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INSECT PATHOLOGY

Two Entomopoxvirus Strains Isolated from the Spruce Budworm, Choristoneura fumiferana (Clem.).—Entomopoxviruses (EPV's) have morphological similarities to vertebrate poxviruses but differ significantly in their genomic structure and protein composition (Arif, Virology 69:626-634, 1976). Moreover, EPV's are occluded in a proteinaceous mass and are transmitted in this occluded form.

EPV's were first described by Vago (J. Insect Pathol. 5:275-276, 1963) and subsequently several were isolated from Diptera, Orthoptera, Lepidoptera and Coleoptera (Bergoin and Dales, pages 169-205 in Maramorosch and Kurstak, eds., Comparative virology, Plenum Press, 1971). An EPV was isolated from the 2-yr-cycle spruce budworm Choristoneura biennis Free. by Bird (J. Invertebr. Pathol. 18:150-161, 1971); and, more recently, J.M. Burke isolated an entomopoxvirus from C. fumiferana (Clem.) that possessed some very large virus inclusion bodies (VIB's). On close examination there appeared to be two distinct populations of virus inclusions in infected larvae. The two types of inclusion bodies did not coexist in any one infected cell, and this indicated that there may be two virus strains in the original isolate from C. fumiferana. One type of VIB (strain I) is oval and larger than the other (strain II), which is more angular and produces a larger number of inclusions per cell (Fig. 1).

These virus isolates were propagated in second-instar larvae reared on artificial diet. Each diet cup received 105 VIB's and the larvae were incubated for 25-30 days. Each larva was diagnosed individually for EPV infection, and the progeny virus was passaged in additional larvae; this was done by extracting the VIB's from individual insects. The concentration was adjusted to 2 x 105 VIB's/mL and 0.5 mL was added to each diet cup. With continuous virus passage in larvae the relative amount of strain II over strain I virus increased. After 7-10 passages no strain I VIB's were detected in infected larvae; this indicated that strain II virus is more virulent. When both strains were propagated in the same insect, strain II interfered with the multiplication of strain I virus and eventually eliminated it. This is further supported by the fact that a pure strain I will always produce strain I progeny regardless of the number of passages in larvae. Likewise strain II gives rise to strain II progeny only.

The inclusion bodies of both strains were semipurified by differential centrifugation in a Sorval centrifuge, and their size was estimated from phase-contrast photomicrographs. The size of strain I VIB's, from measurements of 121 inclusions, is $7.87 \pm 0.29 \,\mu\text{m} \times 11.25 \pm 1.6 \,\mu\text{m}$; a few inclusion bodies as large as $12.2 \times 17.7 \,\mu\text{m}$ and as small as $5.4 \times 7.1 \,\mu\text{m}$ were observed. The size of strain II inclusions by comparison is much smaller. From measurement of 111 inclusions the size was found to be $3.4 \pm 0.55 \,\mu\text{m} \times 4.31 \pm 0.7 \,\mu\text{m}$; the largest and the smallest strain II inclusions were $4.8 \times 5.3 \,\mu\text{m}$ and $2.4 \times 3.4 \,\mu\text{m}$. Although there is considerable variability in the size of strain I inclusions and, to a lesser extent, in those of strain II, the shape of each is characteristic.

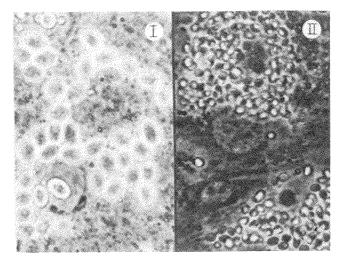


Figure 1. C. fumiferana cells infected with strain 1 and strain 1 entomopoxyirus.

It is conceivable that these two strains of virus exist separately in nature in different spruce budworm populations and that, when these populations merge, a double infection occurs. The more virulent virus will then interfere with the multiplication of the less virulent strain and will either reduce its proportion drastically or eventually eliminate it.—B.M. Arif and Keith W. Brown, Forest Pest Management Institute, Sault Ste. Marie, Ont.

ENTOMOLOGY

Pine Oil Prevents Mountain Pine Beetle Attack on Living Lodgepole Pine Trees.—Pine oil sprayed on bark surfaces of Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) log sections delayed and reduced attacks by ambrosia beetles and appeared also to reduce bark beetle attacks (Nijholt, Bi-mon. Res. Notes 35:22-23, 1979; Nijholt, Can. Entomol. in press).

As an extension of the foregoing, an experiment to determine the effect of pine oil on attacks by mountain pine beetle (*Dendroctonus pondersae* Hopk.) on living lodgepole pine trees (*Pinus contorta* Dougl.) was carried out in a 140-yr-old primarily lodgepole pine stand east of McLeese Lake, B.C., in the summer of 1979.

Twenty uninfested pine trees were selected in an area adjacent to a natural infestation of mountain pine beetle. The lower 2.4 m of the stems of 10 trees were sprayed to the drip point with undiluted Norpine 65 (pine oil, supplied by Northwest Petrochemical Corporation, Anacortes, Wash., 98221, U.S.A.) with a garden-type pressure sprayer (Hudson Manuf. Co. Model #6622) on 5 July 1979. The other 10 trees were left as untreated controls.

Each of the 20 trees was then baited at breast height with two caps containing 0.5 mL of a mixture of trans-verbenol and alpha-pinene (9:1) (Pitman, J. Econ. Entomol. 64:426-430, 1971) and with 5 mL of 95% ethanol in a loosely capped polyethylene Boston bottle to take advantage of possible synergistic effects (Pitman et al., Z. Angew. Entomol. 78:203-208, 1975).

The trees were checked daily from 19 to 30 July, inclusive, and on 14 and 21 August. The first attacks were observed on 23 July on untreated trees. By the end of the experiment, on 31 August, when beetle flight had ended, 8 of the 10 untreated trees were heavily attacked. The remaining two received zero and two attacks, respectively. Nine of the pine oil treated trees remained free from attack. The 10th had 15 attacks above the treated part of the stem and six within the treated area; all galleries in this tree were pitched out.

No evidence of phytotoxicity owing to the treatment was observed on the trees by 9 October 1979, although damage occurred to underbrush near the stem of the treated trees. The pine oil, as applied, was effective in reducing attacks by the mountain pine beetles. The trees will be kept under observation for evidence of resistance to future beetle attack and of phytotoxicity.—W.W.Nijholt and L.H. McMullen, Pacific Forest Research Centre, Victoria, B.C.

Field Test of Swedish "Drainpipe" Pheromone Trap with Mountain Pine Beetle. The mountain pine beetle (Dendroctonus ponderosae Hopkins) is currently causing serious damage to lodgepole pine (Pinus contorta Dougl.) forests in British Columbia. Present control efforts are restricted largely to salvage logging of infested stands. Population and damage reduction is achieved only if green infested trees are cut and the brood is destroyed (Safranyik et al., Environ. Can. For. Tech. Rep. 1, 1974). The use of attractive pheromones for bark beetle mass trapping has been investigated in several species, including the mountain beetle in white pine (Pitman, J. Econ. Entomol. 64(2):426-430, 1971). In 1979, a massive control program against the European spruce beetle Ips typographus L. was undertaken in Norway and Sweden, in which, respectively, 600,000 and 350,000 pheromone-baited traps were deployed in areas where trees were being killed. The aim of the program was to reduce the beetle population to a level below the economic threshold (O'Sullivan, Chem. Eng. News, 57(31):10-14, 1979). The objective of the study reported here was to determine if traps of the type produced in Sweden for I. typographus could be used to trap mountain pine beetle in lodgepole nine stands.

The trap (Fig. 1) consisted of a piece of corrugated black-plastic drainpipe 1.45 m long and 11.5 cm in diameter. Six evenly spaced longitudinal rows of holes 5 mm in diameter (714 in all) were drilled through the pipe wall between the corrugations. A white-plastic funnel and a 2 L plastic widemouthed jar were clamped to the bottom of the pipe. A black-plastic cap at the top of the pipe served to keep out rain. In principle, the pipe simulated a tree-stem silhouette; the holes in the pipe served as exits for the bait pheromones placed inside the trap and as entrances for the attracted beetles that then fell into the jar.

Seven traps were deployed at 7 to 10 m intervals, six in a circle and one at the center, in an infested stand at Riske Creek, B.C., from 17 to 26 July, 1979, inclusive. They were tied with wire to stakes driven into the ground, so that the bottom of the bottles was approximately 30 cm above the ground. Two polyethylene caps, each containing approximately 0.25 mL of "pondelure" (9 parts trans-verbenol, 1 part w-pinene) as bait, were suspended at two levels inside each trap. Pondelure is acknowledged to be a poor attractant for mountain pine beetle on traps in lodgepole pine stands (Pitman et al., pages 165-173 in Kibbee et al. [eds.], Theory and practice of mountain pine beetle management in lodgepole pine forests, Univ. Idaho, Moscow, 1978). Therefore, other materials, as follows, were added to the bait in five of the traps. Each of two traps had a small fresh lodgepole pine bolt (8 x 50 cm), manually infested wth 15 female mountain pine beetles and screened (Fig. 2), as a natural source of pheromones. In each of the three remaining traps were placed two loosely capped polyethylene Boston bottles (5 mL). These were filled as follows: the first pair with 95% ethanol (Pitman et al., Z. Angew. Entomol. 78(2):203-208, 1975) (Fig. 3); the second pair with acetone (Billings et al., Environ. Entomol. 5(1):171-179, 1976); and the last pair with ethanol in one case and

TABLE 1

Catches of mountain pine beetle on pheromone-bailed Swedish "drainpipe" traps

within market account		Numb	er of <i>E</i>). pond	erosae	caught		
Trap	Bait		Pipe trap		Sticky screen		Sex ratio	
1	Pondelure	<i>ੌ</i>	ू ।।	<i>ੈ</i>	្ រ	17	♂:♀ 1:3.0	
2	Pondelure	2	24	2	7	35	1:7.8	
3	Pondelure + ethanol	3	3	7	7	20	1:1.0	
4	Pondelure + acetone	3	7	0	4	14	1:3.7	
5	Pondelure + ethanol + acetone	5	9	0	6	20	1:3.0	
6	Pondelure + Q-infested bolt	15	15	3	4	37	1:1.0	
7	Pondelure + Q-infested bolt	2	2 3	2	1	16	1:3.0	
	Totals	34	80	15	30	159	1:2.4	



Figure 1. Swedish "drainpipe" trap in operation.

Figure 2. Female-infested pine bolt, with pheromone-containing caps below.

Figure 3. Polyethylene cap with 1/4 mL pondelure, and Boston bottle with 95% ethanol.

acetone in the other. To determine if beetles were arriving at the trap but not entering it, a sticky screen cylinder 17 cm in diameter and 22 cm high was attached near the top of each trap (Fig. 1); the ratio of surface areas of the pipe trap (minus the area obscured by the screen) and screen was 3.8 to 1. The traps were checked seven times in the 10-day period.

Although the number of beetles caught was small (Table 1), the test does indicate that the pipe trap is suitable for dead-trapping mountain pine beetles. However, the number of beetles that attacked all surrounding pines, some as small as 8 cm dbh, far exceeded the number

trapped. This indicated that the pheromone bait currently available is not very effective. Further research is needed to produce a bait that is competitive with natural pheromone sources, i.e., mountain pine beetles attacking trees.

Fewer beetles per unit surface area were caught in the pipe traps than on the sticky screens (36.6 vs. 54.7 beetles/m²). This showed that only 67% of the arriving beetles were caught in the traps. The lowness of the catch may have been due to the nature of the baits, which did not offer the beetles a strong enough stimulus to enter the trap through the holes, and/or to the smooth microsurface of the pipe, which caused landing beetles to lose their grip and fall. A brief laboratory test with undisturbed mountain pine beetles indicated that they did indeed have difficulty walking on the vertical surfaces of the pipe trap. Thus a rougher surface may increase catches substantially.

Also, in order to decrease the catch of nontarget organisms, such as predacious insects, e.g., *Enoclerus* (Coleoptera: Cleridae) and spiders, the diameter of the holes in the trap can be reduced from the 5 mm used. A hole diameter of 3 mm would be adequate to accommodate even the larger Douglas-fir beetle (*D. pseudotsugae* Hopkins) and the spruce beetle (*D. rufipennis* [Kirby]).

Gustavsberg Lubonyl Company, Sweden, provided the pine traps through the British Columbia Ministry of Forests, Protection Division, Victoria, B.C.—H.A. Moeck, Pacific Forest Research Centre, Victoria B.C.

MISCELLANEOUS

Surface Sterilization of White Spruce Twigs.—Surface sterilization is an essential routine in the preparation of plant tissue cultures. Its purpose is to kill all microorganisms on the surface of the tissues and at the same time to minimize damage to tissue cells. The most common method of surface sterilization is soaking of the tissues for 10-30 min in a 5% sodium hypochlorite (NaOCl) solution (Gamborg and Wetter, Plant tissue culture methods, National Research Council of Canada, 1975). Generally detergent is added to facilitate penetration of the sterilizing solution into hollows and other difficult-to-reach places on the tissue surface.

Surface sterilization of current-year shoot sections of white spruce, Picea glauca (Moench) Voss, was attempted with 5% NaOCl for 30 min. The result, however, was that most stem sections were insufficiently sterilized. It was therefore decided to determine if better results could be obtained by (1) modifying the NaOCl concentrations and exposure times, (2) adding different detergents, or (3) adding other surface sterilizing chemicals to the NaOCl solutions. A commercial liquid bleach ("Javex," Bristol Myers Canada Ltd., NaOCl 12%) diluted with tap water was used as the NaOCl source. The detergents Decon 75 (BDH), Tween 20, Extran 300 (BDH), Triton x45, and Contrad 70 (S/P Cy) were used in the NaOCl solution at 0.5% v/v. Other germicidal additives to the NaOCl solutions were the fatty acids octanoic, decanoic, oleic, and linoleic acid (Puritch, Can. J. Forest Res. 5:515-522, 1975) at 0.5% v/v; salicyclic acid 5 g/L; Gentian violet 5 g/L; and potassium iodide in combination with polyvinylpyrrolidone (PVP) K-15, each at 16 g/L (GAF Corporation USA Tech. Bull. 9642-070).

Dormant white spruce branches were collected throughout the fall and early winter and stored in plastic bags for 2-14 days at 4°C. The current-year shoots were cut into sections about 20 mm long, and the ends of each section were sealed with molten wax to prevent penetration of the sterilant into the stem through the vascular system. The sections were transferred to 400 mL of sterilizing solution (20 sections per beaker) in 1 L beakers covered with plastic wrap and were placed on a shaker at room temperature. The sterilized sections were washed twice in sterile distilled water. The waxed ends were cut off, and the sections were placed in test tubes (one section per test tube) with nutrient medium (Romberger, Varnell, and Tabor, USDA Forest Serv. Tech. Bull. 1409, 1970) to which had been added 1 mg/L 2,4-D. The cultures were kept at 21°C in approximately 500 lux of fluorescent light for 16 h daily. The cultures were maintained for 6 wk, after which contamination and survival rates were recorded.

In the first experiment, NaOCl was used at 1.2, 2.4, 4.8, and 9.6% for 0.5, 1, 6, and 24 h, with or without detergent (Decon), with 20 twig sections per treatment. The experiment was repeated three times, each time with material from a different collection date. The result of all

TABLET

Effect of various concentrations of sodium hypochlorite, with (*) or without (-) detergent, at 6and 24-h exposures, on contamination and survival rates of white spruce shoot sections cultured for 6 wk on a nutrient medium.

		Number of sections ^a								
		Contaminated		Noncontaminated						
NaOCI	Exposure			De	adb	Aliveed				
e,	(h)	+	-	,			-			
1.2	б	60	60	0	0	0	0			
1.2	24	36	58	0	Ð	24	2			
2.4	6	46	58	0	Ü	14	3			
2.4	24	18	35	9	2	33	23			
4.8	6	3.2	44	Ð	0	28	16			
4.8	24	16	15	15	13	29	3.2			

^a60 sections per treatment.

TABLE 2

Effect of adding various germicides to a low strength (1.2%) sodium hypochlorite solution, at 1-and (24-h exposures, on survival rates of white spruce shoot sections cultured for 6 wk on a nutrent medium.

	Number of sections ^a								
	Contai	minated	Noncontaminated						
			De	ad ^b	Alivet				
Germicides	1 h	24 h	Th	24 h	1 h	24 h			
Decon	60	30	0	12	0	18			
Tween	60	19	0	1	0	40			
Extran	40	28	0	1	20	33			
Triton	60	26	-()	ŝ	0	33			
Contrad	60	22	0	4	0	34			
Octanoic acid	58	13	0	17	2	30			
Decanoic acid	60	17	0	19	0	24			
Oleic acid	60	32	()	10	()	18			
Linoleic acıd	60	42	0	1	0	17			
Saticyclic acid	60	59	0	0	0	ŧ			
Gentian violet	5.5	43	()	3	5	14			
lodide - PVP	49	48	3	Ü	8	12			
NaOCI control	60	57	0	0	0	3			

a 60 sections per treatment

0.5- and 1-h treatments was that all cultures became contaminated. At longer exposure times, the highest NaOCl concentration (9.6%) killed most of the sections. Therefore, only 6- or 24-h treatments with 1.2, 2.4, or 4.8% NaOCl were satisfactory. Table 1 presents the pooled results of these treatments. Contamination rates were lowest at the longest exposure time and the highest NaOCl concentration. The number of noncontaminated but dead sections (needles turned brown) was highest after 24 h exposure to 4.8% NaOCl, indicating that this treatment approaches the maximum exposure to NaOCl that can be tolerated by the sections without excessive mortality. Adding detergent lowered the contamination rate, but may also have increased the toxicity of NaOCI to the sections. The needles of the surviving sections remained fresh green during the 6-wk culture period. About 30% of these sections developed vigorous green callus from either the stem surface or the basal parts of the needles. Some lateral buds on the stems developed and flushed into new shoots. Some of these lateral shoots were dissected and transferred to a fresh medium, where they further elongated and formed new calluses, demonstrating the vitality of the surface-sterilized material.

In the second experiment, different germicidal chemicals were added to a low concentration (1.2%) of NaOCl and the sections were exposed to these solutions for I and 24 h (Table 2). Sterilizing for I him

^bNeedles brown.

⁶Needles and stem fresh green.

d Analysis of variance of the numbers of "alive" sections showed significant differences between concentrations (1% level), between plus and minus detergent (1% level), and between 6 and 24 h (5% level).

b Needles brown.

Needles and stem fresh green.

NaOCl alone resulted in all cultures becoming contaminated. None of the other chemicals used, except possibly Extran, effectively reduced the contamination rate after 1 h sterilization. Sterilizing for 24 h in NaOCl alone again resulted in a high contamination rate, but the rate was reduced by both the detergents and the fatty acids. However, the fatty acids appeared to kill more of the sections than did the detergents. Again, about 30% of the surviving sections developed rapidly growing calluses

In conclusion, it appears that white spruce shoot sections require much longer sterilization in NaOCl than plant parts of most other species, possibly because of microorganisms residing in hollows in the rough bark into which the sterilizing solutions penetrate only slowly. Adding detergent or fatty acid improved the effectiveness of the sterilizing solution in destroying the surface microorganisms, but fatty acid also increased the toxicity of the germicidal solution. The practice of dipping the sections in hot wax before sterilization may not be essential. In subsequent experiments the survival rates of waxed and nonwaxed sections were about the same.—J.M. Bonga, Maritimes Forest Research Centre, Fredericton, N.B.

TREE PHYSIOLOGY AND ANATOMY

A Preliminary Study of Dormancy in Pinus albicaulis Seeds. - Whitebark pine (Pinus albicaulis Engelm.) has good qualities for reclamation projects in British Columbia and Alberta, but seed dormancy is a serious handicap in its utilization. Seed dormancy can be caused by several factors such as impermeability of the seed coats to water and oxygen, underdevelopment of the embryo, mechanical resistance to embryo growth, and endogenous dormancy of the embryo due to a metabolic block (Villiers, pages 220-281 in T.T. Kozlowski, ed., Seed biology, vol. II, Academic Press, New York, 1972). The method prescribed by the International Seed Testing Association (ISTA) to release dormancy of whitebark pine seed is prechilling for 28 days at 3-5°C followed by germination with alternating temperatures of 20 and 30°C (ISTA, Seed Sci. Technol. 4:3-177, 1976). This prescription, however, has proved unsatisfactory to us and others (pers. comm.) and requires improvement. This report describes experiments to determine the type and cause of dormancy of whitebark pine seeds and to develop

better treatments to overcome dormancy and thus improve germination in laboratory testing and field planting.

Whitebark pine seeds from three different geographical sources (Table I) were surface-sterilized with calcium hypochlorite (4% available chlorine) and then washed several times with sterile distilled water. For each experiment three replicates of 50 seeds were used. Seeds were x-rayed to determine their size, condition, and development. They were then classified according to the size and development of the embryos and endosperms (Simak and Kamra, Proc. Int. Seed Test. Assoc. 28:3-18, 1963). Intact seeds of all three seedlots were stratified at 4° C for 28 and 60 days.

Treatments to explore seed-coat permeability included: (1) germinating unstratified seeds after a cut had been made in the middle of the seed coat and the seeds had then germinated while the cut either was exposed to the air or faced the germination paper; (2) making a cut in the middle of the seed coat and stratifying the seeds at 22°C for 30 days and then at 4° C for 30 days (warm-cold stratification), followed by germination as already explained; (3) soaking unstratified seeds in 97% sulfuric acid for 3.5 h before germination testing; and (4) measuring water uptake of intact and decoated seeds and, in the process, using only seeds with full embryos and endosperms. Water uptake was measured on a fresh-weight basis. For hormone treatments, intact seeds were stratified for 28 days at 4°C and then placed in a mixture of Kinetin (1 mg/L) and a gibberellin (GA, at 250 mg/L) for 24 h. Seeds of both treated and untreated seeds were germinated for at least 30 days at 30°C with 8 h light and 20°C with 16 h darkness. Analyses of variance were then performed on the individual experiments to determine the significant variables. Significance of means was determined by Duncan's multiple range test (Steel and Torrie, Principles and procedures of statistics, McGraw-Hill Book Co., Inc., New York, 1960).

The results indicate poor response of the seeds to the treatments performed. Stratification for 28 days at 4-5°C as recommended by ISTA rules or for 60 days gave only slight improvement for two of the seedlots over the unstratified seeds (Table 2). Treatment with sulfuric acid was effective only in seedlot 716553, resulting in 8.7% germination (Table 2). The best results were obtained when a small cut was made in the middle of the seed coat and the seeds were germinated with the cut exposed to the germination paper to facilitate water uptake through the

TABLE 1

Percentage distribution of embryo and endosperm classes of three seedlots of whitebark pine seeds as determined by x-ray analysis (for embryos, Class B = 0) embryos cavity. Class B = 0 embryos smaller than half the embryo cavity. Class B = 0 embryos covering half and three-quarters of embryo cavity; and Class B = 0 embryos. For endosperms, Class B = 0 full-size endosperms, and Class B = 0 shrunken endosperm. The letter "p" refers to "point," or extremely small, embryos)

			A	***************************************								
Pre A			Elevation	Embryo and endosperm class								
Seed source	Latitude	Longitude	(m)	0	1	Hp	HA	IIIA	IVA	11B	HIB	IVB
766450, Bow Cross Forest, Alta.	49°48′	114° 37′	1 675	9	17	west	8	22	19	12	3	9
72586, Smithers, B.C.	54°	126"	1 370	2	12	2	19	18	30	4	7	6
716553. Peyto Lake, Banfi National Park, Alta.	52°	1160		3	14	6	13	21	23	7	0	13

TABLE 2

Effect of physical and chemical treatments on germination of whitebark pine seeds

			Unstratified seeds		Stratified seeds				
						Moist-cold at 4°	C	Warm (22° (?)-cold (4° C)
d rce	Intact H ₂ SO ₄ seed treated	Cut in coat, Cut in coat, exposed to paper air	28 days	60 days	28 days plus hormone treatment	Cut in coat, exposed to paper	Cut in coat, exposed to air		
0	0e	0e	14.7a	0e	2.0d	4.7c	2.0d	12.7b	4.0c
3	2.0e	8.7ъ	10.7a	Of	2.0e	2.0e	4.7d	7.3c	2.0e
6	06	Оъ	0b	06	Øb	2.7a	2 7a	2.0a	0b

^{*}Within seedlots, percentages not followed by the same letter are significantly different at the 5% level.

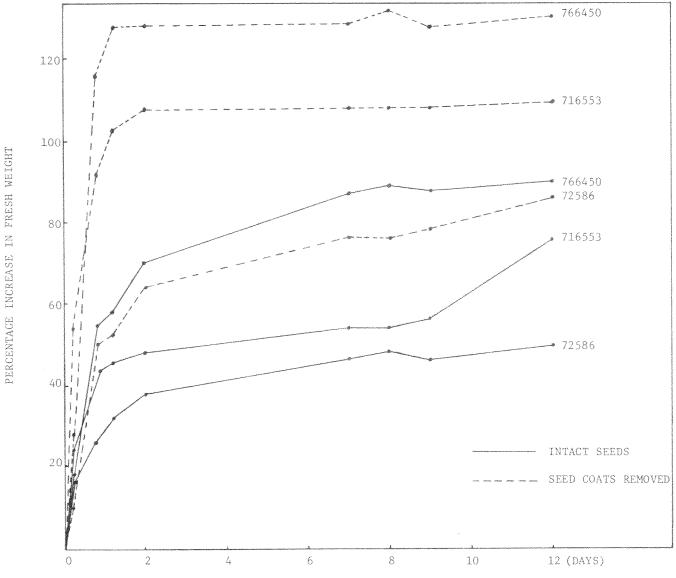


Figure 1. Effect of seed coat removal on water imbibition of whitebark pine seeds.

cut. When the cut was exposed to the air, germination was absent in all seedlots (Table 2).

These results, together with the increased water uptake of decoated seeds as compared with seeds with intact seed coats (Fig. 1), and the results of the sulfuric acid treatment, which digests most of the seed coat, indicate that restriction of water uptake by the seed coat may be a limiting factor for germination. Treatment with a warm (22°C for 30 days) - cold (5°C for 30 days) stratification was effective only when the seed coat was cut, again indicating the importance of seed coat treatment. Treatment of intact stratified seeds with a hormone mixture was not very effective, as it was in the case of western white pine (Pinus monticola Dougl.) seeds (Pitel and Wang, unpublished data), where the same hormones gave a significant increase in germination for both the unstratified and the stratified seeds. One reason for this may be the inability of the hormones to pass through the thick seed coats.

A key factor that might explain the generally low germination was the relatively low number of seeds with full embryos and endosperms, as indicated by Table 1. Seeds classified as 0, I, and IIp do not germinate. High germination is expected only with seeds in classes IIIA, IVA, and IVB (Simak and Kamra, 1963). It would thus not be possible to expect much more than 50% seed germination of this species unless the

underdeveloped seeds could be effectively removed. In view of this, our treatment results with sulfuric acid and cut seed coat are an improvement over the existing method.—J.A. Pitel and B.S.P. Wang, Petawawa National Forestry Institute, Chalk River, Ont.

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