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Effects of Oxygen and Temperature During Imbibition on Seeds of Two Bean Lines at Two Moisture Levels

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Addition index words. *Phaseolus vulgaris*, flooding, cold imbibition

Abstract. **Low temperature and oxygen stresses were imposed during the first 48 hr of germination on 2 lines of snap bean (*Phaseolus vulgaris* L.), stress tolerant (PI-165426-BS) and stress-sensitive ('Goldcrop'). At 22°C, O_2 concentrations of 0%, 1%, and 2% increased leakage from the seeds, delayed emergence, and reduced growth, compared to 5% and 21% O_2. These effects were aggravated by reducing the initial seed moisture from 12% to 8% in 'Goldcrop', but not in PI-165426-BS. At 10°, the effect of oxygen deficiency was minimized. Low temperatures inhibited growth of 'Goldcrop', but not of PI-165426-BS, and increased leakage from seeds of both lines. The survival of seeds exposed to the low temperature decreased when initial seed moisture was reduced from 12% to 8%. Flooding the seeds for 24 hr increased leakage and reduced emergence and growth much more than 24 hr of complete anoxia. Since the effects of anoxia are different than flooding injury, a mechanism of flooding injury not related to oxygen deficiency is discussed.**

Poor emergence of snap bean is a major problem for bean growers. Factors contributing to poor stands can be divided into 2 categories: preplanting factors, such as genotype, initial seed moisture, and mechanical damage (8, 19, 26); and post-planting environmental factors, such as excess or deficit of water, low temperature, soil compaction, and pathogen infestation (12, 19, 24). Soil flooding after planting is not uncommon as a result of heavy rains, which frequently result in poor stands especially when temperatures are low (12, 13). It is often assumed that flooding causes injury through oxygen starvation (20), though other mechanisms of injury have been suggested (16, 29, 30). Susceptibility to these conditions can be influenced largely by initial seed moisture (12, 19) and variety (25, 26).

Recent cultivars of snap beans, such as 'Goldcrop', were developed primarily for disease tolerance, processing quality, and yield (20). With emphasis in these areas, seed quality characteristics did not receive sufficient attention. The U.S. plant introduction, PI-165426-BS (black-seeded selection), is a collection of wild, viney bean seed from Mexico that is superior to most domesticated varieties in its ability to germinate in cold, wet conditions (26).

The objective of this study was to determine the difference in sensitivity between PI-165426-BS and 'Goldcrop' to anoxia, imbibitional chilling, and low initial moisture content of the seed. In addition, the study attempted to reveal the interaction between anoxia, cold temperature, and seed moisture content during germination of bean seeds.

Materials and Methods

Experimental procedures. A factorial experiment was designed to expose seeds of 2 bean lines with initial moisture contents of 8% and 12%; to oxygen concentrations of 0%, 1%, 2%, 5%, and 21%; and to temperatures of 10° and 22°C during the first 48 hr of imbibition and germination. Twelve seeds were planted 2.5 cm deep in each of 50 plastic beakers (400 ml) containing 244 g of acid-washed fine sand with 25% (dry-weight basis) deionized water. The sand was pre-equilibrated to the correct temperature for about 2 hr. Immediately after seeding the beakers were sealed with melted paraffin, and designated mixtures of air and N_2 were passed over the sand surface at a flow rate of 500 ml·min^{-1} per beaker. The treatments were applied in a growth chamber set at either 10° ± 1° or 22° ± 1°. Each 48-hr run (50 beakers) consisted of 5 replications of the 2 lines at 5 O_2 levels for one temperature–seed moisture combination. Four runs were required to accommodate the 2 temperatures and 2 seed moisture levels.

After each 48-hr imbibition treatment, 20 ml of deionized water was added to each beaker and the seeds were removed by floatation. The seeds were replanted 2.5 cm deep in a greenhouse soil mix [1 soil : 1 river sand : 1 peat (by volume)] with 20% water (dry weight) in pans (46 × 25 × 15 cm), 120 seeds

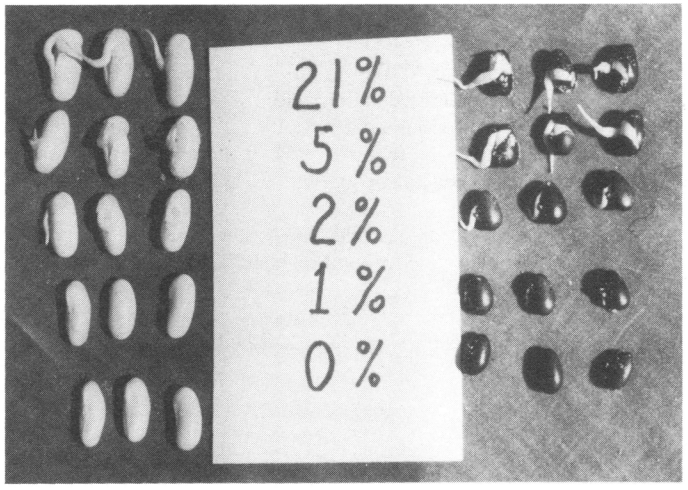

Fig. 1. Germination and radicle elongation of 12% moisture seeds of 'Goldcrop' (left) and PI-165426-BS (right) after 48 hr of O_2 treatments at 22°C.

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Table 1. Emergence from 8% and 12% moisture seeds of 2 bean lines, as affected by 5 O_2 concentrations and 2 temperatures during a 48-hr imbibition period.

	Days to 50% emergence			
	Seed moisture (8%)		Seed moisture (12%)	
	Temperature (°C)			
O_2 (%)	10	22	10	22
	Goldcrop			
0	4.8 ± 0.05[z]	4.6 ± 0.23	4.7 ± 0.11	3.6 ± 0.05
1	4.8 ± 0.11	4.8 ± 0.17	4.7 ± 0.11	3.7 ± 0.07
2	4.9 ± 0.09	3.8 ± 0.08	4.6 ± 0.06	2.6 ± 0.08
5	4.8 ± 0.08	2.6 ± 0.08	4.7 ± 0.07	2.0 ± 0.08
21	4.8 ± 0.17	2.5 ± 0.08	4.7 ± 0.11	1.9 ± 0.12
	PI-165426-BS			
0	4.1 ± 0.05	3.0 ± 0.20	3.7 ± 0.09	3.0 ± 0.11
1	4.1 ± 0.13	2.9 ± 0.12	3.5 ± 0.06	2.9 ± 0.06
2	4.2 ± 0.09	2.4 ± 0.06	3.6 ± 0.04	2.3 ± 0.10
5	4.0 ± 0.17	2.2 ± 0.10	3.6 ± 0.03	1.7 ± 0.08
21	4.2 ± 0.05	2.0 ± 0.08	3.6 ± 0.04	1.6 ± 0.04

[z]Means and SEM.

per pan. After planting, the soil surface was sprayed with 500 ppm Ridomil to prevent infection by *Pythium* spp. The pans were placed in a growth chamber for emergence and growth of the seedlings at day and night temperatures of 25°C for 16 hr and 22° for 8 hr, respectively. Light intensity of 132 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at plant level was provided by VHO fluorescent lamps. Emergence was recorded daily at the appearance of the hypocotyl hook. After a 10-day growth period, seedling tops were harvested and dry weight was measured after 15 hr of drying at 95°.

Estimation leakage. Following removal of seeds, the water was extracted from the sand by vacuum. Conductivity was measured, and sugars and related substances were estimated by phenol-sulfuric acid reaction (9).

Leakage was expressed on a "per seed" basis. No adjustments of leakage to seed size were made. The ratios of the dimensions between seeds of 'Goldcrop' and PI-165426-BS are 1.33:1 by weight, and 1.21:1 by surface area (considering the seeds a sphere).

Oxygen regimes. Five O_2 levels were obtained by mixing atmospheric air and N_2. Each of the 5 gas regimes was mixed and humidified by bubbling through a water column and distributed by a manifold to 10 beakers (5 replications × 2 lines). Air samples were taken above the sand and at seeding depth, and O_2 concentration was determined with a Varian Aerograph gas chromatograph (model 90-P). Oxygen concentrations at seed depth were found to be the same as those above the sand and in the original N_2/air mixtures (determined 5–10 min after sealing the beakers).

Demonstration of the effects of O_2 deficiency and flooding. A completely randomized experiment consisted of 3 treatments in 5 replications: 24-hr imbibition under flooding, under N_2, and under atmospheric air.

'Goldcrop' seeds with 11% moisture were planted in flasks with 300 g of sand containing 75 ml deionized water (15 seeds/flask). The flasks were sealed, and N_2 or air was passed over the sand surface. In the flooding treatment, 36 ml deionized water was added immediately after planting. To the nonflooded flasks, 36 ml deionized water was added after the 24-hr treatments. After treatment, the seeds were removed and the water extracted from the sand by vacuum. Leakage was evaluated as described. The seeds were replanted as described and held in a growth chamber for 14 days.

Plant material. Two bean lines were used: 'Goldcrop', a domesticated cultivar (25); and a black-seeded selection out of PI-165426. Seeds free of mechanical damage were screened for uniform size between 12 and 14 mesh (4.8–5.6-mm width of the screen holes) for 'Goldcrop' and between 10 and 12 mesh (4.0–4.8-mm width) for PI-165426-BS. Average seed weight was 305 and 225 mg per seed for 'Goldcrop' and PI-165426-BS, respectively.

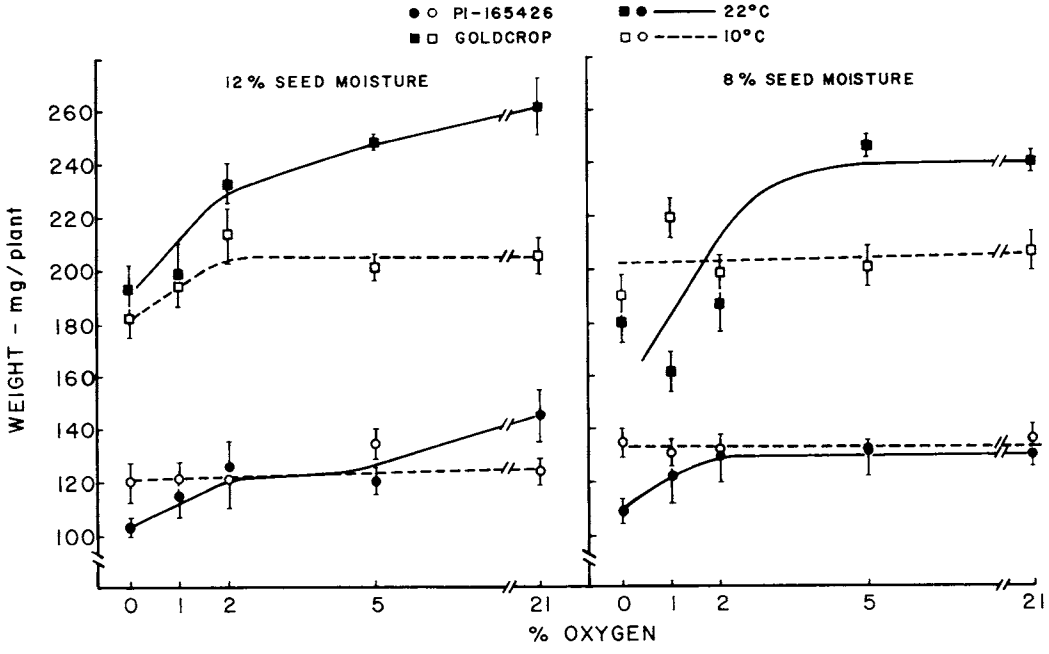

Fig. 2. Dry weights of 10-day-old seedlings from 8% and 12% moisture seeds of 2 bean lines, exposed to 5 O_2 levels and 2 temperatures (bars = SEM).

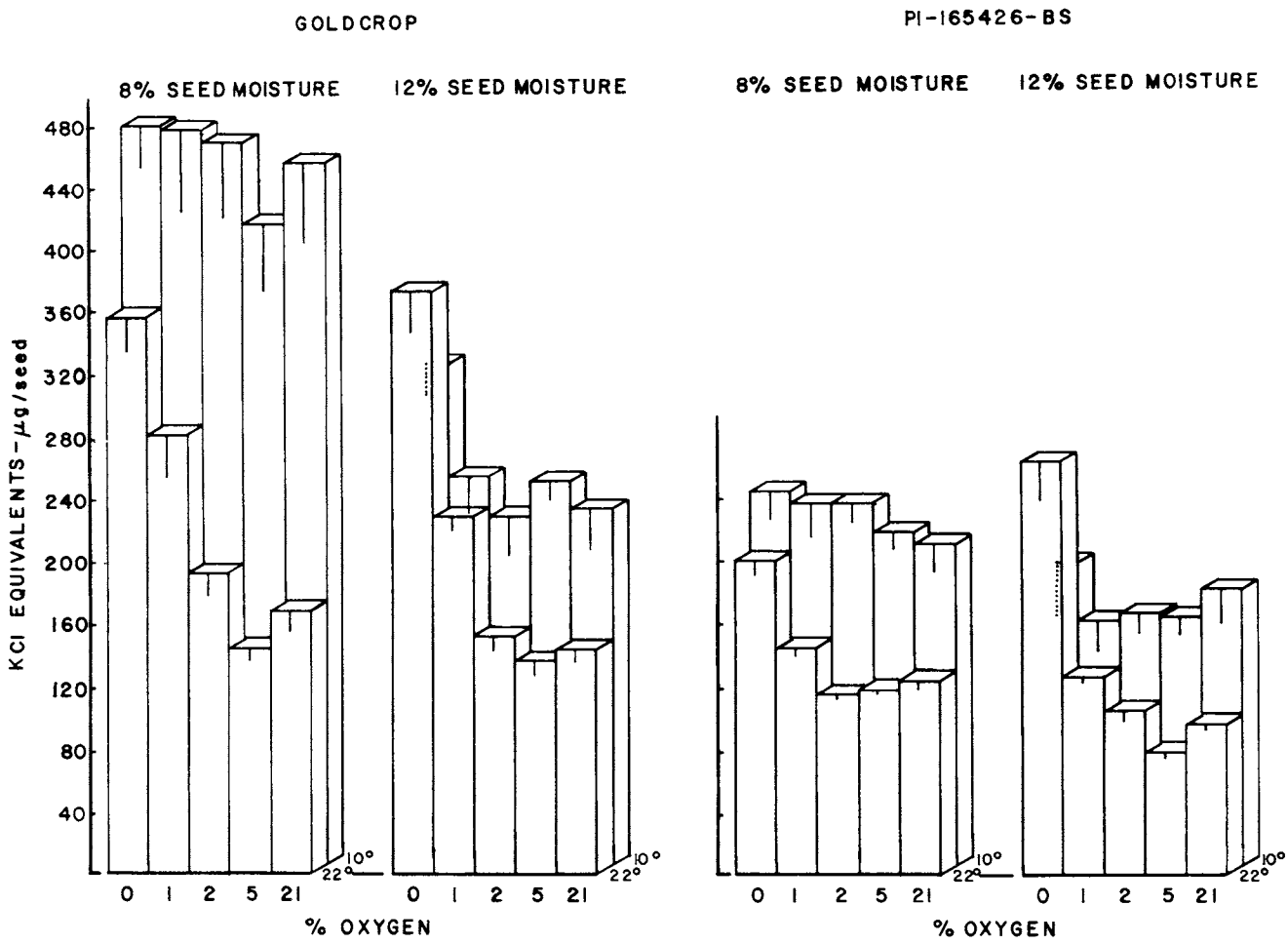

Fig. 3. Electrolyte leakage from 8% and 12% moisture seeds of 2 bean lines exposed to 5 O_2 levels and 2 temperatures (bars = SEM).

Table 2. Effects of anoxia and flooding during the first 24 hr of germination of 'Goldcrop' seeds with 11% moisture content.

Treatment	Fresh wt increase[z] (%)	leakage equivalent of KCl[z] (µg)	leakage equivalent of CH_2O[z] (µg)	Days to 50% emergence	Total emergence[y] (%)	Seedling top wt[y] (mg·plant^{-1})	Normal plants[y] (%)
Air	106 ± 0.3[x]	144 ± 10	21 ± 2	1.9 ± 0.07	100	234 ± 4	98
Nitrogen	92 ± 0.2	411 ± 24	79 ± 13	2.8 ± 0.04	100	208 ± 4	98
Flood	105 ± 0.08	1023 ± 11	313 ± 9	3.8 ± 0.16	92	129 ± 6	46

[z]Recorded at the end of the 24-hr treatment period.
[y]Recorded 14 days after emergence.
[x]Means and SEM.

Initial seed moisture was determined by a Steinlite moisture tester or gravimetrically by drying at 95°C to constant weight. Seeds from storage had 11.6% moisture (fresh weight basis) in both bean lines. To reduce the moisture content, the seeds were dried for 3 weeks at 22°–25° to 7.5% moisture (designated 8%).

Results

Effects of imbibitional stresses on germination and emergence

Germination and emergence as affected by O_2 concentration. Exposure to anoxia during the 48-hr treatment phase affected germination and emergence only at 22°C. Germination and radicle growth were the same for both lines at 5% and 21% O_2 but were reduced at 2% O_2 (Fig. 1). At 1% O_2 only seeds of PI-165426-BS germinated during the treatment phase; and at 0% O_2 neither line germinated during the treatment period (Fig. 1).

Emergence after replanting was affected by O_2 stress at 22°C, but not at 10° (Table 1). Oxygen levels of 0% and 1% resulted in delayed emergence of the 12% seeds by 1.7 and 1.4 days for 'Goldcrop' and PI-165426-BS, respectively. Seeds with 8% moisture were delayed 2.1 days for 'Goldcrop' and 1.0 day for PI-165426-BS. The final percentage of emergence of 12% moisture seeds was not affected by the O_2 treatments (data not shown).

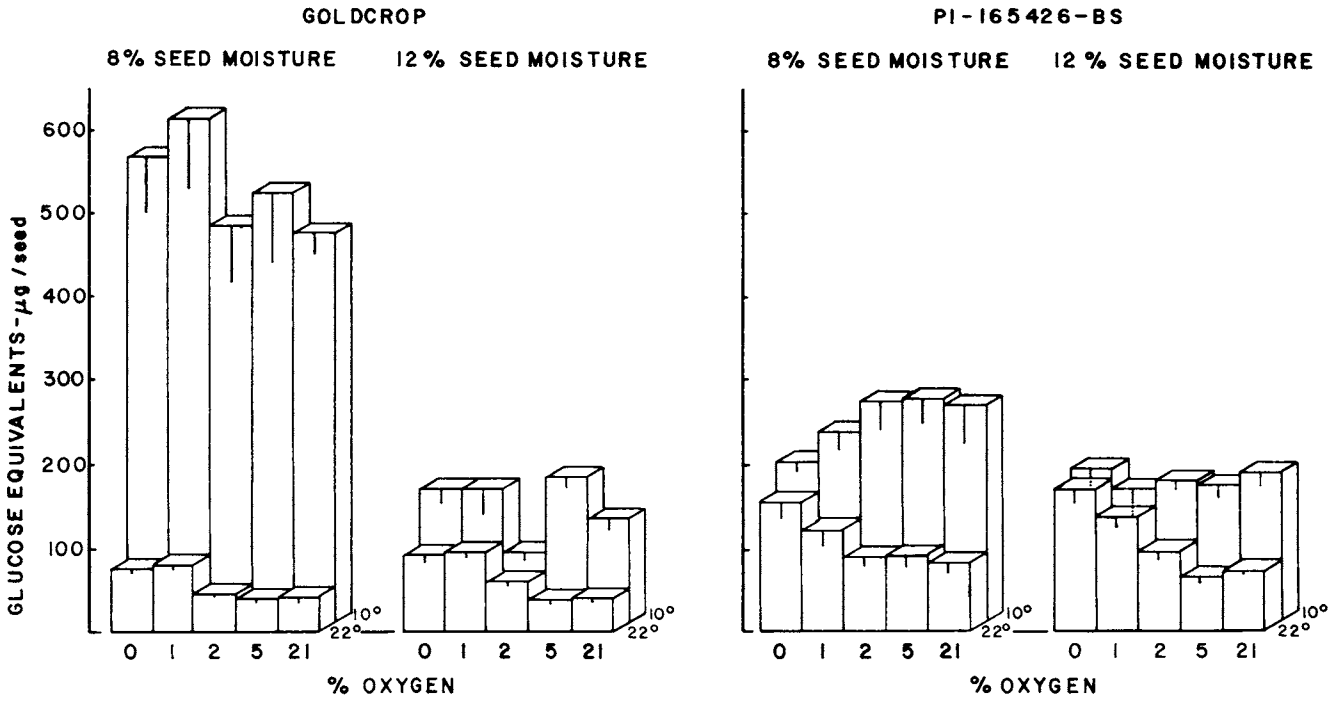

Fig. 4. Leakage of sugars and related substances from 8% and 12% moisture seeds of 2 bean lines exposed to 5 O_2 levels and 2 temperatures (bars = SEM).

Also the emergence of 8% moisture seeds of PI-165426-BS was not affected, but that of 8% moisture seeds of 'Goldcrop' seeds was reduced from 99% to 86–90%.

Emergence as affected by chilling. Exposure of 'Goldcrop' seeds to 10°C during the 48-hr treatment phase delayed emergence of 8% and 12% moisture seeds 2.3 and 2.8 days, respectively (Table 1). Low temperatures did not affect the final emergence of 12% moisture seeds (99%), but reduced emergence of 8% moisture seeds of 'Goldcrop' to 74–88% and survival to 72–80%. Some 'Goldcrop' seedlings that did emerge from 8% moisture seeds, after exposure to 10°, showed loss of geotropism, inability to unfold the hypocotyl hook, loss of the apical meristem, and loss of the cotyledons or primary leaves. Exposure of PI-165426-BS seeds to low temperature delayed emergence of 8% and 12% moisture seeds 2.2 and 2.0 days, respectively (Table 1). Reducing the moisture content of PI-165426-BS seeds did not decrease their final emergence (86–92%) and survival (86–90%) as much as in 'Goldcrop', and only a few seedlings were abnormal.

Effects of seed moisture and bean line on emergence. Reducing initial seed moisture prior to seeding delayed emergence by about 0.5 day for both lines (Table 1). Emergence of 'Goldcrop' seeds at either moisture level was about 0.5 day slower than that of PI-165426-BS. 'Goldcrop' seeds were generally more sensitive to all stresses than PI-165426-BS seeds. Therefore, differences between the 2 lines were larger under stress conditions than under optimal conditions.

Effects of the stresses on seedling growth

Effects of anoxia on growth. Growth was evaluated 10 days after replanting by measuring the dry weight of the surviving seedlings. At 22°C, the seeds exposed to low O_2 produced smaller seedlings than those that germinated in 5% O_2 and in air (Fig. 2), but all the seedlings appeared normal. This anoxia effect was greater for 'Goldcrop' than for PI-165426-BS. At 10°, the O_2 treatments had no significant effect.

Effects of chilling on growth. Exposure of 'Goldcrop' seeds to imbibitional chilling retarded the growth of the seedlings (Fig. 2). Seeds of both moisture levels responded similarly, in agreement with emergence data (not shown), which indicated that 8% seed moisture primarily affected the survival of seeds exposed to 10°C. Seedlings of PI-165426-BS showed no reduction of dry weight as a result of imbibitional chilling (Fig. 2).

Effects of imbibitional stresses on leakage from seeds

Evaluation of leakage from seeds after 48 hr of imbibition and germination is particularly important, because leakage from seeds and roots indicates membrane damage (18, 30) and stimulates pathogen germination and infection (14).

At 22°C, low O_2 concentrations increased leakage of electrolytes and sugars, with the most pronounced effect at 0% O_2 (Figs. 3 and 4). Leakage of electrolytes at 5% O_2 was consistently lower than at 21% O_2, though not always significantly different. At 10°, oxygen treatments had little or no effect on leakage. Imbibitional chilling resulted in higher leakage from seeds of both lines. This low temperature effect was accentuated greatly by low initial seed moisture in 'Goldcrop'. At 22°, leakage was slightly higher from 8% moisture seeds than from 12% seeds of either line. Leakage of electrolytes and sugars from 'Goldcrop' seeds under stress was much more pronounced than that from PI-165426-BS seeds.

The leakage trends of sugars and electrolytes were similar (Figs. 3 and 4), but differed in magnitude. Leakage of sugars from 'Goldcrop' seeds at 8% initial moisture was 10.6 times higher at 10°C than at 22°, while leakage of electrolytes was only 2.7 times higher. However, the increase in leakage resulting from low O_2 was slightly less when estimated by sugars than by electrolytes.

Effects of imbibitional anoxia and flooding on leakage, emergence, and growth

The relatively mild effect of anoxia and its interactions with the other experimental variables indicate that anoxia is either not involved in flooding injury of imbibing bean seeds or is only partially involve. Therefore, a 24-hr flooding treatment was compared to 24-hr anoxia and to control (air) (Table 2). Flooding seeds of 'Goldcrop' resulted in high leakage, delayed and reduced emergence, reduced plant weight, and produced many abnormal plants. The consequence of anoxia matched more closely that of the control than that of flooding. During the 24-hr treatment period, the control seeds and the flooded ones took up significantly more water than seeds in anoxia. However, weight increase of control seeds reflected both imbibition and growth, whereas that of flooded seeds reflected only imbibition, since no growth occurred. The increased influx of water into flooded seeds may be related to flooding injury.

Discussion

The effects of anoxia, low temperature, seed moisture, and bean line

Anoxia had a pronounced effect on electrolyte leakage and a smaller effect on leakage of sugars, whereas temperature stress had a large effect on leakage of sugars compared to electrolytes. These different responses may indicate a different mechanism of leakage. The large release of electrolytes due to low O_2 could reflect increased leakage of K due to reduced ATP availability (22). Leakage of large sugar molecules, which followed chilling treatments, may have resulted from relatively large membrane discontinuities (3, 27).

Increasing the initial seed moisture content gave some protection against temperature stress, especially in 'Goldcrop', which is more sensitive than PI-165426-BS. Therefore, moistening the seeds prior to seeding might reduce injury from adverse field conditions. The impracticality of moistening large volumes of seed and the large differences in stress sensitivity between the lines suggest that incorporating genetic characteristics for stress tolerance into commercial cultivars would be more practical.

The effects of flooding vs. anoxia on seed germination

Despite the adverse effects of anoxia during the first 48 hours of imbibition and germination, the role of O_2 deficiency under flooding is questionable. The present results support the conclusions of investigators who indicate that factors other than O_2 deficiency are involved in flooding injury (1, 2, 10, 13, 29, 30). Three differential responses between anoxia and flooding can be identified: 1) Flooding injury was much more severe than the injury caused by anoxia (Table 2); 2) chilling aggravated flooding injury (12, 13) but reduced the effect of anoxia (Table 1; Figs. 2 and 4); and 3) low seed moisture increased the sensitivity of seeds and embryos to flooding (12, 23) and increased flood related leakage (3), but did not increase leakage as a result of anoxia in either bean line and did not affect the sensitivity of PI-165426-BS to anoxia (Table 1; Fig. 2).

These arguments conflict with evidence that indicates the primary cause of flooding injury is O_2 deficiency (6, 7, 16, 17, 23). A hypothesis that may resolve this apparent conflict assumes that the initial flooding injury during imbibition results from physical membrane damage caused by rapid hydration of the seed (15, 29, 30). At a later stage, when respiration begins, limited O_2 availability may cause additional injury due to ethanol accumulation (7, 16, 30).

Similarities between the effects of flooding and chilling during imbibition may indicate the involvement of a common mechanism. Both injuries occur at the onset of imbibition (4, 17, 21, 28), they are aggravated by low initial seed moisture (Figs. 3 and 4) (2, 12, 29, 23), and both result in high rates of leakage (Figs. 3 and 4; Table 2).

Faulty membrane reorganization at low temperatures has been suggested as the cause of chilling injury to imbibing seeds (3). Since imbibitional chilling injury is irreversible (5, 11), membranes of seeds that imbibe at low temperatures may not reorganize normally even after the temperature increases. When hydrated, membrane phospholipids cannot form a continuous layer across the relatively large quantities of water. Thus, membrane reorganization may be restricted to relatively low levels of seed moisture content. Flooding injury was reported to correlate with the rate of imbibition (2, 29) and could be prevented by soaking in PEG solutions (29, 30). This evidence supports a hypothesis that too-rapid imbibition prevents membrane reorganization because the moisture content quickly exceeds a level that permits membrane reorganization. Thus, flooding injury would be more severe in low-moisture than in high-moisture seeds because more extensive membrane reorganization has to take place. Chilling may intensify the injury because the low temperatures prevent even partial membrane reorganization.

Literature Cited

1. Barton, L.V. 1950. Relation of different gases to the soaking injury of seeds. Contr. Boyce Thompson Inst. 16:55–71.
2. Barton, L. and J. McNab. 1956. Relation of different gases to the soaking injury of seeds: III. Some chemical aspects. Contr. Boyce Thompson Inst. 18:339–356.
3. Bramlage, W.J., A.C. Leopold, and D.J. Parrish. 1978. Chilling stress to soybeans during imbibition. Plant Physiol. 61:525–529.
4. Christiansen, M.N. 1967. Periods of sensitivity to chilling in germinating cotton. Plant Physiol. 42:431–433.
5. Christiansen, M.N. and R.O. Thomas. 1969. Season-long effects of chilling treatments applied to germinating cottonseed. Crop Sci. 9:672–673.
6. Crawford, R.M.M. 1966. The control of anaerobic respiration as a determining factor in the distribution of the genus Senecio. J. Ecol. 5:403–413.
7. Crawford, R.M.M. 1977. Tolerance of anoxia and ethanol metabolism in germinating seeds. New Phytol 79:511–517.
8. Delouche, J.C. 1980. Environmental effects on seed development and seed quality. HortScience 15:775–780.
9. DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350–356.
10. Eyster, H.C. 1940. The cause of decreased germination of bean seeds soaked in water. Amer. J. Bot. 27:652–659.
11. Highkin, H.R. and A. Lang. 1966. Residual effect of germination temperature on the growth of peas. Planta 68:94–98.
12. Hobbs, P.R. and R.L. Obendorf. 1972. Interaction of initial seed moisture and imbibitional temperature on germination and productivity of soybean. Crop Sci. 12:664–667.
13. Kidd, F. and C. West. 1919. The influence of temperature on the soaking of seeds. New Phytol. 18:35–39.
14. Kraft, J.M. and D.C. Erwin. 1967. Stimulation of *Phythium aphanidermatum* by exudates from mung bean seeds. Phytopathology 57:866–868.
15. Ladror, U. 1981. Oxygen and temperature effects on germination of two bean genotypes at two seed moisture levels. MS Thesis, Washington State Univ., Pullman.
16. McManmon, M. and R.M.M. Crawford. 1971. A metabolic theory of flooding tolerance: the significance of enzyme distribution and behavior. New Phytol. 70:299–306.

17. Orphanos, P.I. and W. Heydecker. 1968. On the nature of the soaking injury of *Phaseolus vulgaris* seeds. J. Expt. Bot. 19:770–784.
18. Parrish, D.J. and A.C. Leopold. 1977. Transient changes during soybean imbibition. Plant Physiol. 59:1111–1115.
19. Pollock, B.M. 1969. Imbibition temperature sensitivity of lima bean seeds controlled by initial seed moisture. Plant Physiol. 44:907–911.
20. Pollock, B.M. and E.E. Roos. 1972. Seed and seedling vigor, p. 314–376. In: Seed biology. T.T. Kozlorsky (ed.). Vol. 1. Academic, New York.
21. Pollock, B.M. and V.K. Toole. 1966. Imbibition period as the critical temperature sensitive stage in germination of lima bean seeds. Plant Physiol. 41:221–229.
22. Powell, A.A. and S. Matthews. 1978. The damaging effect of water on dry pea embroys during imbibition. J. Expt. Bot. 29:1214–1229.
23. Roos, E.E. and B.M. Pollock. 1971. Soaking injury in lima beans. Crop Sci. 11:78–81.
24. Schroth, M.N., A.R. Weinhold, and D.S. Hayman. 1966. The effect of temperature on quantitative differences in exudates from germinating seeds of bean, pea and cotton. Can. J. Bot. 44:1429–1432.
25. Silbernagel, M.J. 1973. Goldcrop — a new disease resistant bush wax bean. Annu. Rpt. Bean Improvement Coop. 16:50–51.
26. Silbernagel, M.J. 1977. Seed quality index as an indicator of crop production potential, and a selection tool for the genetic improvement of snap bean quality. Annu. Rpt. Bean Improvement Coop. 20:40–42.
27. Simon, E.W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:377–420.
28. Woodstock, L.W. and B.M. Pollock. 1965. Physiological predetermination: imbibition, respiration and growth of lima bean seeds. Science 150:1031–1032.
29. Woodstock, L.W. and K.J. Tao. 1981. Prevention of imbibitional injury in low vigor soybean embryonic axes by osmotic control of water uptake. Physiol. Plant. 51:133–139.
30. Woodstock, L.W. and R.B Taylorson. 1981. Soaking injury and its reversal with polyethylene glycol in relation to respiratory metabolism in high and low vigor soybean seeds. Physiol. Plant. 53:263–268.

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^{31}P-NMR Monitoring of Ethephon Decomposition in Olive Leaves

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Abstract. **The possibility of using ^{31}P-nuclear magnetic resonance (NMR) spectroscopy to detect ethephon (ET) in olive leaves has been examined. ^{31}P-NMR spectroscopy can be used as a nondestructive technique (tissues excised but not extracted) with the unique attributes of monitoring ET hydrolysis internally and without radiochemicals. A characteristic spectral peak for the parent ET molecule was found 17–21 ppm (a measure of relative frequency, not concentration) downfield from the H_3PO_4 reference, and a nonreactive, minor contaminant spectral peak was found at 26–27 ppm. Absolute spectral peak location ("chemical shift") is pH-dependent. The ET hydrolysis product, orthophosphate, produces a spectral peak at 2 to 3 ppm, which coincides with the broad spectral peak attributed to major endogenous phosphate compounds in leaves, such as inorganic phosphate. The lower limit of ^{31}P-NMR detection of ET in solution was 10^{-3} M; however, spray applications of ET were not detectable in olive leaves unless concentrations of 5×10^{-2} M or more were used, which is far greater than current agricultural use levels for mechanical harvest of olive. Nevertheless, ^{31}P-NMR spectroscopy was useful in following ET uptake and decomposition for more than 48 hr in olive leaves from xylem-fed shoots, and the resolution of the ET spectral peak into separate, adjoining peaks presents the potential to identify and quantify subcellular compartmentalization of ET according to pH-induced chemical shifts. Such knowledge would contribute to understanding long- and short-term in vivo decomposition of ET to ethylene. Chemical name used: (2-chloroethyl)phosphonic acid (ethephon).**

Agricultural scientists have long sought to control the periodic abscission of plant organs. Control of abscission to synchronize fruit harvest is a top-priority concern of growers of olive, one of the world's major tree crops. More than 30 years of research into the specific problem has yielded a promising class of abscission chemicals: the ethylene-releasing compounds (ERCs) such as ethephon (ET). Yet these often induce leaf abscission as well as fruit abscission, which can reduce the following year's crop (3).

Selective stimulation of horticulturally beneficial ethylene responses, such as fruit abscission without leaf abscission or fruit coloring without softening, has been hypothesized to be largely a function of the timecourse profile of ethylene release from ERCs (6, 15). In pursuing the above hypothesis, we have been interested in monitoring the internal ET-to-ethylene hydrolysis reaction in plant tissue without having to rely on standard techniques using mercuric perchlorate trapping of radiolabeled ethylene from ^{14}C-ET. As the ET molecule is hydrolyzed, there is an equimolar change in the phosphorus species from phosphonate dianion to orthophosphate (8). Consequently, ^{31}P-NMR spectroscopy should be able to detect this change in the P chemical environment. This technique would provide an indirect, concomitant measure of the ethylene released within the tissue, as opposed to the radiochemical method, which directly measures the ethylene diffusion from the tissue.

^{31}P-NMR spectroscopy only recently has been applied successfully to studies in plant physiology (10). The inherent insensitivity has been decreased with the advent of Fourier Transform NMR and high field-strength superconducting mag-

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