SOME RESPONSES OF WHITE SPRUCE AND YELLOW BIRCH SEEDS TO ORGANOPHOSPHOROUS INSECTICIDES DURING GERMINATION

by

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RÉSUMÉ

Les insecticides fénitrothion, acephate (Orthène®) et phosphamidon inhibent la germination des graines d'épinette blanche à divers degrés selon la dose appliquée. On a constaté que l'acephate et le phosphamidon favorisaient la germination des graines de merisier aux concentrations de 10 et 100 p.p.m. Le métabolite S-méthyl fénitrothion a retardé la germination aux mêmes concentrations; par la suite, toutefois, les plants ont eu une croissance normale, sans signe de nanisme. On a observé que l'acide gibberellique atténuait considérablement le retard dans la croissance, noté précédemment chez les graines exposées à 100 p.p.m. de fénitrothion. Cet effet de l'acide gibberellique a été minime et s'est limité à la région de l'hypocotyle des jeunes plants de merisier.

INTRODUCTION

Fenitrothion (O,O-dimethyl-O-(4-nitro-m-tolyl) phosphorothioate) is a broad spectrum organophosphate insecticide of relatively low mammalian toxicity (Krehm, 1974). In eastern Canada, the insecticide has been employed extensively since 1969 as a replacement for DDT in controlling forest insect defoliators, chiefly the spruce budworm. The pesticide is generally applied as an oil solution at a dosage rate of 0.14 kg/ha - 0.42 kg/ha (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, the pesticide has been reported to remain at a level of 10% of the original deposit on the foliage of red and white spruce and balsam fir for one year following spraying operations (Yule and Duffy, 1972). Similarly, ecologically vital hardwood species such as red maple have been observed to retain as much as 3 times more fenitrothion on the foliage than similarly treated conifer species (Yule and Varty, 1974). Recent investigations have indicated that following normal spraying procedures, resultant local deposits of fenitrothion may differ between areas by a factor of greater than 10 (Carrow, 1974).

According to Fettes (1968), insecticide applications cannot completely suppress the pest populations and even after massive fenitrothion spraying annually - the spruce budworm survives at about 20%. Thus there is the potential for significant ecological damage following fenitrothion spraying. Buckner (1975) observed significant phytotoxicity and subsequent fall of up to 70% of the total foliage of sugar maple, trembling aspen and white birch following fenitrothion spraying at 1.4 Kg/ha (20 oz/acre); however, the conifer species examined did not show significant effects. Our examinations concerning forest tree regeneration following fenitrothion exposure have confirmed the apparent lack of significant toxicity of the

pesticide on selected conifer species (white spruce, jack pine, and white pine) while indicating the sensitivities of both yellow and white birch species seeds (Pomber et al, 1974 a, b, 1975).

Other organophosphorous insecticides currently employed to control the spruce budworm are Acephate (Orthene ®) and phosphamidon. Both pesticides have shown toxicity to the spruce budworm larva at the fifth instar (Nigam, 1971); phosphamidon and acephate have a relative toxicity (LD_{50}) of 0.39 and 0.42 $\mu g/cm^2$ respectively as compared to an LD_{50} of 0.31 $\mu g/cm^2$ for fenitrothion. Field evaluation studies of acephate have shown 96% budworm reduction following spraying of 0.42 kg/ha (6 oz/acre), followed by 3% defoliation. This compares favorably with fenitrothion which was observed to reduce budworm numbers by 67% with 10% defoliation (Hopewell and Nigam, 1974). Investigations concerning the germination and seedling growth of southern pine seeds have shown the insecticide acephate to be systemic and relatively persistent (Werner 1974), when used in a special spray formulation.

Mixed forests are common in Ontario with yellow and white birch as the most important hardwoods and white pine as the important softwood. For that reason, comparative studies were undertaken to determine the effect(s) of exposing viable seeds of white pine and yellow birch to field concentrations of acephate, phosphamidon and fenitrothion. The toxicity of the carriers Arotex* and Atlox** was also followed. Further, as the S-methyl metabolite of fenitrothion has been implicated in phytotoxicity (Hallett et al 1975) this was also tested in our studies.

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* Arotex an aromatic hydrocarbon produced by Texaco Canada Ltd. Don Mills, Ontario.

** Atlox was produced and provided by the Atlas Chemical Co., Montréal P.Q.

It has been suggested that the plant phytohormones may play an important role in the toxitolerance of some plants to organophosphorous pesticides (Lee and Wilkinson, 1973). Experiments were therefore carried out on interaction between these compounds.

MATERIALS AND METHODS

i) Seeds and Storage

The seeds of white spruce, Picea glauca(Moench) Voss. and white birch, Betula papyrifera, Marsh. were obtained from the Petawawa Forest Experiment Station in late September of 1972 and 1973. Seeds of yellow birch, Betula alleghaniensis Britt., were obtained from this area in October of 1974 and 1975. Seed collection was carried out exclusively in regions not previously sprayed with insecticide. 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

All seeds were routinely sterilized with a 2% hypochlorite solution and washed prior to use. Seeds were prechilled at 5°C for a 21 day imbibition period (stratification) as outlined by Pomber et al (1975). Insecticide exposure was carried out during this phase. Following stratification, all seeds were transferred to dark environmental growth chambers with 12 hour diurnal temperature regimes of 68°F and 86°F for the conifer species and 59°F and 90°F for the birches (U.S.D.A., 1949).

iii) Insecticide Treatments

The organophosphorous insecticides used in this study were fenitrothion (O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate), phosphamidon, (2-chloro-N,N-diethyl-3-hydroxycrotonamide dimethyl phosphate) and acephate (o,S-dimethyl acetylphosphoramidothioate). Two insecticide concentrations were employed, namely 10 ppm (approximating a normal field dosage of 0.28 Kg/ha (4 oz/acre) and 100 ppm. Intermediate fenitrothion concentrations of 25 ppm and 50 ppm were also applied to yellow birch and white birch seeds in order to monitor response levels.

In parallel experiments, yellow birch seeds were exposed to the fenitrothion metabolite, S-methyl fenitrothion (O,S-dimethyl-(3-methyl-4-nitrophenyl)phosphorothioate) at concentrations of 1 ppm, 10 ppm and 100 ppm during the 21 day stratification period.

Yellow birch seeds were additionally exposed to fenitrothion spray formulations. The field formulation consisted of 88% water, 10% fenitrothion, 1% Arotex (the primarly oil base carrier) and 1% Atlox (the emulsifying agent). This field formulation (fenitrothion: Arotex: Atlox = 10:1:1) was extended to the laboratory formulations. Concentrations thus employed consisted of a) 1000: 100: 100: ppm fenitrothion: Arotex: Atlox, b) 100: 10: 10 ppm fenitrothion: Arotex: Atlox, and c) 10:1:1: ppm, fenitrothion: Arotex: Atlox. The effects of the carrier and emulsifier without insecticide was determined in a parallel experiment with a formulation of 100:100 ppm Arotex: Atlox.

Fenitrothion (premium grade)was obtained from Sumitomo Chemical Co., Japan. This was found to be 99% pure and did not contain any impurities detectable by gas chromatography or thin layer chromatography (Hallett et al, 1975). The Arotex and Atlox carriers were supplied by the Chemical Control Research Institute, Environment Canada. S-methyl fenitrothion was synthesized and supplied by Dr. R.G. Greenhalgh, Agriculture Canada, Ottawa Ontario.

iv) Growth Hormone Treatments

Yellow birch seeds were treated with plant growth hormone formulations consisted of (a) 100 ppm gibberellic acid*, (b) 10 ppm gibberellic acid, and (c) 100:10:10 ppm gibberellic acid: Indole-3-acetic acid**:Kinetin**.

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* Gibberellic acid (GA) obtained from General Biological Supply House, Chicago and was identified as Gibberellic acid "100".

** Indole-3-acetic acid (IAA) and Kinetin (G-furfuryl-aminopurine) were purchased from the Sigma Chemical Co., St. Louis, Missouri.

Concurrently, in duplicate treatments seeds were exposed to the growth hormone formulations alone and to growth hormone formulations to which was added 100 ppm fenitrothion.

v) Speed of Germination

Seeds of all species were considered to have germinated when the radicle emerged through the seedcoat. Daily germination percentages, speeds of germination (Maguire, 1962) and germination values were then determined for each treatment group in each species.

Examination of seedling growth was carried out in the birch species exposed to the fenitrothion-Arotex-Atlox formulations, S-methyl and fenitrothion-growth hormone treatments. Seeds were placed in separate filter paper-lined petri plates immediately following germination. Following a seven day growth period, seedlings were carefully blotted dry and weighed. Hypocotyl and root lengths were also determined.

Seedlings of all species from all treatment groups were planted in sterile soil in peat flats and grown under greenhouse conditions. Visual observations of their subsequent development was carefully monitored.

All results were subject to the statistical t-tests and significance was assessed at the 95% probability level.

vi) Water Uptake

The percentage increase in fresh weight of yellow birch seeds during the stratification-fenitrothion exposure period was examined. Eight replicates of 20 seeds were followed for each treatment group (10 ppm, 100 ppm fenitrothion and the control). Seed lots were selected to within 2.5 mg to ensure homogenity and each lot was representative of the entire seed samples.

Fresh weight percentage increase was determined at 1, 2, 5, 12 and 21 days of the stratification period. Seeds were carefully blotted dry prior to the weighing and the seed containers were kept on ice at this time in order to eliminate sudden temperature alterations.

vii) Histochemical Studies of Yellow Birch Seeds Exposed to 100 ppm Fenitrothion During 21 days Stratification

a) Sampling procedure

Due to the minute size of yellow birch seeds, removal of embryos from the surrounding seed tissue was restricted to those seeds in which the internal swelling pressure had ruptured the seedcoat (immediately prior to germination). Embryos were thus obtained at this time, immediately following germination and 2 and 7 days following germination (Avers, 1958).

Small amounts of phosphate pH 6.5 buffer were added during the dissection procedure in order to prevent tissue dessication. A minimum of five embryos from each treatment (control and 100 ppm fenitrothion) were obtained at each sampling period.

b) Tissue preparation

Embryos were fixed in 10% neutral formalin solution for 3 hours and then washed in running water overnight. Samples were dehydrated in a gradedtertiary butyl alcohol-ethanol series prior to infiltration and embedding in paraplast. Ten micrometre sections were obtained on a conventional rotary microtome and adhered to clean glass slides with Haupt's adhesive (Purvis et al 1964). Following rehydration and cytochemical localization, the sections were dehydrated and permanently mounted with Canada Balsam.

c) Localization Procedures

Nucleic acids were localized in tissue sections by the Azure B technique (Flax and Himes, 1952). Sections were rehydrated to water and placed in the Azure B solution (0.25 mg/ml) in 0.2M citrate buffer at pH 4.0 for 2 hours at 50°C. Following a brief rinse in distilled water, the sections were placed in absolute tertiary butyl alcohol for 15 hours.

Total carbohydrates of 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 minutes, washed in water and stained in freshly prepared Schiff's reagent (Jensen, 1962) for 35 minutes. Following a brief water rinse, sections were placed in 2% sodium bisulfite for 2 minutes, rehydrated, then mounted.

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

RESULTS

1) Speed of Germination and Seedling Growth

a) White spruce

Spruce seeds exposed to the insecticides, acephate and phosphamidon during stratification showed an early suppression of germination (Figures 1 and 2). Significantly reduced speeds of germination were apparent following exposures to 10 ppm and 100 ppm of the insecticides, (Table I). White spruce seeds exposed to 100 ppm phosphamidon were most affected, with germination speeds and germination values differing from that of the control at the 1% probability level. Final germination percentages were similar in all groups and subsequent seedling growth was unaffected by acephate and phosphamidon exposure during stratification.

b) Yellow birch

Germination of yellow birch seeds was significantly enhanced by 10 ppm and 100 ppm acephate (Figure 3) and by 10 and 100 ppm phosphamidon (Figure 4) Final germination percentages were significantly greater following exposure to either insecticide (Table II). Speeds of germination of seeds exposed to 100 ppm acephate and 100 ppm phosphamidon were increased twofold (Table II).

Germination of yellow birch seeds was significantly affected by the addition of Arotex and Atlox to the fenitrothion. Field dosage levels (10 ppm fenitrothion: 1 ppm Atlox: 1ppm Arotex) significantly decreased daily germination (Figure 5), resulting in reduced speeds of germination (Table III). Control seeds germinated three times more quickly than seeds exposed to 100 ppm fenitrothion in 10 ppm Arotex and 10 ppm Atlox. Exposure to a mix of 100 ppm Arotex and 100 ppm Atlox without added fenitrothion was also observed to decrease germination and significantly reduce the speeds of germination in yellow birch seeds (Morris 1975).

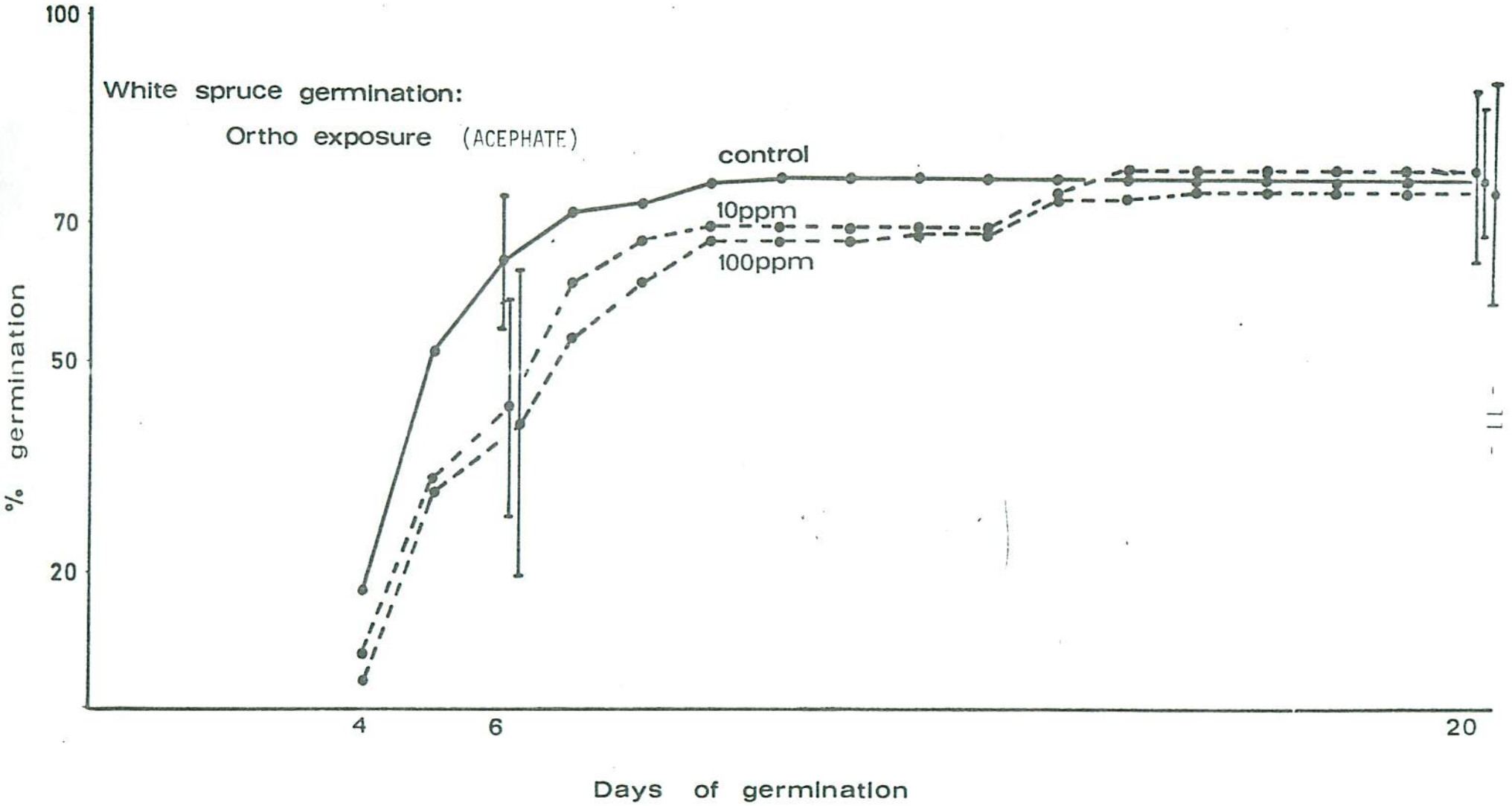

Fig. 1 Effects of acephate on germination of White spruce seeds.

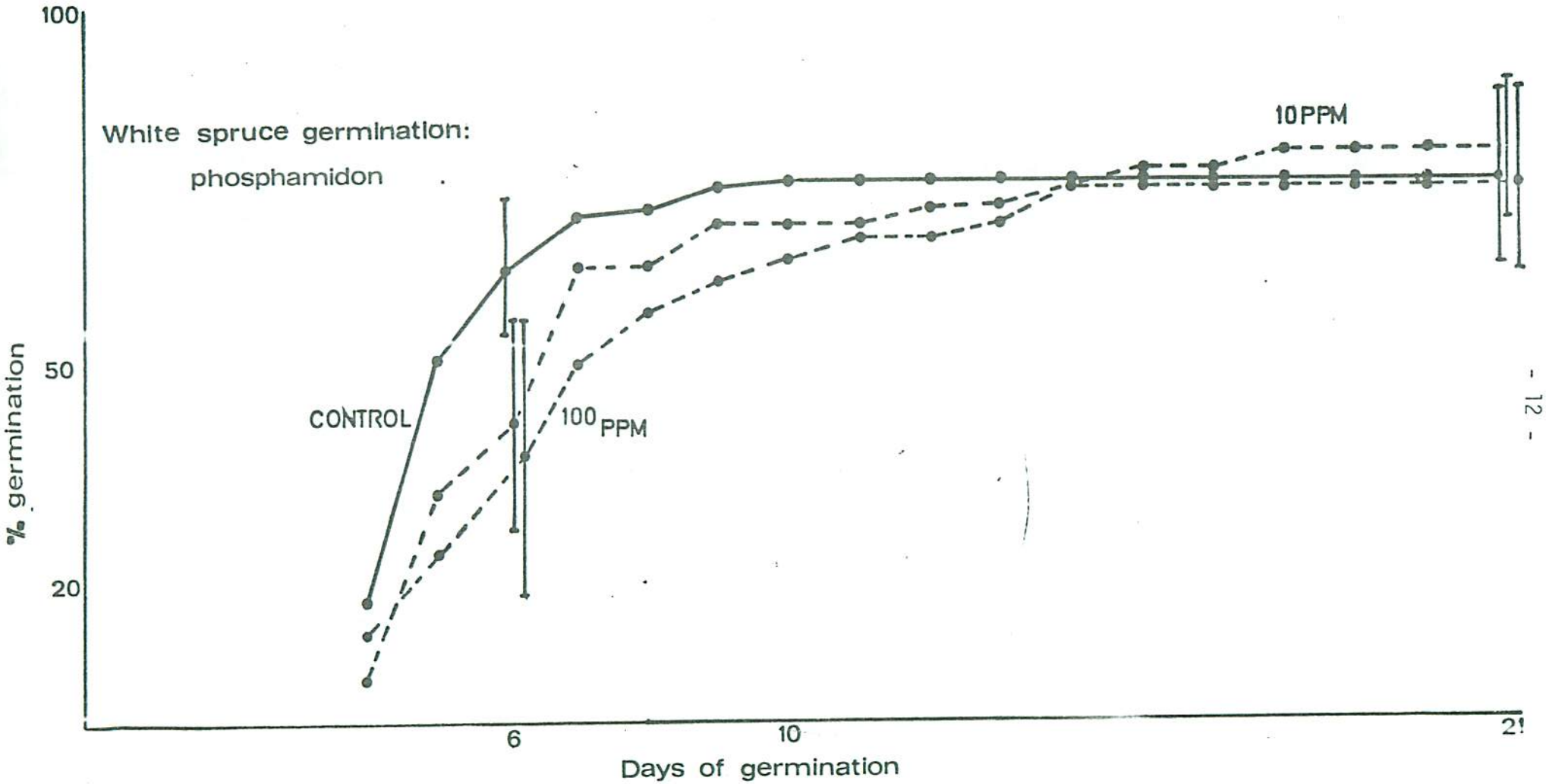

Fig. 2 Effects of phosphamidon on germination of White Spruce seeds

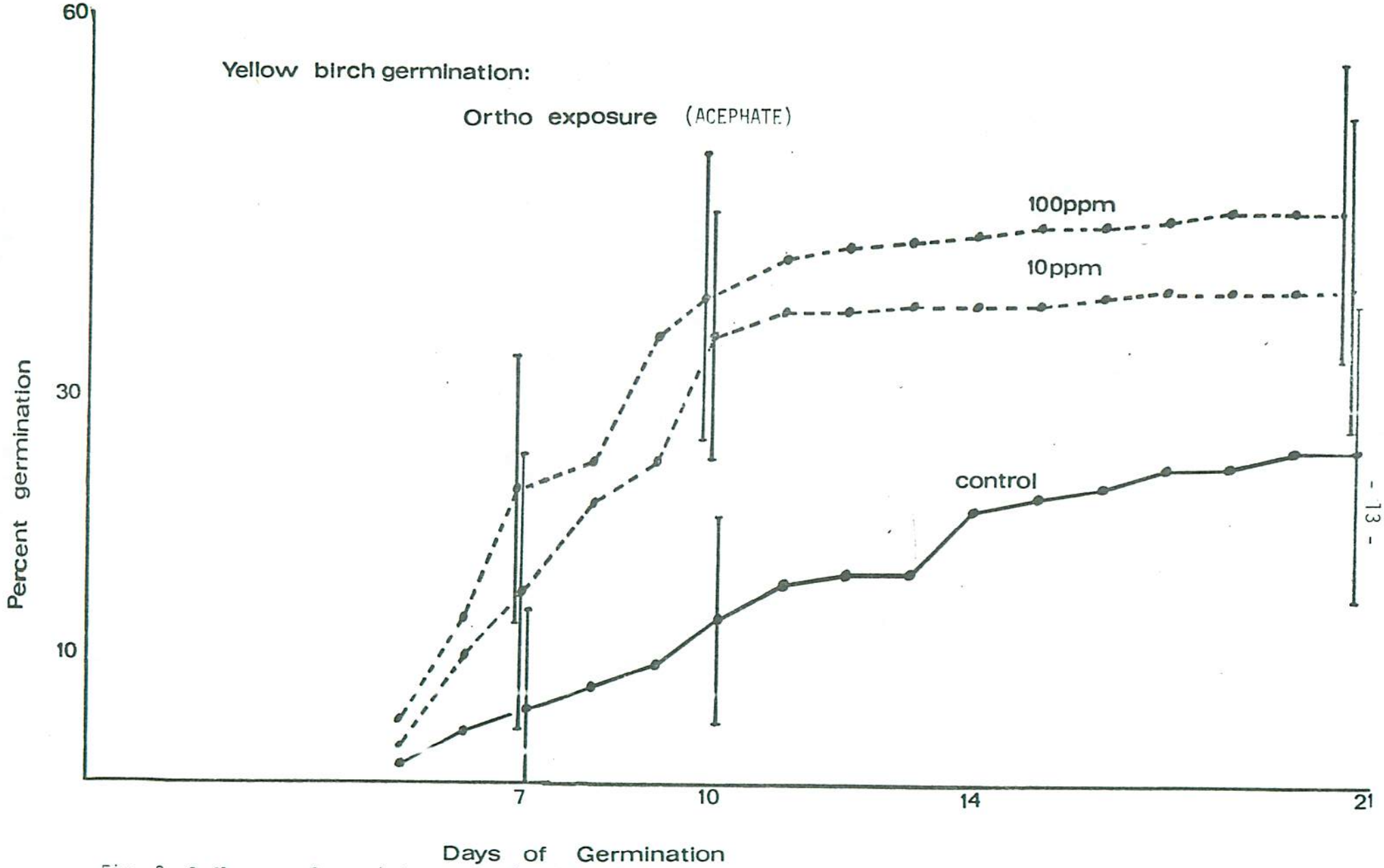

Fig. 3 Influence of acephate on germination of Yellow birch seeds.

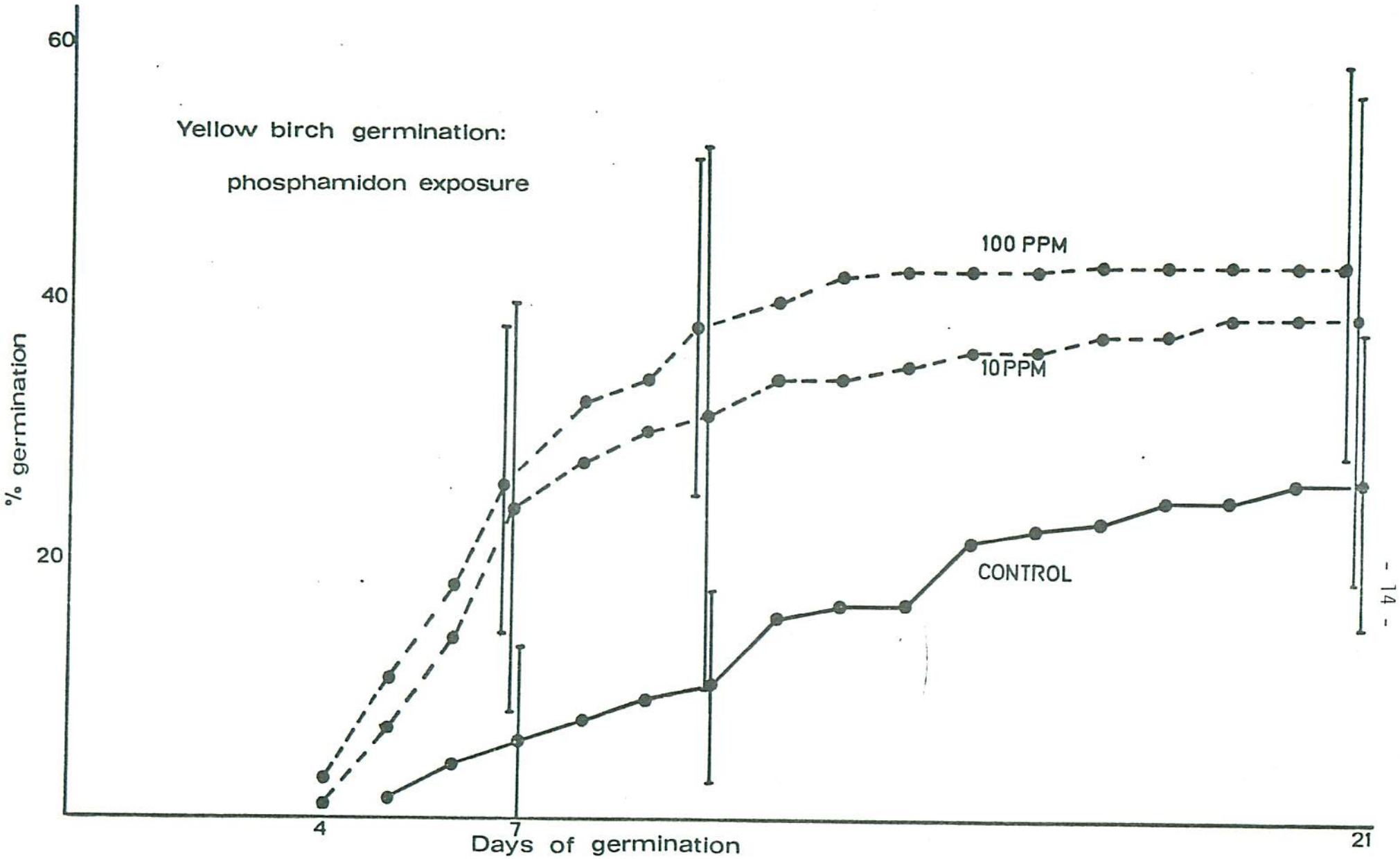

Fig. 4 Effects of phosphamidon on germination of Yellow birch seeds

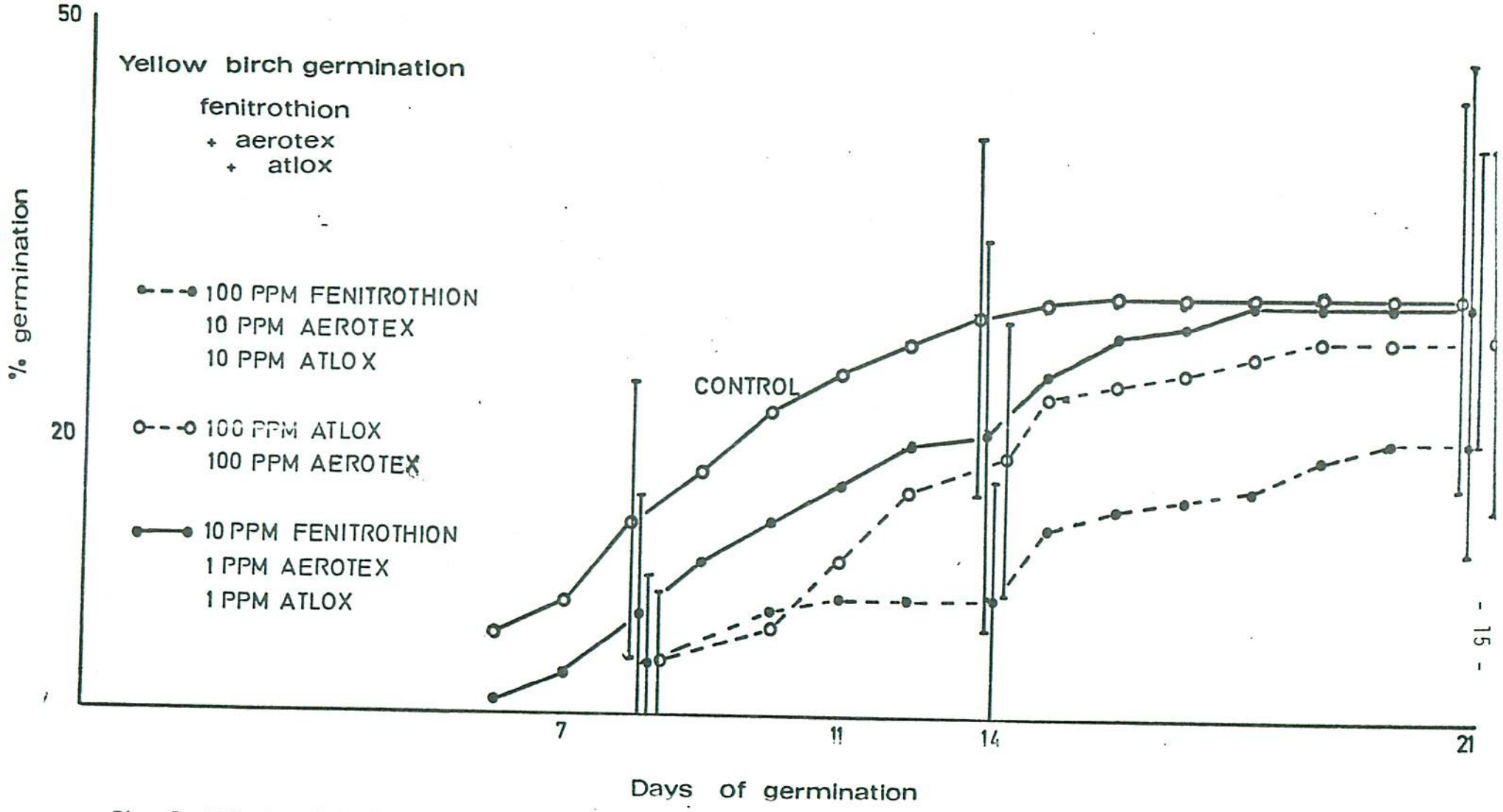

Fig. 5 Effects of fenitrothion and adjuvants on seed germination of Yellow birch

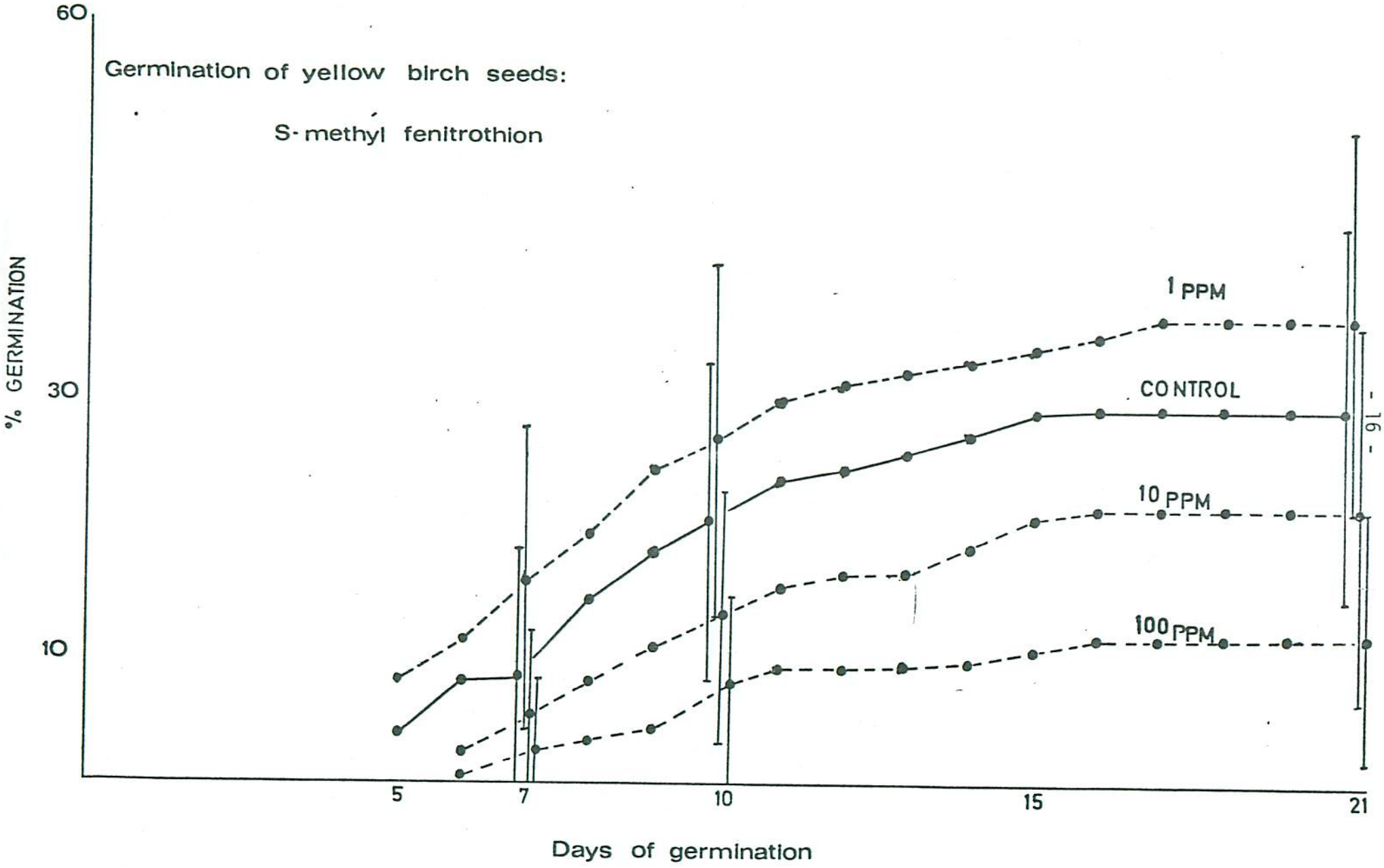

Fig. 6 Influence of a metabolite of fenitrothion (S-methyl fenitrothion) on seed germination of Yellow birch

TABLE I

(a) SPEED of GERMINATION of WHITE SPRUCE SEEDS EXPOSED TO THE INSECTICIDES fenitrothion, phosphamidon and acephate DURING STRATIFICATION at 5^0 C.

	acephate	Phosphamidon	Fenitrothion
CONTROL	1.47 ± .26	1.47 ± .26	1.56 ± .32
10 PPM	1.27 ± .24	1.29 ± .17	1.67 ± .48
100ppm	1.18 ± .31	1.07 ± .30	1.28 ± .199

(b) GERMINATION VALUES OF WHITE SPRUCE SEEDS EXPOSED TO THE INSECTICIDES, fenitrothion, phosphamidon and acephate DURING STRATIFICATION AT 5^oC.

	acephate	Phosphamidon	Fenitrothion
CONTROL	.314 ± .097	.314 ± .097	.314 ± .134
10 ppm	.222 ± .079	.269 ± .101	.336 ± .157
100ppm	.235 ± .172	.162 ± .073	.225 ± .073

TABLE II

GERMINATION CHARACTERISTICS of YELLOW BIRCH SEEDS EXPOSED TO THE INSECTICIDES fenitrothion, acephate and phosphamidon DURING STRATIFICATION AT 5°C.

TREATMENT	Final Germination %	Speed of Germination	Germination Value
25 ppm fenitrothion	18.5 ± 11.6	.15 ± .09	.012 ± .01
50 ppm fenitrothion	18.0 ± 9.5	.15 ± .10	.017 ± .02
100ppm fenitrothion	21.0 ± 10.2	.18 ± .09	.014 ± .01
10 ppm phosphamidon	38.0 ± 19.4	.52 ± .32	.044 ± .03
100 ppm phosphamidon	43.0 ± 15.3	.68 ± .21	.058 ± .03
10 ppm acephate	39.0 ± 11.0	.48 ± .15	.051 ± .03
100 ppm acephate	45.0 ± 11.7	.58 ± .16	.050 ± .02
CONTROL	26.2 ± 11.6	.27 ± .12	.019 ± .01

TABLE III

SPEEDS OF GERMINATION OF YELLOW BIRCH SEEDS EXPOSED TO Fenitrothion SPRAY FORMULATIONS DURING STRATIFICATION

Treatment	Speed of germination
Control	.395 ± .21
10 ppm fenitrothion & 1 ppm aerotex & 1 ppm atlox	.311 ± .17
100 ppm fenitrothion & 10 ppm aerotex & 10 ppm atlox	.164 ± .14
100 ppm aerotex & 100 ppm atlox	.250 ± .117

TABLE IV

GROWTH OF YELLOW BIRCH SEEDLINGS FOLLOWING EXPOSURE TO fenitrothion SPRAY FORMULATIONS DURING STRATIFICATION

Treatment	FRESH WEIGHT	ROOT LENGTH	HYPOCOTYL LENGTH
Control	6.19 ± 1.72mg.	10.3 ± 3.09mm.	23.69 ± 3.47mm.
10 ppm fenitrothion & 1 ppm aerotex& 1 ppm Atlox	5.73 ± 1.51	9.48 ± 3.27	21.97 ± 3.76
100 ppm fenitrothion & 10 ppm aerotex & 10 ppm atlox	2.19 ± 0.67	12.15 ± 1.36	13.98 ± 1.26

TABLE V

CHARACTERISTICS OF ONE WEEK OLD YELLOW BIRCH SEEDLINGS FOLLOWING TREATMENT WITH THE FENITROTHION METABOLITE S- methyl fenitrothion.

Treatment	Fresh Weight	Root Length	Hypocotyl Length
Control	6.02 ± 1.63mg.	9.93 ± 3.27mm.	23.71 ± 3.18mm.
1 ppm	6.05 ± 1.56	9.48 ± 3.46	22.38 ± 3.72
10 ppm	5.91 ± 1.55	9.33 ± 3.52	21.86 ± 3.87
100 ppm	6.35 ± 1.03	8.26 ± 2.31	19.60 ± 3.48

TABLE VI

GERMINATION CHARACTERISTICS OF YELLOW BIRCH SEEDS EXPOSED TO FENITROTHION AND GROWTH HORMONES DURING STRATIFICATION

Treatment	Final Germination %	Speed of Germination
Control	29.35 ± 15.6	.36 ± .20
10 ppm GA	28.18 ± 13.2	.37 ± .19
100 ppm GA	25.88 ± 16.1	.32 ± .25
100 ppm GA & 10 ppm IAA & 10 ppm kinetin	35.00 ± 15.4	.21 ± .11
100 ppm fenitrothion	24.75 ± 10.3	.21 ± .08
10 ppm GA & 100 ppm fenitrothion	23.33 ± 11.1	.21 ± .11
100 ppm GA & 100 ppm fenitrothion	7.14 ± 7.2	.059 ± .05
100 ppm GA & 10 ppm IAA & 10 ppm kinetin & 100 ppm fenitrothion	9.37 ± 7.7	.068 ± .06

TABLE VII

CHARACTERISTICS OF ONE WEEK OLD YELLOW BIRCH SEEDLINGS FOLLOWING TREATMENT WITH fenitrothion AND PLANT GROWTH HORMONES

Treatment	Fresh Weight	Root Length	Hypocotyl Length
Control	6.02 ± 1.63	9.93 ± 3.27	23.71 ± 3.18
10 ppm GA	6.31 ± 2.11	9.30 ± 3.70	22.36 ± 3.70
100 ppm GA	7.01 ± 1.40	10.90 ± 3.61	24.86 ± 5.24
100 ppm GA & 10 ppm IAA & 10 ppm Kinetin	5.75 ± 1.54	3.45 ± 1.80	19.87 ± 3.41
100 ppm fenitrothion	2.44 ± 0.78	2.50 ± 1.23	4.47 ± 1.38
10 ppm GA & 100 ppm fenitrothion	2.85 ± 0.77	3.30 ± 1.74	6.16 ± 2.13
100 ppm GA & 100 ppm fenitrothion	2.51 ± 0.93	3.16 ± 1.61	5.95 ± 1.96
100ppm GA & 10 ppm LAA & 10 ppm Kinetin & 100 ppm fenitrothion	2.83 ± .84	2.94 ± 4.32	5.05 ± 1.43

c) Water Uptake

The increases in seed fresh weight were unaffected by prior exposure for either 10 or 100 ppm fenitrothion (Table IV).

Following exposure of birch seeds to 1 ppm S-methyl fenitrothion, speed of germination was enhanced but with increased concentrations of this metabolite to 10 ppm or 100 ppm, germination speed was significantly reduced (Figure 6). However, of those seedlings which did germinate, no significant differences with untreated controls were apparent in the fresh weight, root or hypocotyl lengths (Table V).

2) Growth Hormone, Treatment

The phytohormones did not significantly reverse suppressed speeds of germination of yellow birch seeds treated with fenitrothion (Table VI). These yellow birch seedlings did not show increases in fresh weights or root lengths (Table VII). However, a significant reversal of 100 ppm fenitrothion imposed hypocotyl dwarfing was noted following application of 10 ppm or 100 ppm G.A.

3) Histochemical localizations in Yellow Birch

Neither, Periodic acid Schiff nor nucleic acid localization techniques indicated any differences between treated and control plants (Plates 1 to 4 and 5 and 6).

Intense localization of protein was observed in the cotyledons of the control and treated seedlings early in germination (Plates 7 and 8). One week old seedlings showed similar distribution of protein localization in root regions (Plates 9 and 10). Large "anomalous bodies", staining protein-positive (verified by protease digestion) were evident in cells of the root and

hypocotyls of 10 ppm fenitrothion treated seedlings (Plate 11). In the cotyledons of one week old 100 ppm fenitrothion-treated seedlings, protein staining was more intense than comparable untreated control seedlings (Plates 12, 13).

PLATE 1

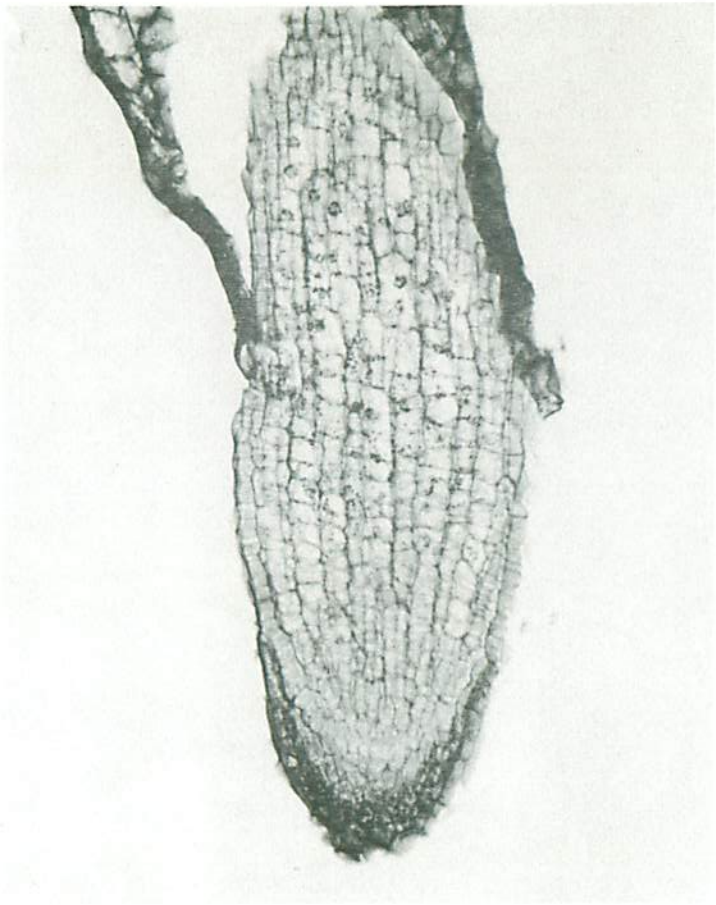

PLATE 2

Yellow birch seedlings from stage 2, localization of starches in root region. Plate 1 is the control, plate 2 is from the 100 ppm fenitrothion treatment.

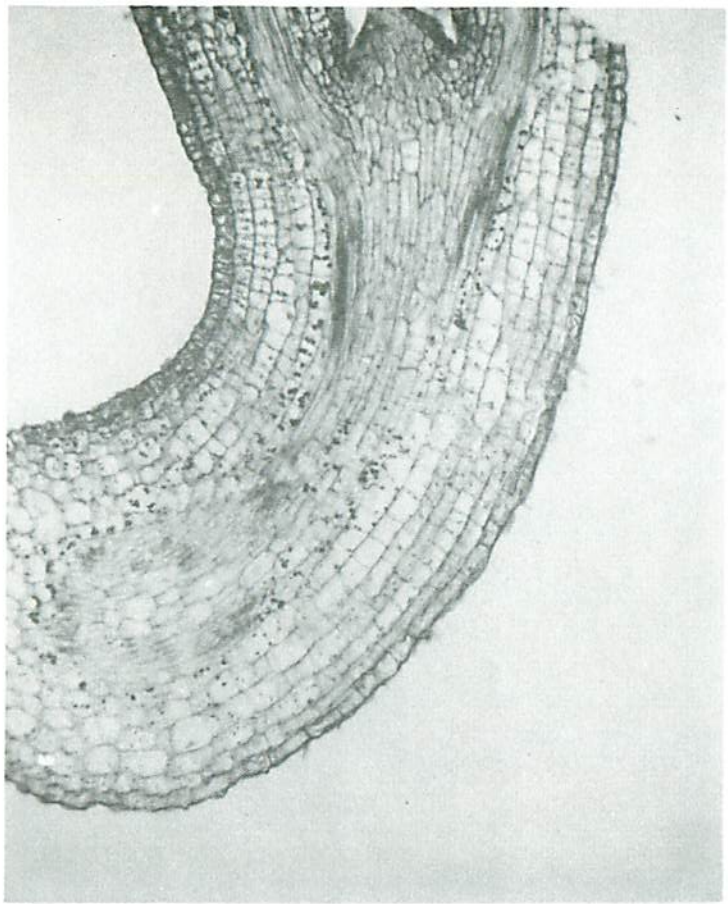

PLATE 3

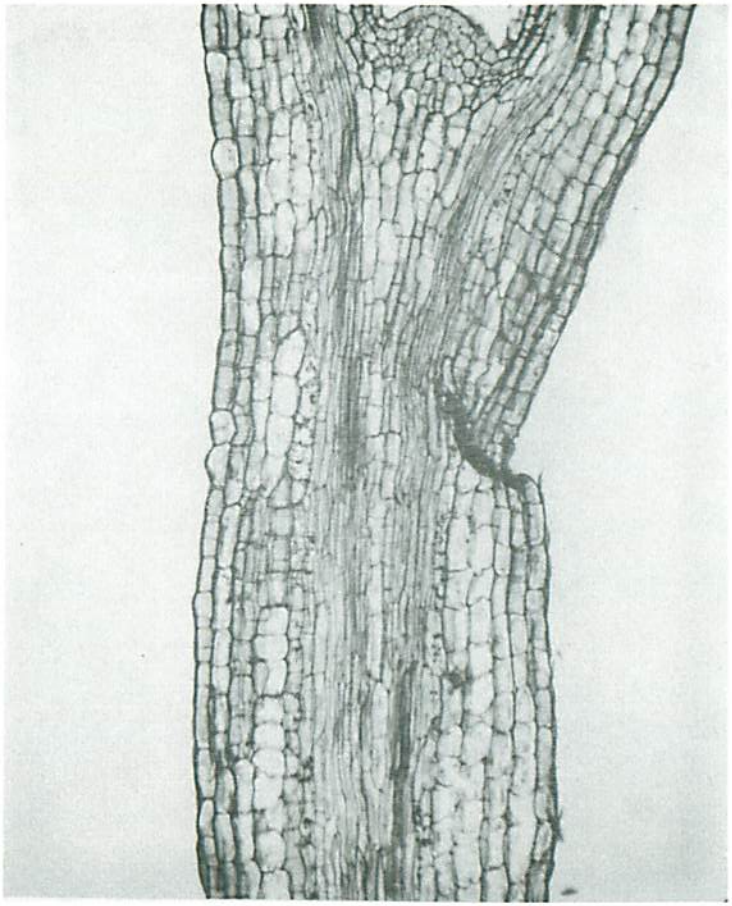

PLATE 4

One week old yellow birch seedlings, localization of starches in the hypocotyl. Plate 3 is the control and plate 4 is the 100 ppm treatment.

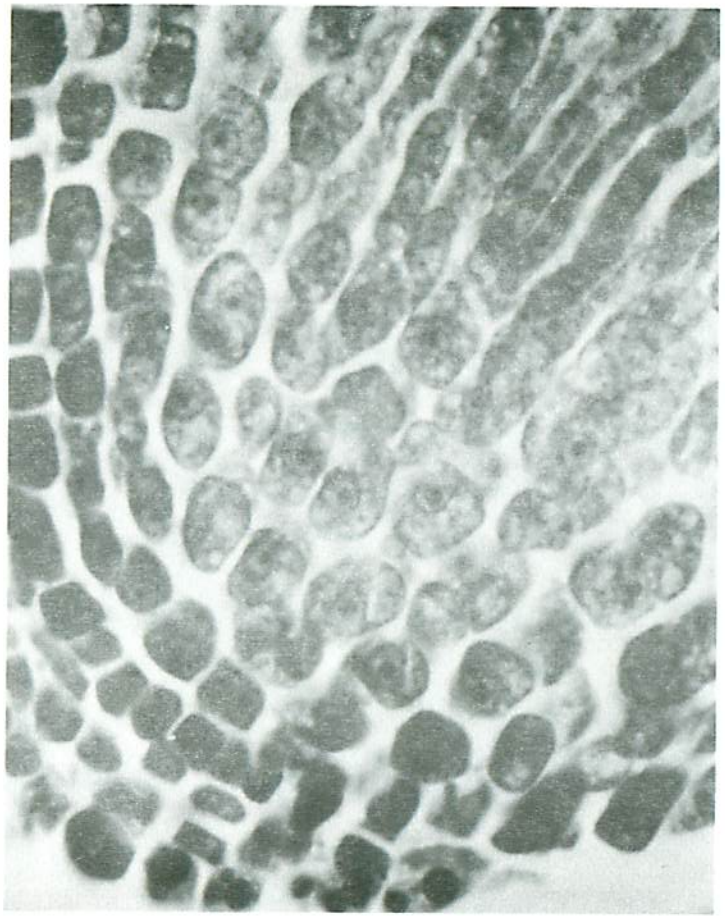

PLATE 5

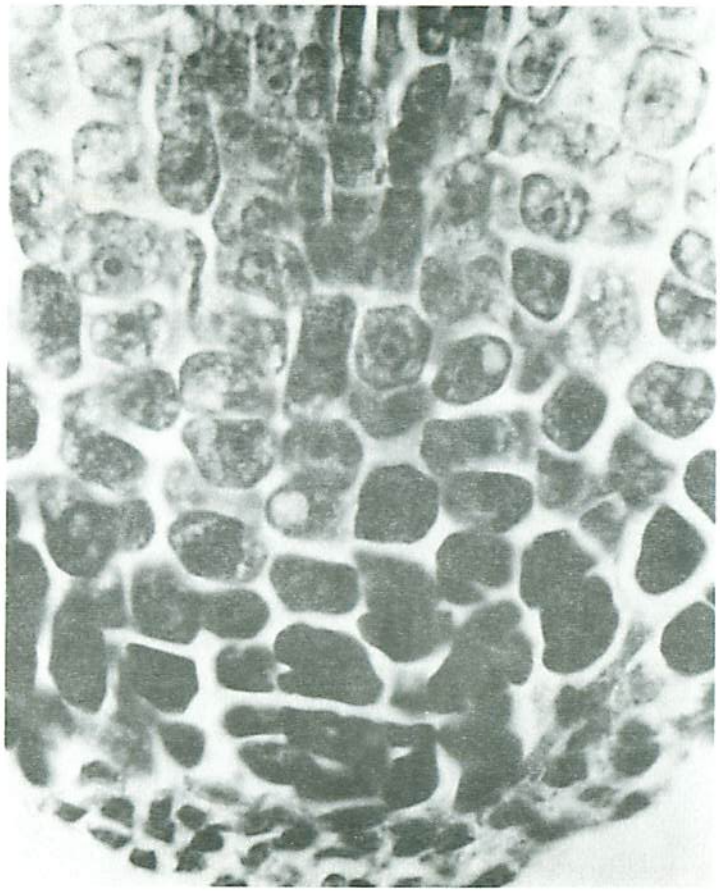

PLATE 6

Localization of nucleic acids in root tip of yellow birch in control (plate 5) and treated (plate 6) embryos prior to germination.

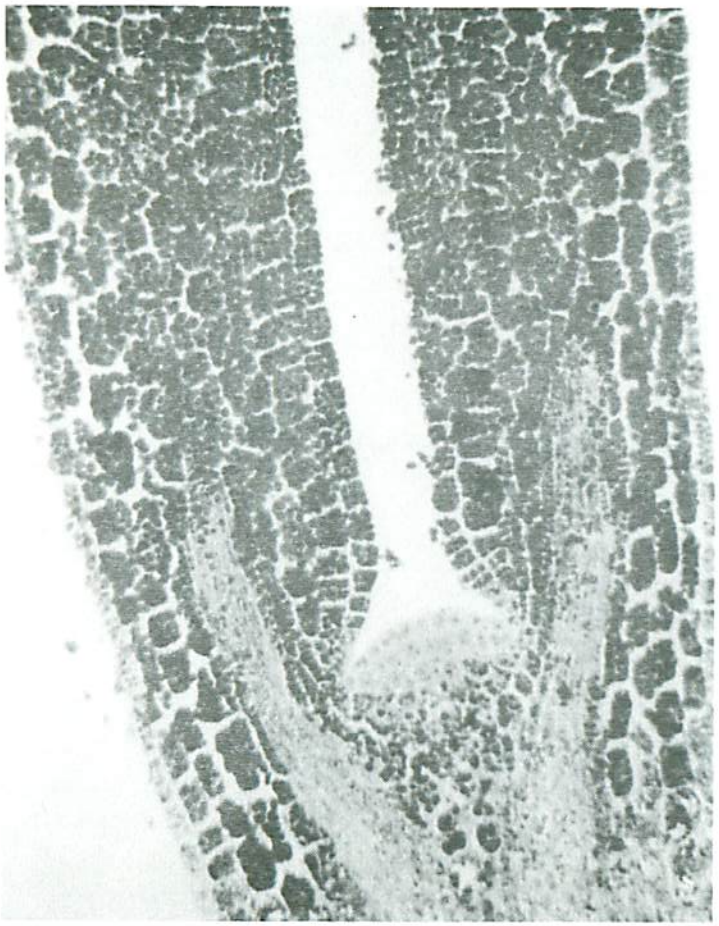

PLATE 7

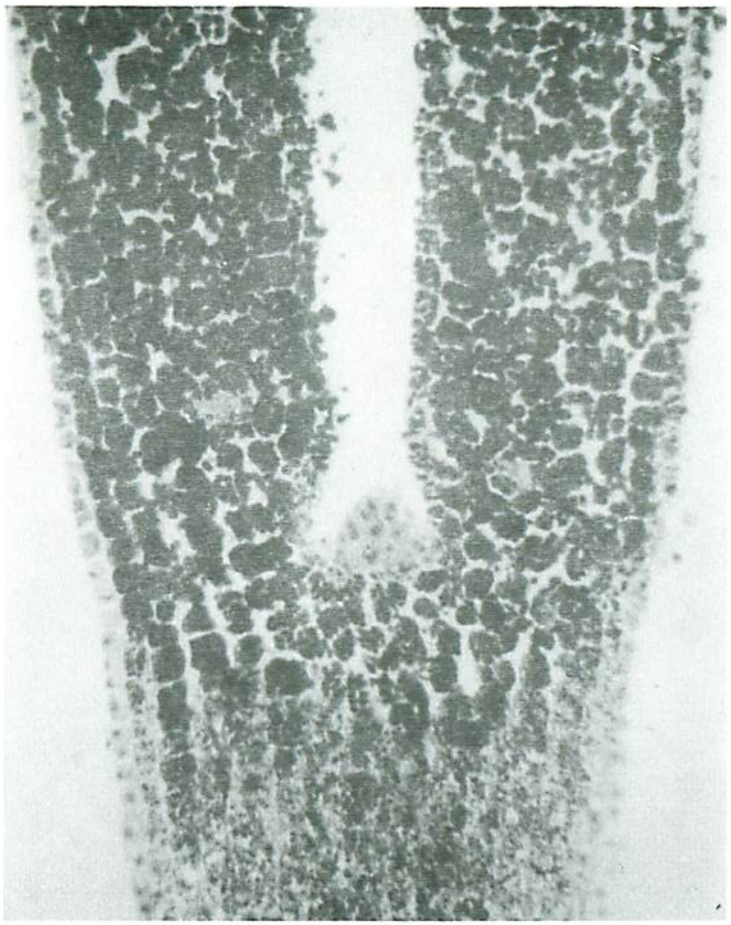

PLATE 8

Protein localization in cotyledon of control (plate 7) and treated embryos of yellow birch (plate 8) prior to germination.

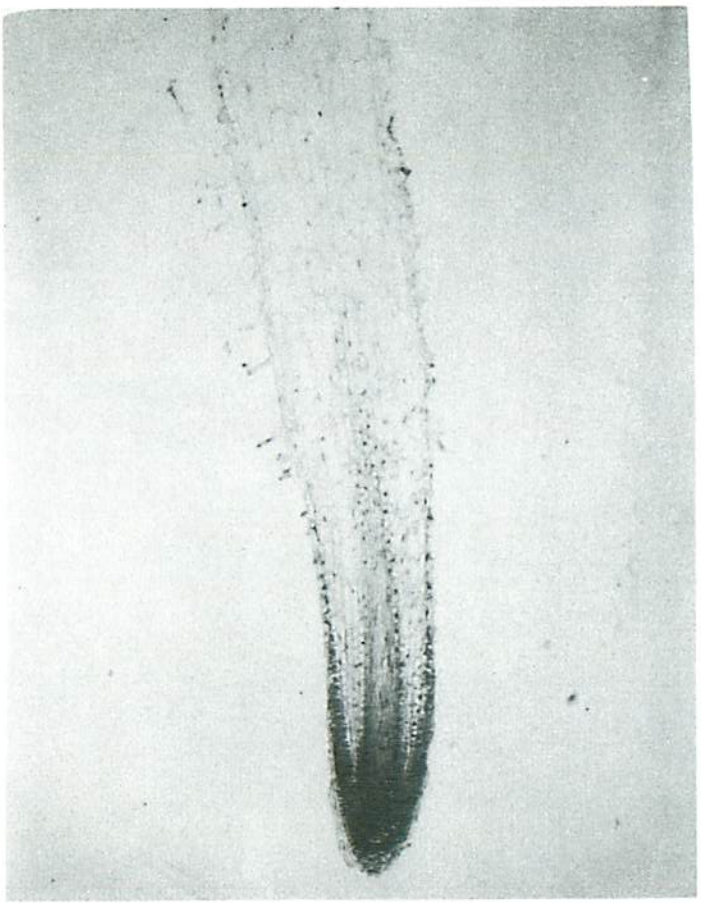

PLATE 9

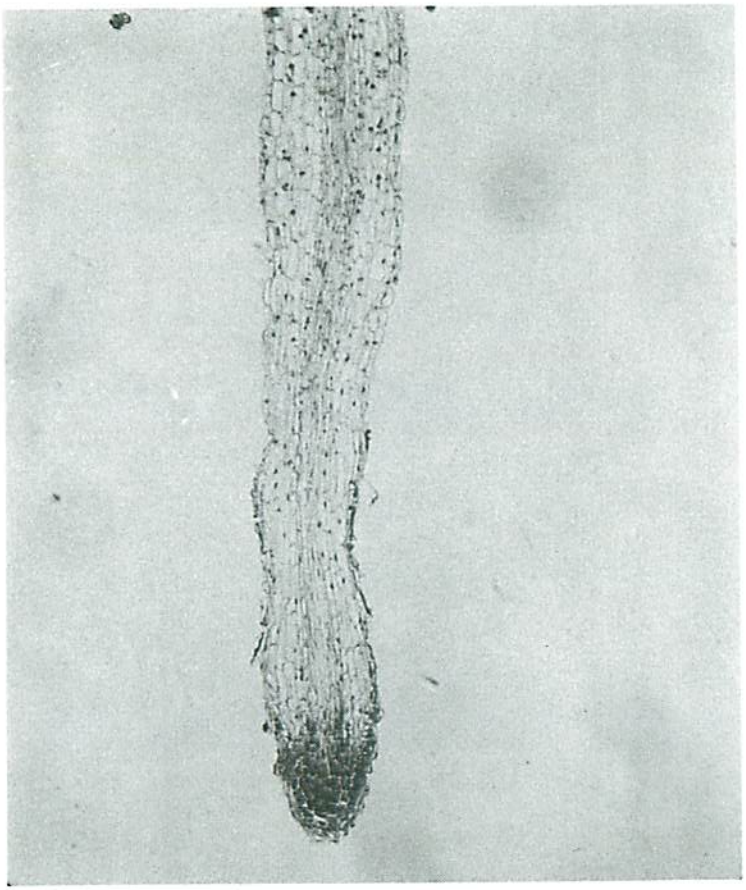

PLATE 10

Localization of protein in yellow birch roots of control (plate 9) and treated (plate 10) in one week old seedlings.

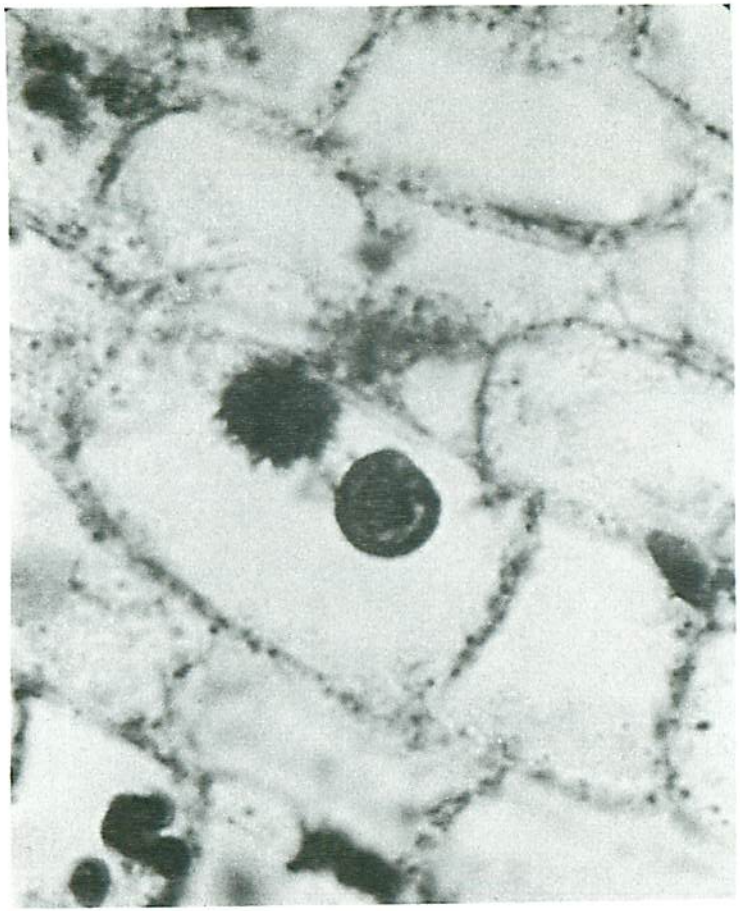

PLATE 11

Protein-positive staining bodies in hypocotyl cells of 100 ppm treated embryos of yellow birch.

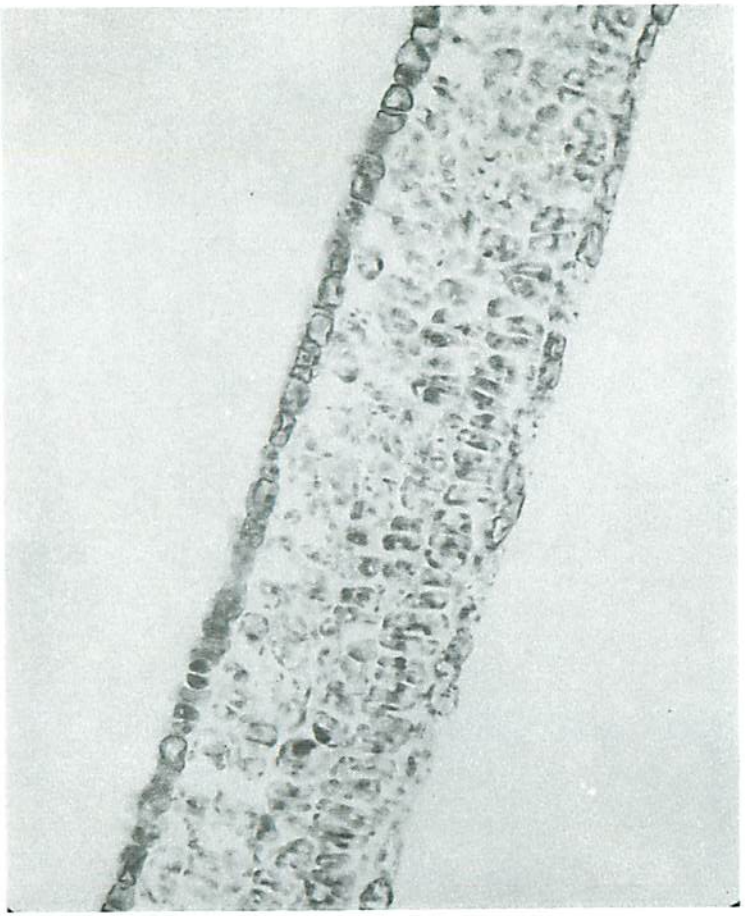

PLATE 12

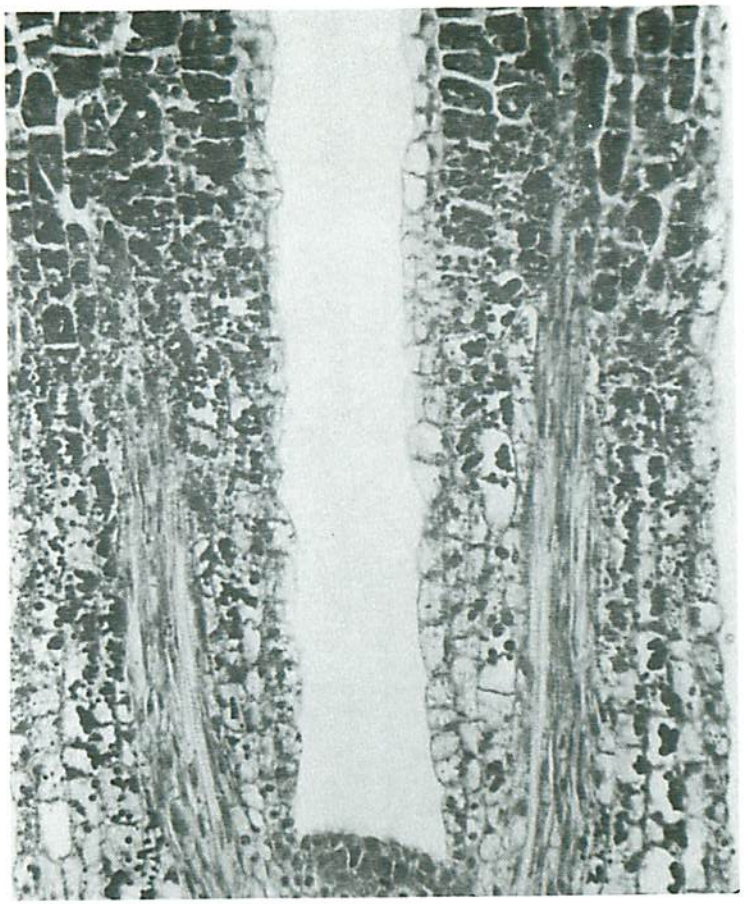

PLATE 13

Localization of protein in hypocotyl and cotyledons of one week old control (plate 12) and treated (plate 13) seedlings of yellow birch.

DISCUSSION

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 and La Mar Anderson, 1972). The suggestion that hydrolysis of storage substances is GA mediated through the activities of various hydrolytic enzymes, including protease (Paleg, 1965) may implicate fenitrothion inhibition of GA activity and/or biosynthesis in yellow birch and accounts for low storage protein utilization in the cotyledons of fenitrothion treated embryos. The treatments of yellow birch seedlings following 10 and 100 ppm GA exposure did not result in seedling sizes different from that of the control, yet application of GA at 10 and 100 ppm concentrations did significantly increase hypocotyl lengths of 100 ppm fenitrothion treated seeds. The inhibitive action of many growth retardant agents has been shown to be reversed by the application of endogenous growth hormones (Chawan and Sen, 1974; Berrie and Robertson, 1972). Similarly, the growth inhibition action of organophosphate insecticides on tobacco callus growth has been observed to be related to levels of growth hormones in the media (Lee and Wilkinson, 1973). The growth retardants may act by inhibiting growth hormone biosynthesis, chiefly GA (Crozier et al, 1973; Reid and Crozier, 1968). In view of this, there is thus a suggestion that fenitrothion may be inhibiting native growth hormone metabolism in yellow birch seeds and this may account for the inhibition of seedling growth.

The increased toxicity effects observed in yellow birch when exposed to spray formulations of fenitrothion-Arotex-Atlox was apparent at field dosage levels whereas fenitrothion alone at this concentration had previously been shown to consistently enhance early germination (Pomber et al, 1974). This finding supports Nigam's (1976) observation that fenitrothion's phytotoxicity was increased in the presence of the carrier and emulsifier. Similarly Atlox has been shown to inhibit the replication of Bacillus thuringiensis at a concentration of 1000 ppm and to significantly supplement fenitrothion toxicity when applied together with this insecticide (Morris, 1975).

The inhibitory action of fenitrothion on the growth of birch seeds is similar to that noted with other organophosphorous insecticides. Chena (1971) observed significant stunting of tobacco growth following application of the insecticide, Dursban®. Similarly, numerous organophosphorous insecticides, most prominently parathion, have been shown to significantly reduce pollen germination and tube elongation in petunia and tomato, whereas application of carbamate insecticides had no affect (Gentile et al, 1971). Our studies, to date, have shown that the effect of fenitrothion on seed germination is a factor of insecticide concentration and the species in question. Comparisons of phytotoxicity of fenitrothion with acephate and phosphamidon have reinforced this observation and indicated the dissimilarity of effects between insecticides. acephate and phosphamidon inhibited germination speeds of white spruce seeds at concentrations of 10 ppm (1.27 ± .24 and 1.29 ± .17 respectively) whereas a 10 ppm fenitrothion exposure slightly enhanced germination speeds (1.67 ± .48) above that of the control (1.56 ± .32). In contrast, acephate and phosphamidon increased germination speeds and final germination percentages of yellow birch seeds above that of the control whereas fenitrothion depressed germination. Werner (1974) observed enhanced germination of slash pine and slash pine seeds following acephate exposure at concentrations as high as 3% aqueous solutions

whereas significant inhibition was obtained employing four other organophosphorous insecticides. Further observations on seedlings treated with acephate indicated no visible adverse effects on subsequent growth. Similar comparative toxicity studies with Bacillus thuringiensis have shown fenitrothion to inhibit replication processes at concentration of 2 ppm whereas acephate had no effect at dosage levels of up to 10,000 ppm (Morris, 1975).

During the germination of yellow birch seeds treated with fenitrothion, the metabolite, S-methyl fenitrothion has been found at levels in excess of 20 ppm (D. Hallett, personal commun.); however, the effects of fenitrothion on the germination of yellow birch seeds do not appear to be strictly the result of S-methyl fenitrothion being formed in the tissues. Although significant suppression of germination was observed in seeds exposed to 10 ppm and 100 ppm S-methyl fenitrothion, all seedlings developed and grew normally. The consistent increase of germination of 10 ppm fenitrothion treated seeds, observed previously (Pomber et al, 1974), may be related to the significant enhancement of germination following 1 ppm S-methyl fenitrothion. Further examinations of the possible growth retardant activities of the fenitrothion metabolites (fenitro-oxon and desmethyl fenitrothion) would be required in order to determine whether it is the insecticide and/or one of its metabolites which is affecting birch germination and subsequent growth and also clarify the role of fenitrothion vis à vis its interaction with the more important plant growth hormones.

SUMMARY AND CONCLUSIONS

The insecticides fenitrothion, acephate and phosphamidon inhibit white spruce seed germination to varying degrees depending on applied concentration. Acephate and phosphamidon were shown to enhance germination of yellow birch seeds at concentrations of 10 and 100 ppm. The metabolite, S-methyl fenitrothion, was observed to decrease germination at concentrations of 10 and 100 ppm, however, the emergent seedlings grew normally and did not show dwarfing effects. Gibberellic acid was observed to significantly reverse the growth retardation previously noted in seeds exposed to 100 ppm fenitrothion. The gibberellic acid mediated reversal was only slight and restricted to the hypocotyl region of the yellow birch seedlings.

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