SEED COAT SURFACE ELEMENTAL COMPOSITIONS OF ACCELERATED AGED SEEDS OF *PINUS TAEDA* AND *PINUS ROXBURGHII* BY ENERGY-DISPERSIVE X-RAY SPECTROSCOPY

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VANANGAMUDI, K., ZOPE, J.S., VOZZO, J.A. & ELAM, W.W. 1998. Seed coat surface elemental compositions of accelerated aged seeds of *Pinus taeda* and *Pinus roxburghii* by energy-dispersive X-ray spectroscopy. Energy-dispersive X-ray spectroscopy of the seed coat surfaces of artificially aged loblolly pine *(Pinus taeda)* and Himalayan pine (*P. roxburghii*) seeds detected more elements in aged seeds than in non-aged seeds. In general, calcium and iron decreased with aging in both species, while potassium, phosphorus, and sulphur increased. Among the elements detected, potassium was more commonly found in the aged seeds of both species. More elements were detected at 96 h aging than at any other periods of aging.

Key words: Energy-dispersive X-ray spectroscopy - *Pinus taeda* - *P. roxburghii* - accelerated aging - elemental composition

VANANGAMUDI, K., ZOPE, J.S., VOZZO, J.A. & ELAM, W.W. 1998. Komposisi anasir permukaan kulit biji biji benih yang dipercepatkan tua *Pinus taeda* dan *Pinus roxburghii* melalui spektroskopi sebaran tenaga sinar-X. Spektroskopi sebaran tenaga sinar-X permukaan kulit biji benih pokok pain lobloli (*Pinus taeda*) dan pain Himalaya (*P. roxburghii*) yang tua secara tiruan didapati mempunyai lebih banyak anasir dalam biji benih yang tua berbanding biji benih yang belum tua. Secara amnya, kalsium dan besi berkurangan dengan penuaan kedua-dua spesies, manakala potasium, fosforus dan sulfur berkurangan. Daripada anasir yang dikesan, kalsium banyak terdapat dalam biji benih tua kedua-dua spesies. Lebih banyak unsur dikesan pada peringkat penuaan 96h berbanding peringkat penuaan yang lain.

Introduction

Energy-dispersive X-ray spectroscopy (EDS) has been used to establish the composition of mature wheat seed *(Triticum aestivum)* (Mazzolini *et al.* 1985) and lodge pole pine *(Pinus contorta)* (El-Kassaby & McLean I983). Electron beam microanalysis is a powerful analytical technique capable of performing elemental analysis of microvolumes, typically in the order of a few cubic microns in thick samples and considerably less in thin sections. The feature of electron beam microanalysis that best describes the power of this technique is its "absolute mass sensitivity". For example, it is often possible to detect less than 10^{-16} g of an element present in specific microvolume of a sample. The speed with which analyses can be conducted makes large sample sizes feasible. Probing of small select areas is possible and differentiation of relatively difficult elements (e.g. K) presents no problem.

Energy-dispersive spectroscopy detects surface elements on seed coats of both good seed and bad seed, but not in sufficient amounts to account for different conductances (Vozzo 1990). Establishment of variations in elemental composition in fresh and old seeds is therefore possible using EDS. Should that be accomplished, identification of good / bad seed lots or fresh / old seeds will be made simple and foolproof.

Against these back-drops, an attempt was made to detect and record the elemental composition from seed coats of accelerated aged seeds of *Pinus taeda* L. (loblolly pine) and *P. roxburgii* Sarg. (Himalayan pine).

Materials and methods

Five seed samples each of loblolly pine and Himalayan pine were aged separately in an accelerated aging cabinet maintained at 41° ± 1°C and 100% relative humidity for 0, 48, 96, 144 and 192 h periods (AOSA 1983). Qualitative elemental composition of the seed coat surfaces was determined by EDS. For the EDS, ten seeds of each source were probed at three random points on the seed coat of each seed. Seeds were mounted on opaque carbon planchetes with carbon paste. Specimens were examined with a Tracer Northern TN-2000 X-ray Energy-Dispersive Spectrometer imaged in a Hitachi HHS-2R Scanning Electron Microscope. Data were collected with the following conditions magnification (35 X), duration of probe (200 s), accelerating potential (20 kV), tilt (0°), condenser lens setting (spot size 3), and working distance (15 mm).

X-ray counts were recorded for each integrated elemental peak and total counts for the spectrum. The elements detected were expressed as a percentage of total counts.

Results and discussion

In *P. taeda,* 13 elements detected at the seed coat surfaces were (in order of ascending atomic number): aluminum (Al), silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), titanium (Ti), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu) and zinc (Zn) (Table 1). Most of the elements were detected in the 96 h aged seeds (Figure 1), while chlorine and zinc, in addition, were detected in those of 0 and 192 h aging (Figure 2). Calcium decreased as aging increased except for a slight increase at 96 h aging. Copper remained constant up to 144 h aging; however, it increased at 192 h aging. Iron increased up to 96 h aging and then decreased. Potassium was common among the elements detected in the seed coat surfaces at all aging periods with a peak at 144 h aging. Phosphorus and sulphur both increased as aging progressed and reached their peak at 192 h aging. Silicon concentration was the highest at 48 h aging and then decreased drastically with further aging. This finding generally follows the pattern reported by Vozzo (1990) for *P. taeda.*

Table 1. Elemental composition of seed coat surfaces from accelerated aged seeds of *Pinus taeda* expressed as a percentage of total counts of spectral energies generated

Element	Accelerated aging (h)				
	0	48	96	144	192
Al	nd	nd	nd	nd	nd
Si	5.2^{c}	28.4^{a}	13.1^{b}	1.8^{d}	2.5^{d}
P	0.0	1.4^{c}	1.9^{c}	4.5^{b}	10.5^{a}
S	4.2^{c}	4.2^{c}	4.4^{c}	6.6^{b}	8.0^{a}
Cl	7.5	nd	nd	nd	1.2
K	33.6^{c}	24.8^{c}	22.1^{c}	59.3^{c}	39.8^{b}
Ca	23.8^{a}	13.3^{b}	21.8^{a}	9.9^{c}	5.3^{d}
Ti	nd	nd	1.0	nd	1.4
Mn	nd	nd	1.0	nd	nd
Fe	22.2^{b}	24.8^{b}	31.9^{a}	15.7^{c}	16.0^{c}
Ni	nd	nd	0.6	nd	nd
Cu	2.4^{b}	3.1^{b}	2.0^{b}	2.2^{b}	14.6^{a}
Zn	1.0	nd	nd	nd	0.7

nd: not detected.
Means not sharing the same letter differ significantly at 0.01 probability by Duncan's Multiple Range Test (DMRT).

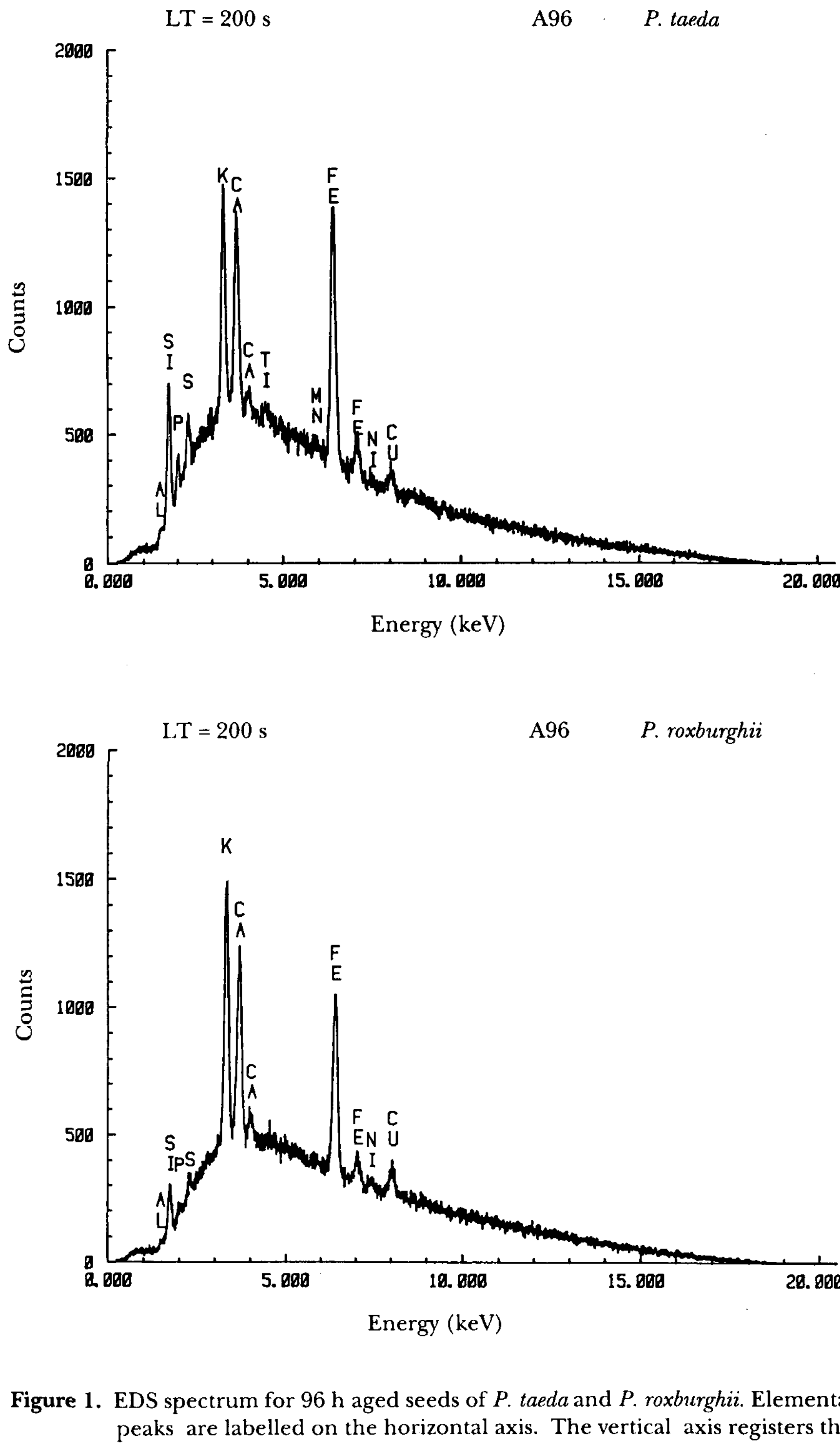

Figure 1. EDS spectrum for 96 h aged seeds of *P. taeda* and *P. roxburghii*. Elemental peaks are labelled on the horizontal axis. The vertical axis registers the number of X-ray counts.

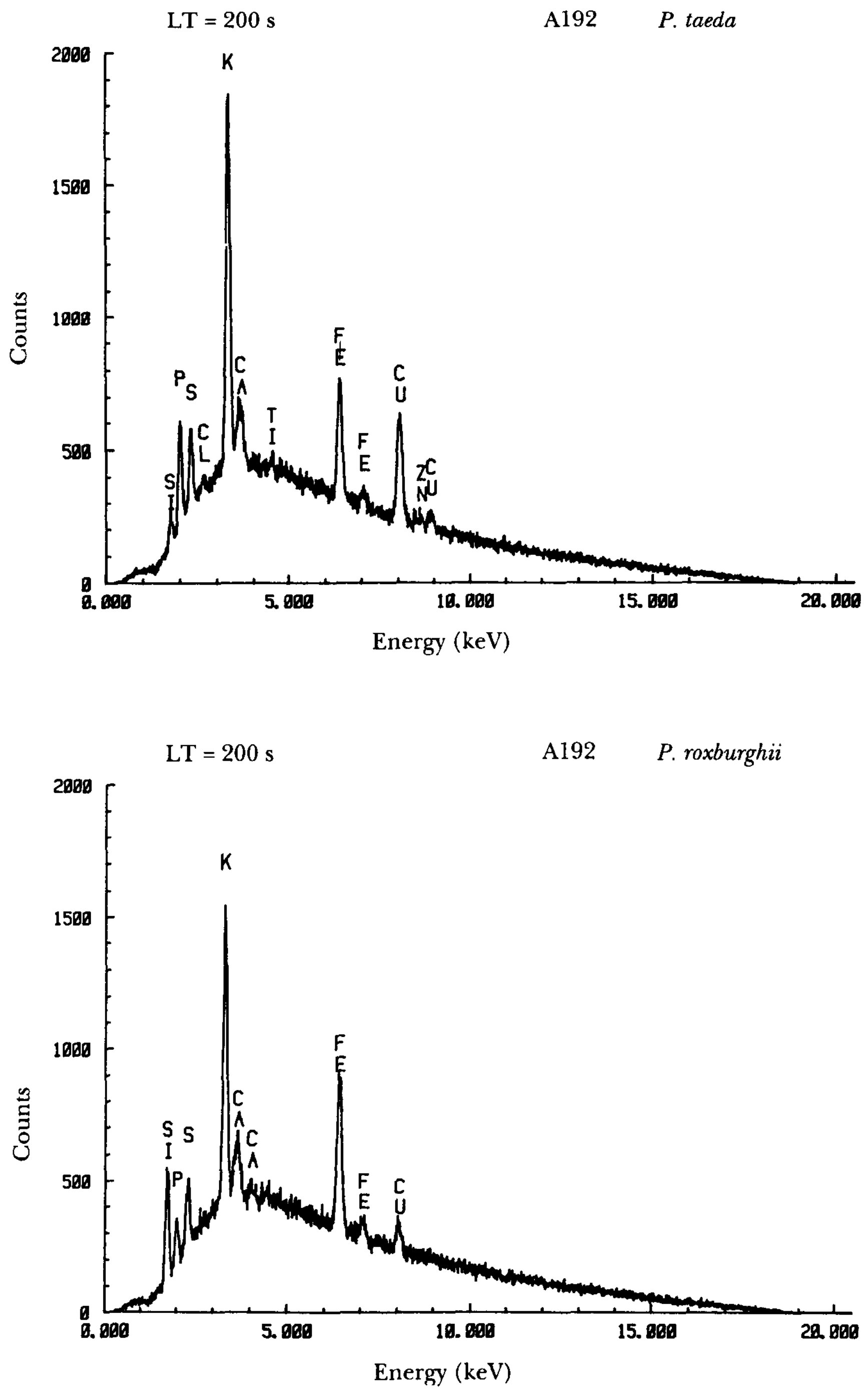

Figure 2. EDS spectrum for 192 h aged seeds of *P. taeda* and *P. roxburghii*. Elemental peaks are labelled on the horizontal axis. The vertical axis registers the number of X-ray counts.

EDS analyses detected 9 elements from the seed coat surfaces of the aged *P. roxburghii* seeds. In order of ascending atomic number, they were silicon (Si), phosphorus (P), sulphur (S), chlorine (Cl), potassium (K), calcium (Ca), iron (Fe), nickel (Ni) and copper (Cu) (Table 2). Calcium concentration decreased with aging after a maximum value at 48 h . Chlorine was detected only in the control. Copper levels were lower in the aged seeds than in the non-aged seeds. Iron concentrations decreased with all aging treatments, while potassium and sulphur increased with maximum levels at 192 h. Silicon concentrations varied irregularly with aging; however, they were higher in the aged seeds than in the non-aged seeds. Aluminum, titanium, manganese and zinc were not detected in the Himalayan pine. Vozzo and Song (1990) proposed that a structural failure or trauma of cell membranes in stressed seeds allows more than usual active and passive translocation of elements. Vozzo (1990) reported a greater amount of ionic loss from poorly germinating seeds than from the good source as detected by atomic absorption. Contrary to results of the present study, Vozzo (1990) reported that bad seeds had twice as much Ca as present in good seeds.

Table 2. Elemental composition of seed coat surfaces from accelerated aged seeds of *Pinus roxburghii* expressed as a percentage of total counts of spectral energies generated

Element	Accelerated aging (h)				
	0	48	96	144	192
Si	2.1^{c}	18.9^{b}	5.5^{d}	24.8^{a}	14.0^{c}
P	0.0	0.5^{b}	0.7^{b}	0.7^{b}	4.4^{a}
S	1.1^{cb}	1.1^{cb}	1.9^{b}	2.9^{b}	8.5^{a}
Cl	1.2	nd	nd	nd	nd
K	19.9^{d}	25.4^{c}	32.7^{b}	20.8^{d}	37.4^{a}
Ca	7.5^{c}	30.9^{a}	26.5^{b}	26.1^{c}	8.6^{c}
Fe	58.6^{a}	21.6^{c}	27.9^{b}	23.1^{c}	23.3^{c}
Ni	1.3	nd	1.3	nd	nd
Cu	8.3^{a}	1.6^{c}	3.5^{b}	1.6^{c}	3.8^{b}

nd: not detected.
Means not sharing the same letter(s) differ significantly at 0.01 probability by Duncan's Multiple Range Test (DMRT).

The hygroscopic nature of the seeds allows them to adjust their moisture content to be in equilibrium with any given relative humidity. If the seed moisture content remains high or reaches higher levels, normal germination may occur; however, if the increased moisture level is not sufficient for germination, the seed deteriorates because of energy expenditure or accumulation of breakdown products. A related group of enzymes, the phospholipases, destroys the membrane structure of seed (Copeland & McDonald 1985).

This study showed clear variations in elemental concentrations during seed aging of both species. Calcium and iron decreased with aging, while potassium, phosphorus and sulphur increased. Among the elements detected, potassium was present at higher concentrations on the seed coat surfaces of aged seeds of both species.

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