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The effect of oxygen concentration on the germination of some weed species under control conditions

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Abstract

Continuous use of heavy machinery in fields and frequent farm traffic sometimes result in soil compaction. Soil compaction reduces the oxygen (O_2) concentration in soil capillaries and hence lowers the O_2 availability for germinating seeds. We investigated how reduced O_2 levels changed germination behavior of weeds to elucidate their potential to adapt to O_2-deficient soils (compacted, compressed, and waterlogged soils and soils with hard surfaces). Two similar laboratory experiments were conducted with five O_2 treatments (20.9%, 15%, 10%, 5%, and 2.5%). The germination percentage of the invasive weed hairy fiddleneck [*Amsinckia menziesii* (Lehm.) A. Nelson & J.F. Macbr. var. *menziesii*] and the common weeds common lambsquarters (*Chenopodium album* L.) and Persian speedwell (*Veronica persica* Poir) was not significantly reduced at 15% O_2. The germination of scarlet pimpernel (*Anagallis arvensis* L. ssp. *arvensis*), silky windgrass [*Apera spica-venti* (L.) Beauv.], catchweed bedstraw (*Galium aparine* L.), and knawel (*Scleranthus annuus* L.) was significantly reduced at 15% O_2. The highest germination was obtained at 20.9% O_2 for blackgrass (*Alopecurus myosuroides* Huds.), *A. spica-venti*, *G. aparine*, annual bluegrass (*Poa annua* L.), wild mustard (*Sinapis arvensis* L.), scentless chamomile [*Tripleurospermum inodorum* (L.) Sch. Bip.], field violet (*Viola arvensis* Murray) and the less common weeds *A. arvensis* and *S. annuus*. Distribution of flora in the landscape may change on O_2-deficient soils by reducing germination of some species such as *A. arvensis* and *S. annuus* and favoring others like *A. menziesii* and *C. album*. The ability to germinate at 2.5% and 5% O_2 may contribute to explain why *A. myosuroides* and *A. menziesii* have become successful as weeds on O_2-deficient soils, as they maintained a germination percentage between 34% and 58% at 2.5% O_2.

Introduction

Agricultural development in many countries of the world has over the last 30 yr resulted in larger farms using bigger and heavier field implements. Continuous use of heavy machinery in fields and frequent field traffic often results in problems with soil compaction in the upper layers of the soil profile (Tullberg et al. 2007). Frequent traffic, even with lightweight tractors, sometimes compacts the topsoil and generates physical soil conditions that prevent seedling emergence (Botta et al. 2006). Soil compaction reduces oxygen (O_2) concentration, influences crop growth, stunts plant root development, and affects the movement of water and chemicals in multiple ways (Lipiec et al. 2003). Soil compaction reduces the oxygen concentration in soil capillaries and hence reduces O_2 availability for germinating seeds.

Atmospheric air typically comprises 79% N_2, 21% O_2, and 0.03% CO_2, while the average arable topsoil atmosphere contains 79% N_2, 20.3% O_2, and 0.15 to 0.65% CO_2. The O_2 in topsoil (usually the top 5.1 cm [2 in.] to 20 cm [8 in.]) has been observed to be as low as 1%, and soil CO_2 has been noted to be as high as 10% under extreme conditions. In soil, O_2 percentage decreases with depth, and the rate of decrease is more rapid in clayey or silty soils than in sandy soils (Brady 1974). Silver et al. (1999) found that O_2 concentrations in soil decreased gradually. O_2 concentration was reduced significantly in 10- to 35-cm soil depths, and in some cases even below 3%. The O_2 level in the soil declined with increasing depth, and at 30-cm depth, the O_2 concentration was less than 0.01 kg m^{-3} soil, which is inadequate for plant growth (Topp et al. 2000).

Under farming conditions, soil compaction is not only caused by frequent use of heavy machinery but also by cattle trampling pastures. Martinez and Zinck (2004) observed that in compact soils, O_2 penetration resistance ranged from 0.45 MPa under forest conditions to 4.25 MPa at 3- to 12-cm depth in 9-yr-old pastures.

In addition to trampling by livestock, rolling also causes soil compaction and limits O_2 concentration under field conditions. After sowing, rolling is often conducted to ensure good contact between seeds and soil particles and thereby improve the water supply and avoid desiccation of germinating seeds. The purpose of rolling can also be to press the stones and soil aggregates lying on the soil surface down into the soil to avoid damage to the combine harvester during harvest. Rolling may cause soil O_2 depletion due to increasing intensity of soil compaction and compression. Soil aggregates can exhibit steep O_2 gradients over tiny distances from the aggregate surface. Sexstone et al. (1985) found that the smallest aggregates with a radius of 4 mm had an anaerobic center. Larger aggregates with a radius >10 mm often had measurable anaerobic centers with irregular O_2 profiles, apparently due to O_2 intrusion often caused by old root channels. These irregular O_2 profiles created by soil aggregates in the topsoil may affect germination of many species like silky windgrass [*Apera spica-venti* (L.) Beauv.], with small seeds germinating from the upper 5 mm of the topsoil.

In compact soils, O_2 deficiency reduces respiration capability of seeds (Benvenuti and Macchia 1995). Deprived soil structure, compressed soil, excess soil water, and greater microbial activity may inhibit gaseous movement within the soil and cause depletion in soil O_2 (Drew 1992; Hodgson and MacLeod 1989; Ishii and Kadoya 1991). Seed germination depends on available O_2 concentration, water potential, and temperature, as well as the dormancy and physiological status of the seeds (Bradford et al. 2007). Most seeds need O_2 to germinate, but some seeds may germinate in the absence of O_2 (Rumpho and Kennedy 1981). Soil compaction may favor the germination of some aggressive weed species that germinate earlier and establish better than a crop. Seeds of velvetleaf (*Abutilon theophrasti* Medik.), tall morningglory [*Ipomoea purpurea* (L.) Roth], and wild mustard (*Sinapis arvensis* L.) buried at compressed soil under low O_2 concentrations switched from aerobic to anaerobic metabolism, resulting in reduced germination (Holm 1972). High O_2 levels were required for the germination of the weed species marsh dayflower [*Murdannia keisak* (Hassk.) Hand.-Maz.], barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], late watergrass [*Echinochloa oryzicola* (Vasinger.) Vasinger], and pale smartweed [*Persicaria lapathifolia* (L.) Delarbre], while monochoria [*Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth] and rock bulrush [*Schoenoplectiella juncoides* (Roxb.) Lye] germinated better at reduced O_2 concentration. At low O_2 concentration, leaf greening and root and plumule elongation of all weeds were inhibited, whereas *S. juncoides* was inhibited by high O_2 concentration (Kataoka and Kim 1978). Seeds of the weed bur beggarticks (*Bidens tripartita* L.) germinated faster at 5% and 10% O_2, and germination was delayed at 20.9% O_2 (Benvenuti and Macchia 1997). Seed germination of *E. crus-galli* was inhibited by the combination of exposure to 20.9% O_2 and light, which prevented surface soil germination (Boyd and VanAcker 2004).

National surveys (Andreasen and Streibig 2011; Andreasen and Stryhn 2008, 2012) indicated that that the occurrence of some weed species was related to the O_2 levels in the soil. We observed that some of the most common weeds grew well even at very compressed soils, while some rarer weeds did not.

The study objective of our research was to investigate how decreasing O_2 concentrations changed germination behavior of some common and less common weed species. We tested the hypothesis that the germination of some successful weed species may be favored by low oxygen concentration, while other weed species, which have become rare, may suffer.

Materials and Methods

Seed Source

Nine broadleaf weeds were selected: one invasive weed (hairy fiddleneck [*Amsinckia menziesii* (Lehm.) A. Nelson & J.F. Macbr. var. *menziesii*]), two scarce weeds (scarlet pimpernel [*Anagallis arvensis* L. ssp. *arvensis*], knawel [*Scleranthus annuus* L.]), and six common weeds (common lambsquarters [*Chenopodium album* L.], catchweed bedstraw [*Galium aparine* L.], wild mustard (*Sinapis arvensis* L.), scentless chamomile [*Tripleurospermum inodorum* (L.) Sch. Bip.], Persian speedwell [*Veronica persica* Poir], field violet [*Viola arvensis* Murray]). We also selected three common grass weeds (blackgrass [*Alopecurus myosuroides* Huds.], *A. spica-venti*, annual bluegrass [*Poa annua* L.]).

A species was defined to be common if it occurred with a frequency above 5% in one of the most common crops in the latest national weed survey in Denmark (Andreasen and Stryhn 2008, 2012). A species was defined to be scarce if it occurred with a frequency below 1%. An invasive plant is a nonnative (alien) that is able to establish on many sites, grows quickly, spreads to the point of disrupting plant communities or ecosystems, and causes or is likely to cause economic or environmental harm or harm to human health. *Amsinckia menziesii* is such a plant in Scandinavia. *Poa annua*, *C. album*, *G. aparine*, *S. arvensis*, *T. inodorum*, *V. persica*, and *V. arvensis* are examples of weeds that have been common for many decades (Andreasen et al. 1996; Andreasen and Stryhn 2008, 2012) while *A. spica-venti* has become common during the last 25 yr (Andreasen and Stryhn 2008). *Alopecurus myosuroides* cannot be characterized as a common species according to the survey from 2004, but farm advisers now consider it a common weed in winter cereals. *Anagallis arvensis* and *S. annuus* are examples of species that have become scarce in common crops (Andreasen et al. 1996; Andreasen and Stryhn 2008, 2012) They have relatively small seed production, and it would probably take a long time before they could become common again, even if their growing conditions were improved.

Weed plants for the experimental study were collected and harvested at maturity from different crop fields at the experimental farms at Hojbakkegaard, Taastrup, Denmark (55.63°N, 12.28°E). Plants were dried in the shade for 3 wk at room temperature (14 ± 4 C). Seeds from plants were threshed using a threshing machine (Wintersteiger LD 350, 4705W, Amelia Earhart Drive, Salt Lake City, UT, USA) and cleaned using a seed cleaner (Westrup LA-LS, Slagelse, Denmark). Cleaned seeds were stored at 5 C for later use. The seed moisture was estimated to be 8% to 12% using a grain moisture meter (Wile 55, model W1910/FLFM, no.1, 3rd Shangdi, Haidian District, Beijing, China).

Germination Experiment

Two similar experiments following a completely randomized design were performed at two separate times. Experiment 2 was performed after completion of Experiment 1. According to International Seed Testing Association (ISTA) procedures for seed testing, fewer than 400 seeds can be tested. In such cases, at least 100 seeds must be tested in replicates of 25 or 50 (ISTA 2011). We used 100 seeds in two replications per treatment level for each species in each germination bioassay. Fifty seeds of individual species were placed on moist filter paper in germination boxes (ISTA 2011) randomly placed in airtight glass containers (50-cm long, 30-cm wide, and 20-cm high) at 15 C under a

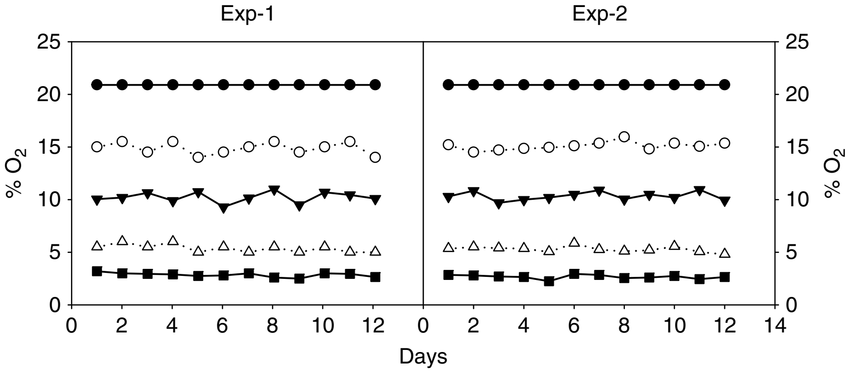


Figure 1. Fluctuation in oxygen concentrations for 12 weed species and eight crops at 20.9% O_2 (filled circles), 15% O_2 (open circles), 10% O_2 (filled triangles), 5% O_2 (open triangles), and 2.5% O_2 (filled squares) in airtight glass containers during germination Experiments 1 and 2.

16-h light/8-h dark photoperiod in a growth chamber (Termaks AS, Nino Lab, Køge, Denmark) containing tube lights on both sides, providing light intensity of 25,000 lx. Many of the weeds in this study were winter annuals (*A. arvensis*, *G. aparine*, *S. arvensis*, *V. persica*, *V. arvensis*, and the three grass weeds, *A. myosuroides*, *A. spica-venti*, and *P. anuua*). However, *C. album* is a summer annual in Denmark. In general, for a standard ISTA test, an alternating temperature regime of 20 C for 16 h and 30 C for 8 h is recommended for some of the species (ISTA 2011). We chose 15 C for all plant species, which is closer to the field conditions during summer and also appropriate for winter species that usually germinate during autumn in Denmark. Keeping the same temperature for all species made it possible to compare the response of the species to reduced O_2 concentrations. Each germination box was irrigated once with 60 ml of water, which was sufficient for every 14-d germination trial. Seeds were assumed to have germinated when either radicle tissue or the cotyledons protruded 2 mm beyond the seed coat. Germination was counted daily following a seedling evaluation protocol (AOSA 1990) until a constant count was achieved in each glass case. A total of 12,000 seeds were tested (5 O_2 treatment levels by 50 seeds by 2 replicates by 2 experiments by 12 plant species). Seeds and seedlings remained in the germination boxes in airtight glass containers for 14 d for observation, and O_2 was added as necessary to compensate for O_2 consumption and adjust the O_2:N_2 balance.

Oxygen Concentration

Five oxygen concentrations (20.9%, 15%, 10%, 5%, and 2.5%) were obtained by mixing N_2 gas with O_2 in airtight glass containers. The O_2 concentrations were kept constant during the experiments (Figure 1). The N_2 gas from a liquid N_2 cylinder was injected into the containers manually. One Gasman Personal Gas Monitor (Crowcon Detection Instruments, Rotterdam, Netherlands) was placed inside each glass container to monitor and keep the O_2 concentrations constant inside the containers during the experiments. N_2:O_2 mixtures were monitored and adjusted daily, and the ratio was maintained inside each glass container. Ambient air contains about 78.09% N_2, 20.95% O_2, and 0.039% CO_2. O_2 is required for germination of most species (Copeland and McDonald 2012). CO_2 concentrations higher than 0.039% retard germination, while N_2 has no influence (Copeland and McDonald 2012). In our experiments, we tried to isolate the effect of O_2 by continuous monitoring and adjustment to maintain a constant O_2 and N_2 ratio in order to find differences between the sensibilities of the species to this specific germination factor. The glass containers used for these experiments had large volumes, and the CO_2 released from germinating seeds inside the glass containers was very small and was assumed to have no influence on the result.

Data Analysis and Statistics

Experiments were analyzed separately. Statistical analyses were performed using the open-source program R v. 3.3.1 (http://www.R-project.org). The seed germination for each experiment was modeled using a cumulative distribution function of the standard three-parameter log-logistic model, allowing a different curve for each treatment using the add-on package drc (Ritz and Streibig 2005), as:

$$F(t) = \frac{d}{1 + \exp\{b[\log(t) - \log(t_{50})]\}} \qquad \text{(Model 1)}$$

where $F(t)$ is the fraction of seeds germinated at time t (days). The upper-limit parameter, d, denotes the proportion of seeds that germinated during the experiment out of the total number of seeds. The parameter b is proportional to the slope of F at time t equal to the parameter t_{50}, where 50% of the total seeds germinated during the experimental period. The parameter t_{50} indicates the germination speed of the population measured in days. The estimation and model-checking procedures were based on treating the data as event times, that is, recording the time it takes for germination (the event of interest) to occur as described by Ritz et al. (2013). An overall test for any differences between d was made by comparing a model with all parameters allowed to vary between curves with a model in which only d was restricted to be the same for all curves using an ANOVA test. In a post hoc procedure, all pairwise comparisons for d were made, and significance levels were assessed by an LSD test.

Results and Discussion

Generally, low O_2 concentrations changed the germination success (fraction of germinated seeds: d) and germination speed (time to reach 50% germination: t_{50}) (Tables 1 and 2). However, the germination percentage for some weed species did not decline at reduced O_2 concentration compared with ambient air (20.9% O_2). In both experiments, the germination percentage of *C. album* was not significantly reduced when O_2 concentration fell to 15%. It declined when O_2 dropped from 15% to 2.5% (Table 1). The highest

Table 1. Estimated regression parameters of the germination curves (Model 1) with SE in parentheses for six weed species.[a]

Weed species	O_2	t_{50} Exp. 1	t_{50} Exp. 2	$-b$ Exp. 1	$-b$ Exp. 2
	%				
Alopecurus myosuriodes	20.9	4.48 (0.11)	5.28 (0.15)	7.83 (0.80)	6.58 (0.64)
	15	4.87 (0.12)	4.97 (0.22)	8.17 (0.85)	4.48 (0.46)
	10	4.96 (0.16)	4.92 (0.23)	6.55 (0.73)	4.58 (0.52)
	5	4.94 (0.17)	4.34 (0.30)	6.71 (0.83)	3.88 (0.57)
	2.5	6.73 (0.36)	4.84 (0.24)	5.75 (0.91)	6.14 (0.93)
Amsinckia micranta	20.9	4.54 (0.16)	5.78 (0.51)	5.36 (0.53)	3.06 (0.42)
	15	4.38 (0.15)	5.23 (0.29)	5.20 (0.51)	3.66 (0.41)
	10	4.91 (0.18)	6.74 (0.72)	5.11 (0.53)	3.01 (0.45)
	5	4.99 (0.25)	7.91 (2.06)	4.25 (0.49)	2.28 (0.48)
	2.5	7.20 (0.37)	5.90 (0.77)	5.28 (0.77)	2.83 (0.52)
Angallis arvensis	20.9	5.18 (0.20)	8.29 (0.55)	6.98 (1.01)	0.09 (2.27)
	15	5.60 (0.25)	7.72 (0.69)	9.44 (1.92)	6.58 (2.04)
	10	6.03 (0.44)	9.10 (1.32)	7.10 (1.88)	7.06 (3.50)
	5	53.02 (82.7)	19.05 (10.01)	1.43 (1.29)	4.67 (4.54)
	2.5	16.11 (3.27)	16.11 (3.27)	7.75 (5.13)	7.75 (5.13)
Apera spica-venti	20.9	5.57 (0.12)	9.05 (0.37)	10.83 (1.37)	9.80 (2.20)
	15	5.65 (0.13)	8.70 (0.39)	11.09 (1.53)	9.30 (2.15)
	10	5.92 (0.17)	9.61 (0.51)	9.76 (1.41)	10.28 (3.10)
	5	7.17 (0.24)	11.05 (0.93)	12.04 (2.55)	15.94 (9.00)
	2.5	9.41 (0.28)	15.54 (4.61)	19.82 (6.28)	8.37 (8.27)
Chenopodium album	20.9	6.13 (0.53)	9.03 (1.62)	3.63 (0.72)	3.34 (0.75)
	15	5.12 (0.33)	8.35 (1.87)	4.20 (0.62)	2.45 (0.50)
	10	4.94 (0.38)	6.97 (1.30)	4.24 (0.79)	2.61 (0.58)
	5	5.01 (0.57)	5.41 (0.63)	3.44 (0.73)	3.14 (0.65)
	2.5	5.86 (0.59)	9.19 (2.46)	4.45 (1.06)	3.75 (1.48)
Galium aparine	20.9	9.13 (0.30)	9.93 (0.58)	14.60 (3.80)	10.48 (3.28)
	15	9.20 (0.30)	10.40 (1.19)	17.78 (5.53)	8.80 (3.61)
	10	9.48 (1.19)	10.84 (1.02)	1.14 (2.72)	15.04 (9.37)
	5	1.04 (1.18)	1.04 (1.18)	1.25 (2.95)	1.25 (2.95)
	2.5	14.70 (6.37)	14.7 (6.37)	11.03 (7.65)	11.03 (7.65)

[a] t_{50} is the time to 50% germination of those seeds that germinated in the research period. *b* expresses the slope at t_{50}.

germination percentage was obtained at 15% O_2 in both experiments (Experiment 1: d = 0.46; Experiment 2: d = 0.66). *Veronica persica* followed the same trend (Table 2).

Galium aparine, *S. arvensis*, *T. inodorum*, and *V. arvensis* were all negatively affected when the O_2 concentration declined (Figures 2–4; Tables 1 and 2). *Tripleurospermum inodorum* exhibited maximum germination at 20.9% O_2 (Experiment 1: $d = 0.87$; $t_{50} = 3.17$; Experiment 2: $d = 0.89$; $t_{50} = 3.84$) (Figure 4; Table 2). The germination percentage decreased gradually with declining O_2 (Table 2; Figure 3) but sustained germination even at the lowest O_2 level (2.5%) (Experiment 1: d = 0.70; Experiment 2: d = 0.71) (Figure 3), which may give a competitive advantage over companion weed species sensitive to O_2-deficient soils.

The germination of *A. menziesii* was significantly increased when the O_2 level dropped from 20.9% to 15% in Experiment 2, but declined when the O_2 concentration fell to 5% and 2.5% in both experiments. An O_2 level of 15% gave the fastest germination and highest germination percentage (Experiment 1: $d = 0.90$; $t_{50} = 4.38$; Experiment 2: $d = 0.93$; $t_{50} = 5.23$), followed by 20.9% O_2 (Figure 1; Table 1). Germination was significantly slower at 20.9% O_2 in Experiment 2, probably because of seed aging. The 2.5% O_2 resulted in slower germination (Experiment 1: $t_{50} = 7.20$; Experiment 2: $t_{50} = 5.90$) and reduced the germination percentage (Experiment 1: $d = 0.58$; Experiment2 : 0.46) (Figure 2; Table 1).

Amsinckia menziesii is an invasive species in Europe and is native to the states of Oregon and Washington, USA. It has become a widespread and aggressive weed in many parts of southern Scandinavia (Andreasen and Streibig 2011). Its success is probably caused by several factors such as fast growth and big seed production over a long period of the growing season. Our experiments also showed that it germinates well at 15% O_2. This may give *A. menziesii* a competitive advantage on O_2-deficient soils compared with weed species that have maximum germination at 20.9% O_2 and reduced germination at 15% O_2 or below. Low O_2 concentration combined with a high CO_2 level in the soil may act as a signal for early completion of the germination phases in *A. menziesii* seeds and may promote synchronized seedling establishment. A similar effect has been reported for *E. crus-galli*, in which an increased level of CO_2 following rainfall acted as a germination signal (Yoshioka et al. 1998).

Anagallis arvensis and *S. annuus* were among the most sensitive species to any drop in O_2 concentration. The germination percentage of these species was relatively low, probably due to seed dormancy, but even if various methods were used to release the dormancy (see Yasin and Andreasen 2015), we would expect that the germination trend would remain the same at declining O_2 levels. For all individual species, the trend was the same in the two experiments, although the germination ability varied between the two experiments. The same trend has also been shown for horticultural species exposed to declining O_2 levels and subsequently having altered germination ability (Yasin and Andreasen 2016). *Scleranthus annuus* and *A. arvensis* have become less common (Andreasen and Stryhn 2008, 2012), probably due to several factors, such as their susceptibility to herbicides and sensitivity to low O_2 content in soil combined with relatively small biomass and seed production.

Alopecurus myosuroides and *P. annua* were less sensitive to decreasing O_2 concentrations (Figures 2 and 3). Germination

Table 2. Estimated regression parameters of the germination curves (Model 1) with SE in the parentheses for six weed species.[a]

		t_{50}		$-b$	
Weed species	O_2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Poa annua	20.9	5.47 (0.07)	9.39 (0.38)	16.06 (1.59)	7.00 (0.93)
	15	5.94 (0.11)	10.15 (0.24)	9.98 (0.96)	11.49 (1.68)
	10	6.07 (0.13)	15.11 (9.76)	8.77 (0.84)	4.90 (1.76)
	5	6.78 (0.20)	10.30 (0.69)	7.02 (0.75)	9.12 (2.58)
	2.5	8.01 (0.44)	11.27 (2.90)	6.77 (1.37)	6.04 (2.72)
Scleranthus annus	20.9	8.63 (0.31)	9.24 (0.53)	11.99 (2.83)	11.00 (4.05)
	15	9.14 (0.61)	7.97 (0.61)	10.66 (4.07)	12.01 (5.88)
	10	19.05 (10.0)	9.05 (0.59)	4.67 (4.54)	16.72 (9.32)
	5	15.54 (4.61)	11.55 (4.54)	8.37 (8.27)	8.63 (8.00)
	2.5	16.11 (3.27)	11.42 (4.00)	7.75 (5.13)	11.36 (12.9)
Sinapis arvensis	20.9	3.04 (0.14)	3.45 (0.13)	5.83 (0.85)	7.40 (1.08)
	15	3.12 (0.12)	3.65 (0.15)	8.18 (1.39)	7.51 (1.19)
	10	3.90 (0.24)	4.36 (0.34)	6.51 (1.37)	5.49 (1.29)
	5	4.97 (0.59)	5.92 (0.42)	6.93 (2.70)	56.18 (302.)
	2.5	7.13 (1.72)	23.50 (16.53)	4.47 (2.37)	1.92 (1.27)
Triplerospermum inodorum	20.9	3.17 (0.09)	3.84 (0.14)	6.85 (0.70)	5.17 (0.50)
	15	3.35 (0.12)	4.22 (0.20)	5.15 (0.51)	4.22 (0.44)
	10	3.69 (0.14)	3.67 (0.11)	5.08 (0.51)	6.67 (0.70)
	5	4.29 (0.27)	4.50 (0.25)	3.77 (0.45)	3.84 (0.45)
	2.5	8.18 (1.43)	7.35 (0.88)	2.68 (0.49)	3.00 (0.49)
Veronica persica	20.9	4.88 (0.08)	5.54 (0.11)	10.65 (1.04)	8.66 (0.83)
	15	4.78 (0.07)	5.40 (0.08)	12.10 (1.17)	12.08 (1.16)
	10	5.55 (0.12)	5.85 (0.13)	8.86 (0.84)	8.07 (0.77)
	5	8.20 (0.17)	9.46 (0.48)	10.01 (1.10)	6.38 (0.91)
	2.5	9.16 (0.18)	10.68 (0.32)	21.11 (4.77)	15.92 (4.23)
Viola arvensis	20.9	4.38 (0.12)	4.97 (0.14)	8.41 (1.05)	9.88 (1.40)
	15	4.53 (0.10)	4.92 (0.18)	14.36 (2.12)	8.83 (1.44)
	10	4.68 (0.11)	4.34 (0.13)	12.66 (1.92)	11.55 (2.04)
	5	6.68 (0.53)	5.32 (0.30)	4.86 (0.96)	6.49 (1.21)
	2.5	8.04 (0.15)	9.50 (0.84)	67.1 (146.8)	9.53 (4.66)

[a] t_{50} is the time to 50% germination of those seeds that germinated in the research period. *b* expresses the slope at t_{50}.

was fastest and germination percentage was largest for *A. myosuroides* at 20.9% O_2 (Experiment 1: d = 0.82, $t_{50} = 4.48$; Experiment 2: $d = 0.89$, $t_{50} = 5.28$). There was a significant reduction in the germination speed and percentage when O_2 was reduced from 20.9% to 2.5% (Table 1; Figure 3). At the lowest O_2 concentration, 40% and 34% of the *A. myosuroides* seeds were able to germinate in Experiments 1 and 2, respectively.

Alopecurus myosuroides and *A. spica-venti* have posed increasing weed problems in Europe the last three decades, but their success seems more to be a result of the immense shift from summer to winter annual crops and the development of herbicide-resistant biotypes (Andreasen and Stryhn 2012) together with their ability to adapt to compacted soils. However, compact soils also ensure better water uptake and reduce soil evaporation, which can be an advantage for species sensitive to drought like *P. annua*, which has become the most common weed in many fields. Our results support the finding that common species like *P. annua* are favored by soil compaction (Warwick 1979) due to low O_2. In our study, the three monocotyledons (*A. myosuroides*, *A. spica-venti*, and *P. annua*) were able to germinate even at 2.5% O_2 concentration. Heichel and Day (1972) also found that some monocotyledons were able to germinate even below 2 kPa O_2. However, as our studies and that of Siegel and Rosen (1962) have shown, the capacity to germinate at low O_2 is not restricted to monocotyledons.

Our results cannot be compared directly with weed behaviors under field conditions, where CO_2 produced by root respiration and germinating plants, soil organisms, and microbial activities sometimes affects seed germination. Many factors, such as root respiration and respiration of soil-dwelling animals and microorganisms, can affect germination conditions in the soil. CO_2 release may lead to the production of increased ethanol in cells, which is toxic to seed metabolism and inhibits seed germination (Thomson and Greenway 1991). We cannot expect the same response under field conditions, but testing under field conditions is normally unsatisfactory, as the results cannot be repeated with reliability (ISTA 2011). Consequently, it makes good sense to make germination tests under control conditions, even though we should keep in mind that many other factors affect germination locally in the field, and O_2 deficiency is often only temporary.

Weed Flora Changes Over Time

Some species decline in abundance, whereas others increase due to the effect of a single factor (e.g., herbicide use, low O_2) or multiple factors like improved agricultural management, changes in the farming system, and use of more broad-spectrum herbicides (Marshall et al. 2003). Boyd and VanAcker (2004) reported that germination of *S. arvensis* increased with increasing O_2 concentration. Fluctuating temperature, light, water, oxygen, ethylene, and nitrate are known to promote seed germination in many species (Yoshioka et al. 1998). The germination of *A. menziesii*, *C. album*, and *V. persica* was significantly favored by 15% O_2. Recent modifications in agricultural practices, that is, enhanced levels of disturbance (soil compaction), favor the most generalist species, leading to biotic homogenization in arable landscapes (Fried et al. 2010).

Our experiments have been able to show the effect of reduced O_2 concentrations on the germination of weed species under

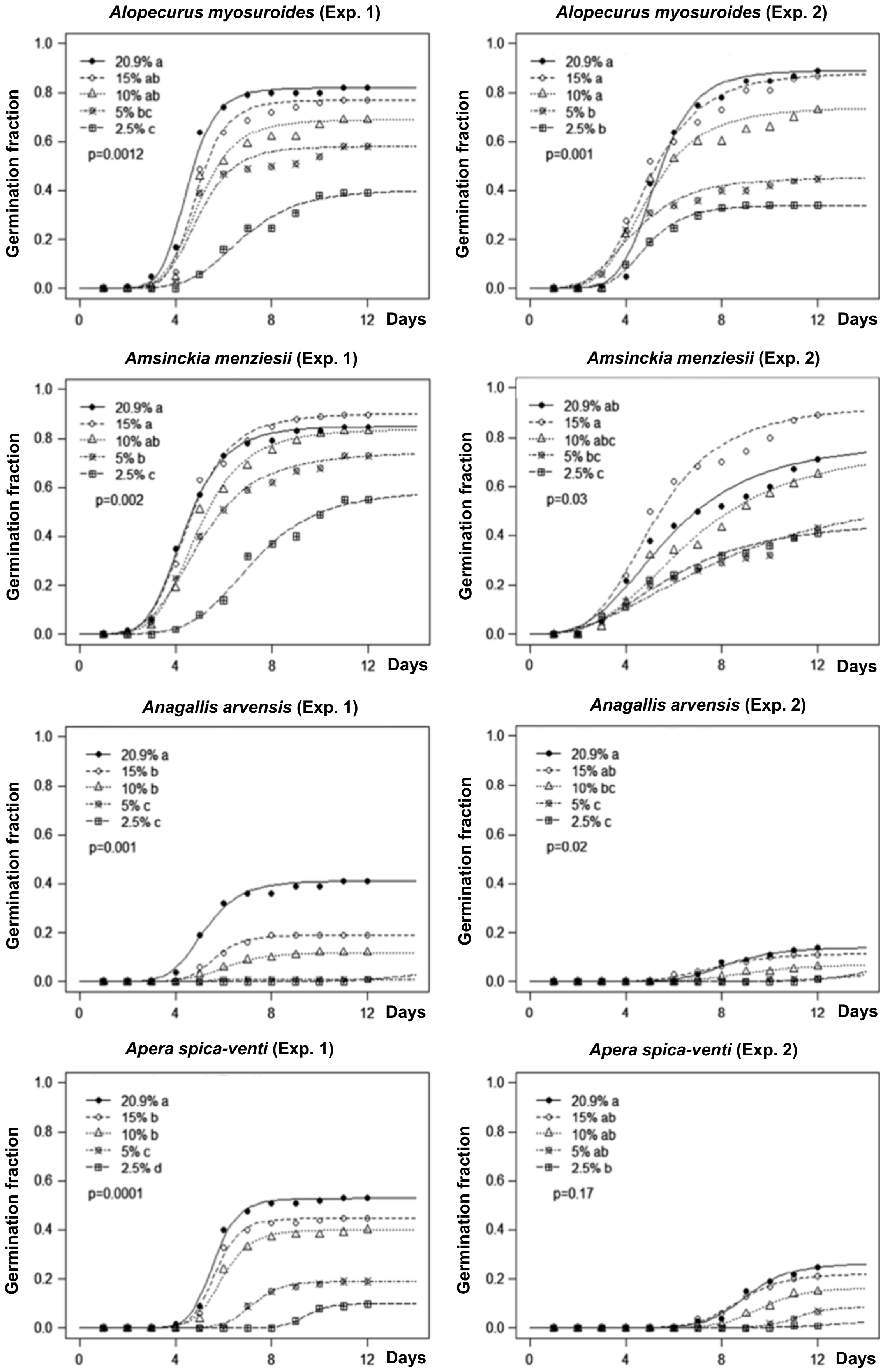
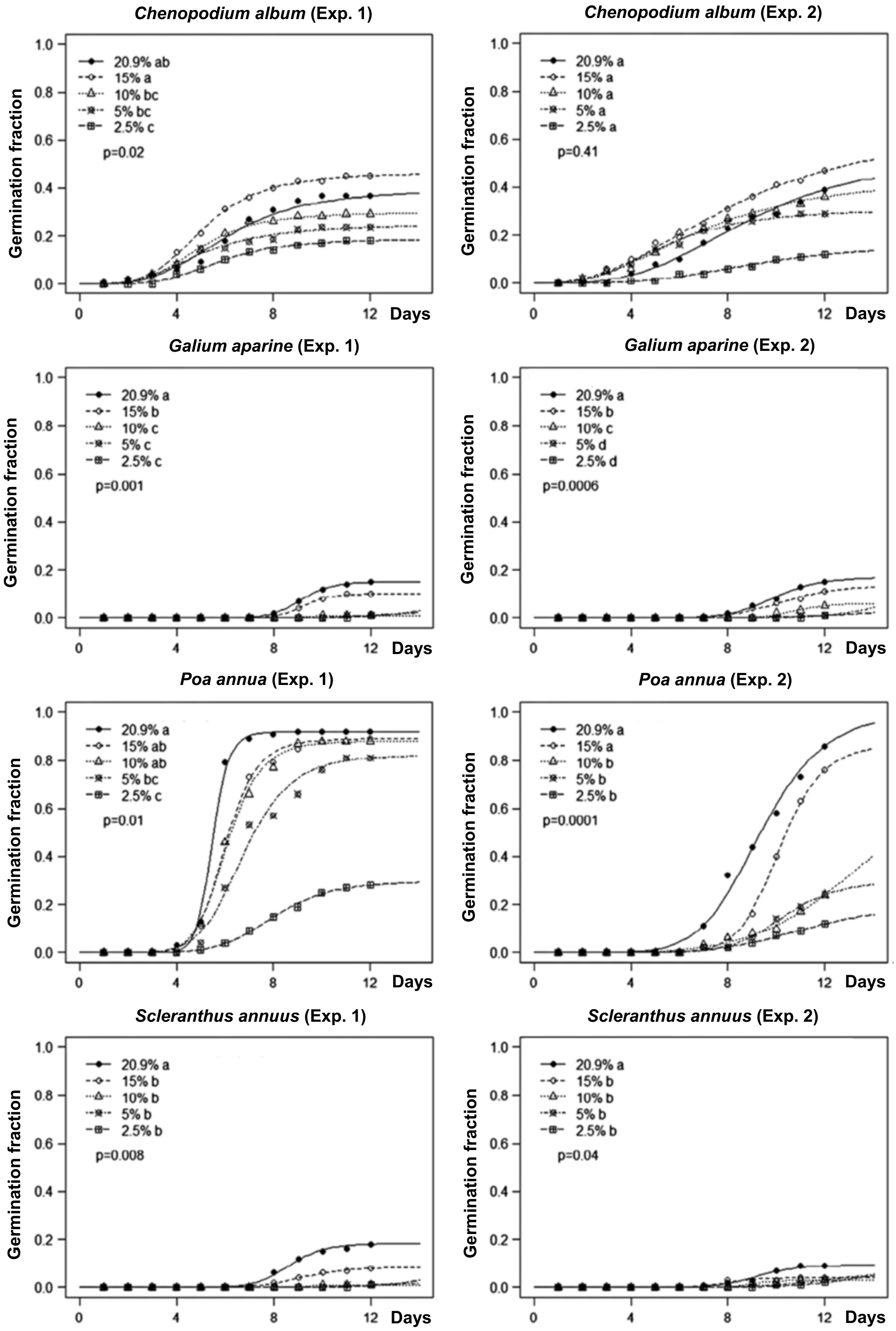


Figure 2. Three-parameter log-logistic dose–response curves of germination over time for the weed species *Alopecurus myosuroides*, *Amsinckia menziesii*, *Anagallis arvensis*, and *Apera spica-venti* at five (20.9%, 15%, 10%, 5%, and 2.5%) O_2 concentrations from two independent experiments. Experiment 2 was performed after completion of Experiment 1. One-way ANOVA and LSD post hoc tests were performed. P-values and small letters beside each % O_2 treatment on the graphs show statistical significance.



Chenopodium album (Exp. 1)

Chenopodium album (Exp. 2)

Galium aparine (Exp. 1)

Galium aparine (Exp. 2)

Poa annua (Exp. 1)

Poa annua (Exp. 2)

Scleranthus annuus (Exp. 1)

Scleranthus annuus (Exp. 2)

Figure 3. Three-parameter log-logistic dose–response curves of germination over time for the weed species *Chenopodium album*, *Galium aparine*, *Poa annua*, and *Scleranthus annuus* at five (20.9%, 15%, 10%, 5%, and 2.5%) O_2 concentrations from two independent experiments. Experiment 2 was performed after completion of Experiment 1. One-way ANOVA and LSD post hoc tests were performed. P-values and small letters beside each % O_2 treatment on the graphs show statistical significance.

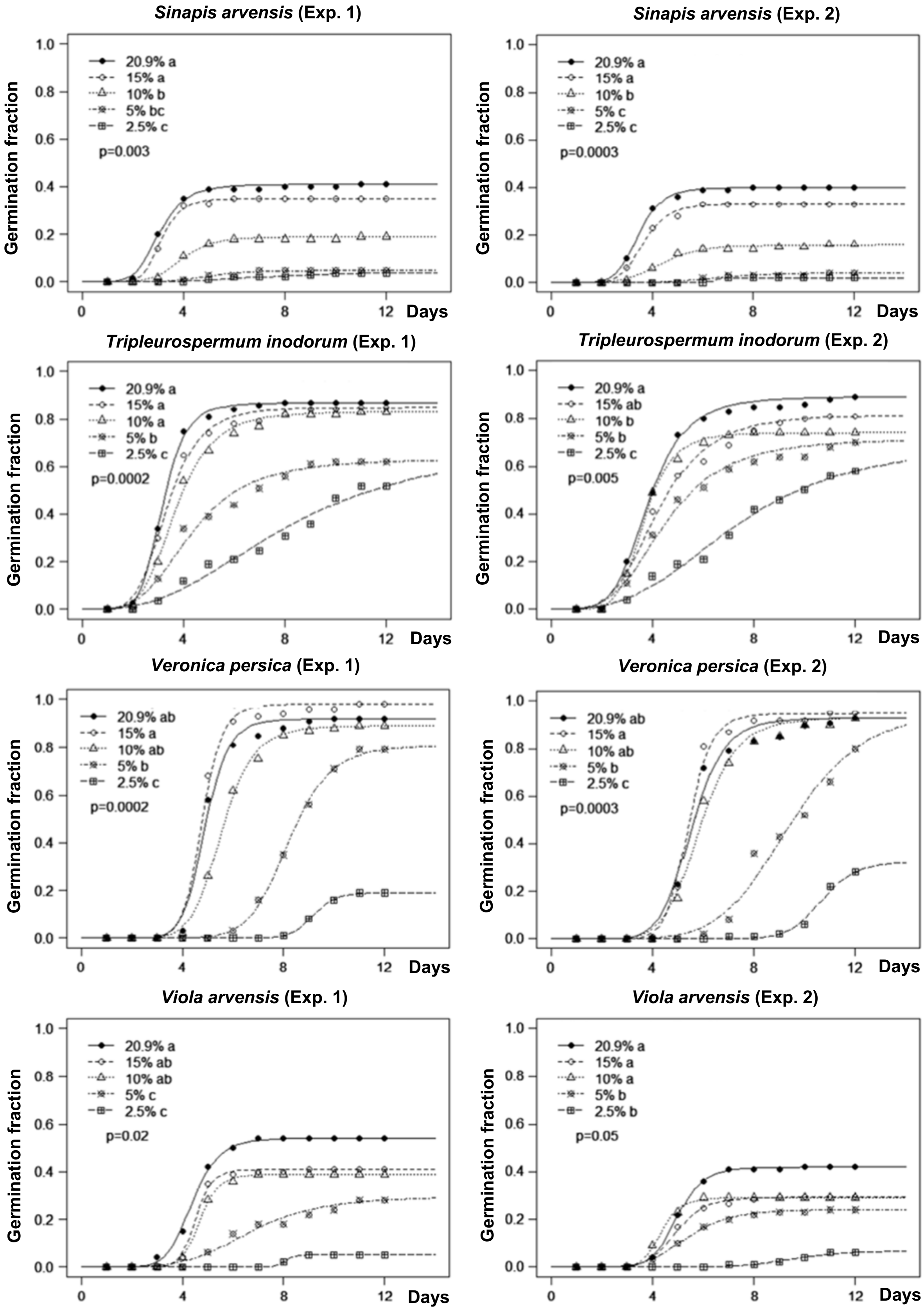


Figure 4. Three-parameter log-logistic dose–response curves of germination over time for the weed species *Sinapsis arvensis*, *Tripleurospermum inodorum*, *Veronica persica*, and *Viola arvensis* at five (20.9%, 15%, 10%, 5%, and 2.5%) O_2 concentrations from two independent experiments. Experiment 2 was performed after completion of Experiment 1. One-way ANOVA and LSD post hoc tests were performed. P-values and small letters beside each % O_2 treatment on the graphs show statistical significance.

control conditions that make comparison possible. Our findings support the hypotheses that reduced O_2 concentrations due to soil compaction may be one reason why the invasive species *A. menziesii* and the aggressive species *C. album* and *T. inodorum* have become very common. It may also contribute to explain why the weed species *A. arvensis* and *S. annuus*, which are sensitive to reduces O_2 levels, have become rare. The ability of the two winter annual grass species *A. myosuroides* and *A. spica-venti* to germinate at 2.5% O_2 may also contribute to our understanding of why they have become extremely successful as weeds on O_2-deficient soils during the last 20 yr in an area where autumn-sown crops have increased significantly in Europe.

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