The Phytotoxicity of Fenitrothion as Assessed by the Germination and Early Growth of *Betula alleghaniensis* Britt.

by

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TABLE OF CONTENTS

ABSTRACT

Seeds of yellow birch were stratified in water or a water-fenitrothion emulsion. Two concentrations of fenitrothion were used, namely 10 ppm (4 oz/acre) and 1000 ppm. Seeds exposed to a concentration of 1000 ppm fenitrothion showed greatly decreased germination rates and dwarfing of the young seedlings. These dwarfed seedlings could not be maintained alive for more than three weeks. The germinability of yellow birch seeds exposed to 10 ppm of fenitrothion differed little from those of the control.

RESUME

On stratifié des semances de bouleau jaune dans l'aeu ou dans une émulsion aqueuse de fénitrothion. On a utilisé deux concentrations de fénitrothion: 10 ppm et 1,000 ppm. Les semences soumises à la concentration de 1,000 ppm de fénitrothion ont connu des taux de germination très réduits et, par la suite un rabougrissement des jeunes pousses. Ces plants rabougris n'ont pas survécu plus de trois semaines. La faculté germinative des semences de bouleau jaune exposées aux 10 ppm de fénitrothion n'a différe que de peu par rapport à celui du groupe témoin.

INTRODUCTION

Important environmental factors controlling seed germination are water, temperature, light, oxygen supply, and biocides. Even if seeds germinate, the establishment of many young seedlings is threatened by several environmental stresses which subsequently develop, as well as attacks by fungi, insects, and higher animals. The use of controlled environmental chambers has considerably reduced the vulnerability of seedling growth during the course of early development and has also minimized the effect of extraneous environmental factors.

Biocides are now widely used in agriculture and silviculture throughout the world. A variety of naturally occuring and applied chemical substances decrease the number of germinants either by suppressing the seed germination process or by being toxic to recently emerged seedlings. Such biocides include insecticides, fungicides, herbicides as well as various chemical inhibitors found in leaves or roots of plants. The influence of biocides on seed germination varies greatly with the specific chemical; the rate, method, time and number of applications, the species, soil type, weather, and other factors (Kozlowski, 1960). A number of insecticides (e.g., dieldrin, aldrin, parathion, DDT, toxaphene, Kilan, chlordane, heptachlor, and octamethyl), at concentrations used to control white grubs in forest nurseries, were non-toxic to growth of pine roots, (Simkover and Shenefelt, 1952).

Infestations of spruce budworm has become a major problem on the American continent. Formerly, DDT had been used as a control agent against the ravages of spruce budworm. However, within the past few years this biocide has been largely replaced by fenitrothion,

0, 0-dimethyl-0-(4-nitro-3-methyl-phenyl) phosphorothioate, (Fettes 1968).

The effects of herbicides on seed germination under natural conditions often are masked because of the manner in which they are applied. Thus, it is important to maintain a clear distinction between real and apparent herbicide toxicity. The absolute toxicity of many herbicides to seed germination cannot be determined in soil cultures because the soil is a barrier between the seed and the applied chemical. Soil-applied herbicides often are lost from seeds by evaporation, leaching, microbial or chemical decomposition and irreversible adsorption on the soil.

High absolute toxicity of 2, 4-D and CDAA to seed germination has been demonstrated (Kozlowski and Sasaki, 1970). When these herbicides were placed in direct contact with Pinus resinosa seeds in petri dishes, they inhibited seed germination at concentrations as low as 50 ppm (Kozlowski and Sasaki, 1968).

The present study was undertaken to assess the impact of fenitrothion applications on germination behaviour and seedling growth of a non-target species, Betula alleghaniensis Britt.* Birches are important deciduous species among the mixed stands of spruces, firs and pines.

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* Betula alleghaniensis Britt . is the revised name replacing Betula lutea Michx. (Maini and Cayford, 1968).

MATERIALS AND METHODS

Seeds and Storage:

The seeds Betula alleghaniensis Britt. (yellow birch) were obtained in late September, 1972 from the Beggar Lake region of the Petawawa Forest Experimental Station, Chalk River, Ontario. The Chemical Control Research Institute had aerially sprayed forest stands within this region with fenitrothion in the spring of 1972 (2-4 ounces/acre). The seeds used in this study were gathered from non-sprayed (c) and sprayed areas (s).

Seeds of yellow birch were removed from the nut by hand and were stored in tightly sealed glass containers at 2^{o} C until required for experimental procedures (Wang 1973).

Conditions of Imbibition and Stratification of Yellow Birch Seeds:

The internal dormancy of these seeds was overcome by placing them in aerated water (or water-fenitrothion solutions) in a constant temperature room maintained at precisely 5^{o} C for 54 days (U.S.D.A., 1949). Following this stratification period, the yellow birch seeds were germinated in ten petri dishes lined with filter paper in growth chambers. During germination light was excluded and a twelve hour night-day temperature regime of 59^{o} F and 90^{o} F was maintained in the growth chambers according to instructions of the U.S.D.A. (1949).

Insecticide Exposure and Concentration:

Yellow birch seeds were only exposed to fenitrothion during the course of their stratification period. Two insecticide concentrations were used, namely 10 ppm and 1000 ppm. Ten ppm approximates a field concentration of 4 oz/acre A.I. of fenitrothion. Only premium

grade fenitrothion was used and this was supplied by the Sumitomo Chemical Company of Japan.

Seed Germination; Germination Rates and Assessment of Seedling Vigor:

All seeds used in this study were routinely sterilized for 10 minutes in a 2% hypochlorite solution and then thoroughly washed.

For the progress of germination, the seeds were examined daily with a dissecting microscope (50 x magnification). They were considered to have germinated when the radicle pierced through the seed coat. Then according to procedure of Macguire (1962), germination percentages and germination rates were determined for each treatment. Three hundred yellow birch seeds were exposed to each fenitrothion concentration during stratification. This represented 30 replicates of 10 seeds each.

Growth Studies:

Yellow birch seeds from sprayed and unsprayed areas were examined following 0, 12, 26, 40 and 54 days of stratification. Seeds which had germinated were placed in separate filter paper-lined petri dishes and grown for one week. Subsequently fresh weights were taken for the root-hypocotyl axis as an index of seedling vigor. Three further sets of seeds stratified for 54 days (control, 10 ppm and 1000 ppm fenitrothion exposed) were germinated in growth chambers for one week from these lots, samples were selected for determinations of fresh weights and lengths of roots and hypocotyls of the one week seedlings. Altogether, there were forty-one seedlings as control, forty as treated with high concentration (1000 ppm) and thirty-nine as treated with low concentration (10 ppm)

Subsequently, the seedlings were planted in a potting medium-containing mixture of soil and vermiculite and placed in a growth chamber

maintained at 20° C. The growth was normal under these conditions and the effect of the treatments could easily be monitored in terms of morphological deviations.

RESULTS

Seed Germination and Germination Rates:

Germination of yellow birch seeds obtained from unsprayed (c) and sprayed areas (s) following specific stratification periods showed no significant differences in germinability (Figure 1). Final germination percentages were essentially similar for the "c" and "s" series following all comparable stratification periods. However increasing the stratification period resulted in significantly higher final germination percentages. The "c" seeds which received no stratification had a final germination percentage (52.00 ± 6.03%), significantly lower ($p<.05$) than those receiving 40 days (78.00 ± 6.9%) of chilling. From Table I, the data clearly show that in all cases the highest germination percentages were obtained in those groups exposed to stratification periods greater than 26 days.

TABLE I

Mean Daily Total Germination Percentages of
Yellow Birch Seeds Stored for 6 Months and
Then Exposed to Fenitrothion During Stratification

Fenitrothion Concentration (ppm)	8th day	10th day	12th day	Final %
0	27.0±11.19	30.3±11.0	32.0±11.6	35.0±14.1
10 ppm	28.1±14.0	31.6±12.1	33.3±12.4	35.0±13.3
1000 ppm	12.3±12.3	16.3±13.0	19.0±12.3	30.3±12.5

The seeds of yellow birch tested for germinability after a six month period of storage were considerably less viable and showed significantly lower germination percentages. Control seeds (c) which had yielded a final germination percentage of 78.5 ± 6.9% in the initial study now germinated to only 35.00 ± 14.08%, (See Figures 1, 2). To determine whether this response was due to a loss of viability or increased quiesence during storage, seeds were placed in 80% ethanol. Yellow birch seeds which floated were found to be empty whereas those which sank were confirmed by dissection to be potentially capable of germination. Thus the germination percentage of the seed lot seemed to have decreased during storage.

Unsprayed seeds (c) and those exposed to a 10 ppm fenitrothion water-emulsion gave similar daily total germination percentages and a similar final germination percentage, (Figure 2, Table 1). However seeds exposed to a 1000 ppm fenitrothion concentration exhibited a

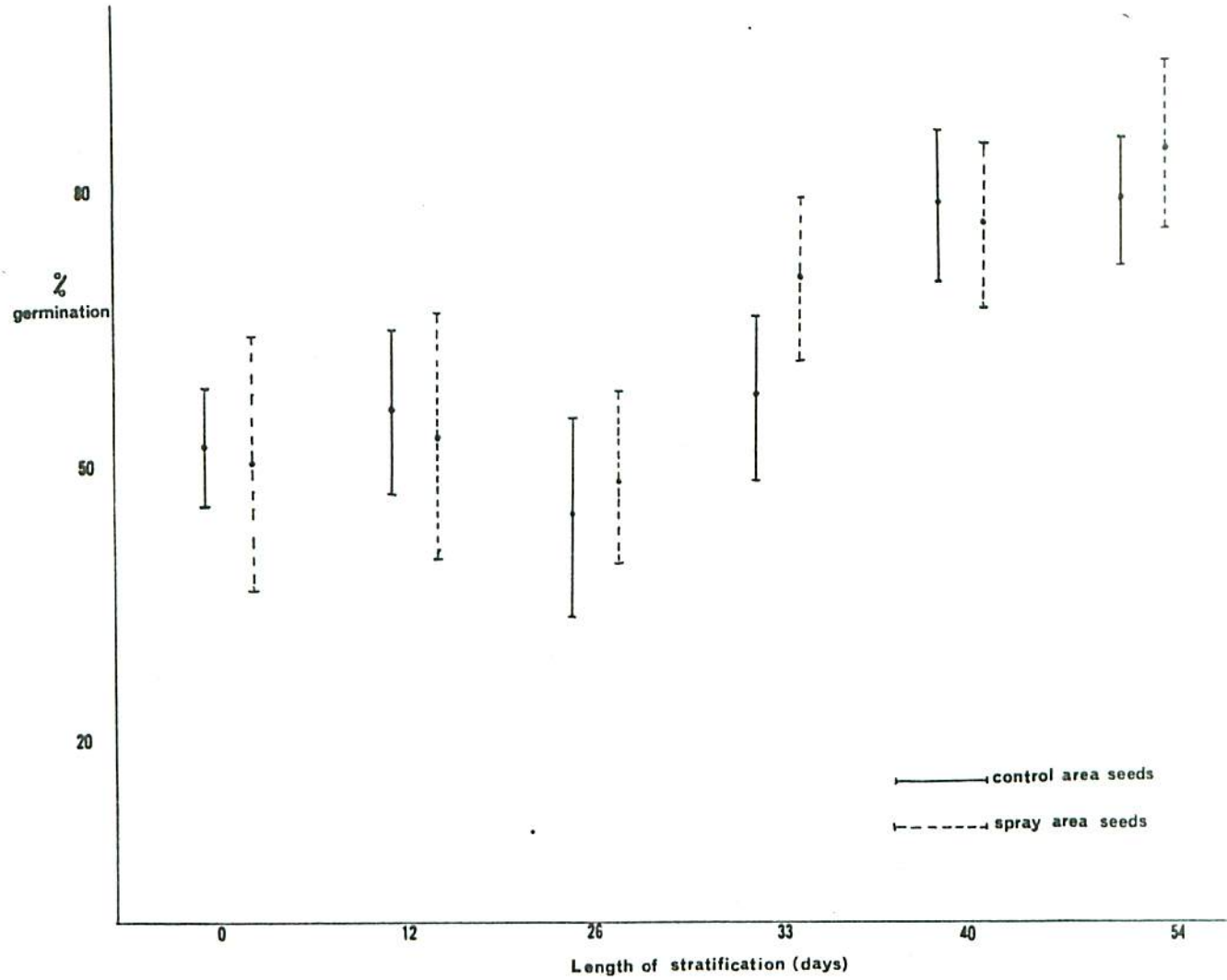

FIGURE 1: Final germination percentages of yellow birch seeds following specific stratification periods.

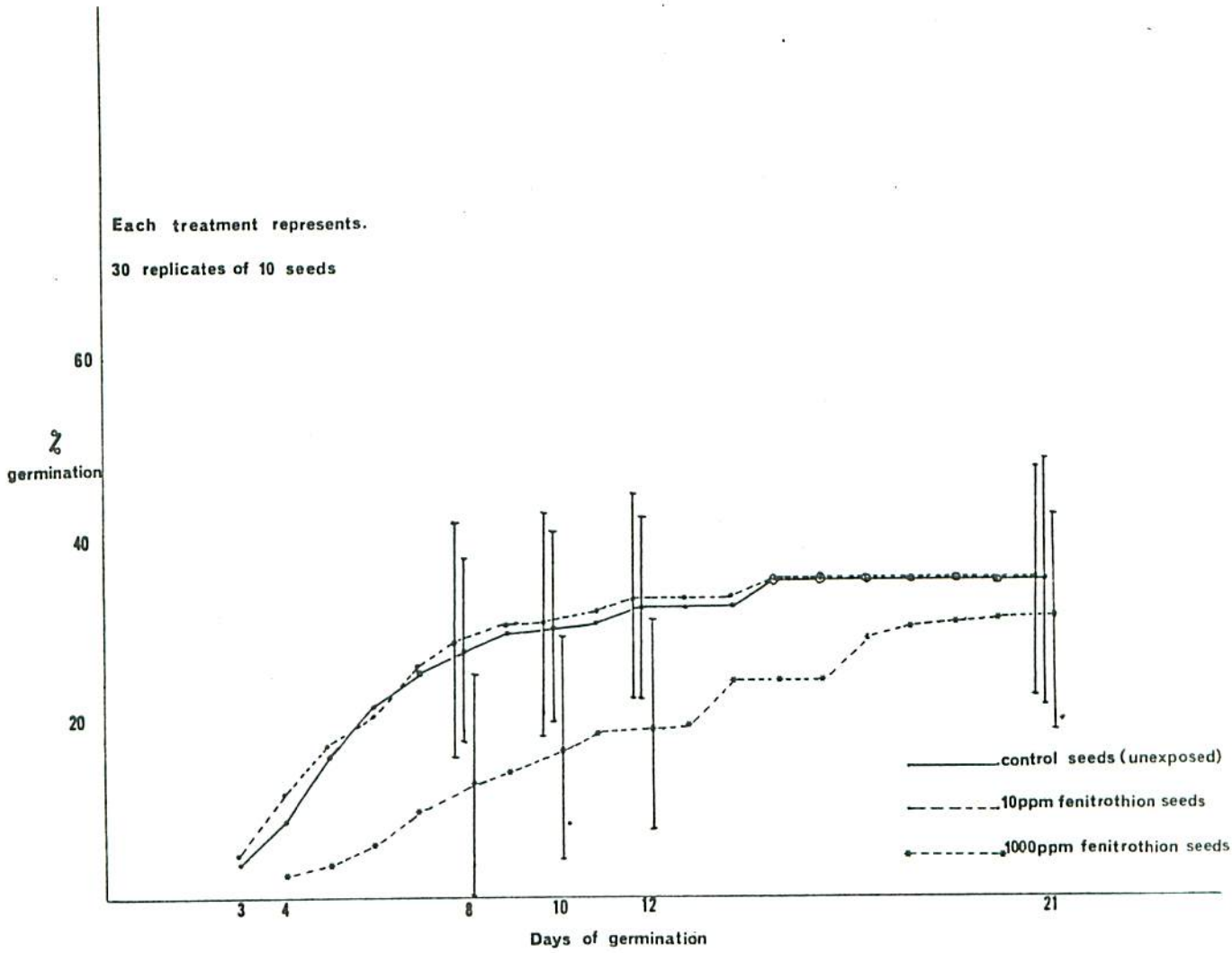

FIGURE 2 Daily accumulative germination of yellow birch seeds following fenitrothion exposure.

significant lag in germination and this lag was somehow persistent from the onset of germination to the final day. Following 8, 10 and 12 days, highly significant deviations from the control and 10 ppm fenitrothion exposed seeds were apparent (Table 1). After 21 days of germination, the germination percentage of the 1000 ppm fenitrothion exposed seeds did not differ significantly from the control or 10 ppm fenitrothion exposed groups.

The overall lag evidenced in the 1000 ppm fenitrothion exposed seeds is best expressed by the speed of germination (Table 2). Although the speed of germination for the control and 10 ppm fenitrothion exposed groups were comparable, both were found to be nearly twice that of the seeds exposed to a 1000 ppm concentration of the insecticide.

TABLE II

Speed of Germination of Yellow Birch Seeds

Exposed to Fenitrothion During Stratification

Fenitrothion Concentration (ppm)	Total Speed of Germination (Seeds per day)
0	0.64 ± .270
10 ppm	0.676 ± 0.270
1000 ppm	0.347 ± 0.200

Growth Studies:

The yellow birch seeds exposed to 10 and 1000 ppm fenitrothion were significantly affected and this was reflected in a drastic decrease in the fresh weights and lengths of the root and hypocotyl of one week old seedlings (Tables 3 and 4).

TABLE III

Effects of Fenitrothion on Root Growth of Yellow Birch Seedlings

Concentration of Fenitrothion (ppm)	Root Weight (mgms)	Root Length (cm)
0	1.77 ± .538	1.45 ± .280
10	1.61 ± .661	1.39 ± .369
1000	0.239 ± .097	0.256 ± .108

TABLE IV

Effect of Fenitrothion Treatments on Hypocotyl Growth of Yellow Birch Seedlings

Concentration of Fenitrothion (ppm)	Hypocotyl Weight (mgms)	Hypocotyl Length (cm)
0	4.73 ± .973	2.33 ± .420
10	4.29 ± 1.30	1.96 ± .484
1000	0.838 ± .328	0.42 ± .131

Seedlings derived from seeds treated with 1000 ppm were considerably smaller than either the control or 10 ppm of fenitrothion (Figures 3 to 4). There was some indication that seedlings exposed to 10 ppm fenitrothion were also smaller than the control but statistically this was not significant at the 95% level. There were gross morphogenetic effects of the fenitrothion (1000 ppm) treatment: general stunting of growth dwarfing effects and complete loss of pubescence on roots were clearly visible. These dwarfed seedlings were planted but could not be maintained alive for more than two to three weeks. Thus the induced effects were irreversible and fenitrothion caused permanent damage to growth and developmental processes of the seeds and seedlings.

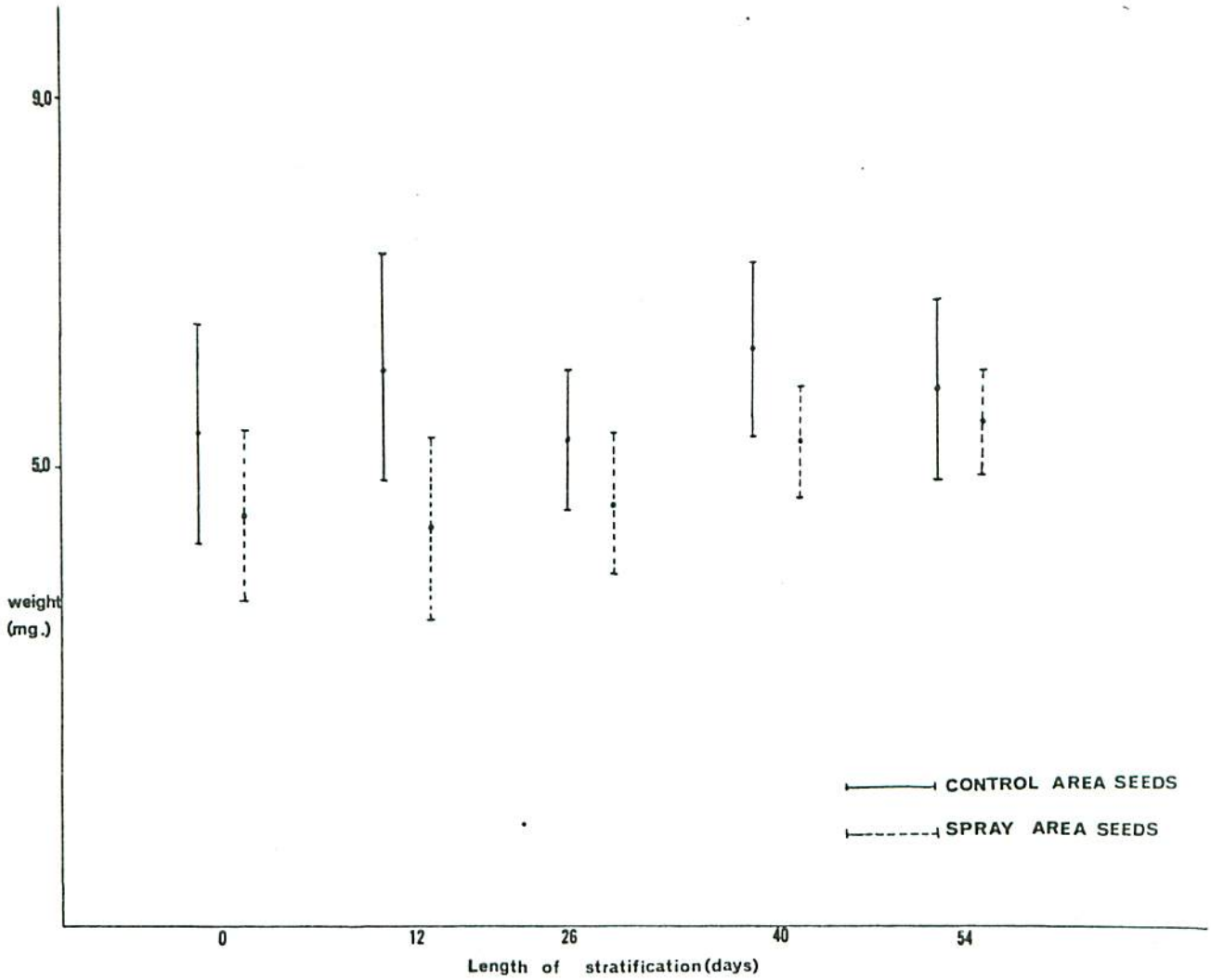

FIGURE 3 Fresh weights of one week old yellow birch seedlings following specific stratification periods.

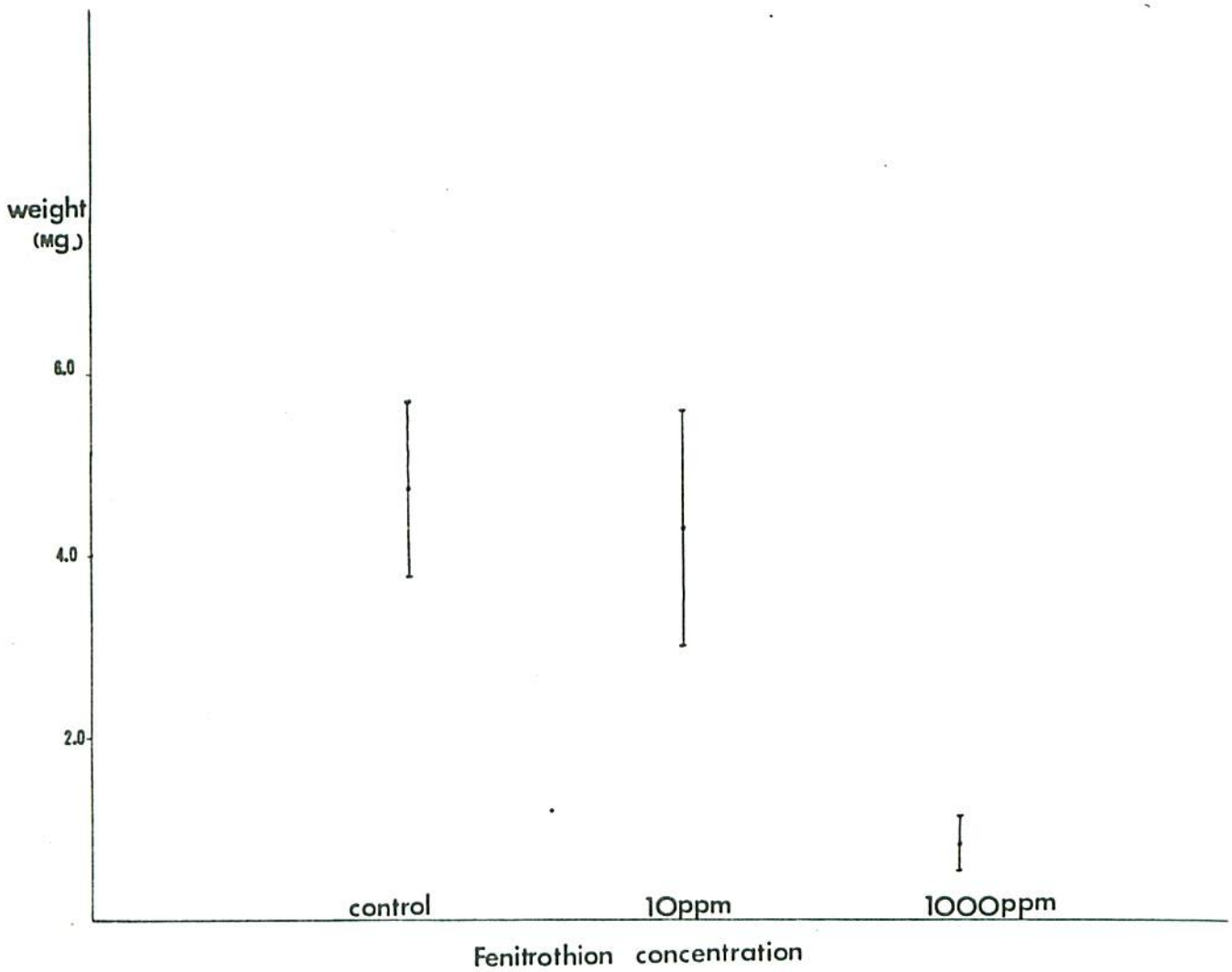

FIGURE 4 Fresh weights of one week old yellow birch seedlings exposed to fenitrothion during stratification.

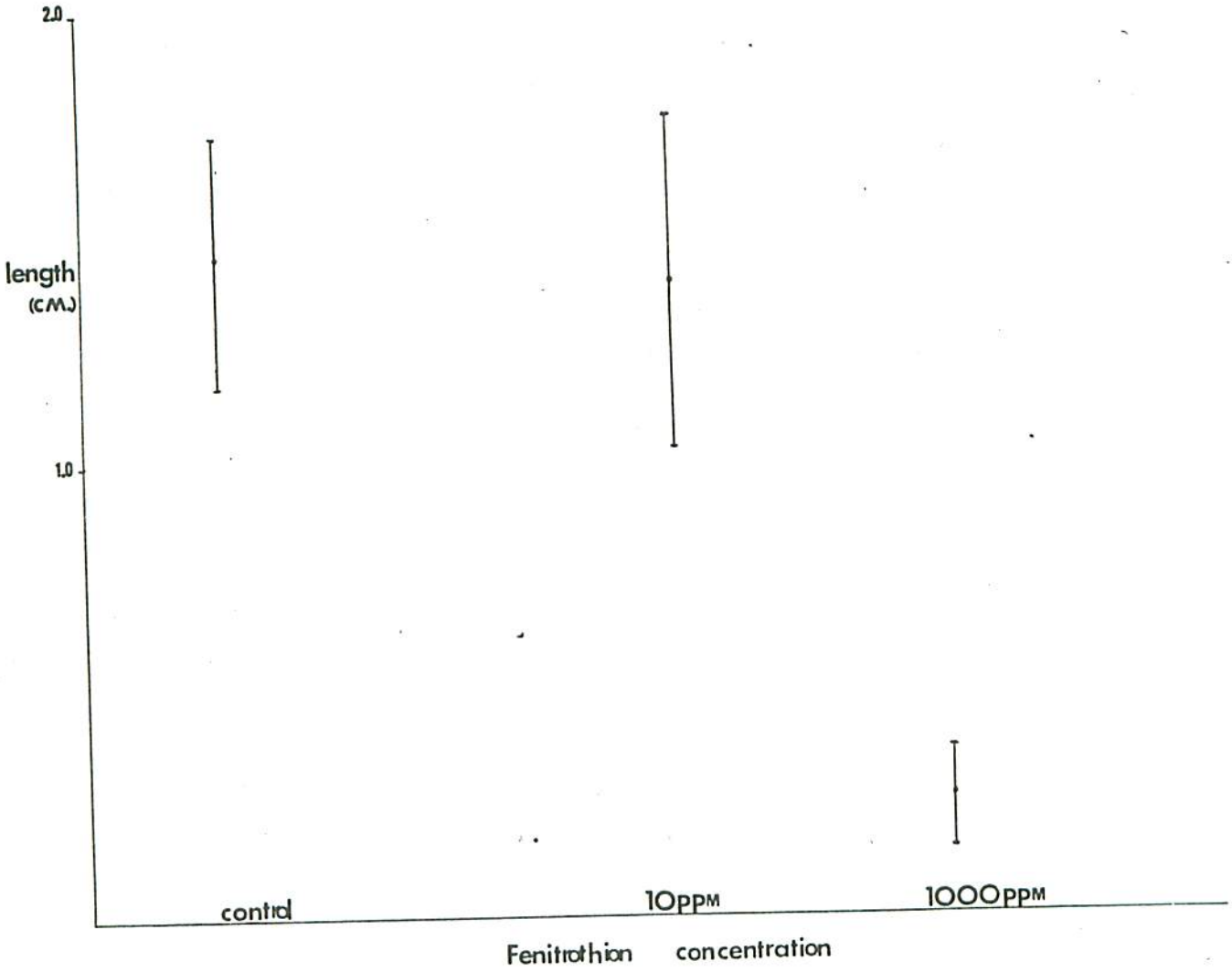

FIGURE 5 : Root lengths of one week old yellow birch seedlings exposed to fenitrothion during stratification.

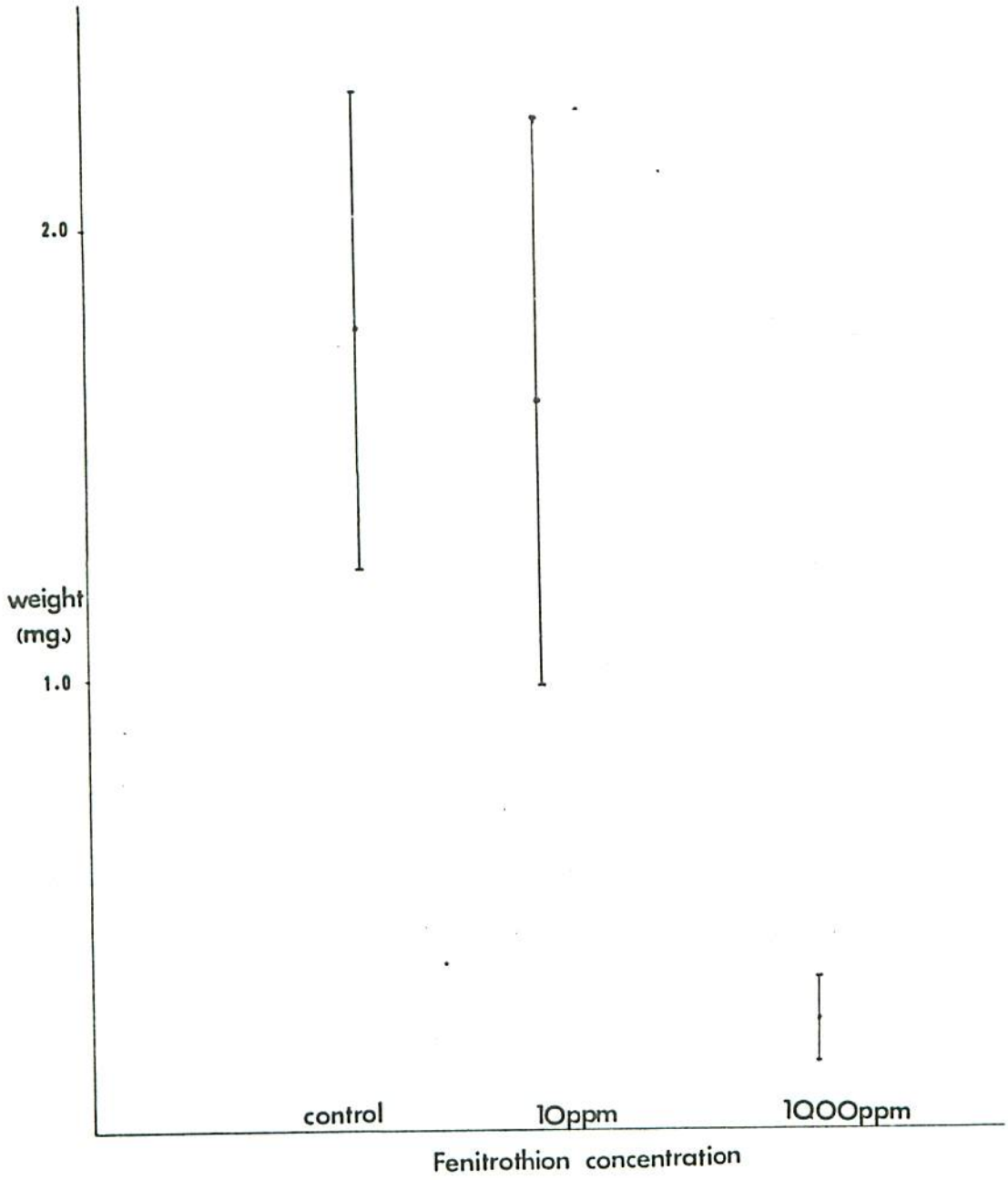

FIGURE 6 Root weights of yellow birch seedlings exposed to fenitrothion during stratification.

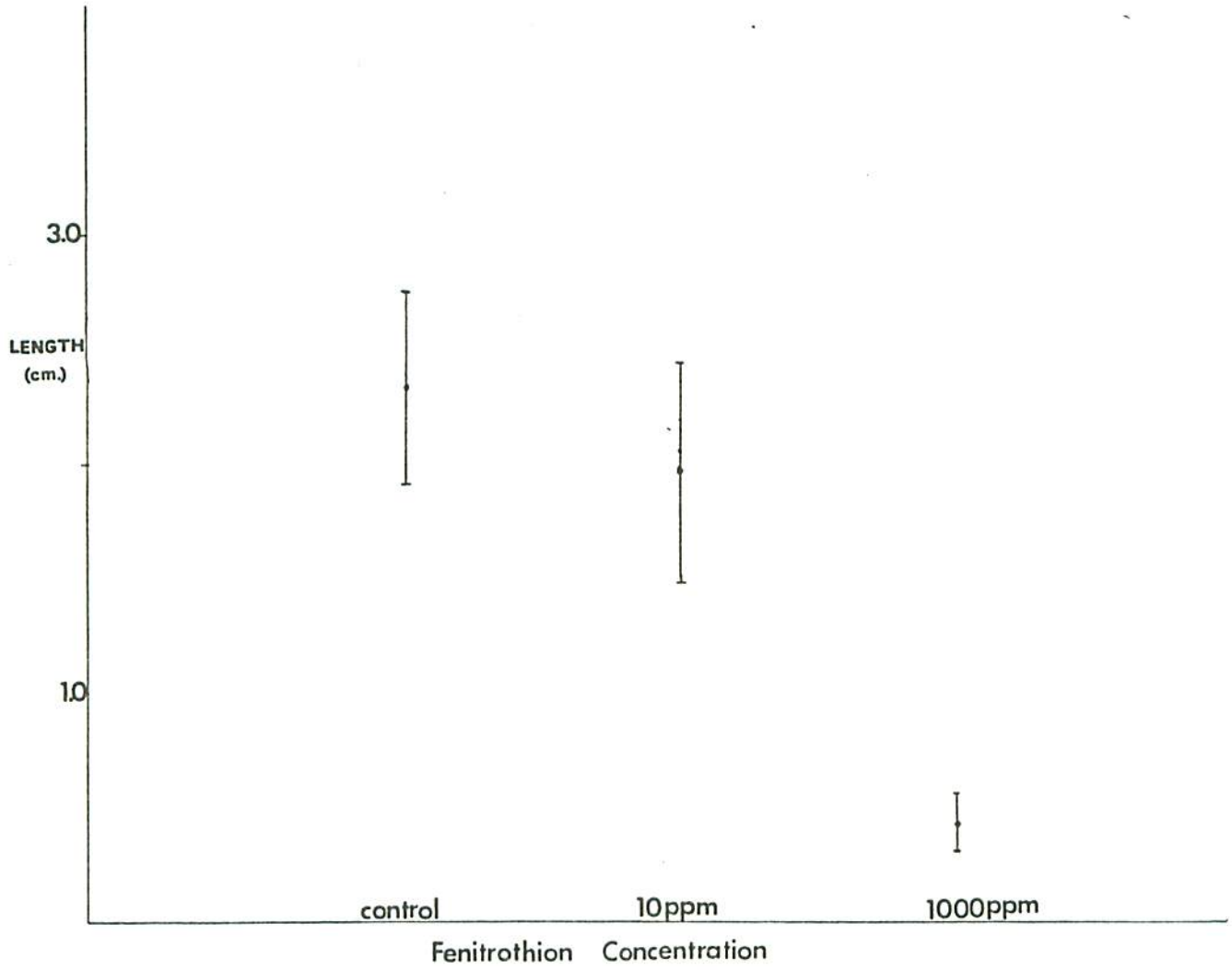

FIGURE 7 Hypocotyl lengths of one week old yellow birch seedlings exposed to fenitrothion during stratification.

DISCUSSION

Yellow birch seeds directly exposed to high concentrations of fenitrothion showed quite drastic deleterious morphological effects. Suppressed germination, reduced size of the young seedling and its subsequent non-viability emphasized the sensitivity of yellow birch seeds to fenitrothion. Control seeds and those exposed to a 10 ppm (field concentration) of fenitrothion germinated in an essentially similar manner and no gross morphological differences could be determined. Seeds derived from spray (s) and non-spray(c) areas did not show significant differences in germination. Evidently, spray exposed seeds at normal field concentration are not vulnerable.

In discussing the modus operandi of fenitrothion on yellow birch seeds and seedlings, it is necessary to emphasize that growth processes (cell division and cell extension) are severely affected by heigh concentration. Since no studies were carried out to determine the fate of fenitrothion in seeds and seedlings, it is not possible to conclude that the observed effects resulted from a degradation product of fenitrothion rather than by the insecticide per se. In this connection, Hallett et al (1974) found that fenitrothion breaks down into 3 metabolites in the pine seeds and that two metabolites were more potent in insecticidal properties than the parent compound. Nevertheless it is a well known phenomenon that many mutagenic agents and pesticides affect cellular enzymes (containing SH groups) involved in meristematic and cell expansion growth (Prasad and Blackman 1964) and it could be postulated that fenitrothion is operating through that mechanism in the birch seedlings. Reduction in root or hypocotyl length results

from retardation in cell division and cell extension and which process is more sensitive to fenitrothion is not known and remains to be ascertained in future studies. Dwarfing effects are also brought about by cessation in meristematic activity and it could be advanced that fenitrothion disrupts the mitotic cycle at some stage during cell proliferation so that the supply of new cells is restricted and thus the linear growth of the root and hyprocotyl is limited by slower pace of mitotic cycle. Some bleaching of chlorophyll or reduction in the synthesis of chlorophyll also took place in the hypocotyl region. This suggests that fenitrothion can exert its action on photosynthesis also and this may explain loss of weight in the treated hypocotyls. Similarly, complete loss of pubescence in the treated roots could result from a reduced cell division rate. Whatever the mechanism of action of fenitrothion it is clear that no phytotoxicity results from lowest concentration (the field concentration 4 oz/acre A.I. = 10 ppm) and therefore operational sprayings are less likely to decimate the deciduous forests and the associated ecosystems. Also since according to Yule and Duffy (1972) considerable proportion of fenitrothion does not persist in the forest environment for longer periods, chances are that no significant damage results to forest communities following the aerial sprays.

SUMMARY AND CONCLUSIONS

Seeds of yellow birch (Betula alleghaniensis Britt) were found to be sensitive to direct exposure of fenitrothion at two concentrations. Exposed to 1000 ppm, seeds gave rise to non-viable seedlings. While at 10 ppm

ppm (equivalent to field concentrations), the seedlings did continue to grow but a consistent suppression of seedling size was observed. However seeds sprayed in the field under natural conditions did not show any deviations from normal growth.

The evidence thus suggests that fenitrothion exposure to a forest environment could affect the regeneration capabilities of members of that community if regions are over-sprayed. Evidently, under normal spraying conditions (4 oz/acre), the effective concentration of fenitrothion does not seem to alter the growth, development and survival of birch forests.

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