

A PRELIMINARY STUDY ON THE FATE OF FENITROTHION IN FOREST SEEDS

II. SOME EFFECTS ON THE MORPHOGENETIC CHARACTERISTICS  
OF GERMINATING WHITE PINE (*Pinus strobus* L.) SEEDS  
IN RELATION TO FOREST REGENERATION

by

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## INTRODUCTION

The insecticide, fenitrothion (0,0-dimethyl 0-(4-nitro-m-tolyl)-phosphorothioate, is widely used in Canada to control attacks of the spruce budworm (Fettes 1968). Two sprayings are generally carried out each spring, (early and late May) to control the feeding larva. It is, therefore, possible that during their ontogeny, conifer seeds developing in the cone and the mature and germinating seeds may be exposed to six separate sprayings of fenitrothion. Spraying concentrations are in the range of 2 to 4 ounces per acre per spray, to a total of 6 ounces, or even 9 ozs per acre per year (Armstrong 1973, Armstrong and Randall 1969, Krehm 1972).

The conifer species *Pinus strobus* L. (the Eastern white pine) is widely used in reforestation projects in North America (Maini 1972). The seeds of this species are mature and released in the fall of the second year following spring pollination. Because of the economic importance of the eastern white pine, the seeds of this species were chosen for the first phase of this study: which was to determine whether exposure to fenitrothion at field concentrations substantially alter seed germination and seedling vigor. In this connection, Hallett, Weinberger & Prasad (1973) reported that considerable quantities of fenitrothion accumulated into the embryo of the white pine seeds following treatments with a field concentration (4 ozs/acre; ca. 10 ppm) of the pesticide and the question arose whether this accumulated concentration would, in any way, affect the germination and seedling vigor. With this objective in mind, experiments were

designed to test the impacts of field sprayed concentrations of fenitrothion on seed germination and seedling vigor of the Eastern white pine.

#### MATERIALS AND METHODS

(i) Seed Samples: The seeds of the Eastern white pine were gathered from the Petawawa Forest Experiment Station in Chalk River, Ontario, during September 1972. Two types of seeds were obtained at this time; those from areas within the Station which had been sprayed extensively in previous years (S) and control seeds from regions in which fenitrothion spraying had never been carried out (C). Only seeds from cones which had fallen to the ground were gathered.

To extract the seeds, the closed cones were placed in the incubator at 28° C for approximately 36 hours and this resulted in complete separation of seeds from the cones. All seeds were then stored in tightly sealed jars in a refrigerator at 2° C until required. Prior to each experiment the seeds were dewinged by hand.

(ii) Conditions of imbibition and stratification: Like many other forest seeds, white pine seeds require prechilling for successful germination and accordingly, seeds from the control and sprayed areas were stratified for periods of 0, 21, and 28 days at the U.S.D.A. prescribed temperature of 10°C. This chilling requirement was carried out in small peat pots, watered to field capacity. Thirty-six lots of ten seeds from each treatment, (S) and (C) were germinated in the dark in Petri dishes. The Petri dishes were placed in environ-

mental growth chambers, maintained at a fluctuating temperature of 68° F for 12 hours. (U.S.D.A. specification, 1949).

(iii) Germination percentages and rates: Seeds were considered to have germinated when the radicle pierced the testa. All seeds were examined daily for visual confirmation of germination with a dissecting microscope (50X magnification). At each stratification period germination percentages and germination rates were computed by the method of Maguire (1962) for both the control and sprayed area seeds.

(iv) Index of seed and seedling vigor: After seven days the fresh and dry weights of the seedlings (radical and hypocotyl) germinated as above and stratified for 0, 14, and 28 days were determined. For the measurement of vigor of seeds, the technique of changes in the water uptake capacity during the period of germination was employed. For this, seeds from the sprayed and unsprayed areas were exposed to water and two levels of fenitrothion (10 and 1000 ppm.) for a known length of time. Germination conditions were comparable to those described previously, except that these seeds were housed in Petri dishes during stratification. The fenitrothion was obtained from the Pesticides Division of Sumitomo Chemical Company Ltd., Osaka, Japan and was tested for 99.5% purity before use.

(v) Experimental Design: A total of 105 replicates representing 1,050 morphologically similar seeds were initially used. Triplicate seed weights were obtained 7 days and 21 days after stratification. Following 4 days of germination the seedlings were reweighed to measure percent water uptake. The seeds were removed

after each weighing and used for other studies. All results were subjected to the t-test analysis. (Snedecor 1956).

### RESULTS

Seeds from all stratification periods that were not skoto (deeply) dormant germinated on or before 21 days, those which had not germinated by that time failed to do so. Seeds obtained from areas which had been previously sprayed compared to those from unsprayed areas demonstrated similar pattern of germination regardless of the length of chilling pretreatment. One exception was evident however, that of non-stratified-sprayed area seeds which had a germination percentage of 40.00 (S.E.  $\pm$  14.114); the means (Fig. 1) differed significantly from sprayed area seeds which had been stratified for 14 days (70%, S.E.  $\pm$  5.48) and also from control seeds exposed to 28 days of stratification (77.15%,  $\pm$  6.29).

The rates of germination of both seed types (C and S) differed significantly. In all seeds at the various stratification periods, with the exception of 21 days stratification, the control area seeds maintained a faster rate of germination (Figs. 2 to 6) so much so that even control seeds which were not stratified (C) began to germinate after 9 days whereas spray-area seeds (S) did not germinate until 14 days (Fig. 2). There was a general trend which seemed to indicate a consistent lag in the germinative capacity of the spray-area seeds. This difference was very clearly marked in the rates of germination (Fig. 7.) Here two major trends were evident. Firstly, there existed a significant difference (90% confidence level) in the germination

rates of unstratified control seeds (C) (rate 10.95,  $\pm$  1.29 per 1,000 seeds) and those from sprayed areas (S) (rate 5.4 S.E.  $\pm$  1.29 per 1,000 seeds). Secondly, there was an indication as if stratification increased the rate of germination to a significantly high level. For example, for the spray area seeds which were stratified for 7 days, the rate rose from 5.4 to 14.80. A similarly accelerated rate is evident in the unsprayed seeds without pretreatment as compared to those which had received 28 days of stratification.

Changes in the fresh weights (Fig. 8) of the hypocotyl axis of the seedlings also suggests two trends. With increasing length of the pretreatment period, the mean fresh weight increases in both seed types. It is also evident, that the mean fresh weights are lower in the sprayed area seedling than those from unsprayed regions. The difference between non-stratified spray-area seedlings with a mean fresh weight of 14.05, (S.E.  $\pm$  7.42 mg) when compared with the seedlings derived from 21 day stratified seeds, with a mean fresh weight 32.27 mg.,  $\pm$  4.67, was highly significant. A similar pattern was indicated in the dry weights of the root hypocotyl axis of these two sets of seedlings, (Fig. 9).

Comparable results were obtained at the termination of the stratification period (Fig. 10). At this time, seeds had been removed from the population which now consisted of a total of 930 seeds (93 replicates). The spray-area seeds had a mean water uptake of 58.45% (S.E.  $\pm$  2.04) which differed significantly from the control-area seeds (53.65%,  $\pm$  1.69) at the range of 90% confidence level. The spray-area seeds, however, did not differ significantly from those control

seeds exposed to the 10 ppm fenitrothion emulsions during imbibition. However, after 4 days of germination following the 21 days of stratification, seeds exposed to a 1000 ppm (fenitrothion solutions) did show a difference in the extent of water uptake over the control-area seeds. Also, the ability to imbibe and take up water at higher concentration (1000 ppm) was different from that of the lower concentration (10 ppm).

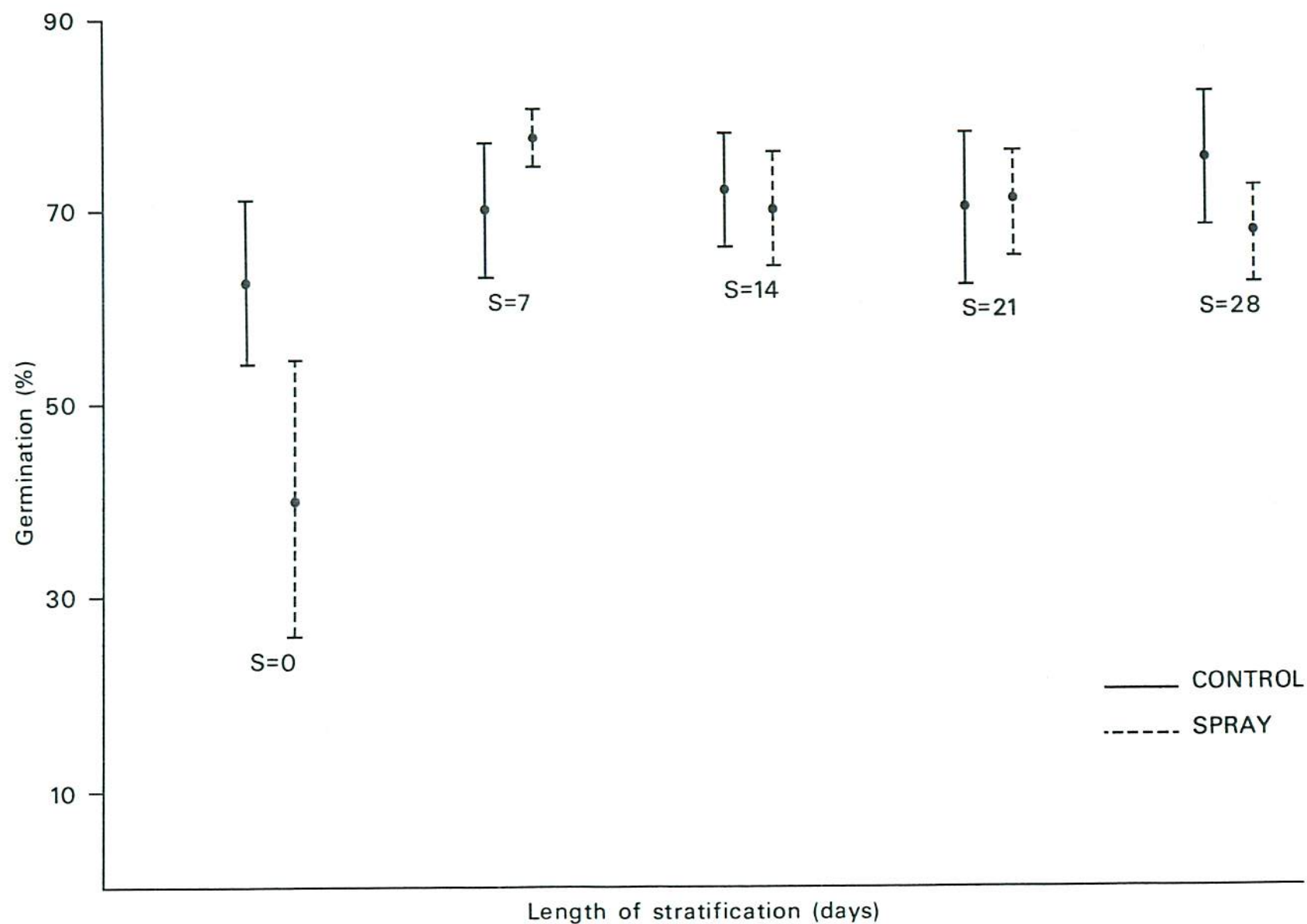


Fig. 1. Effects of various stratification periods on germination of white pine seeds obtained from Petawawa forests treated (spray) and untreated (control) with fenitrothion.

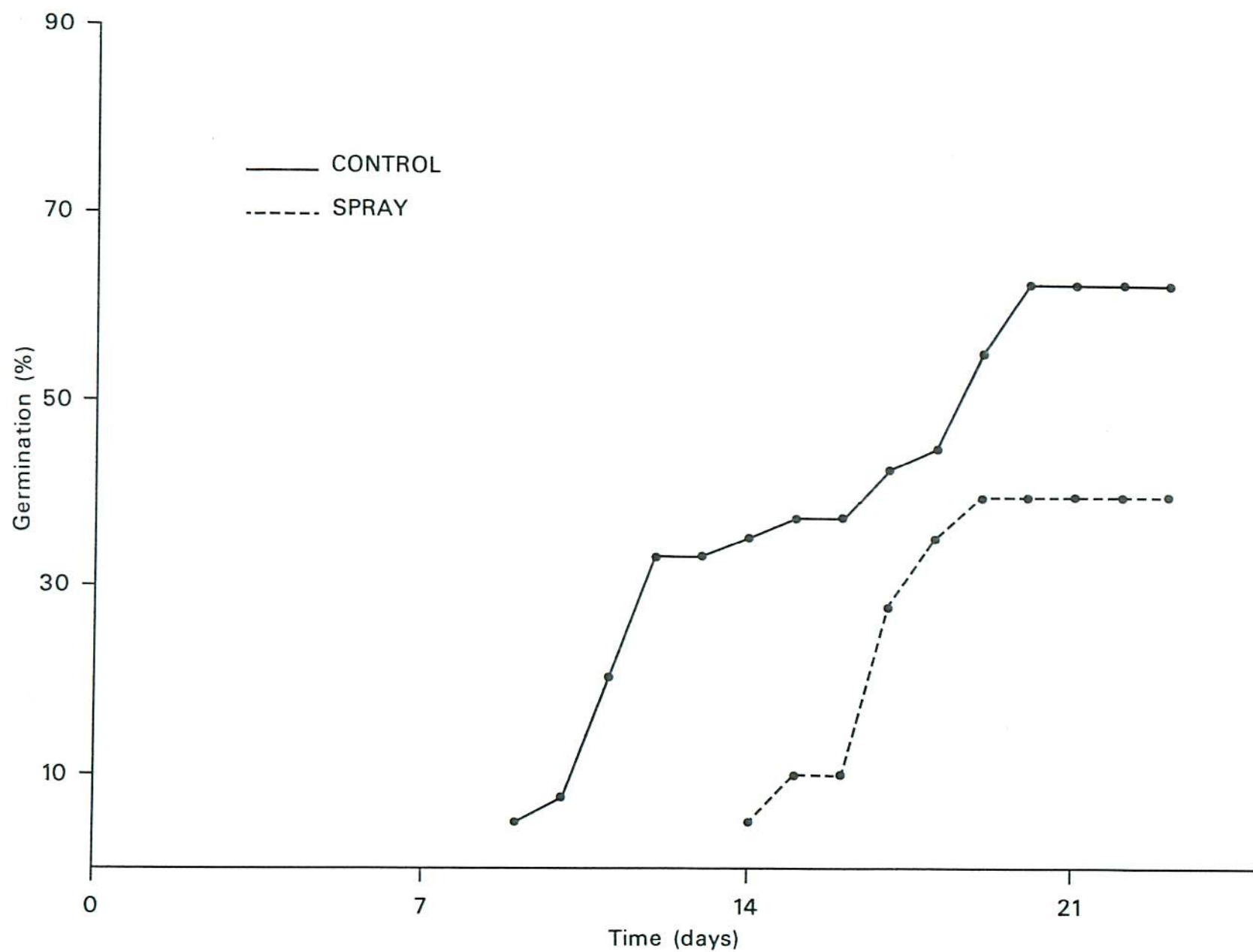


Fig. 2. Germination behaviour of white pine seeds without any stratification of forests treated (Spray) and untreated (Control) with fenitrothion. Note the lag in germination of seeds obtained from the sprayed forests.

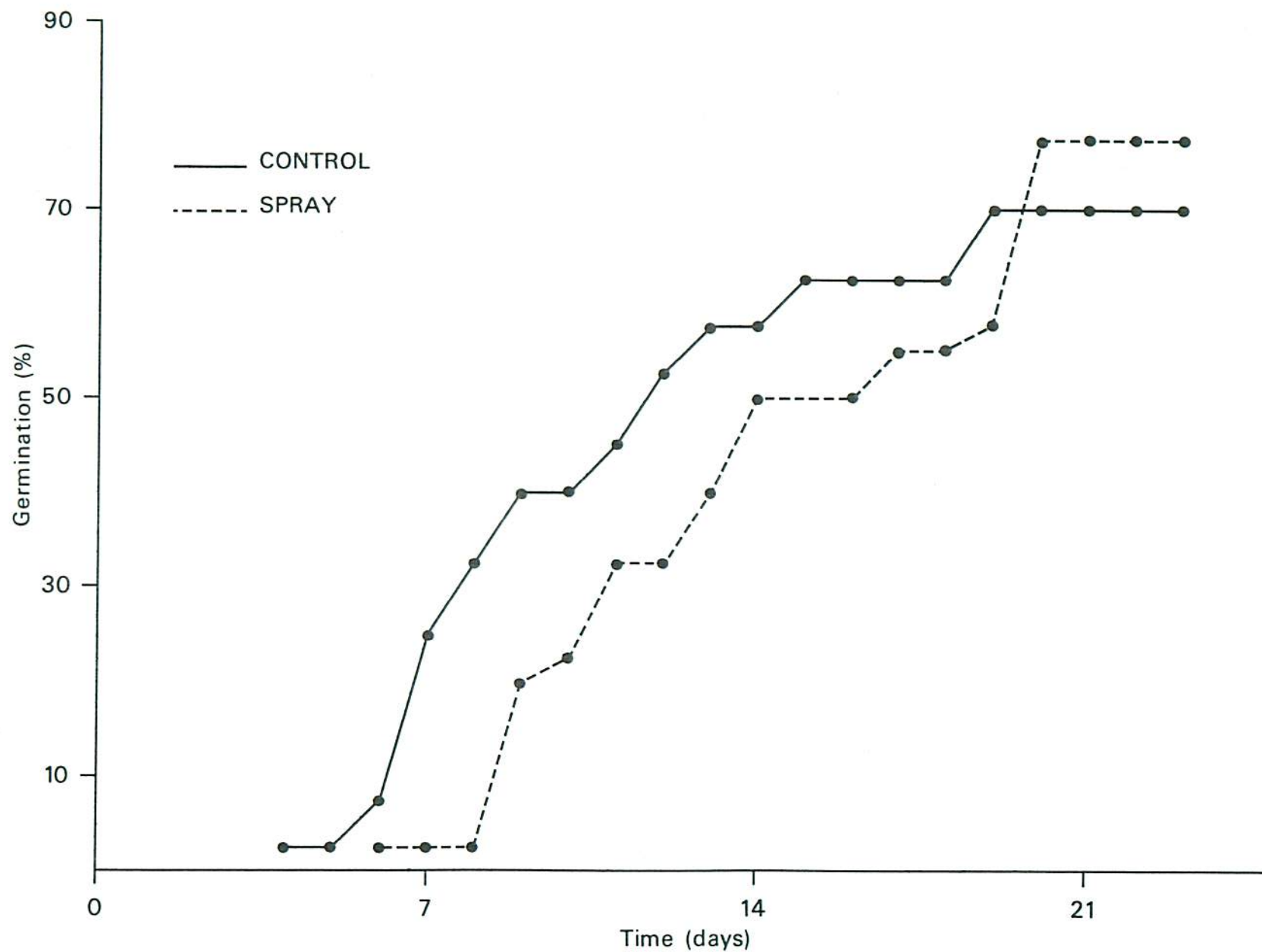


Fig. 3. Effects of 7-day stratification period on subsequent germination behaviour of pine seeds obtained from treated (Spray) and untreated (Control) forests. Note the initial lag in germination is offset later on.

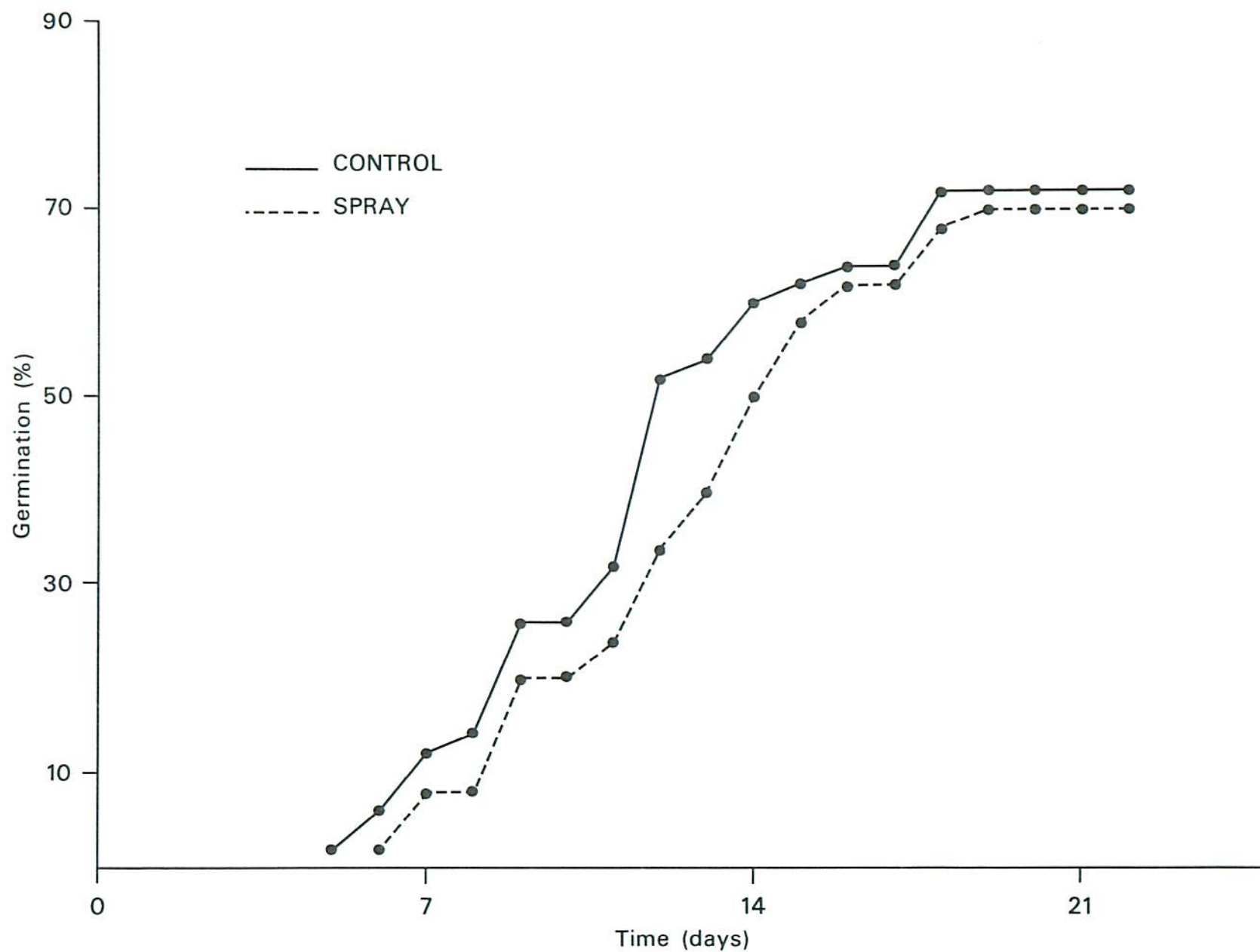


Fig. 4. Effects of 14-day stratification period on subsequent germination behaviour of fenitrothion treated (Spray) and untreated (Control) white pine seeds. Note the consistent lag in germination from the sprayed areas.

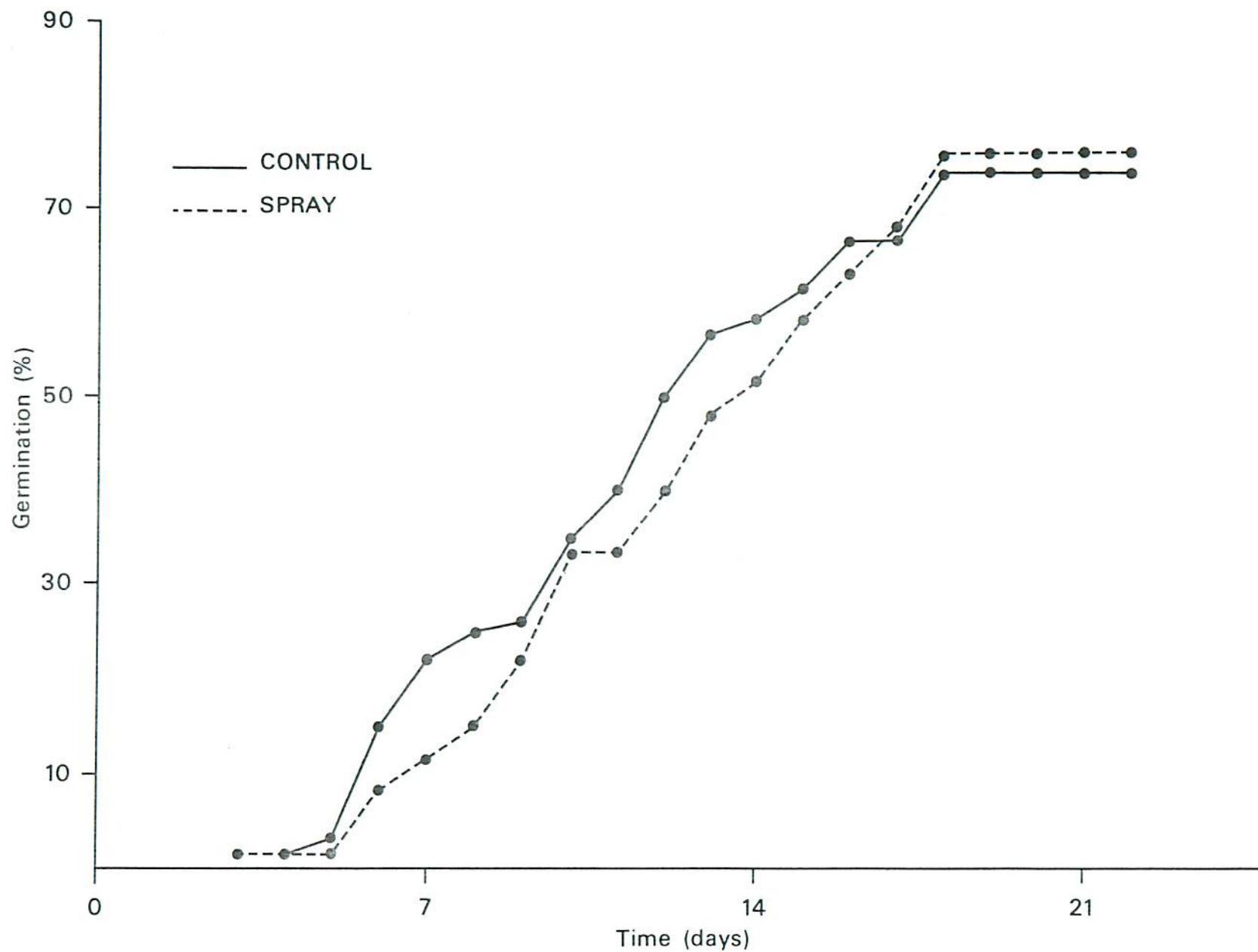


Fig. 5. Germination behaviour of white pine seeds following stratification for 21 days. Note the initial lag phase in germination from sprayed forest begins to catch up with that of the untreated (Control) forests.

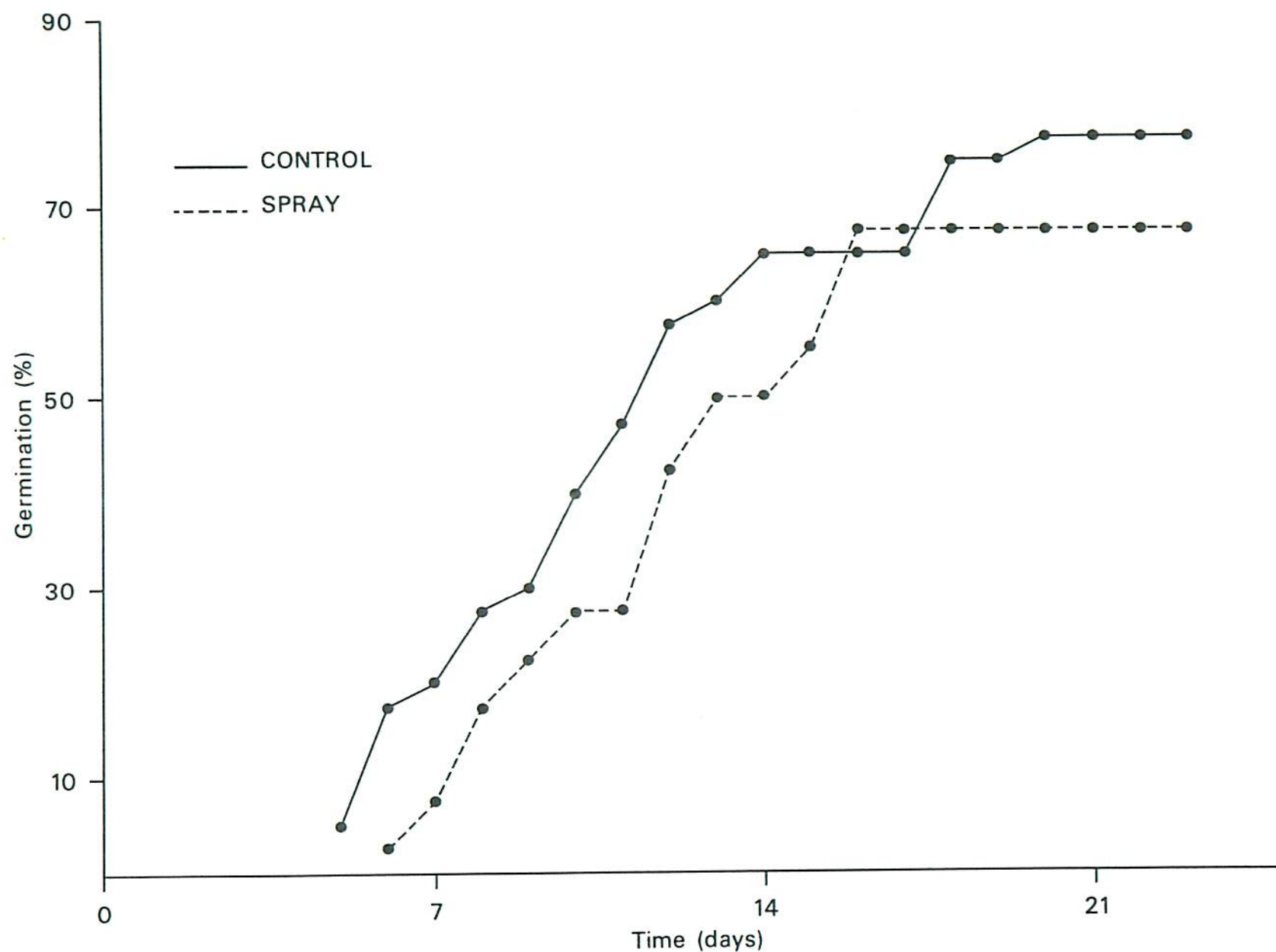


Fig. 6. Germination behaviour of white pine seeds obtained from fenitrothion treated (Spray) and untreated (Control) forests. Note stratification for 4 weeks seems to nullify the effects on germination.

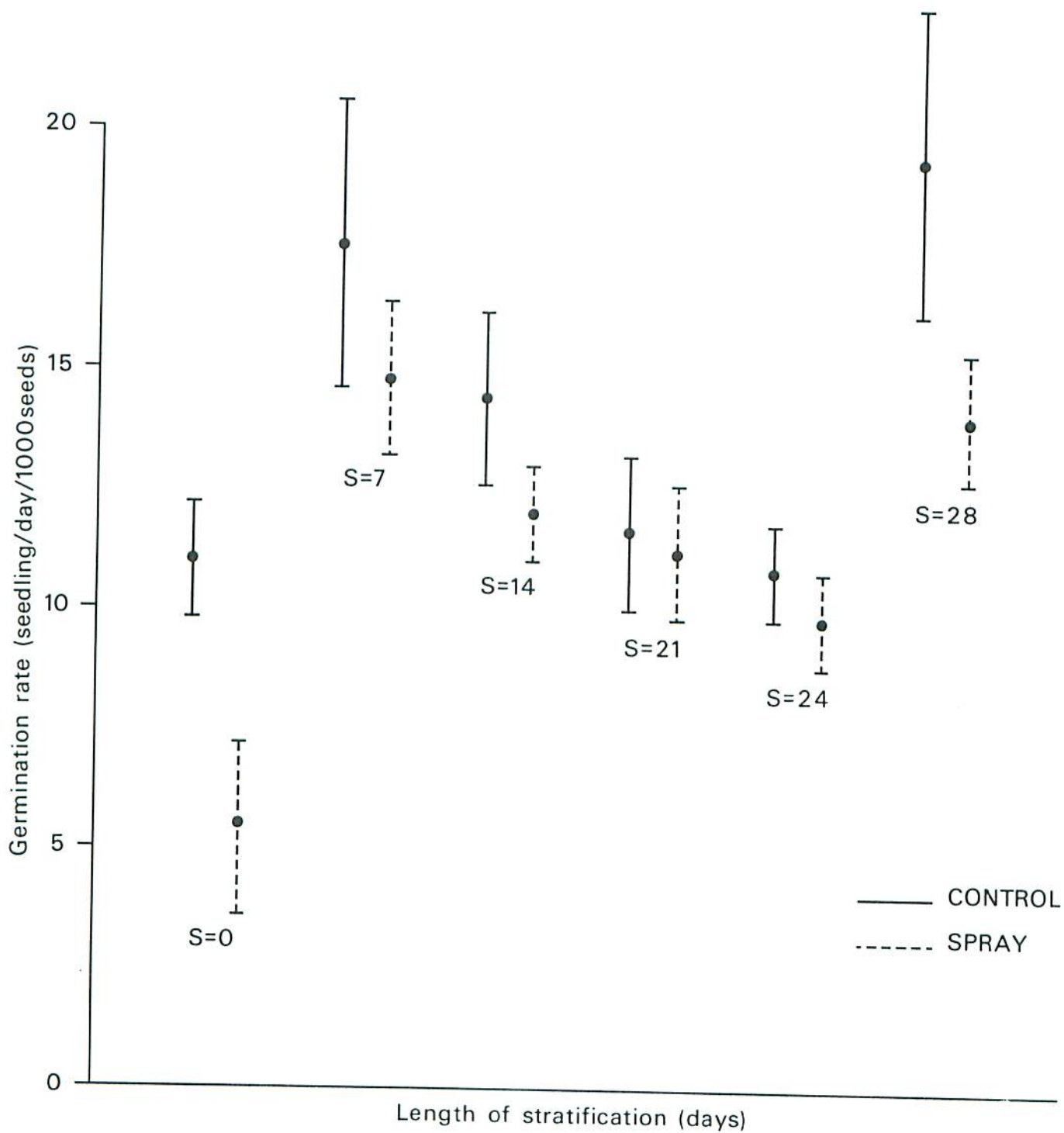


Fig. 7 Influence of stratification on the rate of germination of white pine seeds obtained from fenitrothion treated (Spray) and untreated (Control) forests. Note the marked difference before stratification.

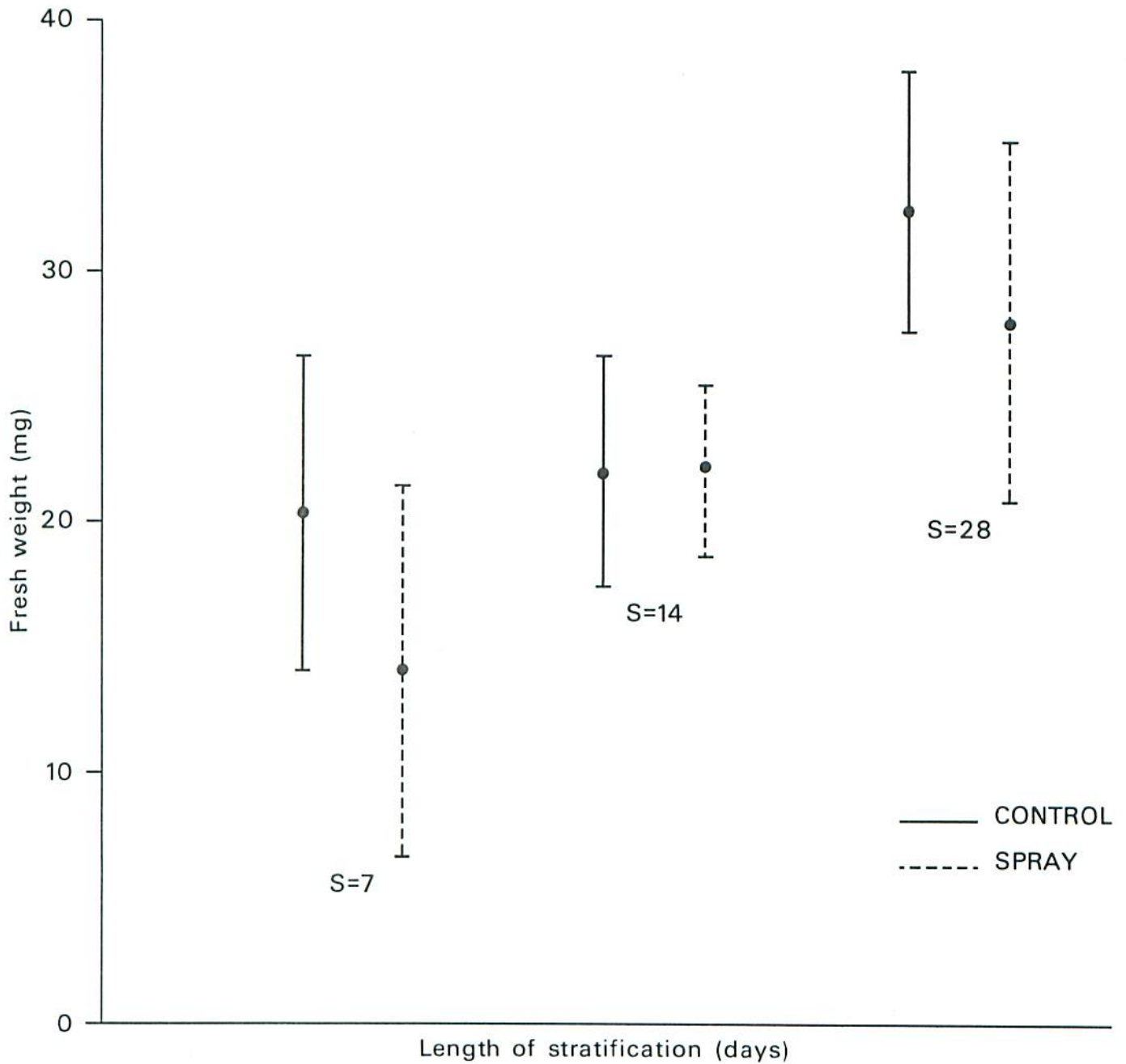


Fig. 8 Effects of stratification on fresh weight changes during germination of white pine seeds obtained from sprayed and unsprayed (Control) forest.

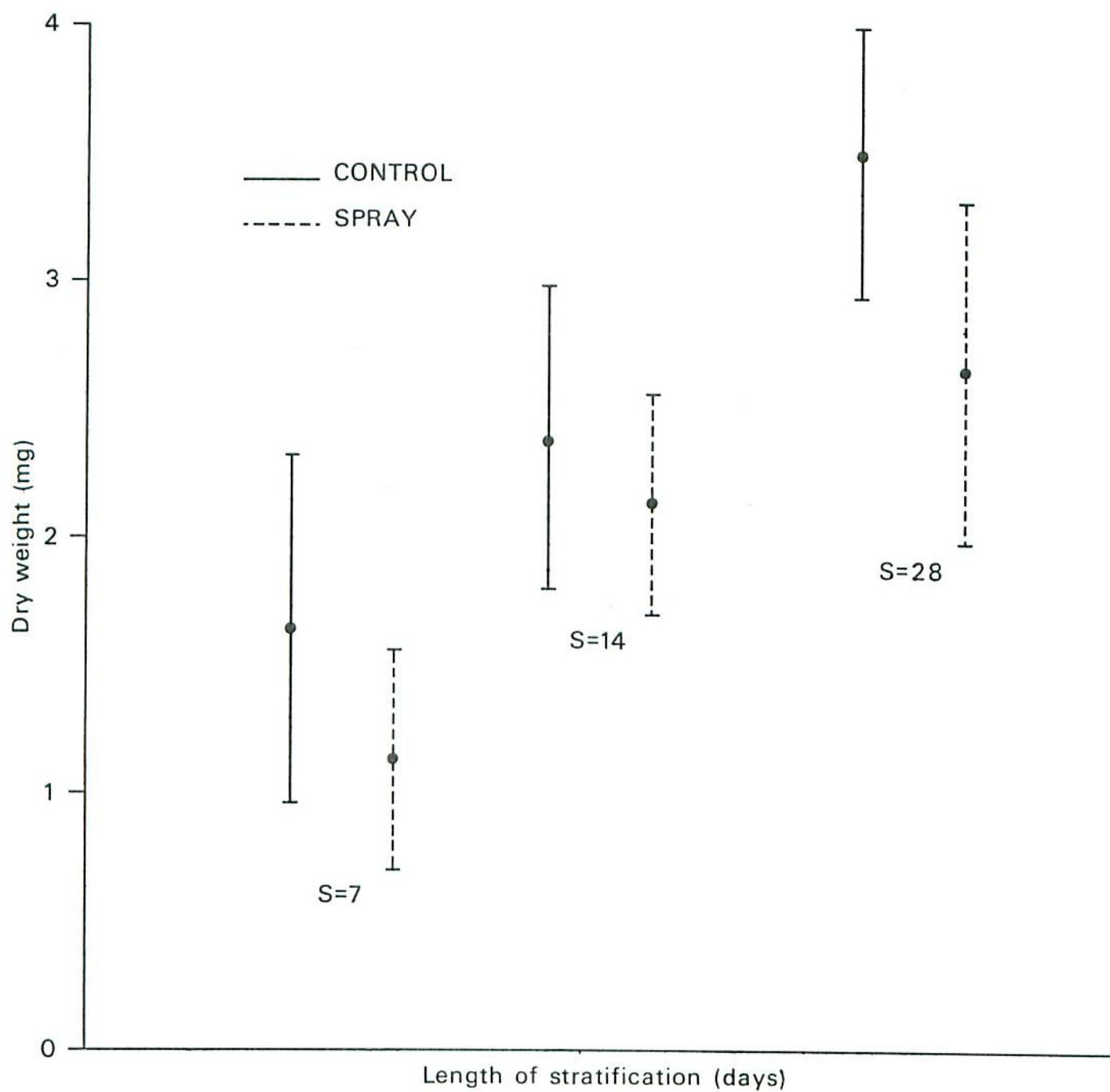


Fig. 9 Dry weight changes following germination of seeds after 28 days period of stratification. Control seeds received no fenitrothion; sprayed plots received at least 4 ozs./acre.

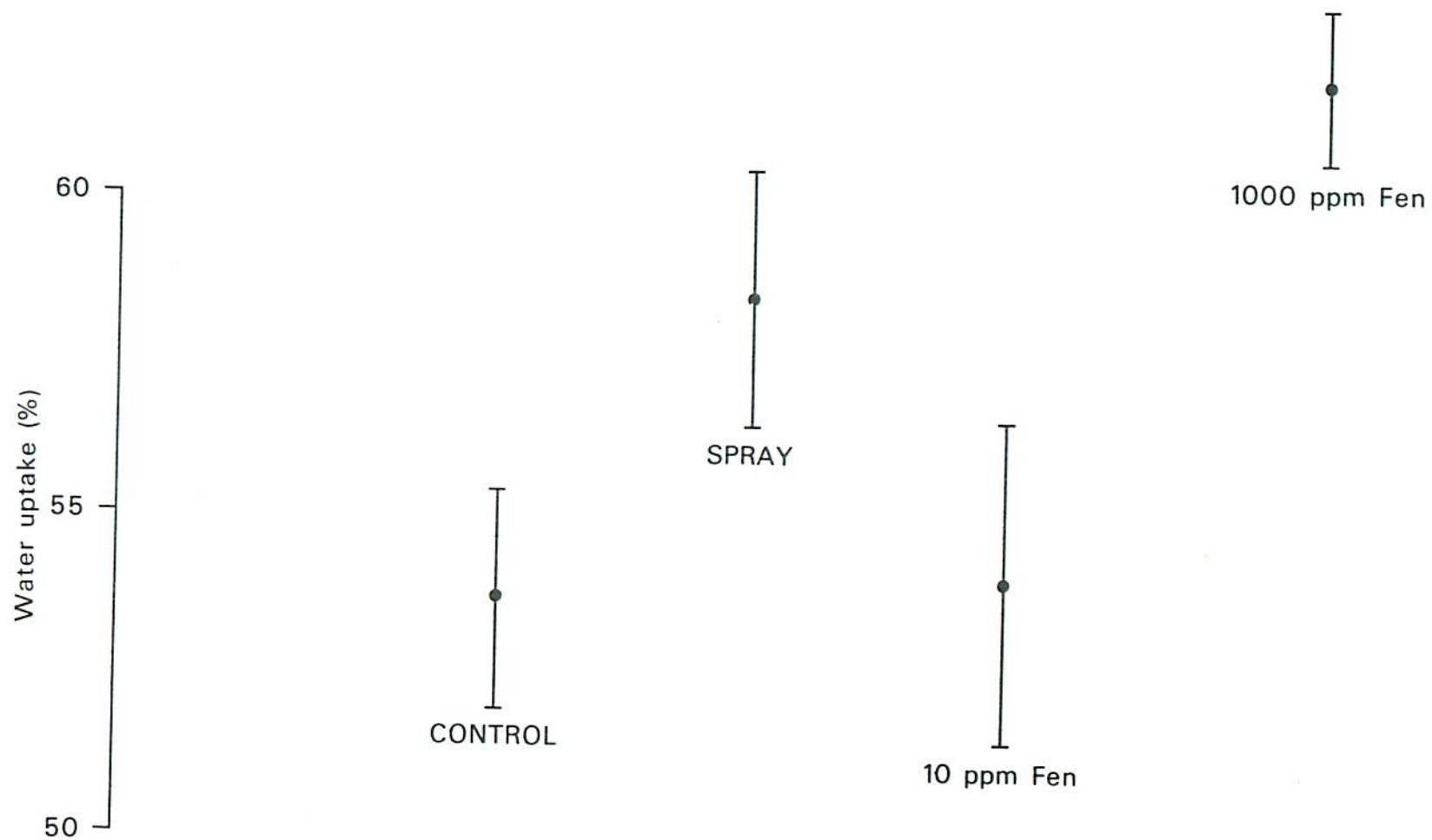


Fig. 10 Changes in water uptake of white pine seeds (stratified for 21 days) following germination for 4 days. Seeds were pre-soaked in fenitrothion emulsion of 10 and 1000 ppm.

### DISCUSSION

A number of trends seem to be evident in these preliminary studies. Firstly, stratification not only seems to increase the percentage of germination but also significantly increases the seedling weight. This information, when applied to the fresh and dry weights of the root hypocotyl axis suggests a possible dwarfism being developed in non-stratified seeds. However, long term growth studies would be required to validate this hypothesis.

Secondly, there appears to be a difference in the germination characteristics of seeds from sprayed areas as opposed to those from the unsprayed region in regard to the parameters studied. The overall germination percentage, germination rates and fresh and dry weights of the root-hypocotyl seem to lag behind those seeds from unsprayed areas. These differences became less significant with increasing the length of the stratification period to twenty one days. Generally, it was observed that the germination and growth characteristics of spray-area seeds was comparable to those of control seeds imbibed with 10 and 1000 ppm emulsion of fenitrothion. Thus it would appear that undue accumulation of fenitrothion or its breakdown product (fenitro-oxon) into the seed parts (endosperm and embryo) as reported by Hallett, Weinberger and Prasad (1973) may not be deleterious to the establishment of white pine forest. Clearly, the initial depression in germination brought about by fenitrothion is, somehow, gradually released and the seedling growth recovers from the disturbances in the amino acid metabolism as was claimed by the above investigators. In this connection, several Japanese workers (Miyamoto and Sato, 1965; Miyamoto, Sato and

Fujikawa, 1965) studied the residues of fenitrothion on cereal grains and cocoa bean seeds during storage and found no adverse effects on palability of the seeds and seed products.

Work is presently underway to determine whether the seeds and seedlings of other forest species, yellow birch (*Betula luscens\** Marsh) show comparable trends in germination capacity and seedling vigour. Enzymatic studies and electron microscopy will complement these studies.

#### SUMMARY AND CONCLUSIONS

Seeds of Eastern white pine (*Pinus strobus* L.) collected from sprayed (with fenitrothion) areas of the Petawawa Forest Experiment Station and tested for germination behaviour under the laboratory conditions, exhibit a general lag in the germinative capacity. The stratification characteristics and seedling vigour are also altered by the spray application but on a long term basis their impact does not appear to be of great consequence to either regeneration or re-establishment of the Eastern white pine.

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\* Also known as *Betula alleghaniensis* Britt. (Maini, 1968)

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