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Temporary Suppression of Radicle Elongation of Germinated Impatiens Seeds by Low Oxygen Concentrations

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Abstract. **Germinated *Impatiens wallerana* Hook. f. 'Super Elfin Salmon Blush' seeds were exposed to subatmospheric O_2 concentrations for 12, 24, or 48 hours at 25C. Suppression of radicle growth during a subsequent 24-hour simulated shipping period was monitored, as was plant growth during a subsequent growth cycle. One percent to 2% O_2 for 12 or 24 hours limited radicle elongation to <1.0 mm during the simulated shipping period (darkness, ambient O_2) and caused no permanent damage to seedlings. Suppression of radicle elongation with low O_2 was greater with a 24-hour than a 12-hour exposure. Oxygen at 0% for 24 hours or at 0% to 1.5% O_2 for 48 hours damaged seedlings irreversibly. These results show that specific subatmospheric O_2 treatments can restrict radicle elongation of germinated seeds during subsequent shipment to a grower and that the low O_2 treatment does not decrease subsequent plant growth.**

Maximum efficiency in plug production systems requires that seeds germinate synchronously and at a high percentage. Seeds of many desirable ornamental species germinate poorly (Cathey, 1969), which precludes their use in plug-system technologies. For several vegetable crops, use of germinated seeds has increased stand establishment substantially (see Finch-Savage, 1984). Germinated seeds that were separated from nongerminated seeds by density gradient differences showed increased vigor and percent emergence when compared with controls (Taylor and Kenny, 1985). Germination of ornamental seeds by a seed company, followed by shipment to and sowing at a commercial greenhouse, could improve plug production efficiency of certain species. Radicle elongation during shipment must be minimized, however, or damage from handling and mechanical sowing would preclude use of germinated seeds.

Radicle growth during storage of germinated seeds can be retarded (Brocklehurst et al., 1980; Finch-Savage and McKee, 1988). However, some methods decreased seedling emergence (Pill and Fieldhouse, 1982), whereas others decreased vigor and increased the number of abnormal seedlings (Frazier et al., 1982). Radicle growth also may be reduced or eliminated by reducing the O_2 concentration in the storage environment. For example, radicle growth of some germinated vegetable seeds stored in 100% N_2 or in evacuated plastic bags was inhibited at 7C but not at 20C (see Ghate and Chinnan, 1987). Eavis et al. (1971) found that radicle elongation of pea (*Pisum sativum* L.) decreased progressively as O_2 concentration decreased. In addition, roots of rice (*Oryza sativa* L.) seedlings did not elongate and shoots grew only slightly when seedlings were transferred to anaerobic conditions (Bertani et al., 1980). Our objective was to determine whether low-O_2 treatment of germinated impatiens seeds could suppress radicle growth during a simulated 1-day shipping period without affecting subsequent seedling growth.

Materials and Methods

'Super Elfin Salmon Blush' impatiens seeds (PanAmerican Seed Co., West Chicago, Ill.) were germinated for 72 h by placing 250 seeds on two layers of water-saturated, Steel Blue Anchor Seed Germination Blotter (Anchor Paper, St. Paul, Minn.) in a plastic box (22.5 × 15.0 × 5.0 cm) sealed in Saran Wrap to prevent evaporation. Germination was conducted in a growth chamber maintained at 25 ± 1C, and the continuous irradiance from the cool-white fluorescent lamps was measured inside the plastic box as 105 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seeds were sown at 0, 24, and 48 h (Expt. 1) or 0, 12, and 24 h (Expt. 2) to allow synchronization of treatments. Germination required 72 h to obtain seeds that possessed the desired radicle length [≤0.25 mm (barely emerged from seedcoat)]. Twenty-five germinated seeds were selected and arranged in a 5 × 5 pattern on two layers of water-saturated germination blotter in a 9.2 × 6.6 × 1.3-cm clear plastic box without a lid in preparation for the modified-atmosphere treatments. The four groups of four seeds in each corner of the pattern (16 total seeds) were retained in the plastic box and used to obtain data at the end of the following periods: modified atmosphere, simulated shipping, and 7-day recovery in air. The nine seeds in the combined middle row and middle column were transplanted into plug trays after simulated shipping and used to obtain data for the 5-week recovery period in the plug-tray section of the experiments.

The plastic boxes were placed in wide-mouth, 1-liter mason jars laid horizontally, and the jars were sealed with lids that contained a gas inlet, a gas outlet, and a sampling port. Each modified atmosphere flowed through its jar at two exchanges per hour. The germination blotter was kept saturated by adding ≈1.5 ml water daily. The germinated seeds were exposed for 24 or 48 h (Expt. 1) or for 12 or 24 h (Expt. 2) to modified atmospheres that contained 0%, 1%, 1.5%, 2%, 3%, or 20% O_2 in N_2. Starting times of the modified-atmosphere treatments were staggered so that treatment periods ended simultaneously. Modified atmospheres were obtained by using a two-stage, capillary-tube mixing system (Diesburg et al., 1989), and composition was verified every 12 h by gas chromatography. Modified-atmosphere treatments were conducted in three growth chambers that were maintained at 25 ± 1C with continuous irradiance of 105 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps. At the end of the modified-atmosphere treatment period, radicle length of the designated 16 seedlings was measured with a ruler. After the modified-atmosphere treatment, the atmosphere in all jars was changed to air for 24 h to simulate shipping. Compressed air was passed through two Balston A92 coalescing filters (Balston, Lexington, Mass.) to remove traces of compressor oil. Twenty-five germinated seeds contained in additional plastic boxes that received no modified-atmosphere treatment, but only simulated shipping, served as controls. Lamps were turned off to provide a dark simulated-shipping period, and the chamber was maintained at 25 ± 1C. After simulated shipping, chamber lamps were turned on, and length of the designated 16 seedlings was measured.

After simulated shipping, the designated 16 seedlings that remained on germination blotter in the boxes were allowed to develop in air in mason jars for 7 days in a growth chamber at 25 ± 1C with continuous irradiance of 105 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Filtered air flowed through each mason jar at two exchanges per hour. At the end of the 7-day recovery period, hypocotyl length and percentage of abnormal seedlings were determined. Abnormal seedlings exhibited thickening and curling of the hypocotyl, negative geotropic growth, loss of cotyledons, or lack of radicle elongation. After simulated shipping, the nine designated seedlings were transplanted into 406-cell plug trays (Blackmore Co., Belleville, Mich.). The growth medium was 3 sphagnum moss (<0.64 cm in diameter) : 3 sphagnum moss (<0.36 cm in diameter) : 2 grade 3 vermiculite : 2 sand-

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finish (small particle diameter) perlite (by volume) amended with $CaCO_3$ at 2.0 kg·m^{-3}. Transplanted seedlings grew in a greenhouse at 23 ± 3C with natural light during spring. Seedlings received tap water once or twice daily during the first week. During the second and third weeks, they received alternating applications of tap water and 50% Hoagland's Solution No. 1 with Sequestrene 330 (FeDTPA) as the iron source (Hoagland and Arnon, 1950). During the fourth and fifth weeks, seedlings received alternating applications of tap water and 100% Hoagland's solution. After the 5-week recovery period, shoots were cut immediately below the cotyledons–cotyledonary leaf scars and oven-dried at 67 to 70C for 72 h. Shoot dry mass per plant was determined.

The 12 treatments and the control that entered at the start of simulated shipping were assigned randomly to three growth chambers (three blocks) in a randomized complete-block design. Each experiment was conducted 3 times. One millimeter was selected as a radicle length that would not incur appreciable damage during subsequent mechanical sowing (Finch-Savage and McKee, 1988). One-tailed *t* tests were used to determine whether radicle length at the end of the simulated shipping treatment was ≤1.0 mm. Treatments that yielded a radicle length ≤1.0 mm were considered successful. For each successful treatment, subsequent growth characteristics were compared by regression analysis and with the control by using two-tailed *t* tests. Data from the common 24-h treatments of Expts. 1 and 2 were combined. The O_2 concentration × exposure duration interaction was tested. Linear analysis was conducted on exposure duration over the range of 0% to 3% O_2 and on O_2 concentration over the range of exposure durations. In addition, regression analysis was done for each exposure duration over the range of 0% to 20% O_2 (preliminary experiments) and 0% to 3% O_2 (combined Expts. 1 and 2).

Results and Discussion

Preliminary experiments. When O_2 concentrations were 0% to 10% for 24 or 48 h, radicle elongation was proportional to O_2 concentration, but elongation in 10%, 13%, and 20% O_2 was similar (Fig. 1). Oxygen concentrations of 0% to 2% inhibited radicle elongation adequately (≤1 mm), but recovery from low O_2 was satisfactory only with 1% and 2% O_2 (data not presented).

Radicle length after simulated shipping. Upon completion of simulated shipping, radicle length was ≤1.0 mm (statistically) at all O_2 concentrations, except 20%. Radicle length responded positively to O_2 concentration, but lack of fit was significant due to an unaccountably high value for 1% O_2 (Table 1), whereas the response to exposure duration was negative (Table 2). The interaction between O_2 concentration and exposure duration for radicle length after simulated shipping was significant (Fig. 2A). Radicle length increased linearly with increasing O_2 concentration for the 24-h exposure (Fig. 2A). Radicle length for the 12-h exposures varied little over the range of 0% to 3% O_2, and the linear effect was absent (Fig. 2A). Radicle length over the 0% to 3% O_2 range was most affected with the 48-h exposure, but the trend was not linear due to an unaccountably high value for 48-h exposure to 1% O_2. Oxygen concentrations ≤2% for all exposure durations restricted radicle growth effectively.

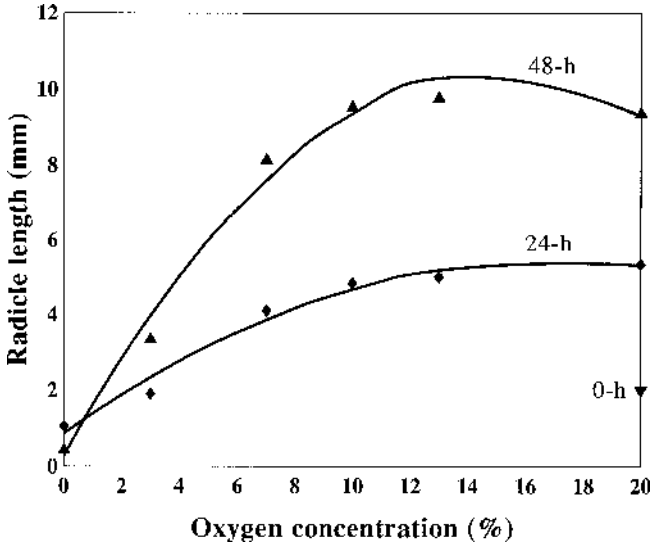

Fig. 1. Effect of O_2 concentration at 24- and 48-h exposure durations on radicle length of germinated impatiens seeds at the end of simulated shipping. Each data point is the mean of three replications over time, with three observations per replicate. Trend analysis is 24-h (◆), $P >$ F: linear, <0.001; quadratic, <0.001; and 48-h (▲), $P >$ F: linear, <0.001; quadratic, <0.001. Control [(▼), 0-h germinated seeds that received no exposure to a modified atmosphere] is the mean of three replications over time, with six observations per replicate.

Table 1. Effect of O_2 concentration averaged over exposure duration treatments on growth characteristics of impatiens after various periods.[z]

		End of recovery period		
		7 days		5 weeks
O_2 concn (%)	Radicle length after simulated shipping (mm)	Hypocotyl length (mm)	Abnormal seedlings (%)	Shoot dry mass (mg)
0	0.6	4.1	22.1	39
1	0.9	4.8	9.6	47
1.5	0.7	4.9	7.9	47
2	0.9	5.0	4.7	46
3	1.1	5.1	4.0	49
20	4.0	5.5	2.5	50
Linear, $P >$ F	0.0001	0.0027	0.0012	0.0345
Lack of fit	0.001	0.187	0.125	0.305

[z]Linear analysis done by O_2 concentration (0% to 3%) on exposure duration. The 20% O_2 means are included for comparison, but they were not a part of the statistical analysis. Each mean represents the average of three (12- and 48-h) or six (24-h) replications over time, with three observations per replicate.

Table 2. Effect of O_2 exposure duration averaged over O_2 concentration treatments on growth characteristics of impatiens after various periods.[z]

Modified-atmosphere		End of recovery period		
		7 days		5 weeks
exposure duration (h)	Radicle length after simulated shipping (mm)	Hypocotyl length (mm)	Abnormal seedlings (%)	Shoot dry mass (mg)
12	1.0	4.9	3.1	51
24	0.8	4.9	6.7	47
48	0.7	4.3	22.1	38
Linear, $P >$ F	0.0005	0.0104	0.0008	0.0034
Lack of fit	0.021	0.095	0.116	0.615
$LSD_{0.05}$	0.066	0.401	6.535	6.192

[z]Linear analysis done by exposure duration on O_2 concentrations of 0% to 3%. Each mean represents the average of three (12- and 48-h) or six (24-h) replications over time, with three observations per replicate.

Seedling growth. Hypocotyl length responded linearly to O_2 concentration and exposure duration (Tables 1 and 2), and O_2 concentration and exposure duration did not interact (Fig. 2B). For 48-h exposures, increasing O_2 concentration permitted a quadratic increase in hypocotyl length during the 7 days after exposure (Fig. 2B). The change in

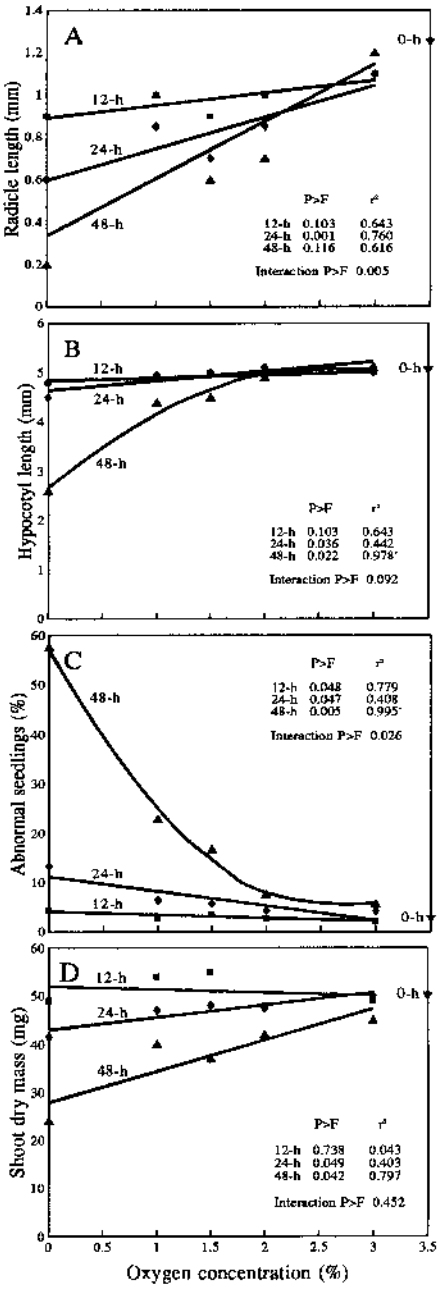

Fig. 2. Interaction between modified-atmosphere O_2 concentration and exposure duration on (**A**) radicle length at the end of simulated shipping; (**B**) hypocotyl length and (**C**) abnormal seedling percentage at the end of the 7-day recovery period; and (**D**) shoot dry mass at the end of the 5-week recovery period. Each data point is the mean of three (12- and 48-h) or six (24-h) replications over time, with three observations per replicate. Data points were compared with the control (six replications over time, six observations per replicate) by using two-tailed *t* tests (statistical analysis for *t* tests not presented). Symbols are 12 h (■), 24 h (◆), 48 h (▲), and control [(▼), 0-h germinated seeds that received no exposure to a modified atmosphere]. The asterisks in panels B and C indicate that R^2 is the appropriate designator, rather than r^2, for the curvilinear response in the 48-h exposure durations.

hypocotyl length was less prominent for 24-h exposures, and for 12-h exposures, there was no effect of O_2 concentration (Fig. 2B). For O_2 concentrations that kept radicle length ≤1.0 mm after simulated shipping, exposure for 12 or 24 h resulted in hypocotyl lengths equal to those of the control and longer than those for the corresponding 48-h treatment (Fig. 2B). Only the 48-h exposure to 0% or 1% O_2, or 24-h exposure to 0% O_2 reduced the hypocotyl length below that of the control. Abnormal seedling frequency decreased with increased O_2 concentration (Table 1), increased with increased exposure duration (Table 2), and exposure duration interacted with O_2 concentration (Fig. 2C). The percentage of abnormal seedlings decreased at all exposures with increasing O_2 concentration, but most for the 48-h quadratic response, presumably because >5 times as many seedlings were injured by 48 h at 0% O_2 (Fig. 2C). Compared with the control, the percentage of abnormal seedlings was greater only with 48-h exposure to 0% to 2% O_2 or with 24-h exposure to 0% to 1.5% O_2. Shoot dry mass responded positively to O_2 concentration (Table 1) and negatively to exposure duration (Table 2), and no interaction occurred between O_2 concentration and exposure duration (Fig. 2D). Shoot dry mass increased linearly with increasing O_2 concentration only with the 48-h exposures (Fig. 2D). None of the 12-h exposure treatments reduced shoot dry mass. Shoot dry mass was less than that of the control only with 48-h exposure to ≤1.5% O_2 or with 24-h exposure to 0% O_2 (Fig. 2D).

Radicle elongation during simulated shipping was arrested by exposing germinated seeds to 1% to 2% O_2 for 12 to 24 h, and these conditions caused minimal seedling damage. Oxygen concentrations greater than ≈7% did not arrest radicle elongation, and O_2 concentrations of 0%, 1%, and 1.5% for 48 h damaged seedlings permanently. Seedling damage increased as the duration of exposure to low O_2 increased. In addition, the growth restriction efficacy of the modified atmospheres decreased as exposure duration decreased; thus, exposure to low O_2 concentration for <12 h may not be effective. These results are similar to those of Siegel and Rosen (1962), who found that radicle growth of cucumber (*Cucumis sativus* L.) seedlings grown in 10% or 20% O_2 was equal. However, Eavis et al. (1971) observed that radicle elongation of pea decreased proportionally as O_2 decreased from 21% to 0%.

We have found that low-O_2 treatments restricted radicle elongation of germinated seeds during simulated shipping. This technology might be commercialized through use of a fluid-drilling seeder (see Pill, 1991), although currently this method is slower than methods used for dry-sown seeds. While fluid drilling is a possibility (Ghate et al., 1984), this method would require modification for use with plug trays. A surface seed-drying treatment (Finch-Savage and McKee, 1988) may allow germinated seeds to be sown with conventional equipment after shipment to the grower. Finally, species specificity, cultivar specificity, and cost efficiency of these procedures must be determined because of variation in germination uniformity between species or cultivars.

Literature Cited

Bertani, A., I. Brambilla, and F. Menegus. 1980. Effect of anaerobiosis on rice seedlings: Growth, metabolic rate, and fate of fermentation products. J. Expt. Bot. 31:325–331.

Brocklehurst, P.A., J. Dearman, and W. Finch-Savage. 1980. Effects of aeration during cold storage of germinated vegetable seeds prior to fluid-drilling on seedling viability. Ann. Appl. Biol. 95:261–266.

Cathey, H.M. 1969. Guidelines for the germination of annual, pot plant and ornamental herb seeds—1. Florists' Rev. 144(3742):21–23, 58–60.

Diesburg, K.L., N.E. Christians, and R.J. Gladon. 1989. A continuous air-exchange roomette and gas-meting system. Crop Sci. 29:344–348.

Eavis, B.W., H.M. Taylor, and M.G. Huck. 1971. Radicle elongation of pea seedlings as affected by oxygen concentration and gradients between shoot and root. Agron. J. 63:770–772.

Finch-Savage, W.E. 1984. The effects of fluid drilling germinating seeds on the emergence and subsequent growth of carrots in the field. J. Hort. Sci. 59:411–417.

Finch-Savage, W.E. and J.M.T. McKee. 1988. A study of the optimum drying conditions for cabbage seed following selection on the basis of a newly-emerged radicle. Ann. Appl. Biol. 113:415–424.

Frazier, D.C., S.C. Wiest, and R.D. Wootton. 1982. Storage of pregerminated seed of snapdragon (*Antirrhinum majus* L.) in hydrophilic gels. J. Amer. Soc. Hort. Sci. 107:660–664.

Ghate, S.R. and M.S. Chinnan. 1987. Storage of germinated tomato and pepper seeds. J. Amer. Soc. Hort. Sci. 112:645–651.

Ghate, S.R., S.C. Phatak, and C.A. Jaworski. 1984. A container design for fluid sowing multitreatments in small research plots. HortScience 19:493–494.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. California (Berkeley) Agr. Expt. Sta. Circ. 347.

Pill, W.G. 1991. Advances in fluid drilling. HortTechnology 1:59–65.

Pill, W.G. and D.J. Fieldhouse. 1982. Emergence of pregerminated tomato seed stored in gels up to twenty days at low temperature. J. Amer. Soc. Hort. Sci. 107:722–725.

Siegel, S.M. and L.A. Rosen. 1962. Effects of reduced oxygen tension on germination and seedling growth. Physiol. Plant. 15:437–444.

Taylor, A.G. and T.J. Kenny. 1985. Improvement of germinated seed quality by density separation. J. Amer. Soc. Hort. Sci. 110:347–349.