

ported from Queensland, Australia. Severe damage was found only on clones 21, 55 and 65.

Thirteen species of pine were checked for scale attack. *C. rubens* was found on six species; considerable damage was present only on *P. caribaea*.

C. rubens attacked the upper crown of *P. caribaea* more frequently than the middle crown. The lower crown portions were least heavily attacked. No correlation was found between height of trees and attack density.

Heavily infested trees were characterized by sparse crowns, considerable darkening of foliage by a dense covering of sooty-molds, and reduced height increment.

Key words: *Ceroplastes rubens*, *Pinus caribaea*, Clonal Resistance, Damage, Sooty-mold, Papua New Guinea.

Zusammenfassung

In einer Samenplantage von *Pinus caribaea* MORELET, in der Nähe von Bulolo, Neu Guinea, wurden im Herbst 1974 starke Schäden durch die rote Wachsschildlaus *Ceroplastes rubens* MASKELL festgestellt. Die Plantage enthält insgesamt 23 *Pinus caribaea*-Klone, von denen drei besonders stark geschädigt waren, insbesondere im oberen Kronenbereich der Pflöplinge. Die eingehende Untersuchung aller Klone der Plantage ergab, daß diese drei Klone, unabhängig vom jeweiligen Standort der randomisiert ausgepflanzten Pflöplinge, als besonders anfällig anzusehen sind und damit ein individualspezifisches Verhalten gegenüber *Ceroplastes rubens*.

Weitere Untersuchungen an 13 Kiefern-Arten ergaben einen mehr oder weniger starken Befall durch *Ceroplastes rubens* bei *Pinus pseudostrobus* LINDL., *Pinus oocarpa* SCHIEDE, *Pinus patula* SCHIEDE et DEPPE, *Pinus cubensis* GRISER., *Pinus radiata* D. DON, *Pinus keseya* ROYLE und *Pinus michoacana* MARTINEZ, der jedoch in keinem Fall so stark war wie bei *Pinus caribaea*. Kein Befall konnte festgestellt werden bei *Pinus merkusii* JUNGH. et DE VRIESE, *Pinus taiwanensis* HAYATA, *Pinus occidentalis* SWARTZ, *Pinus strobus* L. und *Pinus luchuensis* MAYR.

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Effect of Seed Extracts on Radiosensitivity

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(Received October 1974 / May 1975)

Introduction

It is known that soaking seeds in water immediately before treatment with ionizing radiations and chemical mutagens greatly increases the sensitivity of seeds to mutagenic treatment. The increased sensitivity of pre-soaked seeds was attributed to leaching out of radioprotective substances, KAMRA et al. 1960 a, b. The absence of oxygen has been reported to cause chromosomal aberrations in *Vicia faba* (MERZ, 1959) but an aerobiosis is not effective in barley (RIEGER and MICHAELIS, 1958). On the other hand, D'AMATO and HOFFMANN-OSTENHOF (1956) have postulated the production of automutagens inside the seeds after soaking in water which increases the frequency of spontaneous chromosomal aberrations (MICHAELIS and RIEGER, 1958) as well as spontaneous mutations.

Preliminary experiments have demonstrated the radiosensitivity of the 48 hours pre-soaked Douglas-fir seeds is maximum (EL-LAKANY and SZIKLAI, 1969). The present experiments were undertaken to investigate whether the extracts from soaked Douglas-fir and lodgepole pine seeds contain active substance(s) which would modify sensitivity to gamma-irradiation when it is used as a medium of pre-soaking other seed samples of the same species, or the radiosensitivity is affected by in situ systems.

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Materials and Methods

Seeds of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO) collected near Duncan, B.C. in 1968 and those of lodgepole pine (*Pinus contorta* DOUGL.) collected near Shuswap Lake, B.C. (Canada) in 1971 were cleaned, equilibrated for moisture content of 8 per cent, and stored at 0–4° C at George Allen's Tree Seed Laboratory, U.B.C., Vancouver. Seed samples were given the following treatments:

(A) Dry seeds were exposed to different doses of γ -radiation, soaked in tap water for 48 hrs. then germinated.

(B) Seeds were pre-soaked in tap water for 48 hrs., irradiated, then germinated,

(C) Seeds were pre-soaked in seed extracts for 48 hrs., irradiated then germinated. The seed extract was prepared from seeds of the same species that had been soaked for 48 hrs. in water, crushed in a mortar and a paste was made by adding 50 ml of water per 100 seeds. The paste was filtered through muslin cloth then used to soak the seeds immediately,

(D) Seeds were soaked in pre-irradiated extracts prepared by the same method mentioned above, then germinated without further irradiation.

At the end of the pre-soaking period, the seeds were washed thoroughly in running water for 2–3 min. The following doses of γ -radiation were given to the seeds of Douglas-fir and lodgepole pine respectively: 0 (Control), 1, 5 and 10 kR and 0, 1, 5 and 15 kR at 140 R/sec. A220

Gamma — cell that contained ^{60}Co was used as a source of radiation. Every treatment was replicated 5 times. Each replication contained 50 seeds and were placed on the Jacobsen germinating table at the same time.

The germinator was set to provide 12 hrs. of light and 12 hrs. of dark periods per day for the 28 days test period. The temperatures were 25° C during the light and 15° C during the dark periods for Douglas-fir. Corresponding temperatures were 21° C and 15° C for lodgepole pine. Germinants were counted every two days and germination percentages were calculated and analyzed statistically. Germination as a percent of control was also calculated in order to estimate LD 50's (Dose of radiation that reduces germination by 50% of the control). Douglas-fir germinants representing each treatment were grown in a "Percival" growth chamber in order to follow seedling growth and to score the somatic mutations if they appear.

Results and Discussion

The results of the germination are presented in Table 1, and further these average germination values are expressed as percent of the controls in Figures 1 and 2 for Douglas-fir and lodgepole pine respectively. Douglas-fir dry seeds gave a typical response to increasing doses of γ -radiation and their LD₅₀ value was about 5600 R which is close to the LD₅₀ value of 5525 R previously reported by EL-LAKANY and SZIKLAI (1969) for the same species. Soaking the seeds in water before irradiation increased their radiosensitivity sharply with an LD₅₀ value of 900 R. GUSTAFSSON and SIMAK (1958) reported that seed stored in moist air were

more radioresistant than seed stored in dry air. However, SHARMA (1970) found that water pre-soaked barley seeds produced a higher mutation rate than dry ones.

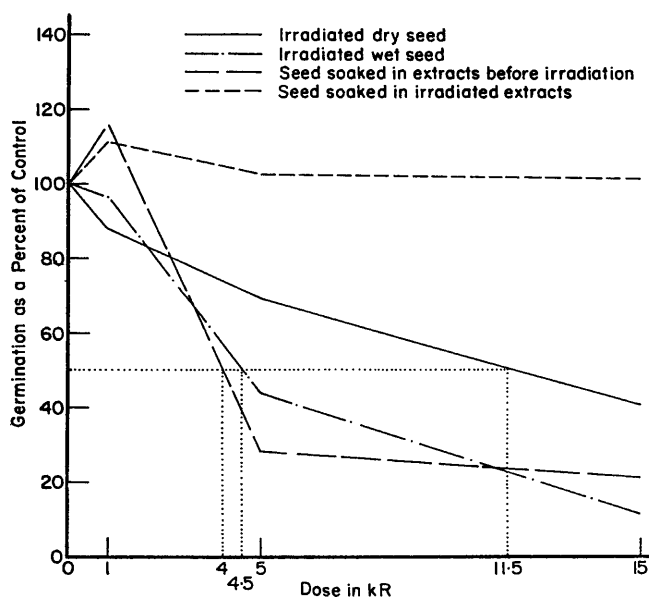


Fig. 2. — Effects of γ -irradiation and pretreatments on seed germination in Lodgepole pine.

Soaking the seed in extracts before irradiation had a variable effect on germination (Fig. 1) depending on the dose. At 1000 R there was a marked increase in germination

Table 1. — Average germination percentages of Douglas-fir and lodgepole pine seeds at the end of 28 days germination period

Treatments	Douglas-fir				Lodgepole pine			
	0 percent	1	5	Dose of irradiation (kR) 10	0	1	5	15
A	68.21	52.51	36.83	27.97	83.74	74.53	58.62	34.33
B	70.63	34.61	04.94	05.65	86.82	84.22	38.20	10.42
C	71.32	91.29	07.85	07.13	81.76	94.84	22.89	17.99
D	69.18	51.89	47.04	46.35	88.12	97.81	90.76	89.88

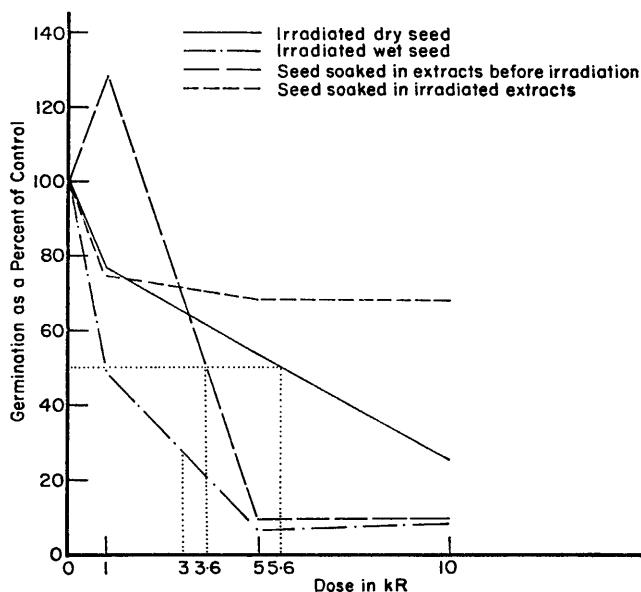


Fig. 1. — Effects of γ -irradiation and pretreatments on seed germination in Douglas-fir.

tion but above that level radiation. This data also reveals that pre-soaking in water resulted in less radiosensitization than pre-soaking in seed extracts, which is the case in the present study. Soaking Douglas-fir seed in irradiated extracts slightly reduced germination and the dose had no effect, however.

The results of lodgepole pine seed germination are shown in Fig. 2. The trend of response was similar to that of Douglas-fir except that lodgepole pine was more radioresistant than Douglas-fir. The LD₅₀'s for dry seed, water-soaked seed and extract-soaked seed were 11,500, 4,500 and 4,000 R respectively. To the best of our knowledge these values are the first reported LD₅₀'s for lodgepole pine (DUGLE and EL-LAKANY, 1971). In lodgepole pine, unlike Douglas-fir, soaking the seed in irradiated extract stimulated the germination slightly irrespective of the dose. SHARMA (1970), proposed that the metabolic activity initiated by soaking seeds gives rise to certain sensitizing substances. It could be added that the progressive decrease in the germination of extract-pre-soaked seed with increasing dose of γ -radiation provides an indication that the phenomenon of sensitization has several components, and the metabolic products of seed extract act on some processes,

which are affected less by water or not affect at all. The sensitization also appears to be related to *in situ* system as it had little or no effect when the extracts were irradiated outside the seed.

Summary and Conclusions

The effects of seed extracts on radiosensitivity were studied in Douglas-fir and lodgepole pine and compared with water-soaked and dry seed irradiation.

At a lower dose of radiation (1,000 R), soaking the seed in extract before irradiation stimulated the germination relative to the control.

Seed extracts appeared to have a radiosensitizing effect at higher doses of radiation in both species. This effect appears to be related to *in situ* processes as it was minimal when the seeds were soaked in irradiated extracts.

Experiments will be conducted to investigate whether these effects are related to systems in the embryo or in the endosperm.

Key words: Radiosensitivity, Seeds, *Pseudotsuga menziesii* (MIRB.) FRANCO, *Pinus contorta* DOUGL. ex LOUD.

Zusammenfassung

Saatgut von *Pseudotsuga menziesii* (MIRB.) FRANCO und von *Pinus contorta* DOUGL. ex LOUD. wurde in verschiedenen Behandlungsarten radioaktiver Bestrahlung ausgesetzt. Insbesondere wurde die Empfindlichkeit von trockenem und gequollenem Samen geprüft. Gleichzeitig wurden

radioaktiv bestrahlte mit Wasser versetzte Saatgutextrakte als Quellungssubstrat benutzt.

Es zeigte sich in einigen Fällen, daß eine niedrige Dosis an radioaktiver Bestrahlung das Keimprozent gegenüber demjenigen von unbehandelten Samen sogar erhöhen kann, während höhere Dosen im allgemeinen eine deutliche Herabsetzung der Keimkraft bewirken.

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Vergleichende zytologische Untersuchungen der Chromosomenstruktur von *Abies borisii regis* Mattf., *A. cephalonica* Loud. und *A. alba* Mill.

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(Eingegangen August / Oktober 1975)

Das natürliche Verbreitungsgebiet von *Abies borisii regis* MATTF. umfaßt ein kleines Areal in Mittel- und Nordgriechenland. Im Süden überlappt es im Zentralpeloponnes mit dem von *A. cephalonica* LOUD. (BASSIOTIS 1956), im Norden dagegen mit *A. alba* MILL. Der in ihrem gesamten Verbreitungsgebiet ausgeprägte Polymorphismus von *A. borisii regis* ließ MATTFELD einen Hybridschwarm aus den im Norden und Süden angrenzenden Arten *A. alba* und *A. cephalonica* annehmen (MATTFELD 1926, zit. nach KLAHN and WINIENSKI 1962), während REHDER (1958) *A. borisii regis* als eine selbständige, *A. cephalonica* verwandte und mit ihr z. T. sympatrische Art beschreibt. Zur Klärung dieser Frage sollen vergleichende zytologische Untersuchungen der Chromosomenstruktur aller 3 Arten aus Material ihres natürlichen Verbreitungsgebietes beitragen.

Das Karyogramm aller 3 Arten stellten Mergen and Burley (1964) nach Untersuchungen an ♀ Gametophyten von Arboretmaterial auf. Sie werteten dazu die jeweils besten 14 Metaphasen aus. Bei allen 3 Arten waren 5 der 12 haploiden Chromosomen heterobrachial und entsprachen der für die Gattung typischen Uniformität des Chromosomensatzes. Die Autoren weisen aber ferner auf zahlreiche sekundäre Einschnürungen und kleinere Strukturunterschiede der Chromosomen hin, die wegen ihrer hohen Variabilität bei dem begrenzten Umfang des Materials für eine Identifizierung der Chromosomen ungeeignet waren.

Störende Umwelteinflüsse auf das teilungsfähige Gewebe, wie sie bei Verwendung des haploiden Endosperms unvermeidlich sind, lassen sich bei der Samenkeimung unter gleichbleibenden kontrollierten Laborbedingungen weitgehend ausschließen. Außerdem ist bei der Verwendung des Meristems der Keimwurzel die Durchsicht eines umfangreicheren Materials möglich, als bei der Einsammlung von

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²⁾ Herrn Forstdirektor Dr. DIMPFMEIER sei an dieser Stelle für die Überlassung des *A. alba*-Saatguts gedankt.