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**Abstract**
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**Keywords**
Biochemistry Biophysics and Molecular Biology

**Disciplines**
Agricultural Science | Agriculture | Agronomy and Crop Sciences | Biochemistry | Molecular Biology | Weed Science

**Comments**
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# WEEDY ADAPTATION IN *SETARIA* SPP. V. EFFECTS OF GASEOUS ENVIRONMENT ON GIANT FOXTAIL (*SETARIA FABERII*) (POACEAE) SEED GERMINATION[1]

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The effects that naturally occurring gases (oxygen, nitrogen, carbon monoxide) may cause in dormant giant foxtail (*Setaria faberii*) seed germination under favorable temperature and moisture conditions were investigated. The germination responses to gas mixtures supported the hypothesis that *S. faberii* germination behavior is regulated by the amount of oxygen taken into hydrated seed over time. *Setaria faberii* seed germination was markedly affected by $O_2$ concentration (in $N_2$) above and below that of air (20% $O_2$): the largest increase in germination (from 37 to 60%) occurred between 20–25% $O_2$; between 0–10% $O_2$, germination increased from 0–30%; and surprisingly germination at 10 and 20% $O_2$ was similar. These observations reveal an asymmetrical response to incremental changes in $O_2$ above and below that typically found in agricultural soils. Carbon monoxide had opposite effects on *S. faberii* germination in air depending on concentration, stimulation, and inhibition: germination increased from 37 to 56% with the addition of 1% CO, but decreased from 37 to 14% with 75% added CO. An explanation may be that there are two separate effects of CO, each occurring in different physiological systems of dormant seeds at the same time. At high concentrations (75%) in air CO inhibited seed germination, probably by inhibiting mitochondrial respiration. But low CO concentrations (0.1 or 1%) in air stimulated seed germination. It was not apparent which physiological system(s) CO and $O_2$ affected. It seems unlikely that CO-stimulated germination arises from effects on the respiratory apparatus, but may be a consequence of CO interactions with an as yet unknown physiological factor in the seed. We provide a model of *Setaria* spp. dormancy consistent with its seed morphology, the gas-germination data, and the hypothesized second physiological factor that may be involved in CO stimulated germination.

**Key words:** carbon monoxide; Graminae; oxygen; respiration; seed dormancy; seed germination; *Setaria faberii*; soil atmosphere; soil seedbanks.

The weedy foxtails (*Setaria* spp.) are an invasive species group colonizing vast areas, especially agricultural fields in the north temperate region of the world (Rominger, 1962; Holm et al., 1979, 1997). The reasons why *Setaria* spp. are so successful in disturbed habitats derives from their biodiversity (Wang and Dekker, 1995; Wang, Wendel, and Dekker, 1995a, b; Dekker, 1997), especially their ability to produce diverse phenotypes from a single parent plant (e.g., Dekker et al., 1996) and their ability to time germination precisely. This results in individual seedlings emerging from the seedbank at different times over large temporal scales (hours to decades; Forcella et al., 1992, 1997). Giant foxtail (*Setaria faberii*) infests vast areas due to its ability to form long-lived soil seedbanks that are the source of future infestations (Rominger, 1962; Dekker, 1999). These seedbanks are reservoirs of highly heterogeneous seed (Dekker et al., 1996; Dekker, 1997) poised to inflict heavy agricultural crop losses.

Previous studies in this series have documented the global genotypic population structure of the weedy foxtails (Wang, Wendel, and Dekker, 1995a, b) and some of the agriculturally important phenotypic traits responsible for its extensive biogeographic distribution (Wang and Dekker, 1995; Dekker et al., 1996). Weedy adaptation in this species-group also depends on the ability to time germination and seedling emergence such that it results in maximum subsequent reproductive efforts.

Individual panicles on a single parent plant produce a diverse array of seeds, each with potentially different after-ripening requirements for germination. The production of seeds with different levels of dormancy (experimentally revealed by the after-ripening dose [e.g., time at 4°C, moist, dark] required for germination) is a function of plant architecture. Earlier-fertilized seeds (both intra- and interpanicle) are relatively more dormant than later-developing seeds. The first seeds on an individual panicle were shown to possess relatively greater dormancy (greater after-ripening requirement) than the last seeds maturing on the same panicle (Haar, 1998). Additionally, primary (1°) panicles produce seeds with relatively greater dormancy than those produced on secondary (2°), and again on tertiary (3°), panicles of the same parent plant. Significant heterogeneity in dormancy states among seeds shed by a single plant allow these species to emerge at appropriate times within a cropping season and in different years (Dekker et al., 1996; Forcella et al., 1997). Soil seed banks consisting of diverse foxtail species and genotypes, each contributing a heterogeneous collection of dormancy phenotypes, reveal a hedge-betting strategy for adaptation to changing conditions within agroecosystems (e.g., Cohen, 1966; Philippi and Seger, 1989).

The wide geographic range of adaptation, heterogeneity in dormancy phenotypes, and genotypic diversity raise the question of what mechanisms in foxtail seeds drive seed behaviors (e.g., seed dormancy, germination, or seedling emergence). A recent review of foxtail seed behavior argued for the possible unifying role that soil water and oxygen may play describing both biogeographic distribution and responses by individual seeds in a soil microsite (Dekker, 2000).

The morphology of foxtail seeds also provides important clues about what environmental factors limit germination and maintain dormancy. Morphologically, the foxtail seed sym-

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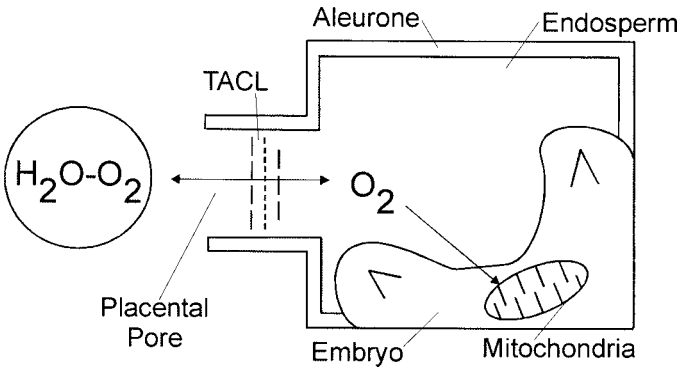


Fig. 1. Schematic model of hydrated, weedy foxtail (*Setaria* spp.) seed parts involved in dormancy and germination and the signal transduction pathway of water–oxygen ($H_2O$–$O_2$) movement from the outside environment, through the placental pore and the transfer aleurone cell layer (TACL; membrane) at the basal end of the seed into the endosperm, aleurone, embryo, and mitochondria.

plast (embryo, endosperm, and aleurone layer) is surrounded by several enveloping layers which control its behavior (Dekker et al., 1996). The symplast is surrounded by the caryopsis coat composed of several crushed cell layers (Rost, 1971, 1973, 1975; Rost and Lersten, 1973). It is filmy, oily to the touch, water- and gastight, and continuous except at the placental pore opening on the basal end of the seed. Although the mature foxtail seed is capable of freely imbibing water and dissolved gases, entry is restricted and regulated by the placental pore tissues, where there is membrane control by the transfer aleurone cell layer (TACL; Rost and Lersten, 1970). Gases entering the moist seed symplast must be dissolved in the imbibed water passing through the narrow placental pore and TACL. The importance of gases entering dry seed is unknown. The morphology of foxtail seeds strongly suggests that seed germination is restricted by water availability in the soil and by the amount of oxygen dissolved in water reaching the inside of the seed symplast to fuel metabolism. Oxygen solubility in the water entering the symplast is an inverse function of the diurnally, seasonally, and annually changing soil temperature. The control by this oxygen-limited, gastight morphology is supported by observations of increased germination when foxtail seed envelopes, including the caryopsis coat, are punctured (Stanway, 1971; Dekker et al., 1996). A graphical representation of the restricting morphology on $H_2O$-$O_2$-time signal is presented in Fig. 1.

Based on these observations we have proposed a hypothesis by which biogeographic distribution, as well as individual seed behavior, may be explained: foxtail seed behavior is regulated by the amount of oxygen dissolved in water taken into the seed over time. When adequate amounts of water and oxygen reach the embryo, sufficient energetic equivalents are generated to support germination metabolism (Bewley and Black, 1994). When inadequate amounts of oxygen reach the embryo, dormancy is maintained or secondary dormancy is induced (Corbineau and Côme, 1995). The global regions that possess soils with suitable qualities of fertility, moisture, temperature, aeration, and disturbance are extensive (e.g., Eurasia, North America).

In agricultural soils the gaseous components, including oxygen, nitrogen, carbon dioxide, water vapor, and volatile metabolites such as carbon monoxide and ethylene, also influence seed behavior (Siegel, Renwick, and Rosen, 1962; Siegel and Siegel, 1987; Corbineau and Côme, 1995). The gaseous environment of the surface soil layers within which seeds germinate is influenced by the structural characteristics of the soil (texture, drainage, aggregation, crusting, and cracking), chemical qualities of the gases, and biological activity within the soil (Pareja and Staniforth, 1985; Pareja, Staniforth, and Pareja, 1985).

Diatomic oxygen is required for germination by almost all plant seeds (Bewley and Black, 1994), including dormant grasses (Simpson, 1990), but exceptions have been observed (Corbineau and Côme, 1995). Oxygen concentrations do not usually limit seed germination, but soil structure can inhibit $O_2$ availability (Pareja and Staniforth, 1985a). Oxygen concentrations in agricultural soils range from 18 to 21% depending on soil depth, drainage, time of year, cropping, and tillage system (James, 1968). The stimulatory effect of oxygen on many plant species has been observed (Atwood, 1914; Côme and Tissaoui, 1972), but not previously in foxtails. High concentrations of oxygen can relieve seed dormancy (Roberts, 1962; Corbineau and Côme, 1995). Nondormant seeds of many species have been shown to be less sensitive to oxygen deprivation than dormant seeds (Gay, Corbineau, and Côme, 1991).

Carbon monoxide (CO) is a naturally occurring diatomic gas present in water at the soil surface–air interface (Siegel and Siegel, 1987), but its role in seedbank dynamics is not understood. Soil fungi, seeds, and seedlings of a wide variety of species produce significant amounts of CO in the upper soil layers in the dark. This CO production is of significant magnitude to contribute to the overall CO flux, especially at night (Siegel, Renwick, and Rosen, 1962; Siegel and Siegel, 1987).

Carbon monoxide has many inhibitory effects on processes important during seed germination. Its toxic effect was recognized in the 17th century (Moray, 1665). Carbon monoxide is an inhibitor of plant respiration and seed germination, acting on the coupled reactions of the mitochondria (Einarsdottir et al., 1988; Sowa, Roos, and Caughey, 1993). Specifically, it is an inhibitor of cytochrome *c* oxidase (Enzyme Commission 1.9.3.1), the terminal oxidase in the mitochondrial electron transfer chain. Often its inhibition of respiration is incomplete because of the alternate (cyanide resistant) respiratory pathway. Carbon monoxide has other roles, including environmental pollutant and inhibitor of terpene and terpenoid biosyntheis and other cytochrome $P_{450}$-catalyzed reactions (e.g., Goodwin and Mercer, 1983; Klaasen, Amdur, and Doull, 1986; Voet and Voet, 1995).

Several studies have demonstrated the stimulatory effects of CO on germination of dormant seed, although no studies showing the effects of $O_2$, $N_2$, and CO over a wide concentration range have been reported previously (Fischnich, Thielbein, and Grahl, 1962; Roberts, 1964; Majors and Roberts, 1968; Esashi et al., 1991). Roberts demonstrated germination stimulation of rice (Roberts, 1964) and of barley (Major and Roberts, 1968). He observed ''the paradox that dormancy could be broken by either increased oxygen tensions or (CO, respiratory inhibitors)'' (Roberts, 1969, p. 173).

Considering foxtail biogeography, genotypic and phenotypic diversity, the restraints imposed by seed morphology, and previous studies on the influence of gaseous atmosphere on seed germination, the objectives of this paper are to determine the effects of the gaseous atmosphere (oxygen [$O_2$], nitrogen [$N_2$], and carbon monoxide [CO]) on the germination of dor-

mant giant foxtail (*Setaria faberii*) seed under favorable temperature and moisture conditions.

## MATERIALS AND METHODS

***Seeds***—A dormant *S. faberii* seed population was used to determine the effects of gases on germination. To detect both stimulatory and inhibitory effects of the various gaseous treatments, the seed source was partially, uniformly, after-ripened to ~35% germination in the conditions described below. Our lot #3731 was derived from seed in soil collected early in 1997 from an agricultural field (historically a corn–soybean rotation) 32 km southwest of Ames, Boone County, Iowa, USA. After seed germination, plants were grown in an unheated glass greenhouse on the Iowa State University campus (Ames, Iowa, USA) during the summer of 1997, and seeds at abscission were harvested on 16 September 1997. Immediately after harvest, the seeds were air-dried overnight on screens at 19°C. None of the seed germinated when it was evaluated at harvest under the conditions described below. On 17 September 1997 the majority of the dried seed was placed in paper envelopes (200 seeds each) and stored at a constant temperature of −20°C. Seeds were removed from these conditions immediately prior to being used in each of the experiments reported here (November 1999 through March 2000).

***Germination***—Several types of apparatus have been used to evaluate the effect of gases on germination, the primary difference between them is whether the gas continuously flows through the germination chamber (e.g., Gay, Corbineau, and Côme, 1991; Sowa, Roos, and Caughey, 1993) or whether a sealed system is used (e.g., Major and Roberts, 1968; Esashi et al., 1991). Each presents its own set of experimental artifacts, but it was felt that a sealed system was more appropriate because it mimicked the soil seed bank environment in which atmosphere exchange is limited by diffusion processes within the soil matrix and because it ensured that moisture was at no time either limiting or excessive. In other studies, the atmospheric content was changed during the course of the assay, confounding the interpretation of any changes in germination (e.g., Major and Roberts, 1968; Esashi et al., 1991). A sealed system also provided us with a known, constant quantity of water that never limited germination. The amount of water in our assay system was ample for germination and seedling growth up to several leaves. The seeds were covered at all times with an ample film of water wicked from the blotter paper on which they rested, but were not submerged. Seed germination in air (control) in 30-, 50-, and 100-mL sealed vials was similar (data not reported), additional evidence that gas and moisture were not limiting.

Germination was evaluated in 30-mL gas-tight vials, with 20 mm outside diameter mouths (Wheaton Science Products, Millville, New Jersey, USA). Two disks of Anchor Blue germination blotter paper (Anchor Paper, St. Paul, Minnesota, USA), 32 mm in diameter, were placed in, and completely covered, the bottom of the vials. Immediately prior to sealing and gassing, 1.5 mL of distilled, deionized water was placed in the vials along with ten dry giant foxtail seeds. After placing seed and water in the vials they were immediately sealed (Wheaton hand crimper, model 22430; Wheaton Science Products, Millville, New Jersey, USA) with neoprene stoppers and an aluminum seal was crimped around the vial neck to ensure a gas- and watertight seal.

***Gas atmosphere***—Combinations of $O_2$, CO, and $N_2$ were used to test our hypothesis. Only combinations of these gases that have physiological relevance were included (e.g., not $N_2$–CO combinations). The single gas, two-gas, and three-gas combinations that were evaluated are summarized in the Table 1.

Gas atmospheres were established by mixing appropriate flows of $O_2$, CO, and $N_2$ through direct reading flowmeters (Cole-Parmer model P-32010; Cole-Parmer, Vernon Hills, Illinois, USA) and injecting them into the vials via 3.8 cm length, 23 guage diameter hypodermic needles and rubber tubing. Each vial had an escape needle (same size) also placed in the neoprene stopper to vent the gases and ensure all vials were at local atmospheric pressure. Each sealed vial with seed and water was flushed and equilibrated with the appropriate gas mixture for 5–10 min at a flow rate of 1 L/min.

TABLE 1. Gas atmosphere concentrations (percentage of volume) used to evaluate the single and joint effects of oxygen ($O_2$), nitrogen ($N_2$), and carbon monoxide (CO) on giant foxtail seed germination.

| Gas atmosphere | Gas concentration (% volume) | | |
|---|---|---|---|
| | $O_2$ | $N_2$ | CO |
| $O_2$ control | 100 | 0 | 0 |
| $N_2$ control | 0 | 100 | 0 |
| CO control | 0 | 0 | 100 |
| $O_2$ + $N_2$ | 90 | 10 | 0 |
| | 75 | 25 | 0 |
| | 50 | 50 | 0 |
| | 25 | 75 | 0 |
| Air control | 20 | 80 | 0 |
| | 10 | 90 | 0 |
| $O_2$ + CO | 90 | 0 | 10 |
| | 75 | 0 | 25 |
| | 50 | 0 | 50 |
| | 25 | 0 | 75 |
| | 10 | 0 | 90 |
| $O_2$ + $N_2$ + CO | 20 | 79.9 | 0.1 |
| | 20 | 79 | 1 |
| | 20 | 75 | 5 |
| | 20 | 70 | 10 |
| | 20 | 55 | 25 |
| | 20 | 30 | 50 |
| | 20 | 5 | 75 |

***Germination conditions***—The sealed vials with appropriate gas atmospheres, germination paper, water, and seed were then placed in a Hoffman (model SG-30; Hoffman Manufacturing, Albany, Oregon, USA) controlled environment seed germination cabinet for 8 d. The daily conditions in the chamber alternated between 16 h light 30°C periods and 8 h dark 20°C periods. Past experience indicates these are ideal conditions for germination of seeds at or near the germination threshold (seeds almost or fully after-ripened).

***Data collection and analysis***—The number of seeds germinated in each vial was recorded each day for 8 d. Germination was evidenced by coleorhiza and/or coleoptile protrusion outside the seed hull. Treatments (Table 1) were arranged in a completely randomized experimental design. Each experiment was replicated 3–4 times; and each experiment was repeated 3–6 times. Each treatment mean is the pooled mean response of 9 replicates, whereas the $O_2$, $N_2$ and CO controls were the pooled mean of 24 replicates. Analysis of variance and appropriate *t* tests were conducted on mean final germination (in percentages) and mean time (in days) to germination. Data are reported as means ± 1 SE.

## RESULTS

***Oxygen–nitrogen effects***—The germination of giant foxtail in air was ~37% ± 17% (Fig. 2). This heterogeneity in germination is typical of a population of partially after-ripened, dormant foxtail seed (Dekker et al., 1996). The mean time to germination was 4.53 ± 0.09 d for those seeds that germinated in air. Seed germination in pure nitrogen was completely inhibited.

Germination increased with all $O_2$ concentrations greater than that of air (>20%). The largest stimulation of germination occurred in response to the first increment of added $O_2$ (from 20% to 25%), with smaller increases observed at higher $O_2$ concentrations (50–75%). Maximum germination (86%) was observed at 75% $O_2$, with no additional germination increases above that concentration. The germination of giant foxtail seed in pure $O_2$ was ~87 ± 15% (Figs. 2 and 3). The time to germination was shorter (~1 d) at all $O_2$ concentrations

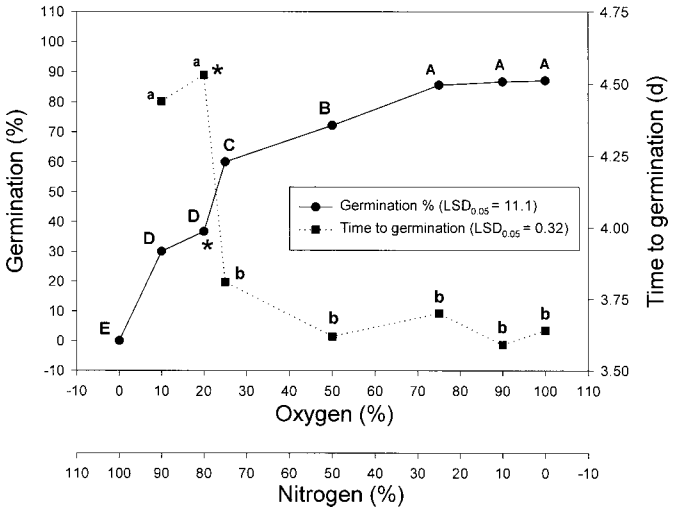


Fig. 2. The effect of oxygen ($O_2$) and nitrogen ($N_2$) on mean final (day 8) *Setaria faberii* seed germination (in percentages) and mean time (in days) to germination (for those individual seeds that did germinate); * air control: 20% $O_2$ + 80% $N_2$; final germination (circles; capital letters) and time to germination (squares; lowercase letters) means, within each parameter only, followed by the same letter are not significantly different using a *t* test at the $P = 0.05$ level; $LSD_{0.05}$ = least significant difference at the $P = 0.05$ level.

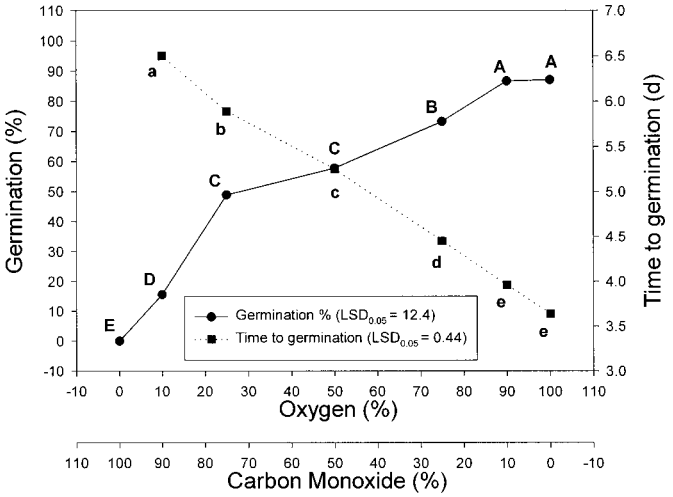


Fig. 3. The effect of oxygen ($O_2$) and carbon monoxide (CO) on mean final (day 8) *Setaria faberii* seed germination (in percentages) and mean time (in days) to germination (for those individual seeds that did germinate); final germination (circles; capital letters) and time to germination (squares; lowercase letters) means, within each parameter only, followed by the same letter are not significantly different using a *t* test at the $P = 0.05$ level; $LSD_{0.05}$ = least significant difference at the $P = 0.05$ level.

greater than that of air (20%). Seed germination and time to germination did not decrease as $O_2$ decreased from 20 to 10%.

***Oxygen–carbon monoxide effects***—An atmosphere consisting of only carbon monoxide completely inhibited seed germination, as did $N_2$, probably due to a lack of $O_2$ (Figs. 2 and 3). Carbon monoxide did not inhibit $O_2$-stimulated seed germination, but it did delay the time it took those seeds to germinate and stunted the size of the embryo axes that emerged (Figs. 2 and 3; Table 2).

Decreasing concentrations of $O_2$ (75–0%) and increasing CO (25–100%) resulted in reduced seed germination as well as delayed time to germination. The range of delay times was wide; seeds germinated in 90% CO (10% $O_2$) took almost 3 d longer to germinate than those in pure $O_2$, and those that did germinate all had severely stunted embryo axes (coleoptile, coleorhiza) but appeared normally white in color. To evaluate if these stunted individuals would recover after the 8 d germination period, we allowed them to remain in the germination cabinet environment for an additional 3 d (11 d total). No additional germination or recovery from this stunting was observed in that additional time period.

When $N_2$ and CO at the same concentration were compared separately in mixtures of $N_2$–$O_2$ or CO–$O_2$ (Figs. 2 and 3) final germination percentage did not differ, but time to germination did (Table 2). Carbon monoxide in these mixtures resulted in a longer time to germination than the same amount of $N_2$ (i.e., 10, 25, 50, 75, and 90% CO or $N_2$) in mixtures with $O_2$. No effects due to CO (and lowered $O_2$ concentration) were observed in the 10% CO–90% $O_2$ mixture, which resulted in a mean seed germination percentage similar to pure $O_2$.

***Oxygen–nitrogen–carbon monoxide effects***—In three gas mixtures with $O_2$ at 20% concentration (air), and $N_2$ completing the atmosphere, CO had a stimulatory effect on final germination at low concentrations (0.1 and 1% CO), a neutral effect at intermediate concentrations (5, 10, 25, and 50% CO), and an inhibitory effect at the highest (75%) concentration, when all were compared to responses in air alone (Fig. 4). The lower concentrations of CO (0.1, 1, 5, and 10%) in these three-gas mixtures resulted in a time to germination similar to that in air. But the higher concentrations of CO (25, 50, and 75%) inhibited the time to germination. The foxtail seedlings from seeds that germinated in 75% CO (20% $O_2$ 5% $N_2$) were stunted: both coleoptile and coleorhiza axes were atypically short and blunt but typically white in color.

## DISCUSSION

***Oxygen stimulates giant foxtail seed germination***—Oxygen stimulation of giant foxtail seed germination and time to germination was observed for the first time (Fig. 2). This observation is consistent with reports of the stimulatory effect of this gas on many other species (Atwood, 1914; Côme and Tissaoui, 1972; Corbineau and Côme, 1995). This observation

TABLE 2. Comparison of the effects of carbon monoxide (CO) and nitrogen ($N_2$) at the same concentration on mean time (in days) to germination (for those individual seeds that did germinate) with constant oxygen ($O_2$) concentration. * = time to germination means within the same concentration of $O_2$ followed by the same letter are not significantly different using a *t* test at the $P = 0.05$ level.

| Gas concentration (% volume) | | | Time (d) to germination* |
|---|---|---|---|
| $O_2$ | $N_2$ | CO | |
| 90 | 10 | 0 | 3.59B |
| 90 | 0 | 10 | 3.96A |
| 75 | 25 | 0 | 3.70B |
| 75 | 0 | 25 | 4.45A |
| 50 | 50 | 0 | 3.62B |
| 50 | 0 | 50 | 5.25A |
| 25 | 75 | 0 | 3.81B |
| 25 | 0 | 75 | 5.89A |
| 10 | 90 | 0 | 4.44B |
| 10 | 0 | 90 | 6.50A |

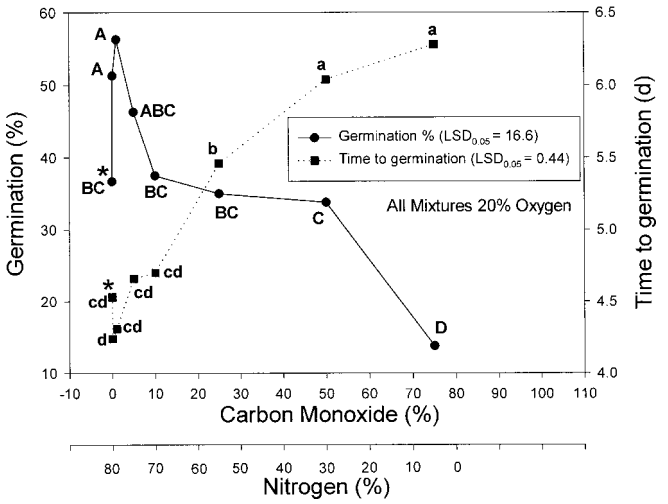


Fig. 4. The effect of constant percentage of oxygen ($O_2$; 20% in all mixtures) and variable percentage of nitrogen ($N_2$) and carbon monoxide (CO) on mean final (day 8) *Setaria faberii* (in percentages), and mean time (in days) to germination (for those individual seeds that did germinate); * air control: 20% $O_2$ + 80% $N_2$; final germination (circles; capital letters) and time to germination (squares; lowercase letters) means, within each parameter only, followed by the same letter are not significantly different using a *t* test at the $P = 0.05$ level; $LSD_{0.05}$ = least significant difference at the $P = 0.05$ level.

provides partial evidence in support of the hypothesis that foxtail seed behavior is regulated by the amount of oxygen dissolved in water taken into the seed over time (Dekker, 2000).

Partially after-ripened, dormant giant foxtail seeds were very sensitive to enriched $O_2$ atmospheres, especially at concentrations immediately greater than ambient air (20% $O_2$). The greatest stimulation of germination (and time to germination) occurred with the first 5% increase in $O_2$ above that of air (from 20 to 25%). Surprisingly, no concurrent decrease in germination (and time to germination) occurred with a decrease in $O_2$ of 10% (from 20% to 10%).

These observations reveal not only the stimulatory effects of $O_2$ on germination or relief of dormancy but a dynamic, asymmetrical response to incremental changes in $O_2$ above and below that typically found in agricultural soils. Foxtails rarely encounter $O_2$ conditions above 20% in natural conditions but may have frequently encountered conditions <20%. Foxtails have evolved a robust, short-term response to low $O_2$ concentrations, but germination remains similar at 20 and 10% $O_2$. Seeds are poised to germinate rapidly once the normal atmospheric level of 20% $O_2$ is exceeded. At abnormal $O_2$ concentrations above that of air, many more seeds germinate with small incremental increases in $O_2$ above 20%. The germination response was saturated at $O_2$ concentrations of 75–100%, conditions probably rarely or never encountered in the evolution of this species.

***Several modes of action of carbon monoxide during giant foxtail seed germination***—Carbon monoxide had three different effects on giant foxtail germination depending on the gas atmosphere composition to which the seed was exposed: it resulted in stimulation, no effect, or inhibition (Figs. 3 and 4).

*Stimulation*—Low CO concentrations (0.1, 1% [20% $O_2$]) stimulated giant foxtail seed germination and time to germination. These observations are mostly consistent with other studies that have demonstrated the stimulatory effects of CO on germination of dormant seed (Fischnich, Thielbein, and Grahl, 1962; Roberts, 1964; Esashi et al., 1991). Low concentrations (1–10%) of CO stimulated cocklebur (*Xanthium pennsylvanicum*) germination by 10–50% (relative to germination in air alone) when seed were presoaked in water and exposed to CO for 44 h, although interpretation of these results was confounded by changing the gas atmosphere during the experiment (Esashi et al., 1991).

More difficult to interpret were the results of Roberts (1964), who observed germination stimulation of rice (*Oryza sativa* L., ssp. *indica*, cv. 'Toma 112') in an atmosphere of 90% CO–9.6% $O_2$–0.4% $N_2$ compared germination in an atmosphere of 9.6% $O_2$–90.4% $N_2$. In the gaseous mixture in our studies that were comparable to those in Roberts (90% CO–10% $O_2$), 90% CO had no effect on germination percentage (but it did increase the time to germination and stunted the emerged embryo axes) relative to germination in our gas mixture of 90% $N_2$–10% $O_2$ (Fig. 3; Table 2). Differences in species, dormancy, physiological system affected, adaptation to aquatic conditions during germination, or experimental technique and equipment may provide an explanation for the differences in observations.

*Neutral effect*—Carbon monoxide had no effect on germination percentage in many of the oxygen-containing gas atmosphere mixtures evaluated in this study (Fig. 3; Table 2). With a given $O_2$ concentration, CO and $N_2$ at the same concentration did not inhibit germination percentage in most mixtures. No effects on either germination percentage or time to germination were observed in the 10% CO–90% $O_2$ mixture. These observations on neutral effects of CO on germination percentage are consistent with results on the effects of CO on germination of *Phaseolus vulgaris* (Sowa, Roos, and Caughey, 1993).

*Inhibition*—Without oxygen to support mitochondrial respiration, pure CO or $N_2$ gas atmospheres completely inhibited germination. The inhibitory effect of CO on the germination process was most apparent in the delay in time it took seeds to germinate and their stunted appearance (Figs. 3 and 4; Table 2) rather than in the final germination percentage. The embryo axes (coleoptile, colerhiza) of those seeds that did germinate in the presence of high CO concentrations were stunted, yet otherwise appeared white and healthy. These reductions in axis growth are consistent with results on the effects of CO on germination of *Phaseolus vulgaris* (Sowa, Roos, and Caughey, 1993). All CO concentrations delayed time to germination when compared to germination in the same $N_2$ concentration, in mixtures with $O_2$ (except 20% $O_2$).

There appear to be two separate effects of CO on foxtail germination, each occurring in different physiological systems of dormant seeds at the same time. These results are consistent with what is known of the inhibitory effects of CO on mitochondrial respiration of the terminal oxidase, but it is unknown whether the alternative (cyanide resistant) respiratory pathway in germinating *S. faberii* seeds allows normal germination in the presence of CO (Goodwin and Mercer, 1983; Einarsdottir et al., 1988; Sowa, Roos, and Caughey, 1993). What remains unknown from these observations is which physiological system(s) are being affected by CO when hydrated foxtail seed germination is stimulated.

The observation that two physiologically active diatomic chemical gases, $O_2$ and CO, both stimulate giant foxtail seed germination provides clues about the nature of the mechanism of seed dormancy regulation. When sufficient oxygen is present in the hydrated seed symplast it stimulates the first events of foxtail germination. The first events in seed germination of domesticated (nondormant) plant species occur when they become hydrated, but the literature is unclear in separating the physiology of seed hydration and the initial processes of germination per se (Fincher, 1989; Bewley and Black, 1994). Foxtail seeds spend considerable time in the soil fully hydrated yet dormant. The exact nature of the first events in hydrated, after-ripened, foxtail seed germination is unknown. But what is the nature of CO stimulation? It is not apparent that it is a consequence of low CO concentrations on the respiratory apparatus, as no reports of CO-stimulated plant respiration exist. If CO-stimulated germination does not arise from respiratory stimulation, then it may be a consequence of CO interactions with an unknown factor in the seed. At low CO concentrations another factor in foxtail seeds may be inhibited, a factor that normally scavenges $O_2$ and prevents it from initiating the first events, thereby delaying the germination process. At low concentrations, CO may poison this $O_2$ scavenging system and thus speed the time until the critical, germination threshold amount of $O_2$ is present in the symplast. The data presented herein are consistent with this alternative explanation.

***An improved model of foxtail seed dormancy***—An inherent problem with seed germination only being regulated by a restriction of $O_2$–$H_2O$ entry (TACL regulation) (Dekker, 2000) is the observation that foxtail seed germination in natural soil habitats occurs several weeks after both favorable temperatures and $O_2$–$H_2O$ are available in the spring (Fig. 1; Forcella et al., 1992, 1997). What is the basis of delayed weed seed germination timing in the soil after adequate temperature and resource conditions are obtained? Hiltner (1910) suggested that inside dormant grass seeds was an oxygen-absorbing substance that would prevent $O_2$ from reaching the embryo. It has since been suggested that polyphenoloxidase-mediated oxidation of phenolic compounds in external seed coat tissues may reduce the oxygen supply to the symplast (Roberts, 1969; Bewley and Black, 1994; Corbineau and Côme, 1995). It is not apparent how these seed hull compounds could influence oxygen uptake into the foxtail seed symplast via the placental pore. Also not apparent is how these phenolic compounds can continue to absorb $O_2$ for the time seeds remain dormant in the soil (1–10 or more years) and how this system could allow dormancy to be reversible allowing reinduction of dormancy in the hot months of summer (Forcella et al., 1992, 1997; Honek and Martinkova, 1992).

A robust seed regulatory system could result from the interaction of the TACL that restricts $O_2$–$H_2O$ entry into the seed and an oxygen scavenging system that delays the transduction of $O_2$ in the symplast. These restrictions in uptake and $O_2$ scavenging could act together to prolong the time before $O_2$ stimulates the first metabolic events of seed germination. Heterogeneous foxtail seeds with differing levels and combinations of placental pore (TACL) size and $O_2$ absorption could also provide the means by which the seedling emergence of different individuals from the same parent plant would occur over the course of an individual growing season and also over many years (Forcella et al., 1992, 1997). Heterogeneous collections of foxtail seeds in the soil would provide a hedge-betting strategy well suited to the disturbances typical of agroecosystems, habitats to which the weedy foxtails are very well adapted (Cohen, 1966; Phillippi and Seger, 1989). This model of foxtail seed behavior is schematically represented in Fig. 5.

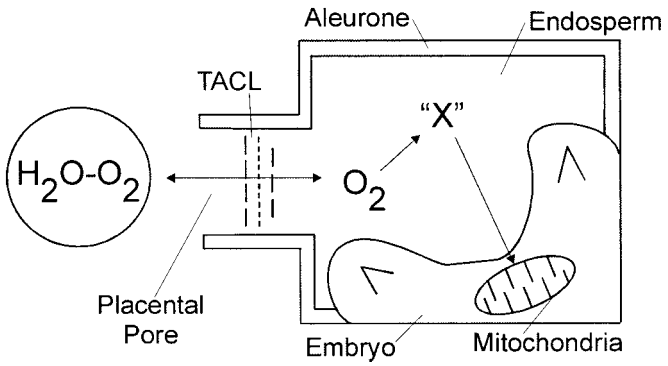


Fig. 5. Schematic model of hydrated, weedy foxtail (*Setaria* spp.) seed parts involved in dormancy and germination and the signal transduction pathway of water–oxygen ($H_2O$–$O_2$) movement from the outside environment, through the placental pore and the transfer aleurone cell layer (TACL; membrane) at the basal end of the seed, into the endosperm, hypothesized oxygen scavenging factor "X", aleurone, embryo, and mitochondria.

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