

STUDIES ON PHYTOTOXICITY OF FENITROTHION TO
SOME FOREST SEEDS AND SEEDLINGS
UNDER LABORATORY CONDITIONS

by

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RÉSUMÉ

Les graines d'épinette blanche (Picea glauca) et de pin gris (Pinus banksiana) ont été traitées par des solutions aqueuses contenant 10 et 1000 ppm de fénitrothion. Celles du bouleau (Betula alleghaniensis) ont été exposées, au cours de la période de stratification ou au début de leur croissance, à des solutions ayant des concentrations de fénitrothion égales à 10, 25, 50, 100 et 1000 ppm, alors que les graines de bouleau à papier (Betula papyrifera) n'ont été traitées que par des solutions aqueuses contenant 100 ppm de fénitrothion. L'évaluation des effets du pesticide a été faite en fonction du pourcentage de germination, de la quantité d'eau absorbée et de la vigueur des semis.

La germination des graines d'épinette blanche traitées au fénitrothion à 100 ppm a été légèrement améliorée alors que celle des graines traitées au fénitrothion à 1000 ppm a été considérablement retardée au cours des 10 premiers jours de la période germination. Passée cette première étape, la germination a repris son rythme normal. L'examen de la croissance des semis n'a révélé aucune différence morphologique entre les deux sortes de traitements.

Comparées avec les graines appartenant au groupe témoin, les graines de pin gris traitées au cours de leur germination par des solutions contenant 10 et 1000 ppm de fénitrothion ont eu une meilleure germination. La croissance ultérieure du pin gris n'a pas été affectée par le traitement au fénitrothion que les graines de cet arbre ont subi au cours de la stratification.

La germination des graines de bouleau à papier traitées par du fénitrothion à 100 ppm a accusé un retard important. Les semis de bouleau

à papier et de bouleau jaune ont considérablement rabougri par suite du traitement au fénitrothion à 100 ppm. Les traitements effectués au moyen des solutions ayant des teneurs en fénitrothion égales à 25 et 50 ppm ont eu pour effet de rabougir les jeunes plants, surtout dans la région de l'hypocotyle.

INTRODUCTION

The recent introduction of biocides to control pest infestation provides new environmental (stress) factors that could influence the normal course of seed germination and growth. The response of plants to biocide application has been shown not only to be species variable but also dependant upon a number of other factors such as biocide concentration (Flater et al, 1974) method of application (Randall, 1974) and plant nutritional and developmental status (Al-Adil et al, 1974). For example, picloram at 1 ppm and 2 ppm significantly reduces germination of alsike clover, stimulates it at 100 ppb but has no effect on the germination of tomato, wheat or fescue (Flater et al 1974). Forest seed germination has been shown to be inhibited by various biocides. Thiram and endrin (seed coat repellents) inhibited germination of seeds of the eastern white pine (Demeritt and Hoher, 1970) and jack pine and white spruce (Dobbs, 1970).

In recent years, organophosphorous insecticides have replaced persistent chlorinated hydrocarbons as crop protectants. These substances are generally considered to be less persistent than organochlorine insecticides (Edwards, 1965), however, they may in some instances be more phytotoxic (Scopes, 1969). Although some organophosphorous compounds have been shown to inhibit seed germination (Gifford et al, 1959), published information concerning their effects on metabolism of germinating seeds is meagre.

In Canada, the organophosphorous insecticide, fenitrothion, has been used mainly to control the spruce budworm. Our study was undertaken to determine whether fenitrothion affects the normal course of germination and/or the seedling vigor of selected forest tree seeds and thereby disrupt natural regeneration processes.

Seeds of five tree species indigenous to the spruce boreal forests of Canada were selected for examination, namely, Picea glauca Moench/Voss (white spruce), Pinus banksiana L. (Jack pine), Pinus strobus L. (Eastern white pine), Betula papyrifera M. (white birch) and Betula alleghaniensis Britt. (yellow birch).

Seeds were exposed to at least two concentrations of fenitrothion (10 ppm and 1000 ppm) during stratification and/or seedling growth. Subsequently, values for speed and percent germination were obtained. Seedlings vigor was also monitored.

Our previous studies indicated that white pine seeds were toxitolerant to high (1000 ppm) concentrations of fenitrothion, while yellow birch seeds were toxisusceptible (Pomber et al 1974). This report provides a more detailed study utilizing intermediate concentrations of fenitrothion (25 ppm, 50 ppm and 100 ppm) to test the toxisusceptibility of yellow birch seeds.

MATERIALS AND METHODS

(i) Seeds Samples:

The seeds of all species were obtained from the Petawawa Forest Experiment Station, Chalk River, Ontario. Seed collection was carried out exclusively in areas not previously sprayed with fenitrothion. All seeds were stored in tightly sealed glass containers at 2°C until required for experimental procedures (Wang, 1973).

(ii) Conditions of Imbibition and Stratification:

The prechilling (stratification) requirements of the seeds differed according to the species. Yellow birch, white birch and white spruce seeds required to be prechilled at 5°C during a twenty-one day imbibition period in order to germinate consistently (U.S.D.A., 1949; Rudolf, 1950; Crossley and Skov, 1951). White spruce seeds germinated well following a 14 days of stratification requirements (Durzan, 1971) and were therefore germinated without a cold pretreatment.

All seeds were routinely sterilized for 10 minutes in a 2% hypochlorite solution and thoroughly washed prior to use.

Seeds requiring stratification were exposed to fenitrothion throughout the entire chilling phase. Stratification requirements were followed except in the case of white spruce where two groups were stratified, one for 14 days (as required) and the other for 21 days, to provide a comparable fenitrothion exposure to the other stratified seed species. Fenitrothion was applied during germination in the case of jack pine seeds.

Following stratification all seeds were transferred to darkened environmental growth chambers with 12 hour diurnal temperature regimes

of 68°F and 86°F for the conifer species and 59°F to 90°F for the birches (U.S.D.A., 1949).

(iii) Fenitrothion Treatment:

Two insecticide concentrations were employed, namely 10 ppm (approximating a normal field dosage of 4 oz/acre) and 1000 ppm. Intermediate fenitrothion concentrations of 25 ppm, 50 ppm and 1000 ppm were also applied to yellow birch seeds in order to monitor dosage response levels. Premium grade fenitrothion was supplied by Sumitomo Chemical Company of Japan and was analysed for purity and quality before use in the experiments.

(iv) Seed Germination and Seedling Growth:

All seeds were examined daily with a dissecting microscope (50 X magnification). They were determined to have germinated when the radicle pierced through the seedcoat (Mayer and Poljakoff-Mayber 1966). Daily germination percentages, speeds of germination (Maguire, 1962) and germination values (Czabator, 1962) were then determined from each fenitrothion treatment in each species.

Longer term effects of fenitrothion exposure were also monitored. Germinated yellow and white birch seeds were immediately placed in separate, with filter paper lined petri plates, replaced in a controlled environment growth chamber with a diurnal day:night temperature 90:59°C on a 12 h. regime. They were then allowed to develop for a further 7 days. Following this period, seedlings were removed, carefully blotted and weighed. Hypocotyl and root lengths were also determined. Measurements were made using a 50 X dissecting microscope with a micrometer eye piece. Seedlings of all five species from each treatment were also planted in sterile soil in peat flats and grown under normal greenhouse

conditions. Visual observation of their subsequent development (i.e. pigmentation, size and morphology of leaves and stems as well as root development) was carefully followed.

All numerical data were subjected to the t-statistic analysis and significance assessed at the 95% probability level.

(v) Water Uptake:

Percentage increase in fresh weight of white spruce seeds during the stratification-fenitrothion treatment and the subsequent four days of germination was examined. Six replicates of 20 seeds were followed for each treatment group (10 ppm, 1000 ppm fenitrothion and the control). All seed lots were selected to within ± 2.5 mg to ensure homogeneity so that each was representative of the entire seed sample. The percentage increase in fresh weight was then determined at 1, 5, 7, 10 and 14 days of stratification and the following 4 days of germination. Seeds were carefully blotted prior to weighing and the seed containers were kept on ice during the rapid weighing procedure in order to minimize sudden temperature alterations.

RESULTS

(i) Toxicity to Jack Pine Seeds:

Jack pine seeds exposed to 10 ppm and 1000 ppm fenitrothion during germination showed no significant differences in daily accumulative germination at the 95% probability level (Figure 1). However, both treatment groups had higher accumulative germination percentages when compared to the control (untreated) group; the significance was evident up to 10 days of germination and was also reflected in the total overall speed of germination and the germination values (Table 1).

From 10 to 21 days of germination no significant differences in these parameters were observed between any of the groups.

Fenitrothion treatment evidently did not affect the longer term growth of jack pine seedlings. Three month old seedlings from all groups were indistinguishable.

(ii) Toxicity to White Spruce Seeds:

Ten ppm fenitrothion treatments did not affect fresh weight gain during the experimental period, however, seeds exposed to 1000 ppm showed a significant increase in wet weight from the fifth day of stratification to 4 days of germination (Figure 2).

Values for the daily accumulative germination (Figures 3,4) and the overall total speed of germination (Table II) obtained for the white spruce seeds following the 14 and 21 day stratification-fenitrothion exposures showed similar treatment relationships. In both cases, the 10 ppm treated seeds germinated similarly to the control and both groups showed germination values significantly above those of the 1000 ppm treatments.

Following three months of growth, white spruce seedlings from all groups had grown with equal vigor and were indistinguishable from one another.

(iii) Toxicity to White Birch Seeds:

One hundred ppm fenitrothion exposure to white birch seeds resulted in a significant suppression of daily accumulative germination percentages when compared to the control group (Figure 5). Final germination percentages were similar, indicating that the insecticide did not affect the germinability of the treated seeds. The overall speed of germination for the control of white birch seeds was more

TABLE I

Germination Characteristics of Jack Pine Seeds Exposed to Fenitrothion During Germination

<u>Insecticide Treatment</u>	<u>Final Germination (percent)</u>	<u>Total Speed of Germination (seeds per day)</u>	<u>Germination Value</u>
Control	82.50 ± 10.69	0.831 ± .184	0.131 ± .082
10 ppm	79.00 ± 12.52	1.229 ± .462	0.170 ± .114
1000 ppm	81.00 ± 12.52	1.159 ± .294	0.178 ± .120

TABLE II

Germination Characteristics of White Spruce Seeds Stratified for 14 Days at 5°C
With and Without Fenitrothion

<u>Insecticide Treatment</u>	<u>Final Germination (percent)</u>	<u>Total Speed of Germination (seeds per day)</u>	<u>Germination Value</u>
Control	81.57 ± 15.0	1.569 ± .3151	0.314 ± .134
10 ppm	83.15 ± 14.5	1.678 ± .480	0.336 ± .157
1000 ppm	75.00 ± 8.88	1.281 ± .199	0.225 ± .073

TABLE III

Germination Characteristics of White Birch Seeds Exposed to 100 ppm Fenitrothion

<u>Insecticide Treatment</u>	<u>Final Germination (percent)</u>	<u>Total Speed of Germination (seeds per day)</u>	<u>Germination Value</u>
Control	49.64 ± 16.66	1.04 ± .37	0.319 ± .24
100 ppm	41.90 ± 17.78	0.45 ± .21	0.101 ± .08

than two fold, and the germination value three fold that of those seeds exposed to 100 ppm fenitrothion (Table III).

One week old white birch seedlings derived from seeds exposed to 100 ppm fenitrothion were drastically dwarfed (Plate 1). This reduction in size was quite apparent in the hypocotyl and root lengths. Control seedling fresh weight was nearly three times greater than the fenitrothion treated group (Table IV).

(iv) Toxicity to Yellow Birch Seeds:

Yellow birch seeds exposed to intermediate concentrations of fenitrothion (25 ppm, 50 ppm and 1000 ppm) were examined one week following germination. Significant reductions, at the 95% probability level, in sizes of the hypocotyls when compared to the control was quite apparent in seedlings from the three fenitrothion treatments (Table V; Plate 2). Control seedling hypocotyls were nearly twice that of the seedlings derived from the 50 ppm treatment and three times that of the 100 ppm fenitrothion seedlings (Plate 3).

Differences in root lengths and total fresh weights were less marked. No significant reduction was observed in total seedling fresh weight in the 25 ppm and 50 ppm treatments when compared with those of the control seedlings. However, control seedlings were twice as heavy (fresh weight) as their 100 ppm counterparts and this was significant at the 95% level. Root lengths of the control and 25 ppm treatment groups were statistically similar. Reduction in root size was apparent at the 95% probability level in both the 50 ppm and 100 ppm fenitrothion treatments when compared to the control.

One week old yellow birch seedlings developed in such a way that the root was attached to the filter paper at the bottom of the petri dish and supported the vertical hypocotyl and cotyledons. This "stance"

TABLE IV

Effects of Fenitrothion on Growth of White Birch Seedlings

<u>Insecticide Treatment</u>	<u>Seedling Weight</u> (mgm)	<u>Hypocotyl Length</u> (cm)	<u>Root Length</u> (cm)
Control	2.7 ± .69	1.34 ± 2.10	0.86 ± .26
100 ppm	1.1 ± .29	0.37 ± .15	0.15 ± .10

TABLE V

Effects of Fenitrothion on Growth of Yellow Birch Seedlings

<u>Insecticide Treatment</u>	<u>Fresh Weight</u> (mgm)	<u>Hypocotyl Length</u> (mm)	<u>Root Length</u> (mm)
Control	6.21 ± 1.88	7.8 ± 2.2	19.7 ± 4.4
25 ppm	4.29 ± 1.44	6.3 ± 2.3	13.6 ± 5.8
50 ppm	4.91 ± 1.36	5.2 ± 2.4	10.0 ± 3.0
100 ppm	3.68 ± 1.54	4.3 ± 2.9	7.1 ± 2.8

was not evident in the severely dwarfed forms (1000 ppm and 100 ppm fenitrothion treatments), these seedlings never showed upright positioning. Twisted hypocotyls were in evidence in seedlings derived from 50 ppm fenitrothion exposure and these seedlings always lay horizontally on the filter paper. All seedlings derived from fenitrothion exposures had unusually large, rounded cotyledons (Plate 4).

The seedlings from all treatment groups were planted and are currently being monitored for possible deviation in subsequent development and growth.

DISCUSSION

The response of forest tree seeds to fenitrothion application varies according to species and pesticide concentration. The conifer species examined in this study, white spruce and jack pine, and also white pine (Pomber, et al 1974) demonstrate that 10 ppm fenitrothion does not result in apparent phytotoxicity during the germination of growth. In fact, this concentration consistently promoted germination in all three species. The significant elevation of germination of jack pine seeds exposed to the two fenitrothion concentrations was unexpected. In order to determine whether this response is due to metabolic differences in the mode of action of fenitrothion on this species or to differences in fenitrothion treatment period, jack pine seeds germination will be examined following a 21 day stratification exposure period (comparable to seeds of the other tree species). The results, however, may well be related to the consistent elevation in germination witnessed following 10 ppm exposure to the other tree seeds examined.

Jack pine seeds in this study were not stratified and were thus exposed to the fenitrothion only during the germination phase; this may

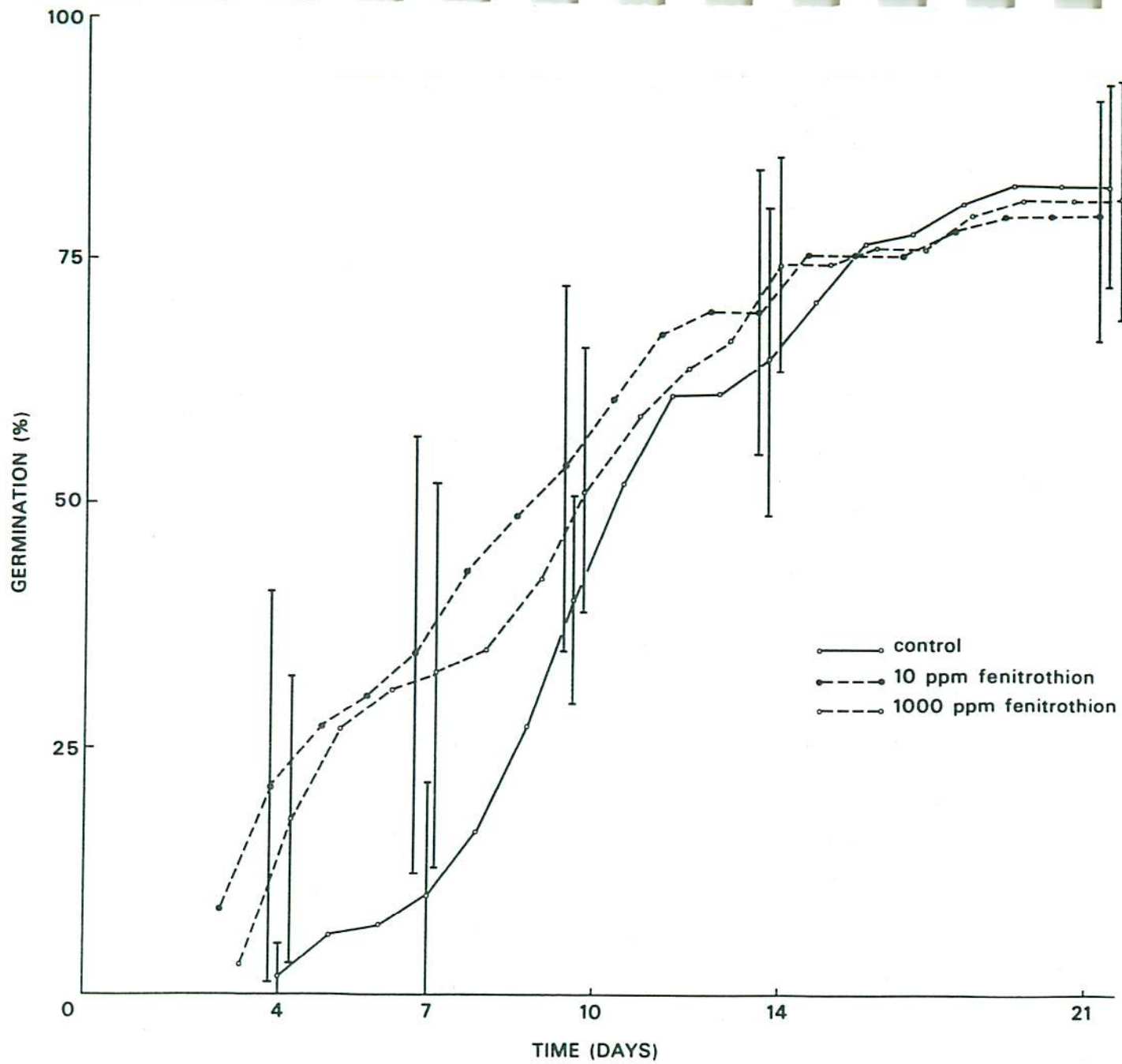


Fig. 1 Germination of jack pine seeds treated with 10 ppm and 1000 ppm conc. of fenitrothion.

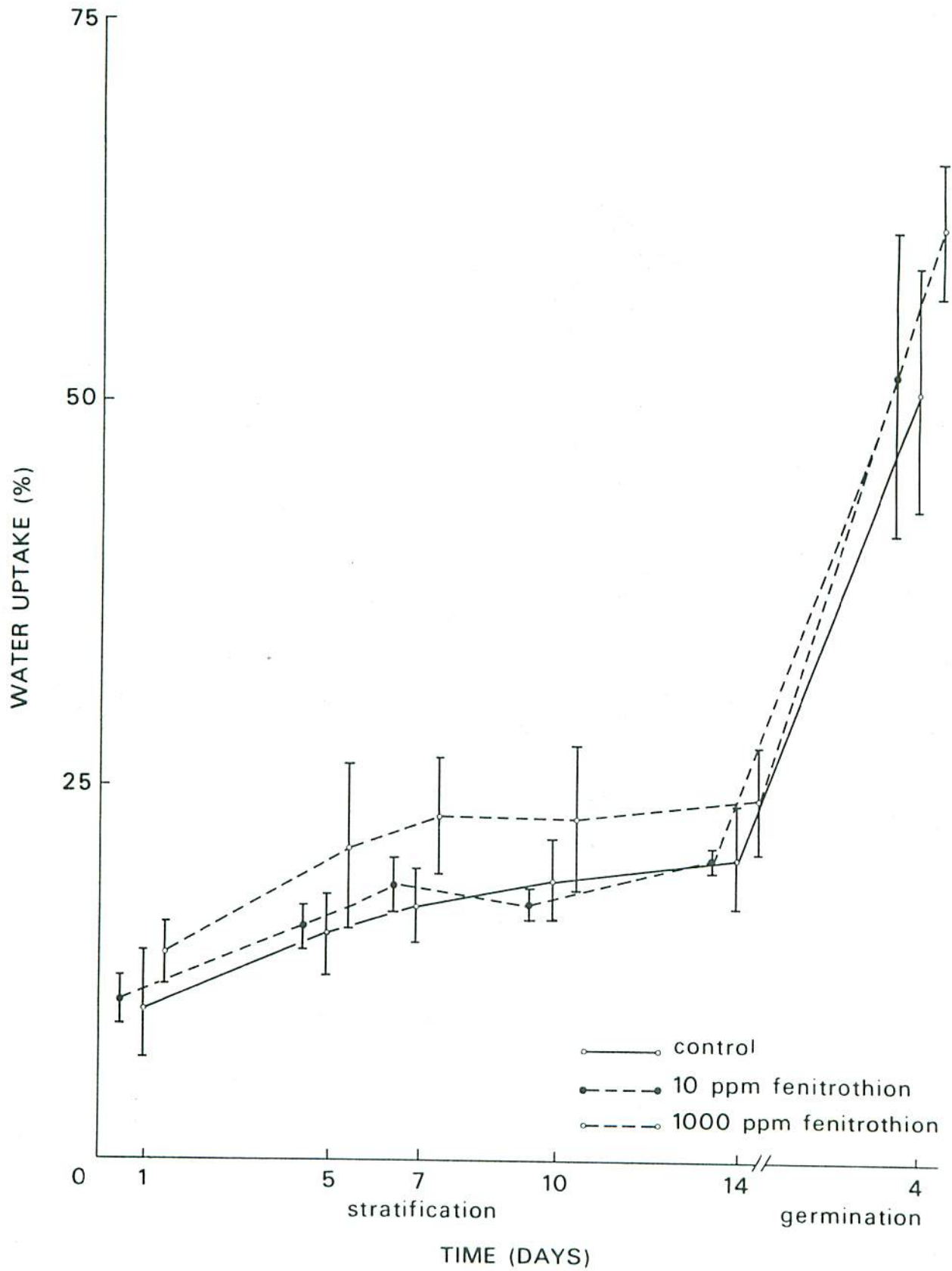


Fig. 2 Fresh weight gain (water uptake) of white spruce seeds exposed to 10 ppm and 1000 ppm conc. of fenitrothion during stratification and germination.

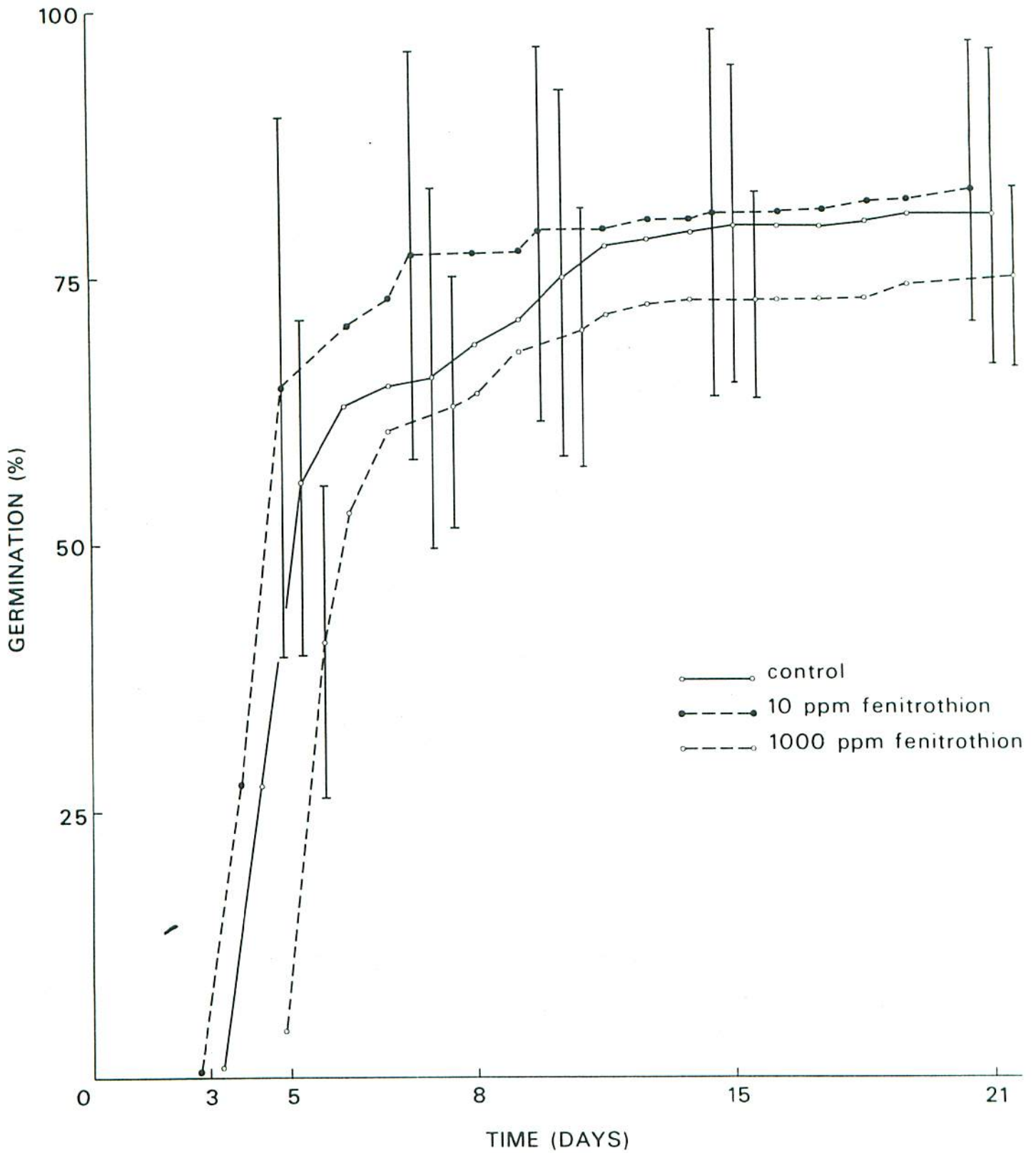


Fig. 3 Germination of white spruce seeds exposed to 10 ppm and 1000 ppm conc. of fenitrothion, following 21 days of prechilling.

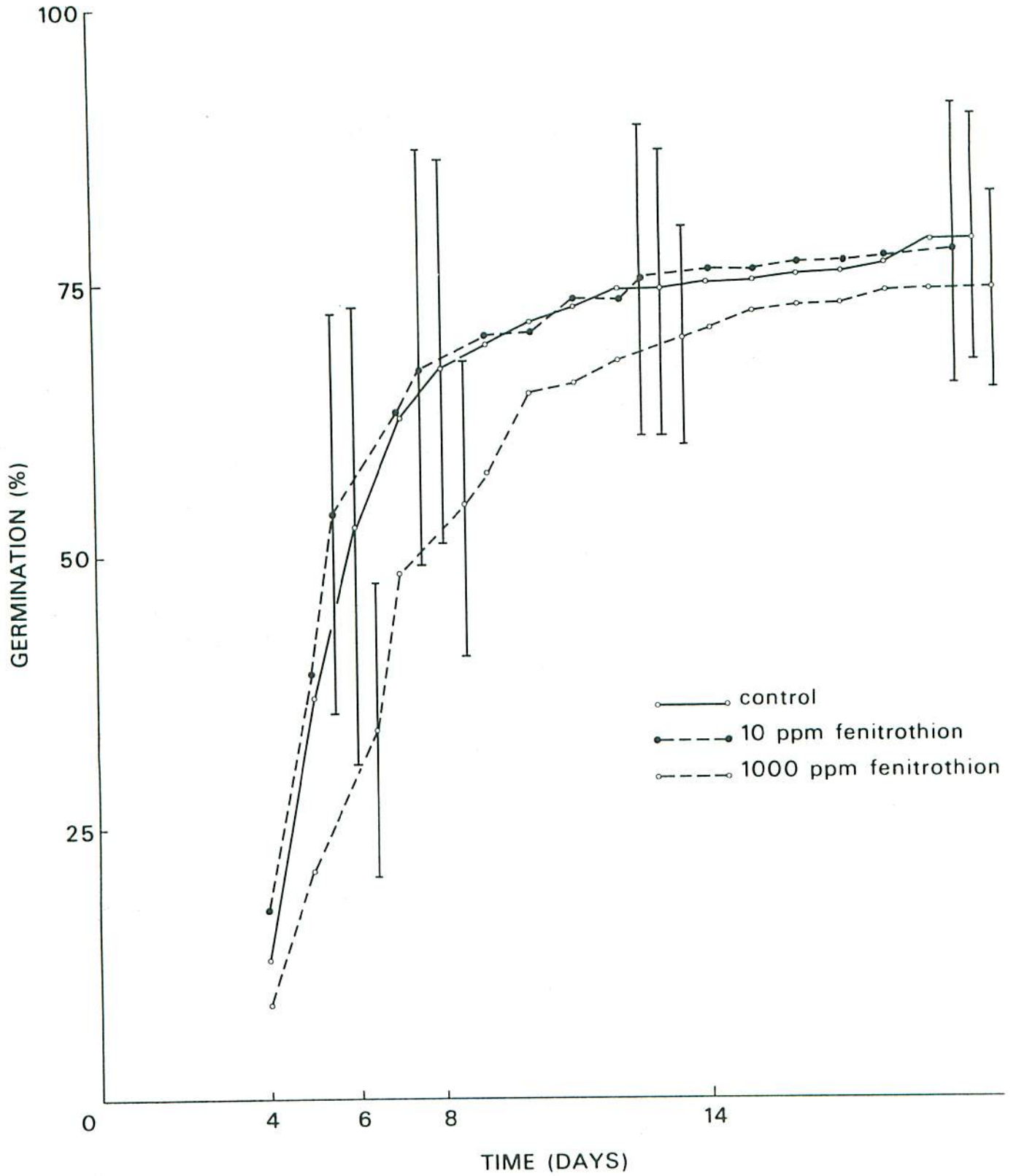


Fig. 4 Germination of white spruce seeds exposed to 10 ppm and 1000 ppm conc. of fenitrothion, following 14 days of prechilling.

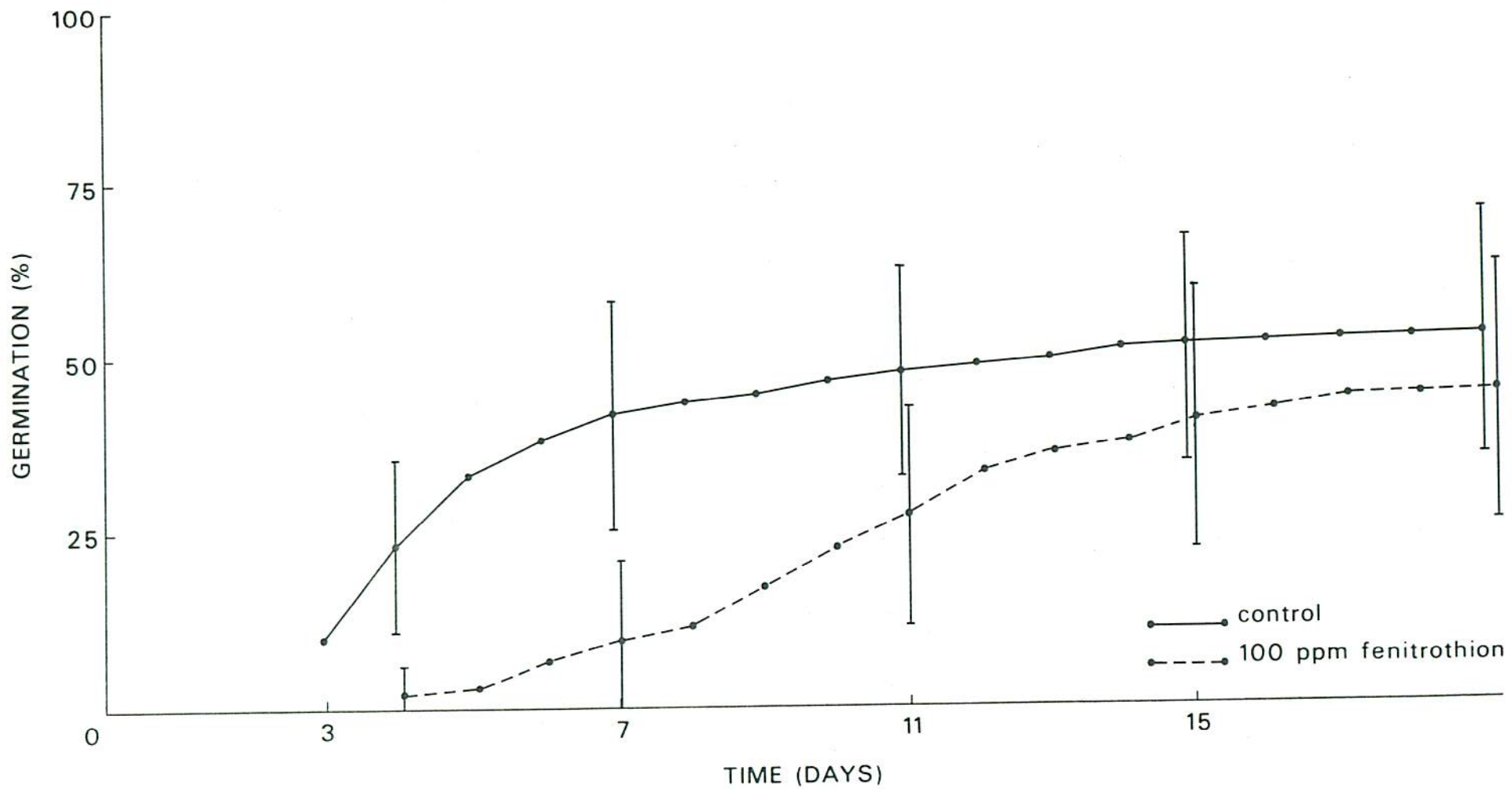


Fig. 5 Suppression of germination of white birch seeds exposed to 100 ppm conc. of fenitrothion.

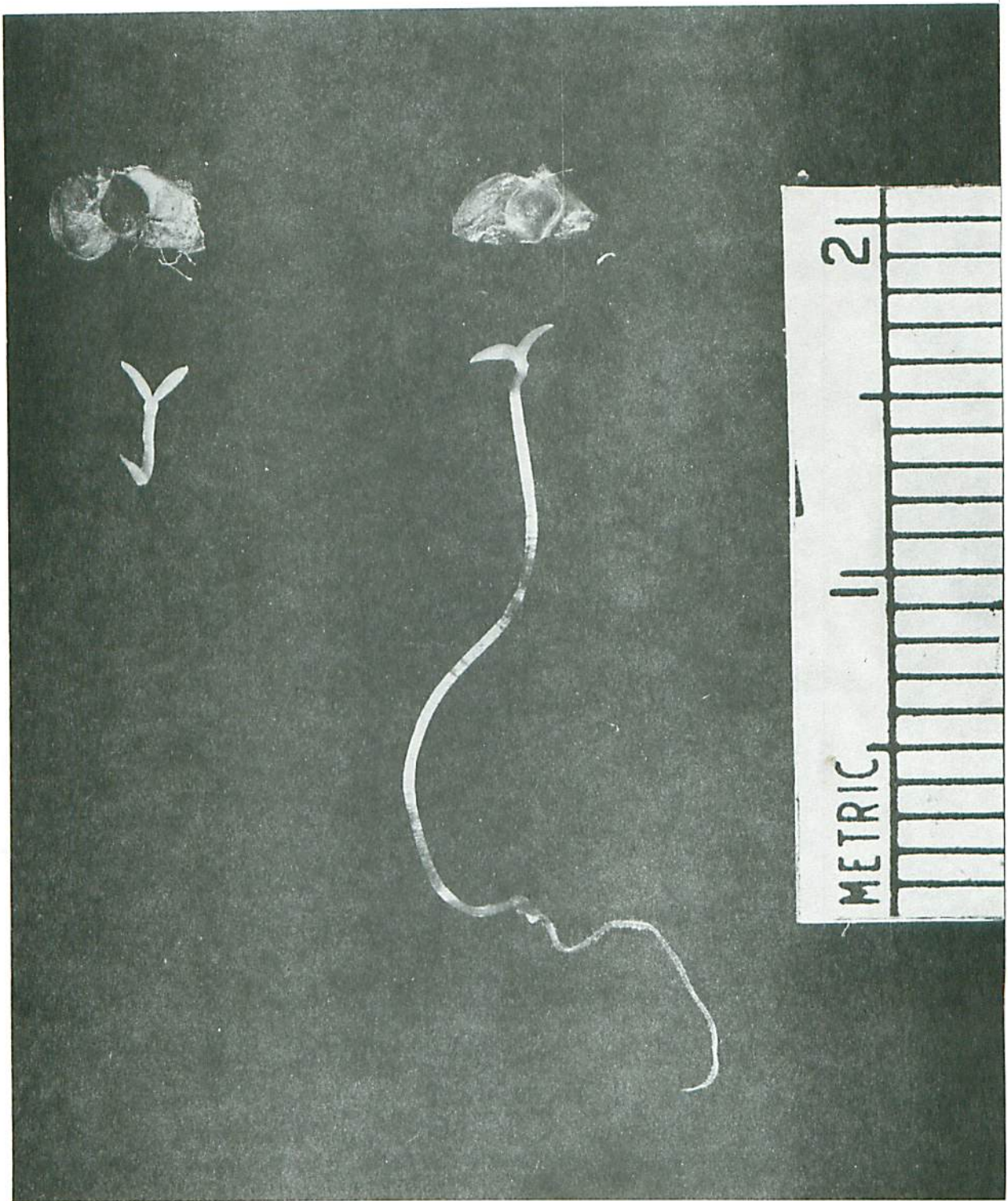


Plate 1 : Effects of fenitrothion on one week old white birch seedlings. The untreated (control) seedling is on the right hand side and the treated (100 ppm) is on the left hand side.

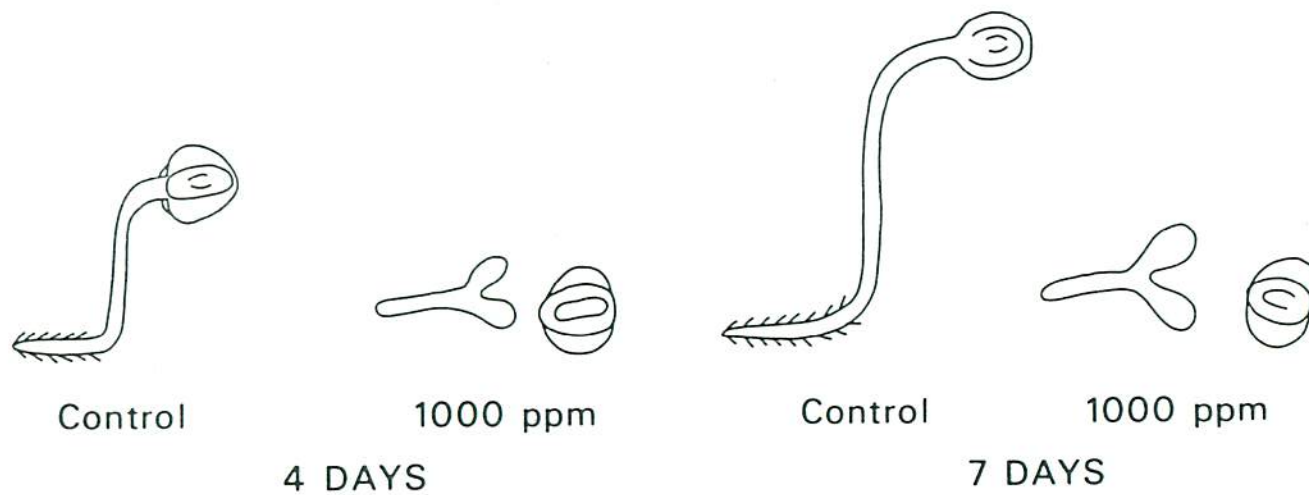


Plate 2 : Scheme of germination in yellow birch seeds with and without treatment with 1000 ppm fenitrothion.

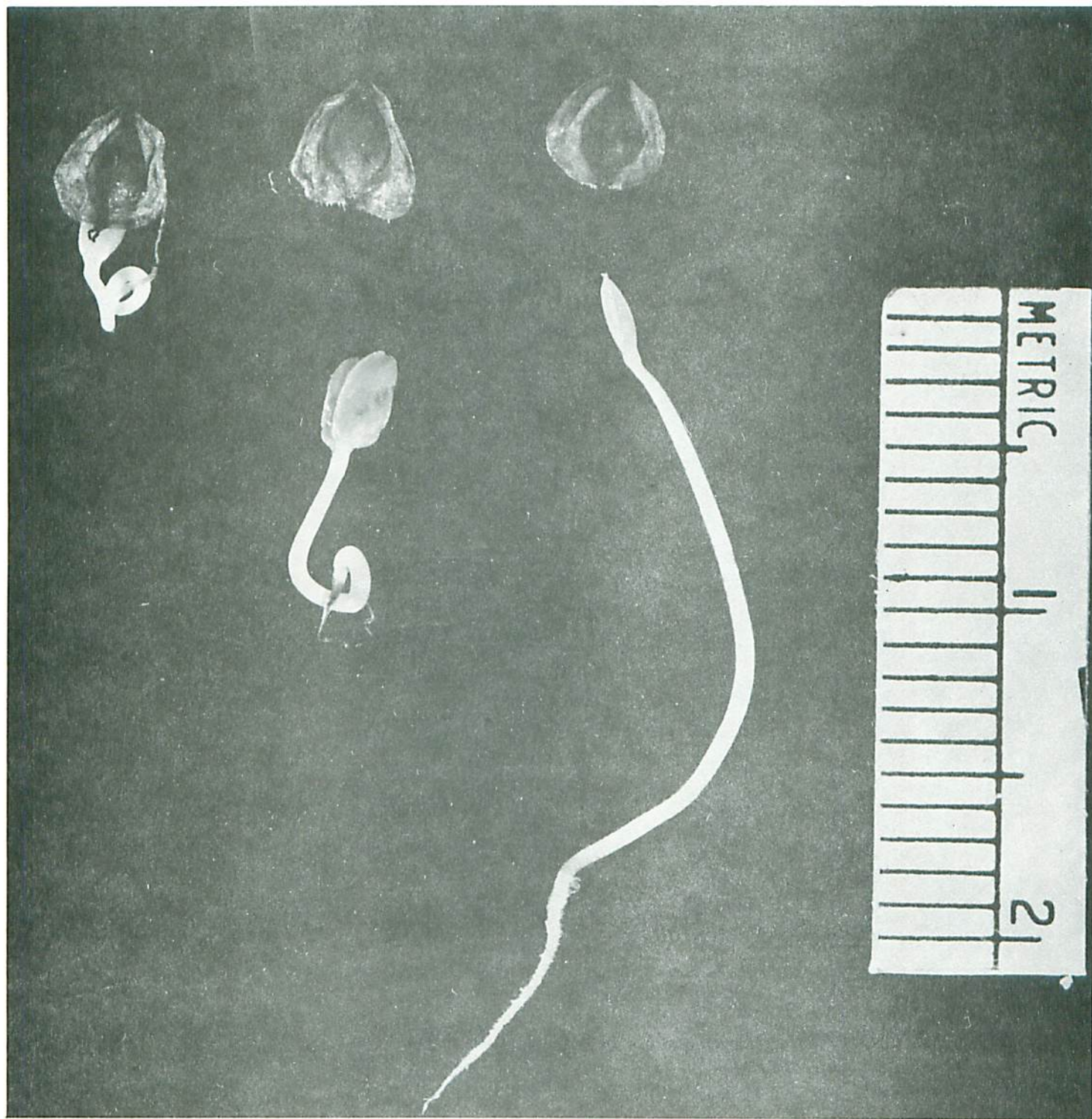


Plate 3 : Phytotoxicity of fenitrothion (50 ppm) to yellow birch seedlings. The control (one week old) on the right does not manifest malformations while the treated (left hand) seedlings show twisted hypocotyls, dwarfing effects and large cotyledons.

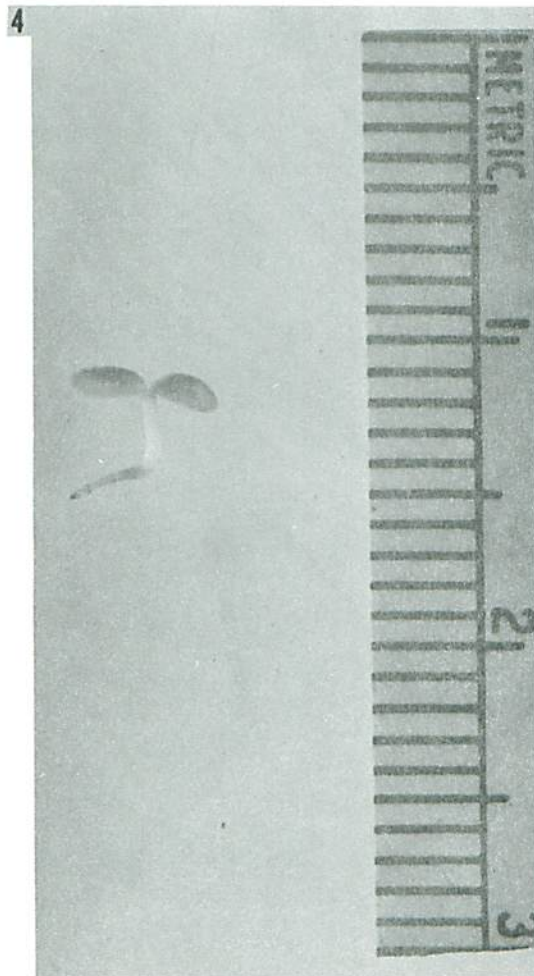


Plate 4 : Dwarfed one week old yellow birch seedlings resulting from 1000 ppm fenitrothion treatment. Similar phytotoxic effects were observed at 100 ppm. Note large rounded cotyledons.

result in endogenous levels of the insecticide that could be comparable to a 10 ppm exposure during a 21 day stratification fenitrothion application period. Holm and Miller (1972) have shown that small amounts of acetylcholine and eserine (a specific acetylcholinesterase inhibitor in animal systems) produces promotive germination responses. Although it remains to be shown that acetylcholine actually is a metabolic component of these forest tree seeds, the fact remains that fenitrothion (an acetylcholinesterase inhibitor) exposure does result in increased germination speeds under some of our conditions.

Evidence of possible fenitrothion interactions with membranes is also suggested from our data. Vinyl organophosphate insecticides such as azodrin have been shown to bind to membrane components (Hague et al 1973) and to cause alterations in membrane structure in plant systems (Lee and Wilkinson, 1973). Increased water uptake in white spruce seeds and white pine seeds (Pomber et al 1974) gives a preliminary indication of somewhat similar reactivity with respect to fenitrothion.

In all conifer tree seeds examined, there is no evidence that fenitrothion exposure during early seed imbibition is deleterious to subsequent growth. The normal growth and development of the conifer seeds exposed to fenitrothion at concentrations ten fold normal field concentrations indicates that this insecticide does not pose an ecological threat in terms of regeneration by these species.

However, the birch species show a marked sensitivity to the insecticide. Application of 1000 ppm fenitrothion to yellow birch seeds results in complete seedling senescence within three weeks following germination (Pomber et al 1974). Dwarfing effects were especially evident

in the hypocotyl region with exposures of insecticide concentrations upwards from 25 ppm. The data reveal an interesting situation, in the sense that roots and hypocotyl lengths are reduced in size in the 25 ppm and 50 ppm groups when compared with control, yet significant reductions in fresh weight are not coincidentally apparent. Two explanations may be possible, either an increase in fresh weight:seedling volume ratio takes place on fenitrothion exposure in this species (similar to that observed in the percent fresh weight increases in fenitrothion exposed imbibing white spruce and white pine seeds) and this would further implicate membrane alteration effects with fenitrothion; or, growth occurs in the cotyledons of fenitrothion treated seedlings at the expense of normal hypocotyl growth processes.

Abnormal cotyledon development was observed in these seedlings and this aspect will be further investigated.

The twisted condition of seedling hypocotyls and apparent loss of geosensitivity in fenitrothion treated seedlings of yellow birch may well be related to low starch and carbohydrate localization in the root-hypocotyl axis. Gordon (1971) has shown increased carbohydrate levels to be related to increased geosensitivity in oat seedlings. Thus fenitrothion may be involved in blocking some aspects of essential carbohydrate metabolism. However, abnormal hypocotyl development may be the result of insecticide disruption of growth hormone metabolism. Organophosphate insecticides have been shown to inhibit cell culture callus growth and this effect to be negated with IAA supplemented media. Gibberellic acids and IAA have been shown to not only induce hypocotyl elongation (Sawhney and Srivastava, 1974; Atsmon and Lang 1968) but to be involved in geotropic responses (Iwami and

Masuda, 1974) through production and migration in and from cotyledons (Lam and Lepold, 1968; Gillespie and Thimann 1963). This problem too will be further examined.

A persistent general esterase inhibition (Pomber et al 1975) was accompanied by morphological dwarfing and early seedling senescence in yellow birch. Increased insecticide levels may result in acetylcholinesterase inhibition and a resultant growth retardation due to native acetylcholine accumulation in yellow birch seeds. Acetylcholinesterase inhibitors such as Phosfon-D and AMO-1618 generally result in root growth retardation (Riov and Jaffee, 1973; Crozier et al 1973). These inhibitors appear to act in a similar manner to that of fenitrothion application of birch seeds. It is interesting to note that these substances do not result in growth retardation when applied to conifers (Cathey and Stuart, 1961).

SUMMARY AND CONCLUSION

Using a range of concentrations (0, 10, 25, 50, 100 and 1000 ppm) the phytotoxicity of fenitrothion was tested on two species of conifers (Picea glauca and Pinus banksiana) and two species of deciduous seedlings (Betula papyrifera and Betula alleghaniensis) during germination and early stages of growth. The effects were monitored in terms of germination rate, water uptake and seedling vigour. A marked differential effect was noticed: coniferous species were least or not affected, while both deciduous species exhibited stunting, dwarfing and distorted growth and development patterns. However, since no aberrant growth pattern was noticed at the lowest concentration (10 ppm) which is widely employed to control the spruce budworm under operational conditions, it is concluded that forest sprayings of fenitrothion at 4 ozs/acre are unlikely to decimate selectively birch populations in a mixed forest environment.

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