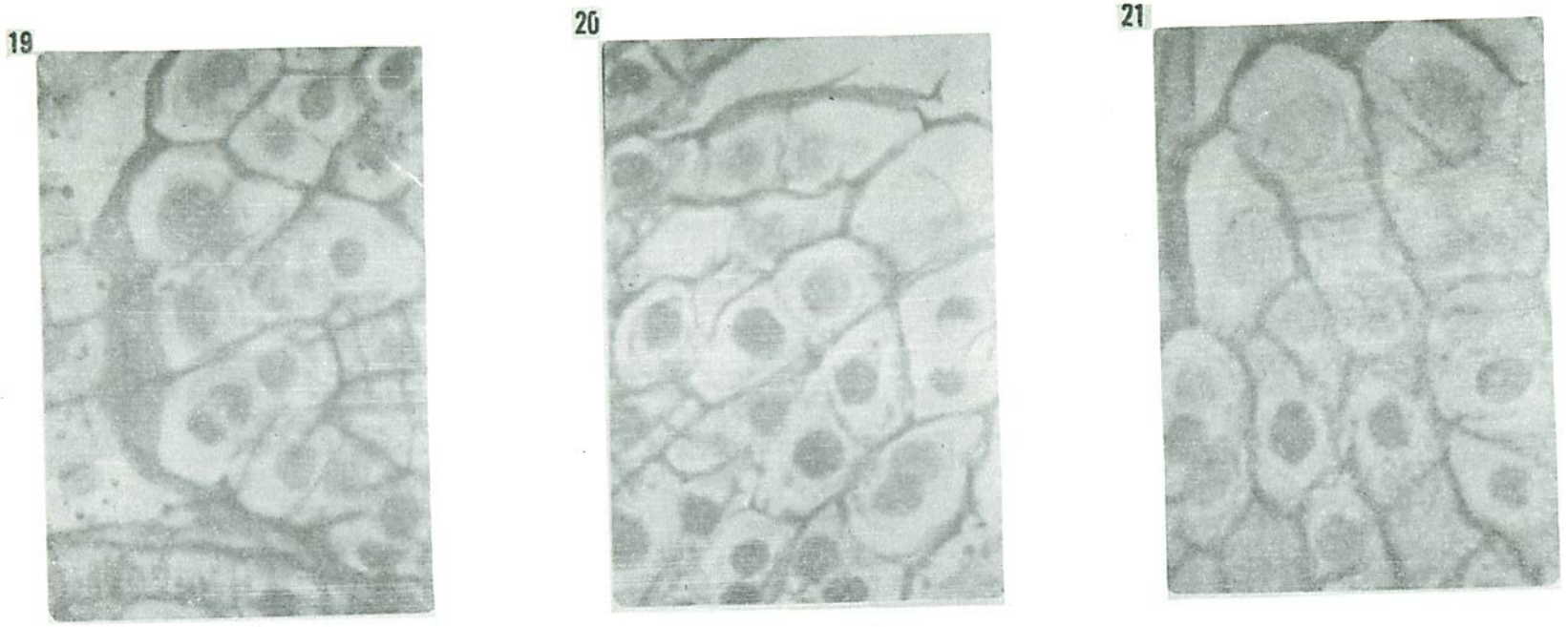
Unlike our earlier observations with white pine embryos (Pomber et al 1974) and those of jack pine (Durzan et al, 1971) basic proteins in white spruce embryos were confined to the nucleus, no histone protein was observed in the cytoplasm. Intensity of staining of the basic nucleo-protein was observed following stratification and during germination in the cells of the root apex, shoot apex and cotyledons of embryos from the three treatments. No significant differences were apparent between treatments.

Similarly, intensity of DNA staining in the nuclei of white spruce embryos did not show appreciable differences between treatments following stratification and during subsequent germination.

TABLE I

PERCENTAGE OF NUCLEI IN WHITE SPRUCE COTYLEDONS SHOWING MITOTIC FIGURES FOLLOWING FENITROTHION TREATMENTS

CONCENTRATION	3 DAYS	6 DAYS	10 DAYS
Control	7.00 ± 2.79	7.79 ± 3.77	8.57 ± 2.07
10 ppm	7.40 ± 2.44	8.40 ± 2.66	8.08 ± 1.86
1000 ppm	4.08 ± 1.49	4.59 ± 1.39	7.34 ± 1.34



(a) (b) (c)

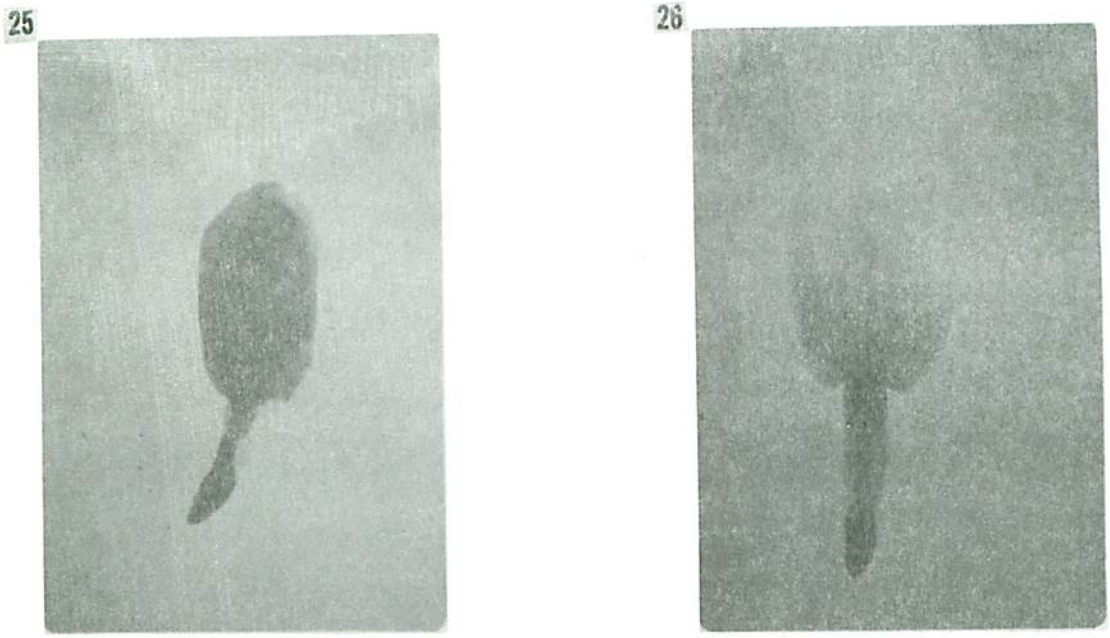
Fig. 1. Effects of fenitrothion on cell division (Fuelgen staining of nuclei) in white spruce embryo 6 days after germination. (a) Control; (b) Fenitrothion treated 10 ppm; (c) Fenitrothion treated 1000 ppm.

Consistently, mitotic figures were absent in the nuclei of the cotyledons immediately following the stratification. Following 3 and 6 days of germination (when mitotic figures are first evident) no significant differences were observed between the control and 10 ppm treatments (Table I). However, at these periods both treatments showed significantly higher percentages of dividing cells than the embryos treated with 1000 ppm fenitrothion (Figs. 1, a, b, & c). Following 10 days germination, differences between treatments were no longer significant.

B. Effects of Fenitrothion on Yellow Birch

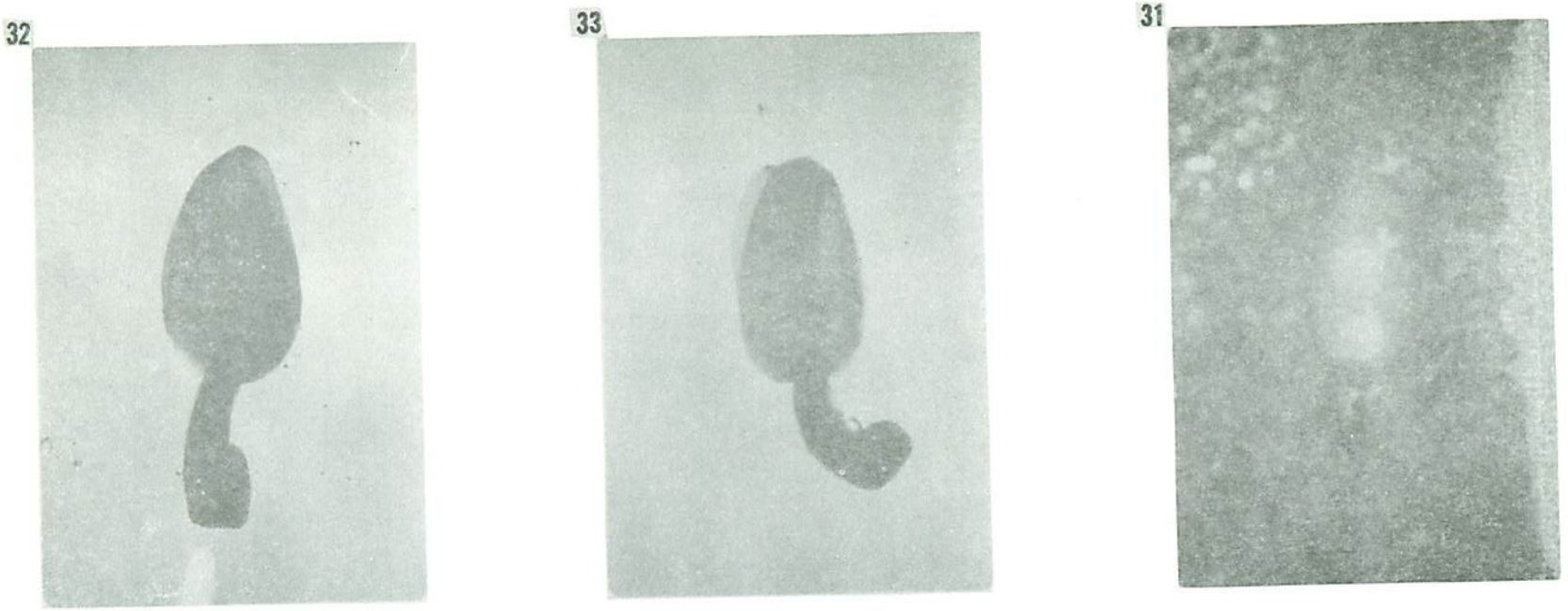
Our earlier report documented the suppressed germination and subsequent seedling dwarfing effects of yellow birch seeds following exposure to 1000 ppm fenitrothion (Pomber et al, 1974). Subsequently, we have examined metabolic parameters of yellow birch germination with regards to fenitrothion treatments.

Histochemical localization of succinic dehydrogenases showed effects in the embryos following fenitrothion exposure. In control embryos, after 4 days of germination, intense localization of the enzyme was apparent throughout the radicle axis with less intense positive localization being confined to the meristem region. Following 6 days germination, control embryos showed more intense localization in the cotyledons, whereas 1000 ppm treated embryos showed enzyme localization to be most pronounced in the radicle with only light positive staining in the cotyledons (Fig. 2 a & b). Ten and thirteen days after germination, succinic dehydrogenases were restricted to the root apex in the control, whereas the enzyme was apparent throughout the seedlings derived from the 1000 ppm treatment. Intensity



(a) (b)

Fig. 2. Effects of fenitrothion on distribution of succinic dehydrogenases in yellow birch embryos after 6 days of germination. (a) Control, (b) 1000 ppm treatment.



(a) (b) (c)

Fig. 3. Effects of fenitrothion on distribution of non-specific estrases in yellow birch embryos and cotyledons. (a) Control; (b) 10 ppm and (c) 1000 ppm treatment.

and distribution patterns of the enzyme localization in the 10 ppm treatment embryos were similar to those of the control at each sampling date.

Non-specific esterases were also shown to be affected by fenitrothion exposure to the seeds. Control (untreated) embryos, following 4 days germination showed extensive and deep enzyme staining. Less intense localization was observed in the cotyledons of the 1000 ppm exposed embryos. Control and 10 ppm treatments showed similar distribution patterns and localization intensities following 6 days of germination. Non-specific esterases were, however, confined to the root tip and procambial tissues in seedlings of the 1000 ppm treatment. This pattern was also quite apparent following 10 and 13 days of germination, i.e. intense positive staining took place in the control as well as 10 ppm treated samples. Seedlings with less pronounced enzyme activity were observed in those of the 1000 ppm group. Inhibition of the enzyme was definitely most apparent in the cotyledons. (See Fig. 3, a, b & c).

The Fuelgen reaction does not provide an intense DNA localization in yellow birch embryos. It was apparent, however, that DNA localization in control embryos exceeded that of the 1000 ppm fenitrothion treated embryos. High carbohydrate levels were associated with the nuclei of control embryos in this region whereas much less staining was observed in cells of the 1000 ppm treatment.

DISCUSSION

In white spruce, low esterase activity was registered following stratification and treatment with fenitrothion but soon the enzyme activity was restored to normal levels very early in germination. Similarly, the high concentration (1000 ppm) of fenitrothion treatment did not seriously disrupt metabolism in the post stratification period in this species. Succinic dehydrogenase levels were unaffected by either 10 or 1000 ppm fenitrothion applications. This suggests that metabolic processes were essentially unaltered. On the other hand, yellow birch seeds showed positive response to fenitrothion treatments. For example, yellow birch nuclei showed very faint Feulgen staining. This condition has been observed in other plant species (Jensen, 1962). Thus, findings may be related to low DNA levels (Hillary, 1939) or to the presence of tanins and/or proteins (Ishida, 1961) which block the reaction. Staining of proteins at pH 2.1 with Fast Green FCF indicated high protein levels associated with the yellow birch nuclei. Less intense DNA localization in the 1000 ppm treated embryos may be related to one of these factors.

The gross morphological effects resulting from yellow birch exposure to fenitrothion as opposed to the conifer species may be due 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 and Kozlowski, 1973). Our enzyme localizations 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 seeds following exposure to various pesticides (Dalvi et al, 1974; Dhillon and Anderson, 1972). Thus it is possible to speculate that differential responses of birch and the conifer seeds to the insecticide are due to the extensive surrounding gametophytic tissue in the conifers in contrast to the confined nutrient material in the embryos of birch (i.e. the cotyledons). Secondly, there may be intrinsic variations in the level of resistance among enzymes (such as general esterases) in coniferous seeds and those of yellow birch seeds. Such organophosphorous resistance mechanisms have been observed by Motoyama and Dauterman (1974).

The work to date has enabled us to observe the effects of fenitrothion application in the germinative process of representative forest tree species. Information concerning the metabolites of the insecticide and their levels within the seeds has recently been determined for these species (Hallett et al, 1974). Thus a more detailed examination of key enzyme systems coincident with toxicity and toxitolerance of the insecticide and its metabolites in these species is now being planned in order to elucidate the differential mechanisms of response. Attempts will also be made to determine acetylcholine levels in the toxitolerant and toxisusceptible species currently being studied.

SUMMARY AND CONCLUSION

Histochemical localization techniques were employed to study the effects of two levels (10 and 1000 ppm) of fenitrothion on succinic dehydrogenases, general esterases, basic nucleoproteins mitosis and DNA contents in seeds of yellow birch (Betula alleghaniensis Britt) and white spruce (Picea glauca) during the course of stratification. In white spruce embryos, esterase levels were initially depressed by the low concentration (10 ppm) but they were restored to normal levels as soon as germination took place. The same pattern was observed with yellow birch seeds too. However, following treatment with the high concentration (1000 ppm) of fenitrothion, significantly lower levels of DNA, general esterase and succinic dehydrogenases were obtained and these low levels were maintained throughout germination and early seedling growth. There was no apparent effect on the rate of cell division at lower concentration in both species. Therefore, it is safe to conclude that fenitrothion applications do not cause undue hazard to vegetative growth at low concentrations.

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