Effect of Thiram on Germination of Douglas-fir Seed

W. J. Bloomberg and J. Trelawny

Forest Pathologist and Technician, respectively, Forest Research Laboratory, Canadian Forestry Service, Department of Fisheries and Forestry, Victoria, British Columbia.

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ABSTRACT

In low viability Douglas-fir seeds, treatment with 25% by wt thiram killed 12% of the seeds and delayed germination of the remainder. Germination of treated seeds was further delayed if seed coats were injured. An interaction between thiram treatment and stratification treatment affected germination percentage. In seeds with high viability, germination percentage was not affected, but germination was increasingly delayed by greater thiram dosage. Germination was further delayed if seeds were treated before stratification rather than after. Emergence percentage in nursery beds was lower than germination percentage; it was increased by 12.5 and 25% thiram treatment, but not by 50% treatment. Emergence failure was due equally to disease and inhibition of germination. Significant correlation occurred between emergence percentage and emergence speed. The correlations for treated and untreated seeds had the same slopes but differed in elevation. An interaction between seed density and thiram dosage affected emergence percentage in nursery beds. Phytopathology 60:1111-1116.

Although thiram (tetramethylthiuram disulphide) has been widely used as a fungicidal protectant for forest tree seeds (22), it has sometimes produced detrimental effects; e.g., reduction of germination in spruce and pine (10, 21) and in Douglas-fir (11, 18, 19), delayed germination in pine (7, 17), reduction of respiration in spruce germinants (15), and deformation of pine germinants (14). These effects are serious enough to create doubts as to the usefulness of the treatment, and indicate a need for greater understanding of them.

Little is known about the importance of seed condition in thiram treatment of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), although Allen (1) showed that viability and seed-coat injury had great influence on germination and vigor. He also showed that stratification treatment was important. Furthermore, little is known about the effect of sowing density of thiram-treated seeds in nursery beds, although this must influence the concn of thiram in the soil.

The investigation reported herein was designed to evaluate the above factors and their interactions for their effects on germination, emergence, phytoprotection, and phytotoxicity.

MATERIALS AND METHODS.—One seed lot of Douglas-fir obtained from cold storage had low viability (germination 50%), and about 40% of the seeds had cracked coats; another lot had high viability (germination 74%), and about 10% of the seeds had cracked coats. The cracks, detected by microscopic examination, were attributed to the seed extraction process.

Stratification treatment consisted of soaking the seeds in water for 24 hr, surface-drying, then storing in a loosely-closed jar at 2 C for 21 days (2). Unstratified seeds were soaked in water for 0.5 hr, then surface-dried.

Thiram treatment consisted of tumbling seeds in a rotating flask with 1% methyl cellulose sticker (Dow Chemical Co., Methocel), followed by thiram (E.I. Dupont de Nemours, Arasan, 75% active ingredient, WP) dosages of 12.5, 25, or 50% wt of thiram to wt of seeds. Some seeds received no treatment, or methyl cellulose alone.

Germination tests consisted of sowing 50 seeds in a sterile petri plate containing moistened Sponge Rok (Paramount Perlite Co.). Plates were placed in sterile polyethylene bags and incubated in darkness at 25 C for 30 days. Tests were replicated four times. Germination was defined as the production of a radicle at least 5 mm long. At the end of the test, ungerminated seeds were dissected and examined microscopically. They were classed as morphologically normal, diseased, or empty. Seeds were considered diseased if they were discolored, had lesions, or were decayed. Tissue from within diseased seeds was placed on pimaricin agar (12) to detect phycomycetes, and on 2% malt extract agar for other fungi. Squash preparations of diseased seeds were stained with cotton blue and examined for fungi. Embryos of normal nongerminants were tested for viability by tetrazolium chloride (6). Germination, disease, and normal nongerminants were expressed as percentages of germinable seeds (total number of seeds minus empty ones). The germination speed was calculated as the number of days of incubation required for 50% of germinable seeds to germinate.

Seedling emergence was tested in nursery beds filled with 20 cm crushed rock topped with 15 cm nursery soil. The beds ($0.9 \times 4.8 \times 0.37$ m) were partitioned into two blocks of 48 plots, 0.9×0.6 m. The soil was maintained in a moist condition. Soil temp, measured by Colman thermistors (8) inserted at 2.5-cm depth, varied throughout the experiment from 17.5 to 35 C with a mean of 28 C. Seeds of the high viability lot were stratified, then treated with thiram or methyl cellulose, or left untreated. They were broadcast in the plots at seed densities of 20, 40, 60, 80, 100, and 120 seeds/ft^2 (215, 430, 645, 860, 1,075, and 1,290 seeds/m^2). Emergence (appearance of any part of the seedling aboveground) was recorded daily. Seven weeks after sowing, seeds were recovered from the soil in selected plots (4), classified, and examined for fungi. Emergence, disease, and nonemergence were expressed as percentages of germinable seeds. Emergence speed was expressed as the number of days required for 50% of germinable seeds to emerge. Seedling emergence was also tested at two nurseries in plots 1 ft^2 using several seed lots. Seeds were stratified, then treated with 12.5%

thiram and broadcast in the plots at seed densities ranging from 30 to 125 seeds/ft^2.

Data were analyzed in original and transformed form. Tables and graphs show original data. All differences cited were significant at the 95% level or higher by Duncan's multiple range test unless otherwise stated.

RESULTS AND DISCUSSION.—In seeds from the low viability lot, germination percentage of unstratified seeds but not of stratified was reduced by 25% thiram treatment (Table 1). Apparently, stratification killed the thiram-sensitive seeds. In this respect, thiram seems to act like stratification in killing low viability seeds (1). Germination percentage was not related to seed-coat injury. Untreated seeds germinated earlier than those treated with thiram (Fig. 1-A). Seeds that were injured, stratified, and treated with thiram germinated last, whereas those that were injured, stratified, and untreated germinated first. Evidently, while seed-coat injury did not enhance the effect of thiram on germination percentage, it did so on germination speed. Stratification may increase penetration of thiram through injured seed coat because it causes the seed to swell and its coat to split.

Disease was increased by stratification but reduced by thiram (Table 1). Seed-coat injury increased disease in thiram-treated seeds only, suggesting that thiram prevented seed-coat fungi from penetrating the seeds, but did not affect fungi which had already penetrated the seeds through cracks in the coat. Disease was unrelated to germination percentage, and was confined mainly to seeds that were not treated with thiram. Normal nongerminants, though protected by thiram, were all nonviable as shown by tetrazolium test. Thus, disease appeared to be confined to non- or poorly viable seeds (5). Fungi in diseased seeds were mainly *Aspergillus* spp., *Trichoderma viride*, and *Penicillium* spp.

Germination percentage of the stratified high viability seeds averaged 74% and was not affected by thiram, whether applied before stratification or after. Methyl cellulose alone increased germination by 2.2%. Germination speed decreased with increasing thiram dosage (Table 2), and was also reduced when thiram was applied to seeds before stratification, in effect causing seeds to be in contact with thiram for 21 days longer than those treated after stratification. The effect was least at the 50% dosage, which may have been sufficient to supersede partly the effect of longer contact.

Daily germination increased abruptly, then decreased abruptly, in untreated seeds (Fig. 1-B). The increase and decrease became less abrupt as thiram dosage increased. This change in distribution could only occur because thiram selectively delayed germination of individuals with the potential for early germination; i.e., stratified injured seeds (1) (Fig. 1-A). If, on the other hand, it equally delayed all germinants, the distribution would not change shape but would merely be shifted to the right along the time axis. Thus, it is reasonable to attribute thiram-effect to penetration through seed-coat cracks, thus selectively delaying the germination of otherwise early germinants.

Emergence percentage from seeds of the high viability lot sown in nursery soil was less than germination percentage (Table 3). It was increased by 12.5 and 25% thiram dosages, but not by 50% dosage or by methyl cellulose. Emergence failure of untreated seeds appeared to be due mainly to disease, whereas failure in thiram-treated seeds appeared to be due equally to disease and inhibition. Over 90% of the diseased seeds were infected by *Fusarium oxysporum* Schlecht., a proven pathogen of Douglas fir germinants (3, 20). All inhibited seeds were viable by tetrazolium test.

Emergence speed decreased as thiram dosage increased (Table 3). It was much slower than germination speed in corresponding treatments, and the range between replicates was greater, but changes in the shape of emergence distribution as dosage increased were similar (Fig. 2-A). Distributions in individual plots within a treatment varied in height but not in width (Fig. 2-B).

There was a significant negative, linear correlation between emergence percentage and emergence speed within the plots of each treatment (Fig. 3-A), except the 12.5% in which it was obscured by sampling variation. The correlation slope was the same for treated and untreated plots, but the 25% slope had a higher elevation than the untreated slope. The slope of the 50% treatment overlapped that of the 25% treatment, and was displaced to the right of it.

Although this correlation could have arisen in several ways, the most logical explanation is that *F. oxysporum* selectively killed or thiram selectively inhibited the earliest germinants so that the germination speed, and thus the emergence speed, of the surviving population was slower. The greater the proportion of the earlier

TABLE 1. Effect of stratification, seed-coat injury, and 25% by wt thiram treatment on percentage germination and disease of Douglas-fir seeds

		Germination		Nongerminants			
				Diseased		Normal	
Pretreatment	Seed-coat condition	Thiram, 25%	No thiram	Thiram, 25%	No thiram	Thiram, 25%	No thiram
Unstratified	Injured	38.5 ax[a]	50.5 ay	18.5 ax[a]	49.5 ay	43.0 ax[a]	0 ay
	Uninjured	39.0 ax	51.8 ay	8.9 bx	48.2 ay	52.1 ax	0 ay
Stratified	Injured	21.1 bx	23.2 bx	29.7 cx	76.8 by	49.2 ax	0 ay
	Uninjured	17.5 bx	24.3 bx	19.7 ax	71.8 by	62.8 ax	3.9 by

[a] Means of seed-coat condition read vertically are significantly different ($P < .05$) if followed by different letters a, b, or c. Means of thiram treatment read horizontally are significantly different if followed by different letters x or y.

germinants killed or inhibited, the fewer and later the emergents. Seed-coat injury could account for both causes of failure by the greater sensitivity to thiram and greater susceptibility to fungus pathogens (13) of injured seeds; i.e., those with potential for earliest germination.

The partial ineffectiveness of thiram as a seed protectant, even at the highest dosage, suggests that other factors contributed to increased emergence at 12.5 and 25% dosages. One possibility is that if the infective period of a disease was short, the delaying action of thiram would increase the proportion of germinants

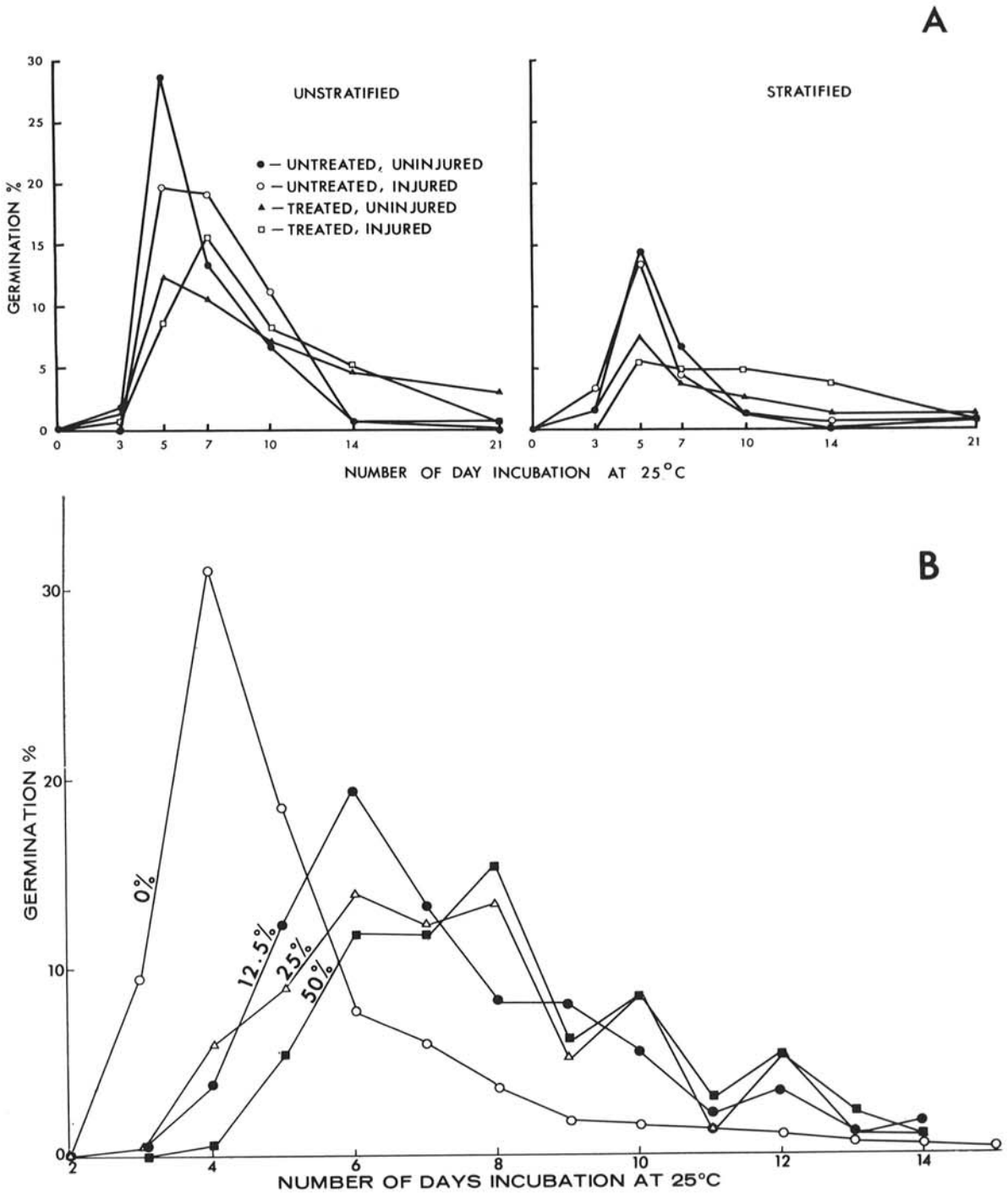

Fig. 1. Daily germination of Douglas fir seeds. **A)** The effects of stratification, seed-coat injury and thiram treatment. **B)** The effects of different thiram dosages.

TABLE 2. Days required for germination of 50% of germinable seeds treated with three different dosages of thiram applied before and after stratification

Dosage of thiram	Time of thiram application			
	Before stratification		After stratification	
%	*Avg*	*Range*	*Avg*	*Range*
0	4.1 ax[a]	1.2	3.1 ax	0.8
12.5	6.3 bx	1.8	4.1 ay	0.4
25	6.8 bx	1.2	5.1 by	1.2
50	7.4 cx	0.8	6.7 cx	0.8

[a] Dosage means read vertically are significantly ($P < .05$) different if followed by different letters a, b, or c. Application time means read horizontally are significantly different if followed by different letters x or y.

that would escape infection. There is some analogy for this situation in *Fusarium* diseases of other hosts (9, 16). The upper limit for thiram effectiveness in this respect would occur when inhibition cancelled disease reduction. Inhibition was probably an extreme form of germination delay, because the greatest incidence of both occurred at the greatest thiram dosage. Inhibition occurred in soil but not in germination tests, possibly because the higher max temp of the soil enhanced the thiram effect. Also, seed coats bearing the thiram could less easily be thrust away from germinating tissues in soil.

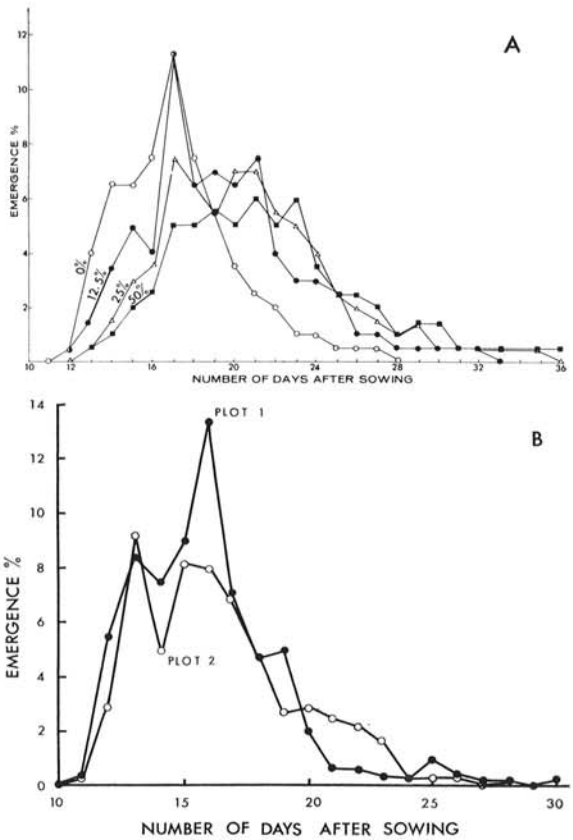

Fig. 2. Daily emergence in Douglas fir seeds. **A)** The effect of different thiram dosages. **B)** Comparison between plots with different emergence percentage and emergence speed. Plot 1, 67.0% emergence; speed = 17.9 days. Plot 2, 57.7% emergence; speed = 19.1 days.

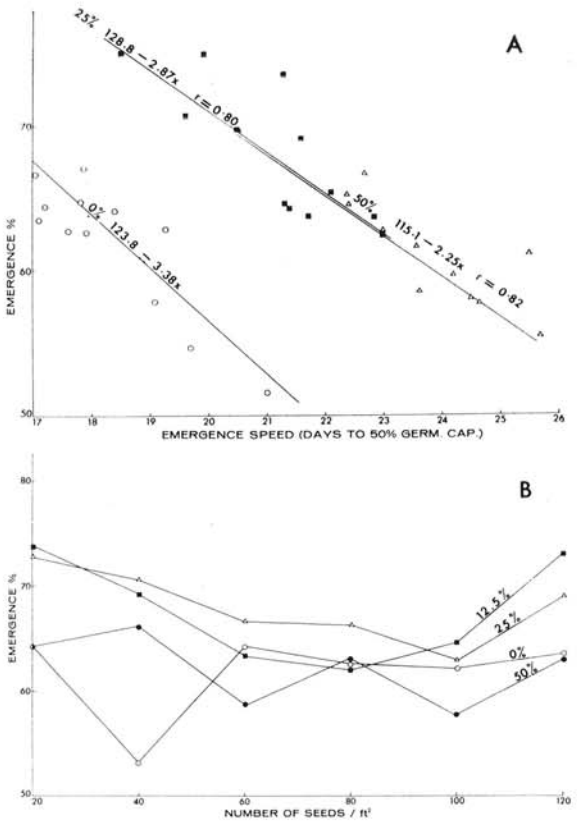

Fig. 3. Effect of thiram dosage on emergence in Douglas-fir seeds in plots. **A)** Correlations between emergence percentage and emergence speed. **B)** Interactions of thiram dosage with sowing density.

Other explanations for the correlation seem to be less probable; e.g., some germinants might have been delayed but not killed by *F. oxysporum*. The change in distribution of daily emergence that should have accompanied the decreased emergence, however, did not occur (Fig. 2-B). Germination speed might have varied by chance so that some plots had a greater proportion

TABLE 3. Percentage emergence and disease and number of days required for 50% emergence in seeds treated with three different thiram dosages

Dosage of thiram, % seed wt.	Emergence			Diseased seeds, %
	%	Days[b] Avg	Days[b] Range	
0	61.9 a[a]	18.3 a[a]	4.0	83.9 a[a]
12.5	67.9 b	20.7 b	5.9	51.0 b
25.0	68.1 b	21.1 b	4.5	
50.0	62.1 a	23.6 c	5.2	53.8 b

[a] Dosage means read vertically are significantly different ($P < .05$) if followed by different letters.

[b] Number of days required for 50% of germinable seeds to produce seedlings that emerged from the soil.

of late germinants than others and *F. oxysporum* might have selectively killed them. This seemed unlikely because random variation in germination speed between replicates was small (Table 2).

Emergence percentage of untreated seeds at different seed densities appeared to vary randomly, whereas that of seeds treated with 12.5 and 25% decreased as density increased to 100 seeds/ft², then increased at 120/ft² (Fig. 3-B). These changes could be ascribed to a thiram concn effect. Broadcasting seeds results in aggregations with increased likelihood of thiram contact with germinating tissues. The increase in emergence percentage as seed density increased from 100 to 120/ft² was accompanied by an increase in the later emergents (Fig. 4-A), suggesting an enhanced thiram effect. This may have been due to a relatively complete distribution of thiram in the soil, with a consequent reduction of *F. oxysporum*. On the other hand, 50% dosage only caused random variation in emergence percentage at different seed densities, so that it may have been great enough to inhibit germination regardless of sowing density. The interaction of seed density with 12.5% thiram treatment in several seed lots at two nurseries produced the same effect on emergence percentage as in the previous experiment, although lots showed individual differences (Fig. 4-B). The effect therefore seems to be general.

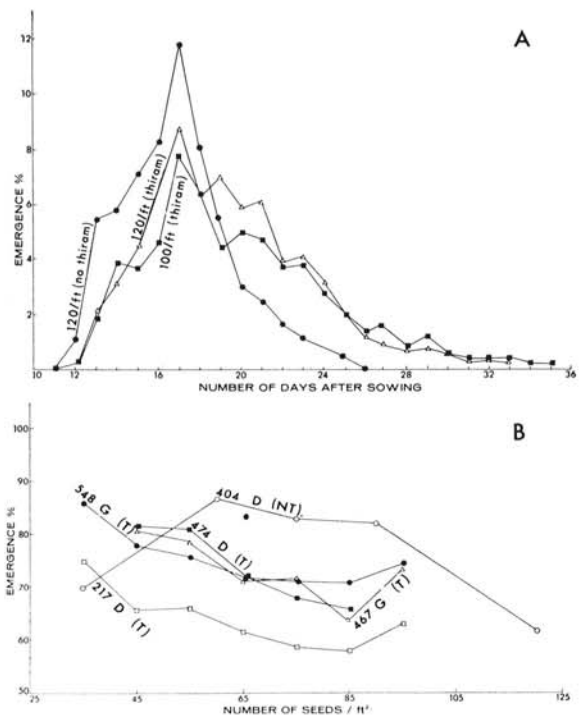

Fig. 4. Emergence in Douglas fir seeds in plots. **A)** The effects of thiram treatment and sowing density on daily emergence. Note greater emergence at 18 to 22 days in treated seeds sown at 120/ft² compared to those at 100/ft². **B)** The effects of sowing density on thiram-treated seeds and untreated seeds of different lots sown at 2 nurseries. D = Duncan nursery; G = Green Timbers nursery; (T) = 12.5% thiram-treated seeds; (NT) = untreated seeds. Numbers refer to seed lots.

Recommendations for the use of thiram on Douglas-fir seeds will necessarily be qualified by individual conditions. However, examples of a general nature can be given: (i) Seed lots with low viability are poor risks for thiram treatment. If these seeds must be stratified despite ensuing loss of germination, however, they should be treated with thiram, because little additional loss should occur due to this treatment and some fungicidal protection will be gained; (ii) Seeds with seedcoat injury probably gain more by fungicidal protection of thiram at moderate dosages than they lose by germination inhibition; (iii) Seed lots which do not need stratification, e.g., in fall sowing, if treated with thiram will probably lose more due to phytotoxicity than they would gain due to fungicidal protection; (iv) In vigorous seed lots not adversely affected by stratification, thiram dosages up to 25% seem to be beneficial with Arasan 75 formulation; (v) Aggregation of thiram-treated seeds may lead to phytotoxic concentrations in the soil.

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