HORTSCIENCE 26(5):597–599. 1991.

Anesthetic Storage of Recalcitrant Seed: Nitrous Oxide Prolongs Longevity of Lychee and Longan

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Additional index words. *Litchi chinénsis, Dimocarpus longan*

Abstract. **Seeds of the recalcitrant species lychee *(Litchi chinénsis* Sonn.) and longan *(Dimocarpus longan* Lour.) were stored near 100% relative humidity at 8 to 10C in air, 80% nitrous oxide (N_2O) plus 20% oxygen, or 100% nitrous oxide. The combination of anesthetic and oxygen extended storage longevity of both species. Seeds stored in 100% N_2O lost terminability at the same rate as those stored in air. Lychee seeds retained 92% of initial germination after 12 weeks under 80% N_2O/20% O_2, while those under air retained only 44%. Longan seeds failed to germinate after 7 weeks under air, yet retained 70% of their initial germination under 809” N_2O/20% O_2. The combination of anesthetic and oxygen atmospheres could provide a new approach to recalcitrant seed storage.**

The long-term storage of recalcitrant seeds has not been accomplished using conventional maintenance techniques. Most tropical seeds are desiccation- and/or temperature-sensitive, precluding the usual low-moisture/low-temperature storage methods employed for orthodox seeds. Several critical factors have been identified for the storage of recalcitrant seed: temperature, moisture, and oxygen (King and Roberts, 1980). Conditions of relatively high temperature (above 0C), high humidity, and a level of O_2 sufficient to support respiratory metabolism are complicated by the potential for both microbial growth and seed germination during storage (King and Roberts, 1980).

The effects of atmosphere on traditional storage of orthodox seed are reported as variable (Bass and Stanwood, 1978; Priestley, 1986). Natural and artificial inhibitors (e.g., hormones, abscisic acid, methyl-1-napthalene acetate) of germination have been applied in recalcitrant seed storage, with uncertain results and with possible deleterious side effects (King and Roberts, 1980). Kidd (1914) reported increased longevity of rubber seeds stored under 40% to 4.5% CO_2; however, this effect has not been confirmed (King and Roberts, 1980).

The biochemical effect of the gaseous anesthetic nitrous oxide (N_2O) has been demonstrated in plants as a reversible, dose-dependent inhibition of mitochondrial respiration, probably by targeting the key bioenergetic enzyme cytochrome *c* oxidase (Sowa et al., 1987); N_2O binding causes small structural changes in the enzyme and, therefore, noncompetitively reduces catalytic activity (Einarsdóttir and Caughey, 1988). In addition to these effects on isolated organelles and enzyme, N_2O also has been shown to affect respiration by inhibiting dioxygen utilization of germinating whole bean seeds (Sowa et al., 1990).

Here we applied a novel approach to recalcitrant seed storage, maintaining seed in a high-moisture, anesthetic atmosphere to reversibly inhibit respiratory (and possibly other) metabolism.

Samples of lychee and longan were collected at the National Clonal Germplasm Repository, Hilo, Hawaii, and shipped in moist sphagnum moss within 48 h of harvest to the National Seed Storage Laboratory, Fort Collins, Colo. The 500 seeds of each species were divided into samples of 30 seeds each (25 for germination, five for individual moisture determinations) and placed in porous nylon netting. Seeds were immediately planted in moist vermiculite at 25C. Germination was evaluated weekly for up to 4 weeks. Initial germination was 100% for lychee and 8570 for longan. Initial moisture contents were obtained on individual seeds by drying at 90C for 5 days.

Samples were stored in air, 80% N_2O/20% O_2, or 100% N_2O in large (250 mm id,) desiccators with tabulated covers. About 2.5 cm of water was kept in the bottom of the desiccators to maintain near 100% relative humidity during storage. Atmospheres were exchanged through inlet/outlet tubes in the cover tabulations. Gases were mixed using precalibrated flowmeters and washed through distilled water before being introduced into the desiccators. Desiccators were purged for sufficient time to exchange 3 to 4 volumes of atmosphere, sealed, and placed in dark storage at 8 to 10C.

Samples were removed at various intervals for germination and moisture testing. Lychee seeds were sampled over a total of 12 weeks of storage, while longan sampling extended over 7 weeks. The desiccators were regassed after each sample removal. Germination percentages were transformed to probit values (Roberts, 1972) before regression analysis. Data are plotted as transformed values.

Moisture content of the longan seeds did not change during storage, remaining close to 42% (fresh-weight basis) (Table 1). The lychee seeds, however, gained water slowly during storage in the same containers with the longan seeds, increasing from an initial moisture content of 45% to an average of 52% after 12 weeks in storage (Table 1). This moisture increase may reflect changes in the physiological state of the lychee seed during storage.

Anesthetic storage extended the viability

Table 1. Percent moisture content (fresh-weight basis) ± SE of lychee and longan seeds after storage under various atmospheres.[z]

Duration of storage (weeks)	Air	80% N_2O/20% O_2	100% N_2O
		Lychee	
0	45.1 ± 2.0	---	---
1	41.5 ± 0.9[y]	42.7 ± 1.0[x]	43.1 ± 1.7[y]
3	44.4 ± 0.2[y]	45.0 ± 0.7	44.9 ± 1.5
5	45.1 ± 0.9[y]	46.7 ± 1.4[y]	44.8 ± 1.5
9	49.5 ± 1.6[x]	51.9 ± 5.2	50.2 ± 2.7
12	54.8 ± 2.0[y]	49.4 ± 3.7[y]	51.3 ± 0.5[x]
		Longan	
0	42.1 ± 1.2	---	---
2	42.7 ± 1.2	44.9 ± 1.3	44.0 ± 1.4
3	43.6 ± 1.4	43.5 ± 1.1	44.3 ± 1.5
4	42.4 ± 1.7	43.3 ± 0.9	44.0 ± 1.5
5	45.0 ± 1.3	42.3 ± 1.6	43.4 ± 1.6
7	45.0 ± 1.7	43.5 ± 1.8	43.1 ± 1.6

[z]For controls, n = 10; all others, n = 5.
[y]n = 4.
[x]n = 3.

Received for publication 19 Jan. 1989. Supported by state and Hatch Act funds allocated to the Georgia Agr. Expt. Stas. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement solely* to indicate this fact.
[1]Professor.

Table 2. Regression parameters for relationship between weeks in storage (x) and germination probits (Y).

Atmosphere	$\hat{Y}$, x = 0	Slope ± SE	R^2
	Lychee		
Air	7.62	−0.24 ± 0.02	0.98[z]
80% N_2O/20% O_2	7.27	−0.10 ± 0.04	0.55
100% N_2O	7.68	−0.27 ± 0.02	0.97[z]
	Longan		
Air	6.28	−0.45 ± 0.10	0.83[z]
80% N_2O/20% O_2	5.50	−0.08 ± 0.09	0.15
100% N_2O	5.52	−0.43 ± 0.13	0.73[z]

[z]Significant at $P = 0.05$.

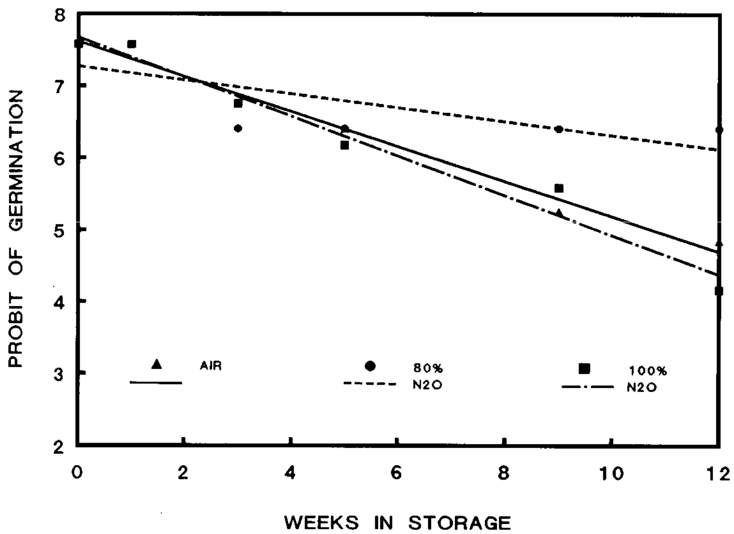

Fig. 1. Probits of percent germination of lychee seed after storage under various atmospheres for 12 weeks. To obtain a probit value for 100% germination, a value of 99.5% was arbitrarily used.

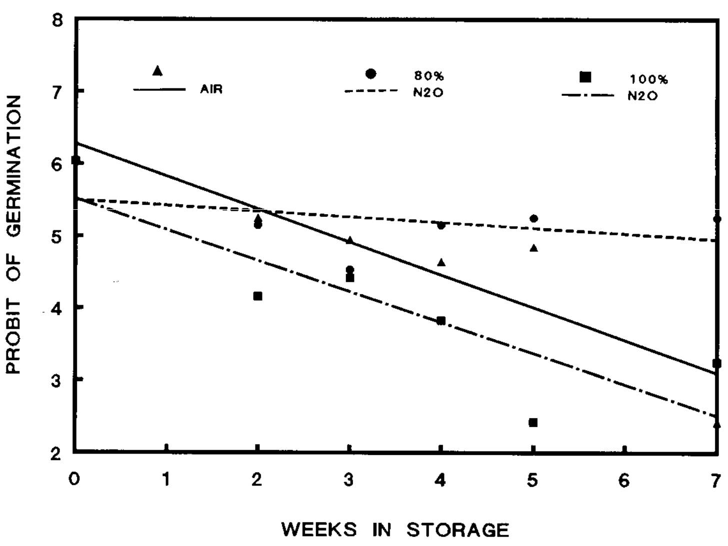

Fig. 2. Probits of percent germination of longan seed after storage under various atmospheres for 7 weeks. To obtain a probit value for 0% germination, a value of 0.5% was arbitrarily used.

of these recalcitrant species (Figs. 1 and 2). Deterioration rates of both species stored under 80% N_2O/20% O_2 differed significantly from those in air or 100% N_2O (Table 2). There was no significant relationship between percent germination and storage time for seeds stored in 80% N_2O, while for seeds stored in the other atmospheres, a negative correlation existed between percent germination and storage time. Also, the standard errors for the slope of the regression lines indicated significant differences between 80% N_2O and the other storage conditions (Table 2). After 12 weeks in storage, lychee seeds stored under 80% N_2O/20% O_2 maintained 92% of their initial germination, while seeds stored in air were at 44%. After 7 weeks under the 80% N_2O/20% O_2, longan seeds maintained 70% of their initial germination, while viability under air was completely lost. This result confirms preliminary data from the 1988 season with a smaller sample of longan seed (S. S., unpublished data).

Most germination occurred within 2 weeks of planting. An assessment of seedling development for each sample was also made after 3 weeks of germination, during which plants that had formed well-defined stem and leaf structures were counted and compared to seeds that merely exhibited root emergence. The number of developed seedlings at each sampling time was highest for those seeds stored under the 80/20 conditions.

Our experiments demonstrate increased storage longevity of lychee and longan seeds under a nitrous oxide/oxygen atmosphere. At present, we do not know the maximal effects of these storage conditions, or how long the storability of recalcitrant seeds can be extended. However, this type of storage does have immediate application for short-term use, such as in transportation or intermediate storage; it might also be used in conjunction with other methods (e.g., cryopreservation) being researched to store recalcitrant germplasm.

Nitrous oxide is particularly suited to storing whole seeds or other plant tissues as it is nontoxic at low concentration and, as a gas, readily permeates tissue. The concentrations used in our experiments are not expetted to induce chromosomal damage, although high specific activity of N_2O (high--pressure application of 100% anesthetic gas) has been shown to alter chromosome numbersin some plant species. For example, 4 atm N_2O was reported to produce eupolyploids and aneuploids in wheat and barley (Dvorak et al., 1973) and aneuploids in oat (Dvorak and Harvey, 1973).

The presumed inhibition of respiratory metabolism by N_2O is expected to slow germination during moist storage and also to inhibit the metabolism and, therefore, growth of contaminating (aerobic) microorganisms.

The application of anesthetic gas is a comparatively short-term solution for prolonged recalcitrant seed storage. It does, however, offer a new perspective to the biochemical considerations of seed storage of some tropical species.

Literature Cited

Bass, L.N. and P. C. Stanwood. 1978. Long-term preservation of sorghum seed as affected by seed moisture, temperature, and atmospheric environment. Crop Sci. 18:575–577.

Dvorak, J. and B. L. Harvey. 1973. Production of aneuploids in *Avena sativa* L. by nitrous oxide. Can. J. Genet. Cytol. 15:649-651.

Dvorak, J., B.L. Harvey, and B.E. Coulman. 1973. The use of nitrous oxide for producing eupolyploids and aneuploids in wheat and barley. Can. J. Genet. Cytol. 15:205-214.

Einarsdóttir, O. and W.S. Caughey. 1988. Interactions of the anesthetic nitrous oxide with bovine heart cytochrome *c* oxidase. J. Biol. Chem. 263:9199-9205.

Kidd, F. 1914. The controlling influence of carbon dioxide in the maturation, dormancy and germination of seeds. Part II. Proc. Royal Soc. (London) sect. B, 87:609-624.

King, M.W. and E.H. Roberts. 1980. Maintenance of recalcitrant seeds in storage, p. 53–89. In: H.F. Chin and E.H. Roberts (eds.). Recalcitrant crop seeds. Tropical Press Sdn. Bhd., Kuala Lumpur, Malaysia.

Priestley, D.A. 1986. Seed aging. Comstock Publishing Assoc., Ithaca, N.Y. p. 59-62.

Roberts, E.H. 1972. Storage environment and the control of viability, p. 14-48. In: E.H. Roberts (ed.). Viability of seeds. Chapman and Hall, London.

Sowa, S., A. Dong., E.E. Roos, and W.S. Caughey. 1987. The anesthetic nitrous oxide affects dioxygen utilization by bovine heart and bean seed mitochondrial particles. Biochem. Biophys. Res. Commun. 144:643-648.

Sowa, S., D. Gerving, W.S. Caughey, and E.E. Roos. 1990. Respiratory effecter molecules and dioxygen utilization in germinating bean seeds. Annu. Rpt. Bean Improv. Coop. 33:141-142.