METABOLISM OF THE INSECTICIDE, FENITROTHION, BY SEEDS AND SEEDLINGS OF PICEA GLAUCA (MOENCH/VOSS), PINUS STROBUS L. AND BETULA ALLEGHANIENSIS BRITT.

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CC-X-134

A Volunteer Paper Presented at the Second FAO/IUFRO World Technical Consultation on Forest Diseases and Insects, New Delhi, India

April 7 - 12, 1975

SUMMARY

Metabolism of the Insecticide, Fenitrothion, by Seeds and Seedlings of *Picea glauca* (Moench, Voss) *Pinus strobus* L. and *Betula alleghaniensis* Britt.

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Fenitrothion (0,0-dimethyl-0-(4-nitro-3-methyl-phenyl)-phosphorothioate) replaced DDT in 1969 as the insecticide of choice for the control of spruce budworm (*Choristoneura fumiferana* Clem.) in Canadian forests. Some 16 million acres of Eastern Canadian forests have been sprayed annually with this pesticide and even through the rate of dissipation is faster, about 30% residues persist on the coniferous foliage for 2 weeks. Therefore studies were required to show the effect of the pesticide on the forest environment. As a part of this program, laboratory experiments were carried out to examine the fate of fenitrothion in two coniferous (spruce & pine) and one deciduous (yellow birch) species during germination of seeds.

Employing modern methodology of pesticide residue analysis (gas liquid, thin layer chromatography, mass spectroscopy and liquid scintillation spectrometry) it was found that all three species absorb, accumulate and degrade the insecticide molecule mostly in the embryos. While the patterns of accumulation and metabolism remained similar between the coniferous and deciduous species the rates were faster for the yellow birch. For example, yellow birch formed metabolites, fenitro-oxon, desmethyl fenitrothion and s-methyl fenitrothion at a faster rate than the white spruce and pine seedlings and this factor finally accounted for differential phytotoxicity to yellow birch. Whether the same pattern holds good under field conditions remains to be studied. The implication of these findings in relation to forest regeneration and ecology are discussed.

RÉSUMÉ

Le métabolisme intermédiaire du fénitrothion dans les semences et les jeunes pousses de Picea glauca (Moench, voss), Pinus strobus L. et Betula alleghaniensis Britt.

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En 1969, le fénitrothion (thionophosphate de O, O-diméthyle O-(méthyl-3 nitro-4 phényle)) remplaçait le DDT comme insecticide efficace dans la lutte contre la tordeuse des bourgeons de l'épinette (Choristoneura fumiferana Clem.) des forêts canadiennes. Cet insecticide a fait l'objet d'une pulvérisation aérienne annuelle sur environ 16 millions d'arpents de forêts de l'est du Canada. Sa rémanence a été inférieure à celle du DDT mais environ 30% du composé ont persisté pendant deux semaines sur le feuillage des conifères. Il est donc devenu essential d'étudier les effets de l'insecticide sur l'environnement forestier. Une partie du programme consistait à mener des expériences de laboratoire afin de déterminer ce qu'il advenait du fénitrothion chez deux conifères (épinette et pin) et un feuillu (bouleau jaune) au cours de la germination des graines.

Les méthodes modernes d'analyse des résidus d'insecticides (chromatographie en phase gazeuse et sur couche mince, spectrographie de masse et spectrométrie à scintillation en milieu liquide) ont révélé que les trois espèces d'arbre pouvaient absorber, accumuler et dégrader les molécules de l'insecticide, principalement dans les embryons. Les processus d'accumulation et de transformation ont été semblables pour les conifères et l'essence feuillue, mais chez le bouleau jaune, ils se sont révélés plus rapides. Ainsi, par exemple, la production de métabolites, de fénitro-oxon, de fénitrothion déméthylé et de S-méthyl fénitrothion a été plus rapide chez le bouleau jaune que chez les jeunes pousses de pin et d'épinette blanche; c'est ce facteur qui a finalement expliqué la différence de toxicité pour le bouleau jaune. Il reste encore à confirmer ces résultats par des études sur le terrain. Nous étudions donc ici la signification de ces résultats relativement à la régénération des forêts et à leur écologie.

RESUMEN

Metabolismo del Insecticida Fenitrotión en Semillas y Plantones de Picea glauca (Moench, Voss), Finus strobus L. y Betula alleghaniensis Britt.

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En 1969 se eligió al fenitrotión (0,C - fosforotioato de bimetilo - 0 - nitro 4 - metilfenilo 3) para sustituir al DDT, en la lucha contra la oruga del cogollo de la picea (Choristoneura fumiferana Clem.) en los bosques canadienses. Anualmente se han rociado con este pesticida unos 41 millones de kilómetros cuadrados de bosques del este de Canadá y, aunque su ritmo de disipación es elevado, todavía queda un 30% de residuos en el follaje de las coníferas. Por lo tanto, hubo que realizar estudios sobre el efecto de este pesticida en el medio forestal. Como parte del programa, se llevaron a cabo experimentos de laboratorio para analizar la presencia de fenitrotión en dos especies de coníferas (picea y pino) y una especie de hoja caduca (abedul amarillo) durante la germinación de las semillas.

Utilizando métodos modernos de análisis de residuos de pesticidas (gas licuado, cromatografía de capa fina, espectroscopia de masa y espectrometría por escintilación de líquidos) se descubrió que las tres especies absorben, acumulan y descomponen la molécula del insecticida, sobre todo en los embriones. Si bien el proceso de acumulación y metabolismo fue similar entre las especies de coníferas y de joja caduca, su velocidad fue más elevada en el abedul amarillo. Por ejemplo, el abedul amarillo formó metabolitos, fenitro-oxon, fenitrotión bimetílico y fenitrotión metílico s. a un ritmo más rápido que los plantones de pino y picea blanco, factor que finalmente explicó la fitotoxicidad diferencial del abedul amarillo. Resta por comprobar si subsiste la misma tendencia bajo condiciones de campo. Se discuten las implicaciones de estos resultados en relacion a la ecología y regeneración forestal.

INTRODUCTION

Fenitrothion (0,0-dimethyl-0-(4 nitro-3-methyl-phenyl)-phosphorothioate) replaced DDT in 1969 as the insecticide of choice for the control of spruce budworm (Choristoneura fumiferana Clem) in Canadian forests. Applied at the rate of 2-4 oz/acre (AI), it prevented defoliation by lepidopterous insects and in this way, at least 16 million acres of eastern Canadian forests have been sprayed with this pesticide. Therefore, studies were required to show the environmental impact produced by such a large scale treatment of forests. Yule and Duffy (1971) were the first to investigate the mode of persistence and dissipation on the coniferous foliage and they reported that 50% of the residues deposited by the aerial spray was lost in 4 days but 15-30% persisted for 2 weeks. As a part of a program on fate of the residues in vegetative components of the forest environment, Hallett et al (1975) examined the effects of fenitrothion treatment on forest seeds. Seeds and cones constitute food sources for smaller wildlife (rodents, game birds etc), seeds serve as the starting material for generation of new forests and thus metabolic and phytotoxic effects of the pesticide could be easily examined during the rapid growth of young seedlings with a minimum of extraneous variables.

MATERIALS AND METHODS

Most of the experiments were performed with seeds and seedlings of the eastern white pine first and then investigations were extended to white spruce and birch seeds. The coniferous seeds are fertilized during the first year and are released from the cones during the second year; after wintering on the forest floor they germinate in the spring of the third year. Seeds were collected in the second year and stratified at 10°C for 21 days to break seed dormancy. Duplicate sets of 30 seeds were then germinated in an aqueous solution of 10 and 1000 ppm in the dark. At various time intervals, the seeds were divided into seed coat, endosperm and embryo and analysed for fenitrothion and its metabolites by gas liquid, thin layer chromatography (G.L.C. and T.L.C.) and mass spectroscopy as described by Hallett et al (1974).

Fenitrothion and its metabolites were extracted from the seed parts with acetonitrile. Toxic metabolites, fenitro-oxon, and S-methyl fenitrothion were separated from the parent compound by column chromatography. Residues were detected with a Pye GC fitted with a thermionic alkali flame ionization detector (AFID) and were confirmed using TLC. Structures of metabolites were confirmed by mass spectroscopy. Metabolism was followed using $OC^{14}H_3$ labelled fenitrothion. Radioactive metabolites and conjugates were examined by a TLC radioactive scanner and were quantitated using a scintillation counter.

RESULTS AND DISCUSSION

Preliminary results showed that fenitrothion was absorbed from a 10 ppm aqueous solution, approximating field concentration, by stratified and non-stratified germinating white pine seeds.

Two metabolites, fenitro-oxon, and S-methyl fenitrothion were detected in late stages of germination (Hallett et al 1974). The mode of toxicity of fenitrothion to mammals and insects is via cholinesterase inhibition. Fenitro-oxon has been shown to be 10 to 100 times and S-methyl fenitrothion 100 to 1000 times more active as anticholinesterase agents than the parent compound (Kovacicova et al. 1973).

Germination studies were carried out on treated stratified white pine seeds to quantitate the formation of fenitro-oxon, S-methyl fenitrothion and desmethyl fenitrothion and to examine the pathway of conversion of fenitrothion to the more toxic S-methyl isomer not previously detected as a biological metabolite.

Our previous studies showed that fenitrothion treatment did not produce any long term gross morphological defects in white pine seedlings after 6 months growth. White spruce, the target tree protected by the sprayings was also shown to resist toxic effects. Both of these species are coniferous gymnosperms. Yellow birch, a deciduous angiosperm, prevalent in sprayed forests, showed slight effects on treatment with 10 ppm fenitrothion, and gross stunting after 10 days of germination treated at 1000 ppm fenitrothion (Pomber et al 1974). This effect was not evident in pine or spruce. Similar residue and metabolic studies were therefore carried out in whole seeds of birch and spruce to examine any difference in absorption, metabolism and detoxification of the pesticide.

White pine seeds were then dissected into seed coat, endosperm and embryo, to differentiate between biological metabolism in the actively growing embryo and absorption of chemical contaminants. The concentration of fenitro-oxon and S-methyl fenitrothion rose to 2.5 ppm in the embryos of young seedlings after 14 days of germination. Trace amounts of the metabolites were found in the seed coat and endosperm of the seeds throughout germination. The desmethyl form of the pesticide was also found to rise in the embryos from 2 to 36 ppm during 8 to 14 days of germination (Figures 1 to 4).

Fenitrothion has been shown to be dealkylated by an alkyl transferase using glutathion, a common tripeptide, as substrate to form desmethyl fenitrothion, and S-methyl glutathion in mammalian liver and kidney (Hollingworth et al. 1967). Desmethyl fenitrothion which is non-toxic was found in the pine embryos and was suspected to be an intermediate in forming the toxic S-methyl isomer. Therefore $OC^{14}H_3$ labelled fenitrothion was used to treat seeds between 12 and 15 days of germination. C^{14} labelled S-methyl glutathion was recovered from the seedlings as well as labelled S-methyl fenitrothion (Table 1). The structure of S-methyl glutathion was confirmed by mass spectroscopy and amino acid analysis. The desmethyl form is likely remethylated in the S position by either fenitrothion, fenitro-oxon, or free methyl groups in the cell, to form the toxic S-methyl isomer.

Absorption and metabolism of fenitrothion by whole pine, spruce, and birch seeds was compared. By 4 days of germination,

yellow birch had absorbed 160 ppm of fenitrothion, 1.5 times more than pine and 2 fold higher than spruce (Figure 5). This level fell rapidly in birch but only slightly in spruce and increased in pine during later stages of germination. Fenitro-oxon (Figure 6), reached a high of 11 ppm by 4 days of germination in birch whereas a high (75 ppm) in spruce was not reached until 10th day of germination or 1.0 ppm at 14th day in pine. S-methyl fenitrothion (Figure 7) rose rapidly and reached a high of 5 ppm by 10th day germination in birch. Much higher levels were found in pine. Desmethyl fenitrothion (Figure 8) was found in high levels (15 ppm) after 4th day germination in birch and this increased to 30-37 ppm by 16th day. Lower levels (9.3 ppm) were found in spruce. The levels gradually declined to 5 ppm by 21st day of germination. On the other hand the levels increased to 11.2 ppm during germination of pine seedlings.

Birch seeds treated with 1000 ppm of the fenitrothion absorbed a maximum of 5000 ppm after 4 days of germination. Thus there was a 32 fold increase in concentration absorbed over the 10 ppm treatment. Levels of metabolites increased only 3 times (32 ppm) in the case of fenitro-oxon and S-methyl fenitrothion (16.7 ppm) and 5 times (115 ppm) for desmethyl fenitrothion.

Fenitrothion inhibits lipid metabolism via lipoxidase enzymes in forming fenitro-oxon (Rowlands, 1966). Fenitro-oxon is formed in high levels at early stages of germination in birch. Inhibition of the lipoxidase pathway, therefore, is likely a factor in the stunting of birch. Detoxification via dealkylation is also more evident in birch. The alkylation of glutathion by the pesticide may also alter birch seed metabolism. Glutathion has been implicated in influencing activation of enzymes in the first phase of pea seed germination. In conclusion fenitrothion presents no environmental hazard to conifers but high dosages may prove deleterious to deciduous spp (yellow birch).

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APPENDIX I

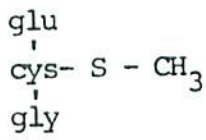
TABLE 1

Thin Layer Chromatogram of Extract from White Pine Seedlings Treated with $OC^{14}H_3$ Fenitrothion

(Ninhydrin Positive Substances)

COMPOUNDS	Rf Values SOLVENT SYSTEM A STANDARD	EXTRACT	HYDROLYSIS PRODUCT	SOLVENT SYSTEM B STANDARD	EXTRACT
S-methyl Glutathion	.55	.55		.59	.59
Glutathion	.47	(1,500dpm)		.55	
Cysteine	.46		.49	.54	
S-methyl Cysteine	.59	.59	.59	.62	
Glutamic Acid	.375	(500dpm)	.38	.53	
Glycine	.325		.32	.27	

S-methyl Glutathion



APPENDIX II

FENITROTHION IN PINE SEEDS DURING GERMINATION

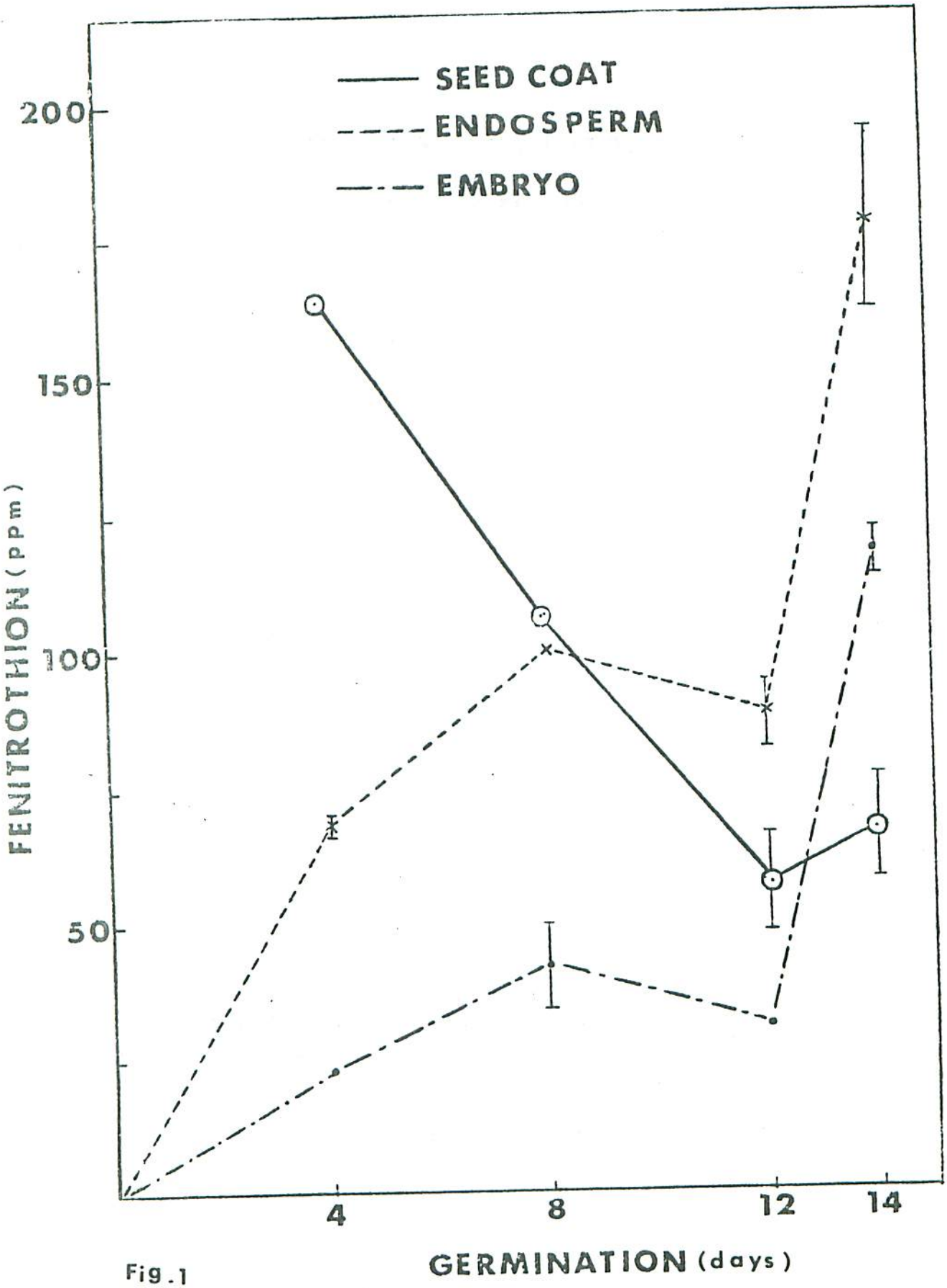


Fig.1

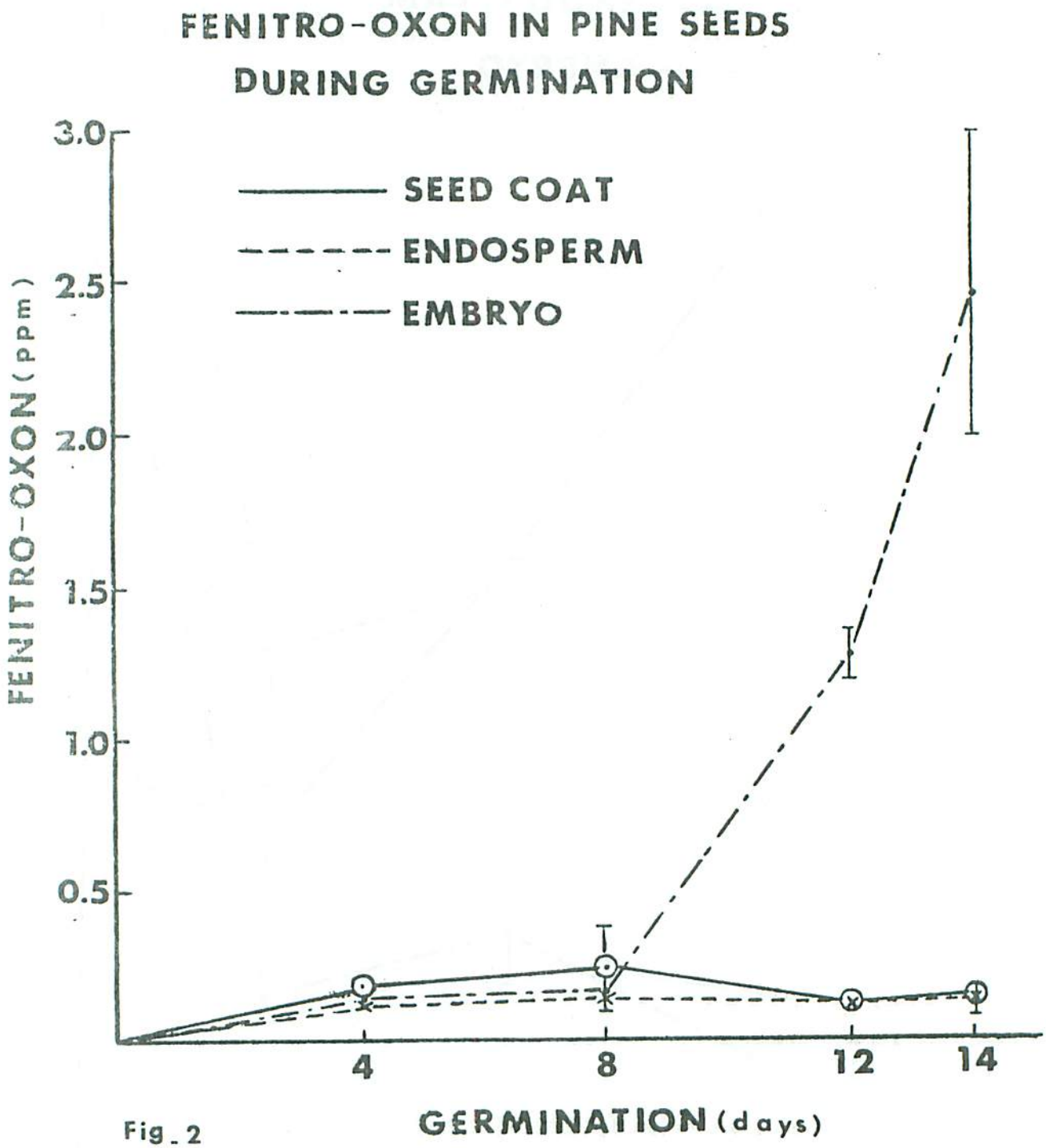

FENITRO-OXON IN PINE SEEDS
DURING GERMINATION
SEED COAT
ENDOSPERM
EMBRYO
FENITRO-OXON (ppm)
3.0
2.5
2.0
1.5
1.0
0.5
4
8
12
14
GERMINATION (days)

Fig. 2

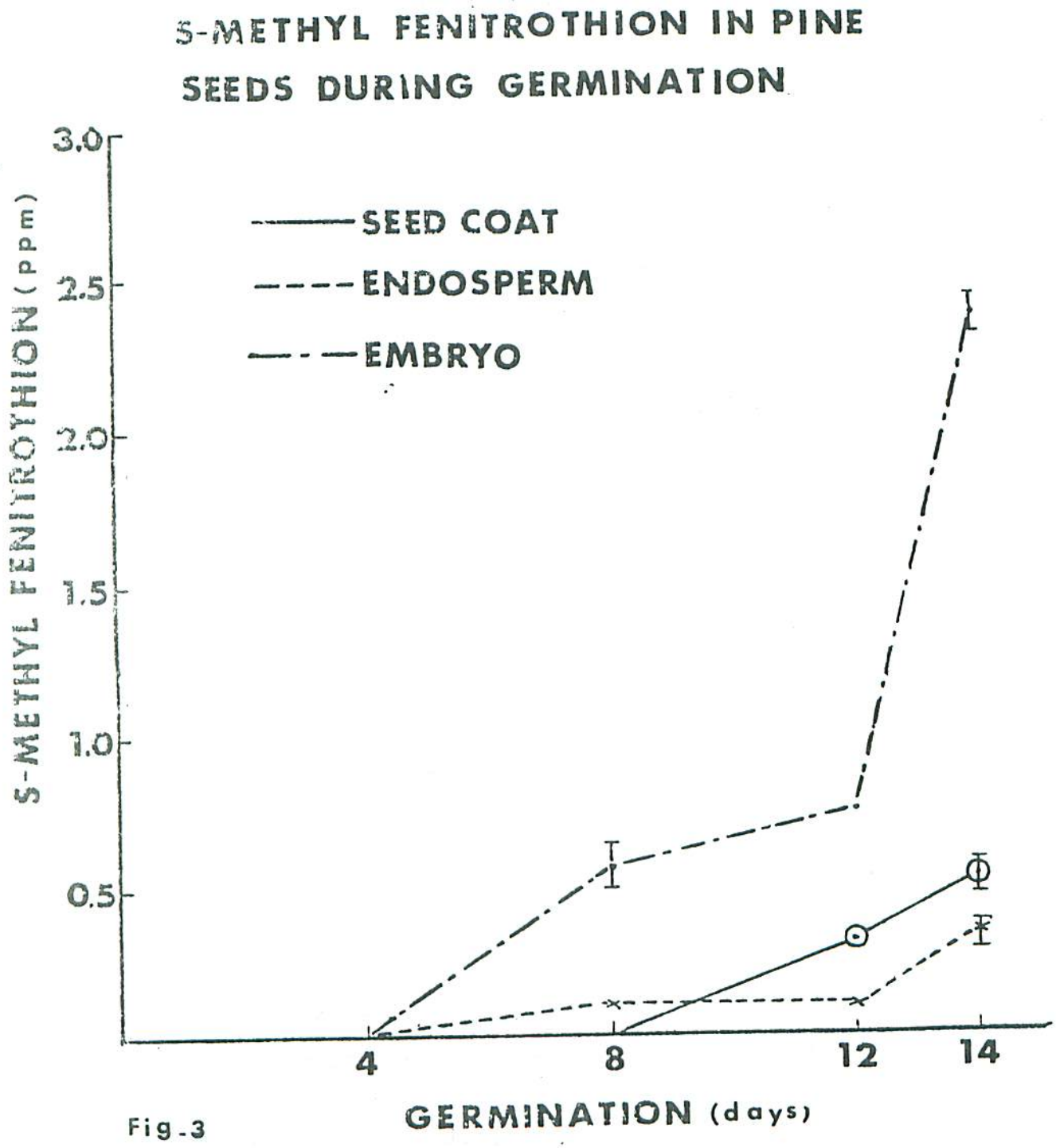

S-METHYL FENITROTHION IN PINE SEEDS DURING GERMINATION
SEED COAT
ENDOSPERM
EMBRYO
S-METHYL FENITROTHION (ppm)
3.0
2.5
2.0
1.5
1.0
0.5
4
8
12
14
GERMINATION (days)

Fig.3

DESMETHYL FENITROTHION IN PINE SEEDS DURING GERMINATION

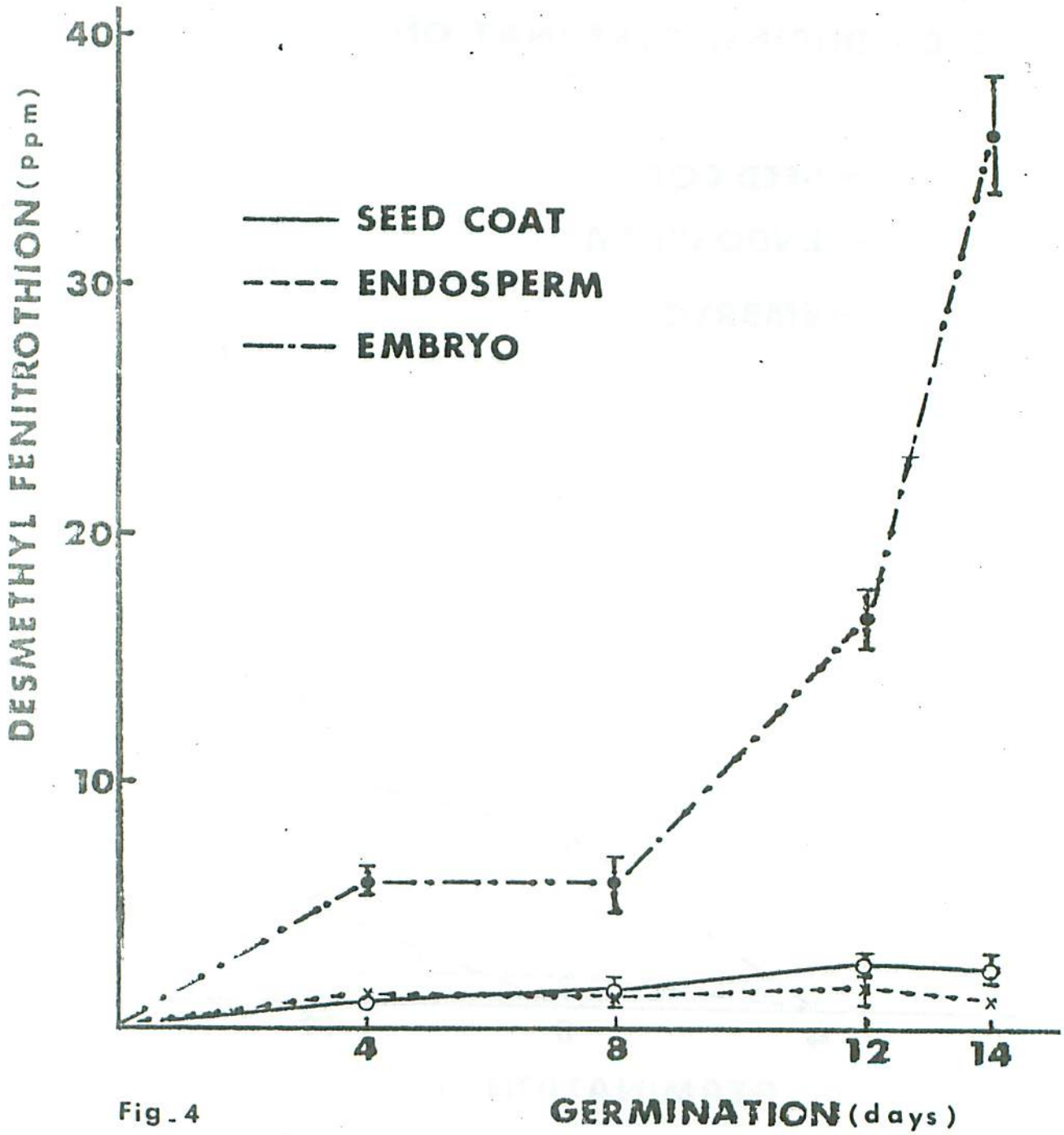


Fig. 4

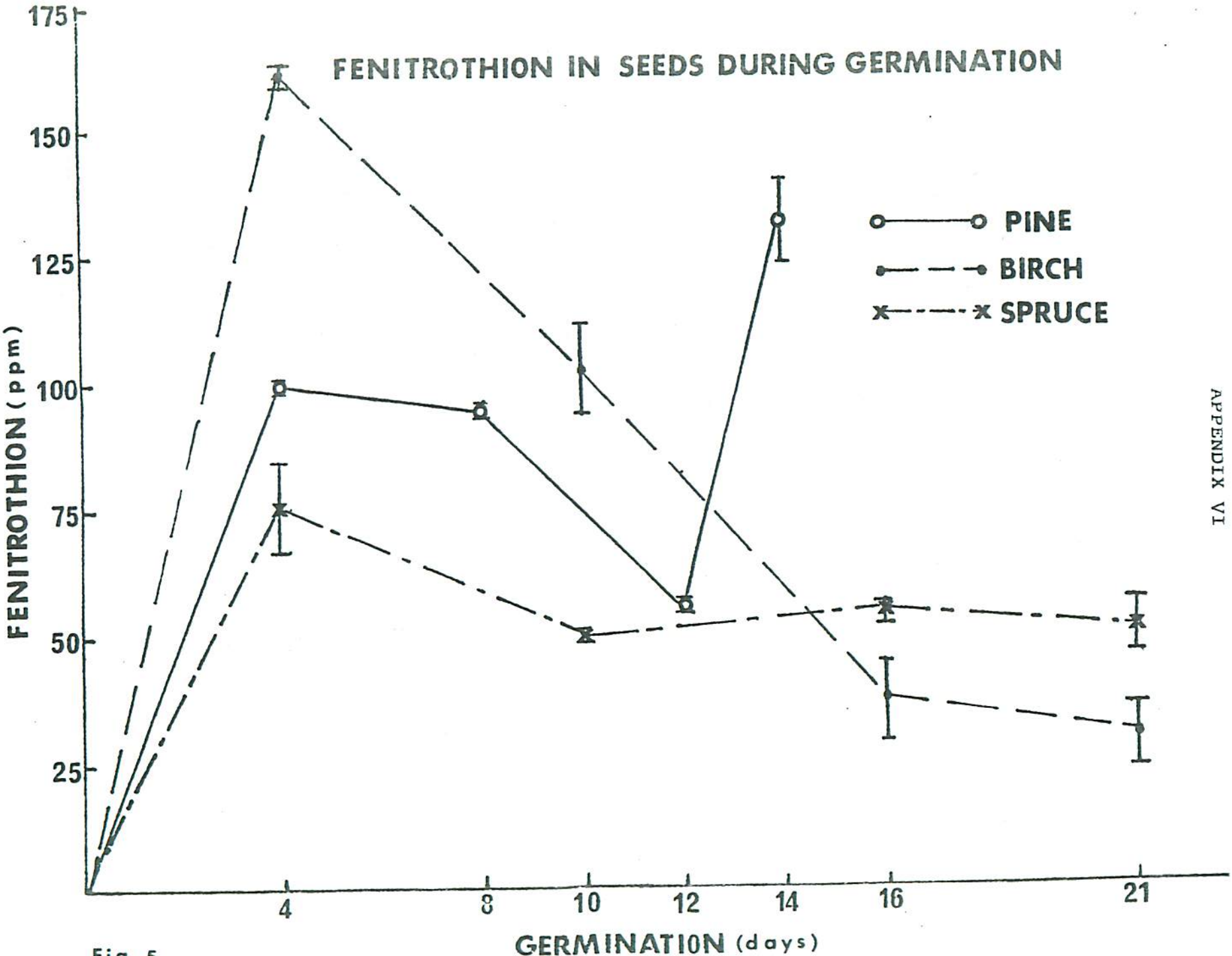


Fig. 5

APPENDIX VI

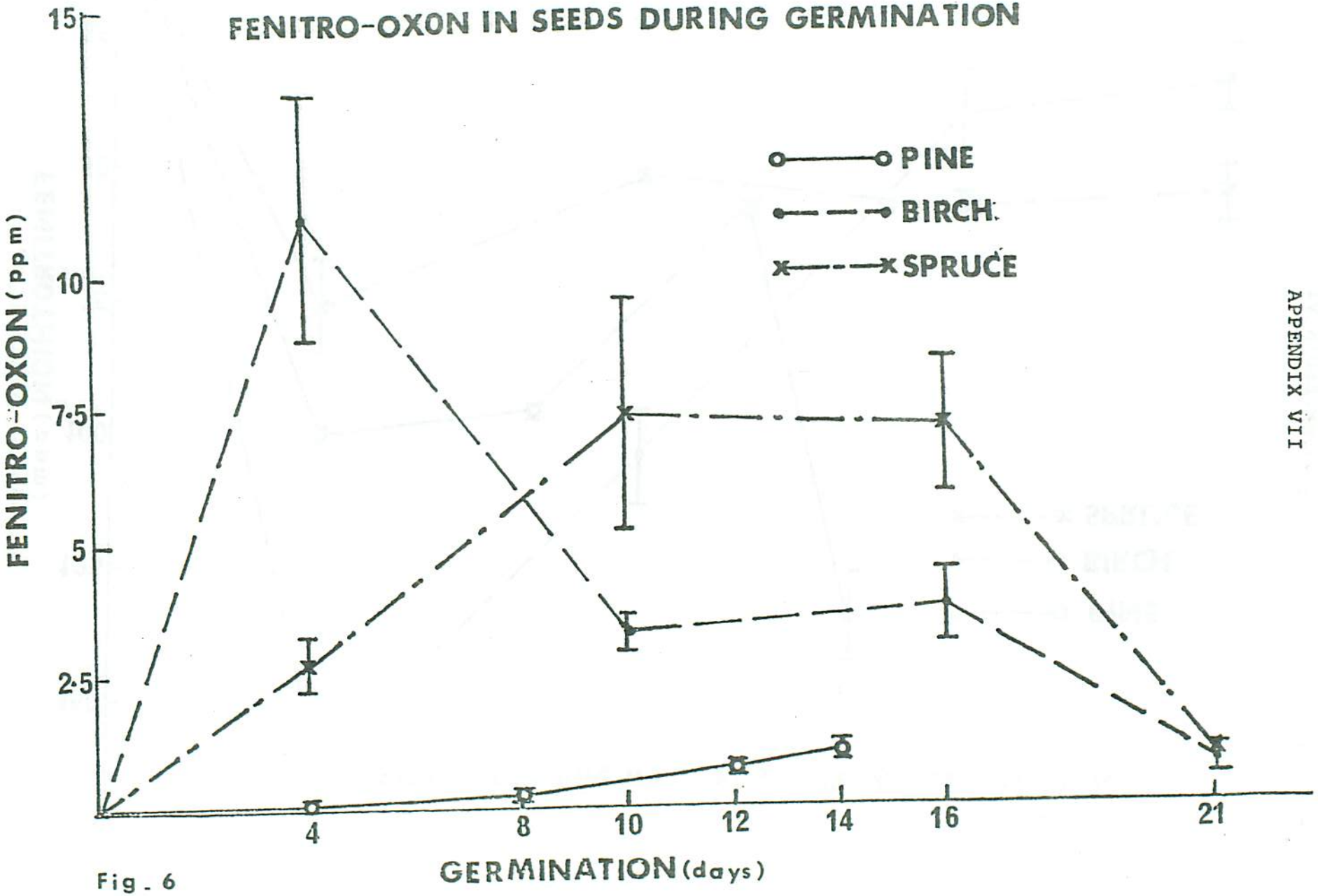

FENITRO-OXON IN SEEDS DURING GERMINATION
PINE
BIRCH
SPRUCE
FENITRO-OXON (ppm)
15
10
7·5
5
2·5
4
8
10
12
14
16
21
GERMINATION (days)

Fig. 6

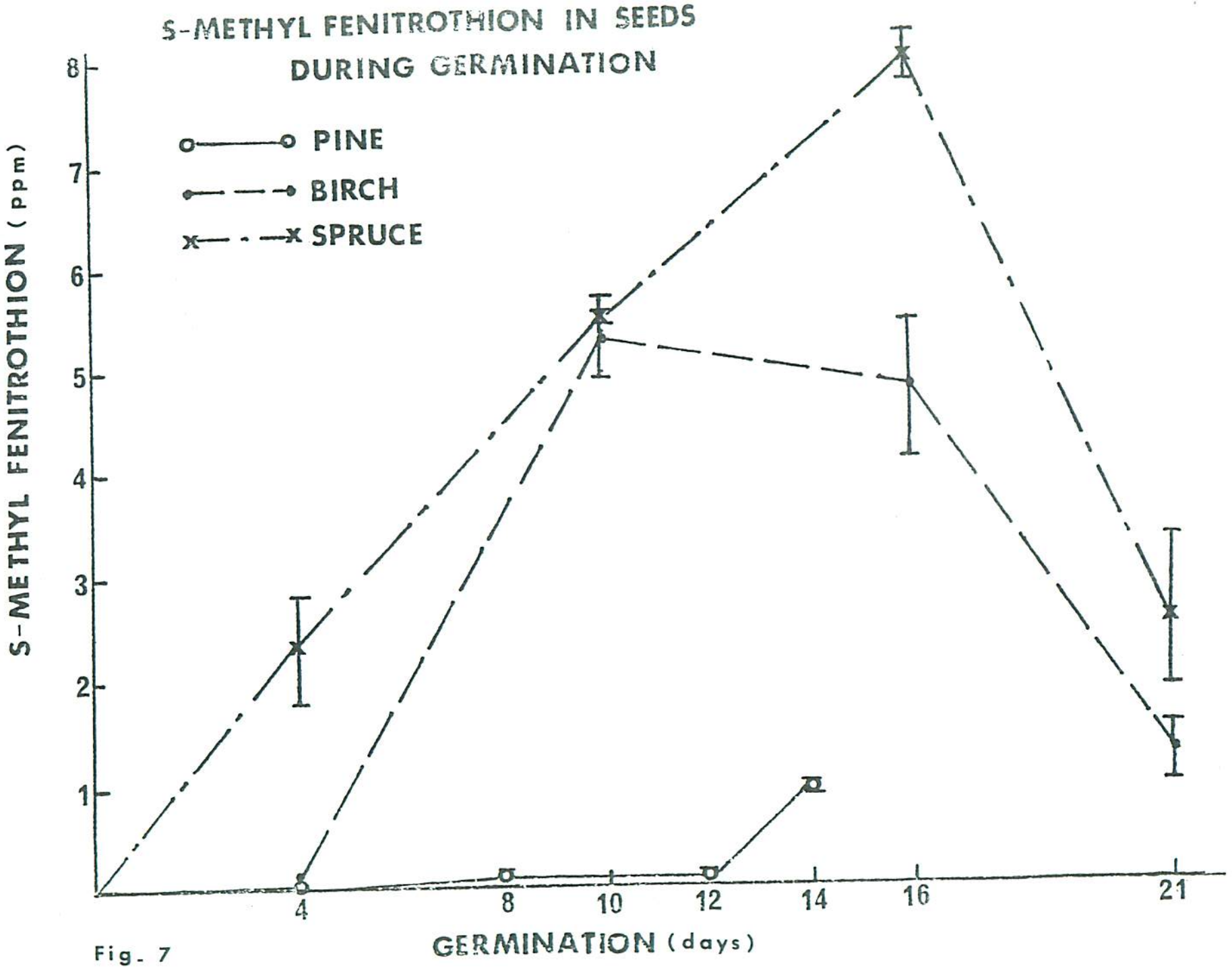

S-METHYL FENITROTHION IN SEEDS
DURING GERMINATION
PINE
BIRCH
SPRUCE
S-METHYL FENITROTHION (ppm)
1
2
3
4
5
6
7
8
4
8
10
12
14
16
21
GERMINATION (days)

Fig. 7

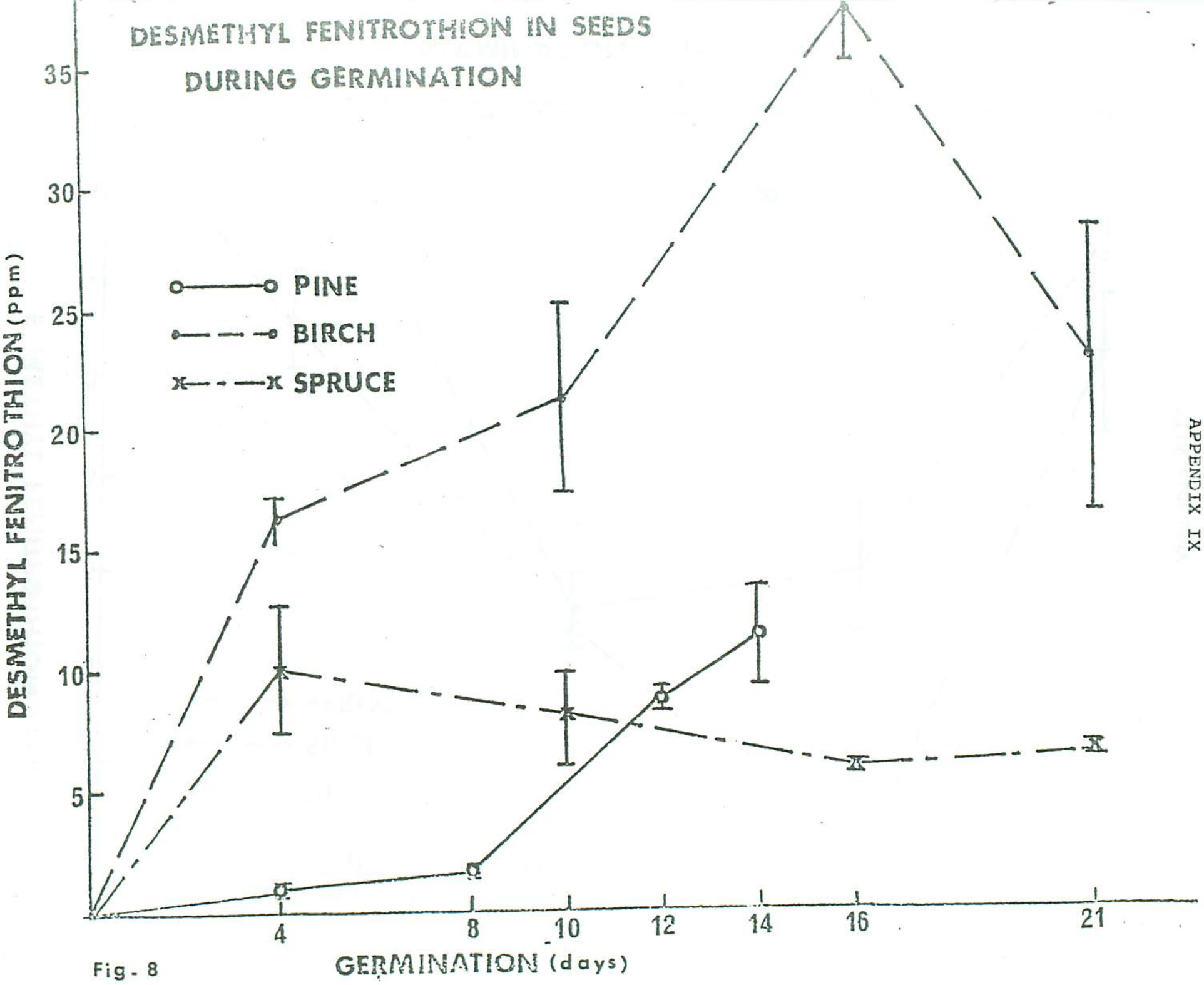

DESMETHYL FENITROTHION IN SEEDS
DURING GERMINATION
PINE
BIRCH
SPRUCE
DESMETHYL FENITROTHION (ppm)
35
30
25
20
15
10
5
4
8
10
12
14
16
21
GERMINATION (days)

Fig - 8