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Studies in Wild Oat Seed Dormancy

I. THE ROLE OF ETHYLENE IN DORMANCY BREAKAGE AND GERMINATION OF WILD OAT SEEDS (*AVENA FATUA* L.)

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ABSTRACT

Seed of *Avena fatua* were shown to exhibit a characteristic loss of dormancy during dry storage at 25 C, whereas similar seed stored at 5 C maintained dormancy. 2-Chloroethylphosphonic acid was shown to increase germination of partly dormant seed imbibed under certain temperature regimes; a similar effect could not be established for fully dormant or fully nondormant seed. Using gas-liquid chromatography, natural ethylene levels were followed during imbibition of fully dormant and nondormant seed. A large peak in production was observed in the period prior to radicle emergence in the case of the nondormant seed. Measurements of ethylene production taken at 15 C, following periods of after-ripening in moist soil at either 5 or 25 C, indicated that endogenous production was unlikely to be a main cause of dormancy breakage in this species. The possibility that endogenous ethylene could play a role in natural dormancy breakage in aged seeds is discussed. The practical possibilities of 2-chloroethylphosphonic acid as a dormancy breaking agent in a field situation are outlined.

The presence of viable dormant wild oat seeds in cultivated soils constitutes an insidious menace in most notable grain-producing areas. Any method of reducing the reservoir of these soil-stored seeds is of obvious importance.

Previously, little attention has been paid to the ability of chemicals that are able to break the dormancy of soil-stored seeds, resulting in a flush of seedling emergence at one predetermined time. Control of *Avena fatua* then could be completed by the application of a conventional herbicide or other treatment.

The reports of dormancy breakage and/or stimulation of germination by C_2H_4 (1, 2, 6, 7, 19, 20) directed us to look at the effect of this gas and the commercially available C_2H_4 producing compound Ethrel (2-chloroethylphosphonic acid) upon seed of *A. fatua* L.

Little is known about the effects of these compounds upon the germination of wild oat seed. One study reports (4) that Ethrel was ineffective in increasing germination of one batch of dormant seed imbibed at 23 C, but no other temperatures or seed samples at different stages of after-ripening were investigated. In a second report (23), Ethrel partially suppressed the post-harvest dormancy of wild oat caryopses, increasing germination from 20% as in the control to over 40% in the most effective treatment.

Since many species produce C_2H_4 during the germination process (11, 16, 19), several reports have suggested the possibility that production of C_2H_4 during early imbibition may contribute to the breaking of dormancy (9, 19). Takayanagi and Harrington (17) considered that C_2H_4 production in rape seeds acted as a stimulant for germination and embryo growth. Van Staden *et al.* (21) also suggested that C_2H_4 production which increased prior to germination in seed of *Spergula arvensis* caused cytokinins to increase to a level allowing germination to take place.

Information concerning the role of hormones in the dormancy mechanism of *A. fatua* seed has been based largely on observations of the effects of exogenously applied phytohormones (13). Recent interest has also been centered around the role of volatile fatty acids (3). At present, very little is known concerning the mechanism by which endogenous C_2H_4 acts in dormancy breakage and early germination of this species.

Here, evidence is presented which suggests that C_2H_4 is an important natural hormone involved in the breaking of one stage in the complex and serial dormancy mechanisms of *A. fatua* seed. The importance of its role can be seen to change with duration of after-ripening and imbibition temperature. The study suggests that soil application of Ethrel at specific times of the year could result in a moderate increase in germination of the soil stored seeds; such an application could be useful in wild oat control.

MATERIALS AND METHODS

Seed Storage and Germination Tests. *A. fatua* L. seeds were obtained from plants grown as a selected strain at the Weed Research Organization, Oxfordshire. Owing to the original site of collection, this strain will be known as Begbroke 1978. After shedding naturally, the collected seed was divided into two lots and stored dry in the dark at 5 or 25 C for periods of up to 18 months. For routine germination tests, four batches of 50 seeds were individually imbibed with the appropriate solution in glass vials (5.1 × 0.127 cm) to prevent cross-stimulation and/or infection spread. In all tests, darkened incubators set at 15 C were used to prevent any complicating interaction with light; 15 C had been previously found to be the optimum temperature for germination in this strain (unpublished data). Counts of visibly protruding radicles were taken daily for a period of 15 days. At this time, the remaining seed was dehusked and incubated in a 0.1 mM GA_3 solution and a final count of germination was made 10 days later; this final value is taken to be an estimation of the total viability of the seed stock.

Chemical Treatments. Ethylene gas was obtained from a standard calibration gas mixture containing 1.05% of the gas in N_2. To test the effect of this gas upon the seed stocks, and to follow gaseous evolution during imbibition, germination tests of 20 seeds were carried out in 50-ml flasks, each fitted with a gas-tight subaseal stopper through which gas samples could either be injected or removed for analysis. The flask volume was considered to be large enough to dilute any possible CO_2 concentration effects similar to those previously observed (6, 10). Prior to imbibition, the seeds

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were given a 30-s wash in 1% mercuric chloride followed by 10 washes in distilled H_2O before blotting dry. This treatment removed surface microorganisms which had previously been shown to produce low levels of C_2H_4 (unpublished data). The growth regulator Ethrel was used as another source of C_2H_4 (22). Germination tests were carried out as described above. The pH of solutions in the controls was adjusted with HCl or NaOH to match those of the appropriate Ethrel treatment.

GLC Analysis. Analysis of gas samples was carried out in a Pye 204 GLC fitted with a flame ionization detector. The glass column (1 m × 6 mm), was packed with Porapak R (80–100 mesh). The chosen carrier gas (N_2) flow rate was 10 cm^3 min^{-1}. The column inlet port and detectors were kept at 60 C. Standard C_2H_4 peaks and retention times were obtained using a dilution series made from gas samples taken from the standard gas mixture of 1.05% C_2H_4 in N_2. At the conclusion of the experiment to test the effect of C_2H_4 upon germination, samples were taken from the flasks and analyzed to establish the presence of the initially added gas. Standard tubes, which showed that the apparatus did not release C_2H_4 during the experiment and that injected C_2H_4 was not adsorbed, were also set up.

Soil-stored Seeds. To ascertain whether a natural increase in the capacity to produce C_2H_4 could coincide with the loss of dormancy in soil-stored seed, the following experiment was designed. Dormant deawned primary seeds (proximal position in the spikelet) in lots of 400 were sown 3 cm below the surface and in a 3-cm band in 15-cm pots containing John Innes No. 1 potting compost. The soil moisture content was adjusted to 20% with the addition of distilled H_2O to simulate an average field situation; this level of moisture was sufficiently large to prevent any fluctuation in seed moisture content, thus eliminating any problems that drying out of the seed could cause. The pots then were stored in darkened growth chambers at either 5 or 25 C for periods up to 7 months. At regular intervals, three replicates were removed from each chamber and the seed was recovered, cleaned, and sterilized. The seeds then were reimbibed in 15 C germination tests in either H_2O or Ethrel (100 μg l^{-1}). Samples of seed were also set aside for the measurement of the C_2H_4 evolved after the first 5 h reimbibition at 15 C. Throughout the period of the experiment, the soil moisture was kept at a value close to 20% using a weighing technique; at each check, a count of seedling emergence was made.

RESULTS

Seed Germination. An initial investigation revealed that seed after-ripened in dark dry conditions at 5 C maintained complete dormancy, whereas similar stocks kept at 25 C lost their dormancy slowly over the 18-month period studied (Fig. 1). Continued incubation with the caryopses alone in GA_3 solutions revealed that the viability of stocks remained over 95% during the period of storage at 5 and 25 C.

Seeds from various stages of after-ripening at 25 C were imbibed with a range of Ethrel concentrations (Fig. 2). Partly after-ripened seed samples taken at 12 months were the most sensitive to all concentrations tried. Seed from this storage period was used to investigate the effect of incubation temperature on the extent of stimulation by C_2H_4. The optimum germination temperature for the control stock was 15 C; C_2H_4 was also shown to be most efficient at increasing germination at this temperature (Fig. 3).

C_2H_4 Evolution. Inasmuch as certain seed batches imbibed under optimum temperature conditions show a marked stimulation of germination by C_2H_4, the questions arise whether natural metabolic C_2H_4 is produced by these seeds, and is this involved in the release for dormancy? To investigate this, seed that had been dry after-ripened for 18 months at 5 C (dormant) or 25 C (nondormant) were used in an experiment to measure the rate of C_2H_4 evolution during imbibition. Special interest was directed to the very early stages of imbibition because it could be the C_2H_4 produced at this time which might have an effect in overcoming a dormancy block. In the case of the dry after-ripened seed (nondormant), a large peak in C_2H_4 evolution can be detected during the first few hours of imbibition which compares with a very low emission from the dormant seed (Fig. 4). A second smaller peak occurred prior to radicle emergence only in the nondormant seed. The percentage of the two seed stocks that had germinated during the 130 h studied is shown in Figure 4a.

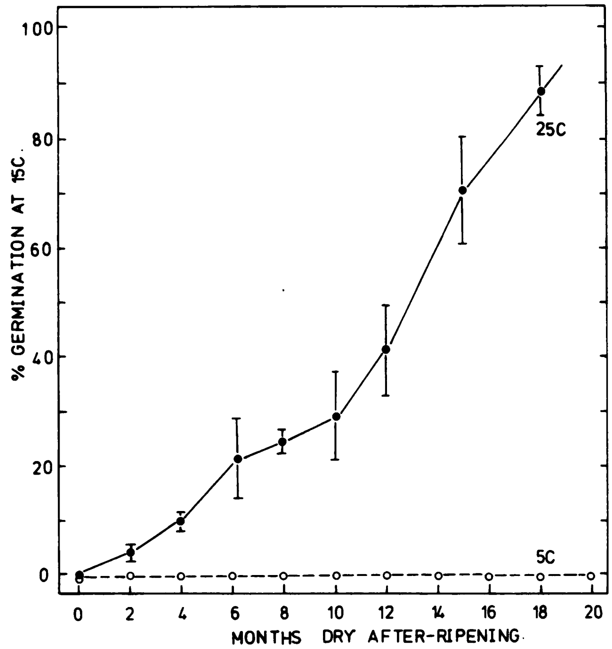

FIG. 1. The change in percentage germination of seed imbibed for 15 days at 15 C in darkened incubators, following periods of dry storage at 5 C (○) and 25 C (●). Vertical lines represent ± SE.

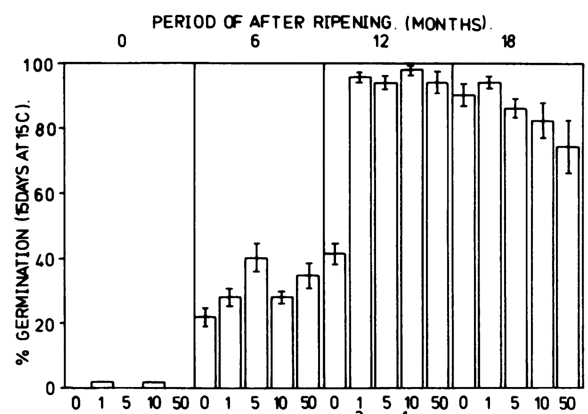

FIG. 2. The effect of Ethrel concentration upon germination measured after 15 days at 15 C dark incubation. The seed used had been previously after-ripened dry at 25 C for the periods stated. Vertical lines represent ± SE.

Dormancy Breakage and C_2H_4 Evolution. To establish whether the C_2H_4 evolved by the nondormant seed was related to the removal of a dormancy block in a field situation, the rate of C_2H_4 evolution was measured from an initially dormant stock of seeds that was placed under environmental conditions that would slowly remove dormancy. Figure 5 shows that moist incubation in soil at 25 C has an effect on reducing dormancy similar to, but more rapid than, that of warm dry after-ripening; seed stored under soil at 5 C maintained dormancy. C_2H_4 evolution during early imbibition at 15 C, from seed taken at various times from these two

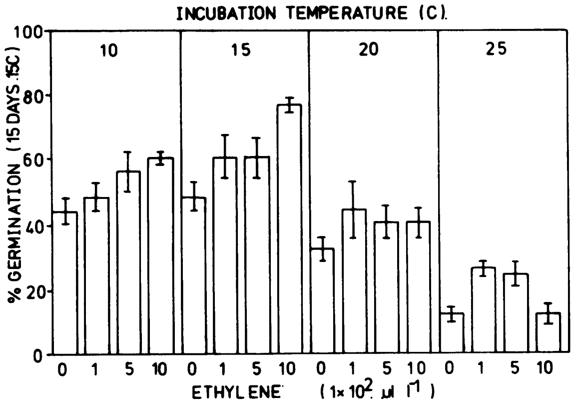

FIG. 3. The effect of C_2H_4 concentration upon germination of seed that had been dry after-ripened at 25 C for 12 months. The percentage germination was measured after 15 day dark incubation at the temperatures stated. Vertical lines represent ± SE.

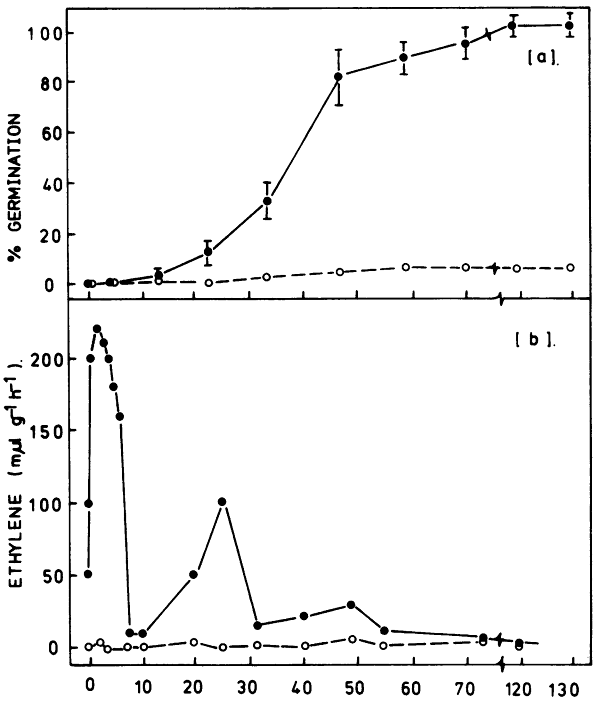

FIG. 4. a, the time course of germination for the seed stocks shown in b. b, the time course of C_2H_4 evolution for imbibed seeds, measured at 15 C. The seed had been previously dry after-ripened for 18 months at either 25 C (nondormant) (●) or 5 C (dormant) (○).

storage treatments, indicate that there was no direct correlation between the C_2H_4 production and the change in the capacity of the seed to germinate when placed under optimum conditions (15 C). Seedling counts taken from the pots during the two storages revealed that very little germination occurred at 5 or 25 C.

The effect of Ethrel (100 $\mu g\ l^{-1}$) was tested on the seed that had been removed from the soil pots at the various time periods (Table I). There was no effect of Ethrel treatment on dormant seed stored at 5 C but small effects became evident after 6 months storage and significantly more seed germinated after 7 months storage at 25 C when Ethrel was added. This sample was already capable of about 50% germination.

DISCUSSION

Previous evidence (13) has shown that the control of dormancy by phytohormones in wild oat seed is very complex. The investigation reported here has identified C_2H_4 as another important component of this system and interesting effects have been observed under certain temperature regimes and with populations of seeds at different stages of after-ripening.

Tests on seed after-ripened in dry storage for various periods reveals that Ethrel can overcome dormancy and is most effective on seeds that have been stored at 25 C for a period of 12 months

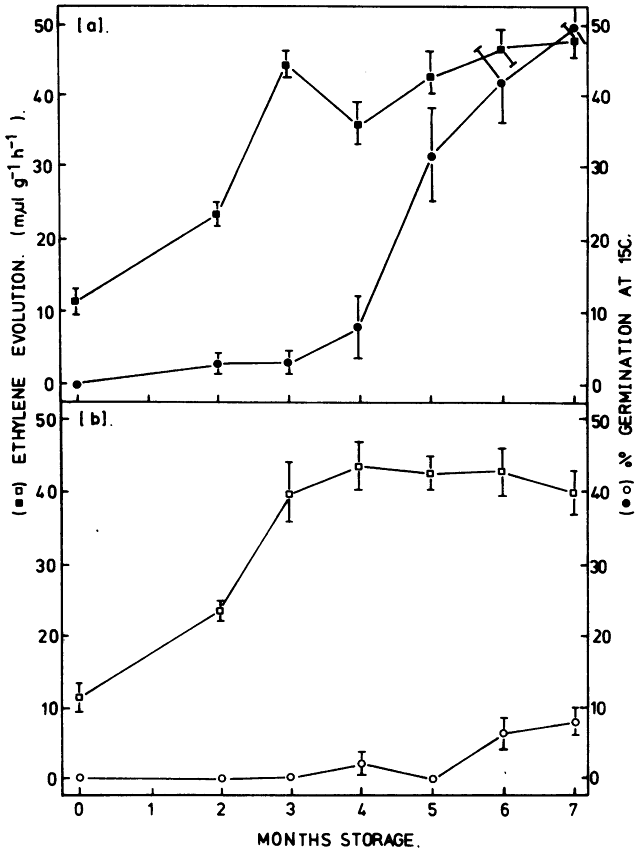

FIG. 5. a, 15 C, 15-day germination percentages for seed after-ripened at 25 C in moist soil for the lengths of times stated (●) and the rate of C_2H_4 evolution occurring during the first 5 h of reimbibition of the seeds at 15 C that had been after-ripened for various times at 25 C in moist soil (■). b, the germination percentages (○) and C_2H_4 evolution (□) for similar tests carried out on seed imbibed at 5 C. Vertical lines represent ± SE.

Table I. *Percentage Germination of A. fatua Seed*

The percentage of germination was measured after 15 days at 15 C imbibition in Ethrel and water. The seed had previously been kept in pots of moist soil at either 5 or 25 C for periods of up to 7 months. Viability of all seed samples tested was over 95%. All results are shown with ± SE.

Period of Soil Storage	Germination at Following Seed Storage Temperatures			
	5 C		25 C	
	Water	Ethrel (100 µg l⁻¹)	Water	Ethrel (100 µg l⁻¹)
months	%			
0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
2	0 ± 0.0	0 ± 0.0	3 ± 1.7	1 ± 1.0
3	0 ± 0.0	0 ± 0.0	3 ± 1.7	2 ± 1.0
4	2 ± 1.7	0 ± 0.0	8 ± 4.4	6 ± 1.7
5	0 ± 0.0	0 ± 0.0	32 ± 6.4	40 ± 4.0
6	7 ± 2.0	0 ± 0.0	42 ± 6.0	48 ± 2.0
7	8 ± 2.0	0 ± 0.0	48 ± 6.2	62 ± 2.0

or so. The compound possibly has an inhibitory effect on completely nondormant seed but little or no effect upon deeply dormant seed. Similar results have been previously demonstrated for C_2H_4 on completely dormant and partly dormant lettuce (1) and mature and immature peanut seeds (19).

Further results with C_2H_4 gas indicate that the stimulation effect noted with the partly dormant seed was most effective at cooler temperatures (10 and 15 C) and least effective at warmer ones (20 and 25 C). If temperature is critical to the action of C_2H_4, as these results suggest, then this may explain why a previous study (4) reported Ethrel to have no effect in breaking wild oat dormancy. The possibility that certain incubation temperatures would stimulate additional endogenous C_2H_4 production which could modify the effect of the exogenous C_2H_4 or Ethrel was not investigated.

These results indicate that C_2H_4 may be a more effective dormancy breaking agent than previous reports suggest (12, 18). The inability of earlier tests to demonstrate the effectiveness of C_2H_4 may be due to the use of unsuitable temperatures or, more probably, to the stage of after-ripening of the seeds at the time of the test. The results also show why some species are shown to be inhibited from germinating by C_2H_4 (4); if the applied dose is too high or the seeds are at an advanced state of after-ripening, then an inhibition similar to that observed here may result.

Several reports have suggested that natural production of C_2H_4 in imbibed seeds may contribute to the breaking of dormancy. The investigation here has shown that nondormant seed dry after-ripened at 25 C produced a small amount of metabolic C_2H_4 when still dry; however, upon imbibition production increased 5-fold before dropping to a low level after about 8 h. A similar response could not be followed with the dormant seed of the same age. A second peak in production was noted after 24-h imbibition, coinciding with the beginnings of visible radicle protrusion. Production rate after 40 h possibly decreased due to the build-up of C_2H_4 in the chamber having a feedback effect upon production. Over the 6-day period, evolution from dormant seed remained very low.

The germination data (Fig. 4a) show that the first period of high C_2H_4 production noted in the nondormant seeds occurred before any visible germination processes were initiated. However, it was clear from a second experiment (Fig. 5) that, when the dormancy of a population of seeds had naturally declined by warm, moist soil storage, the germination capacity of these seed, when placed at 15 C, increased irrespective of C_2H_4 production. Seed kept at cold temperatures which maintained dormancy had a pattern of C_2H_4 evolution similar to that of the nondormant seed. It is concluded that this early period of C_2H_4 production noted during the imbibition of the dry after-ripened seed is not related to dormancy breakage.

We suggest that, in young, freshly shed seed, germination is prevented by one or several dormancy-inducing systems, the sum of which cannot be overcome by natural or exogenous C_2H_4. As the seed ages under conditions that are well above 5 C, the effect of exogenous C_2H_4 or Ethrel (Table I) becomes manifest because a limiting system has been reduced or removed. Naturally produced C_2H_4 may now have a decisive effect, tilting the balance of dormancy-inducing systems and germination-promoting systems in favor of germination. The possibility that there is an interaction with other natural promotors is advocated and the existence of a possible interaction with endogenous gibberellin is at present under investigation.

Since C_2H_4 is a natural component of the gaseous environment of soils (14) and has been shown to reach physiologically active levels (15), it is of interest to consider whether this C_2H_4 coupled with endogenous seed C_2H_4 may be involved in the breaking of dormancy of aging seeds in a field situation.

So far, very little attention has been paid to the possibilities of wild oat control involving the depletion of the soil seed reservoir by chemicals that can either break dormancy or bypass it. The problems of applying C_2H_4 to soils are self-evident and, in an effort to avoid these, the study presented here has looked at the effect of a widely available C_2H_4-producing compound, Ethrel. From the initial results obtained, it could be tentatively speculated that soil applications of Ethrel would stimulate the germination of some wild oat seeds present in soils. Second, from counts taken on root abnormalities caused by Ethrel treatment, it may be assumed that a large percentage of these stimulated seeds would not give rise to healthy seedlings surviving the period of early competition on emergence. It is therefore proposed that the application of Ethrel to infested soils would not only cause germination of some seeds but would ultimately lead to the death of a large number of these. A similar dual effect has been previously described for C_2H_4 (5). It may also be assumed that this treatment would be most effective on seed that had been present in the soil for some time and least effective on newly shed seed. It would also be most effective when soil temperatures are around 15 C or 10 C, which characterize the spring and autumn, and not so at the warmer temperatures found in the summer. Soil application at an incorrect level or in the inappropriate season would result in poor wild oat germination (8). The possibility that Ethrel could be used in a commercial way to control wild oat under certain conditions deserves further investigation.

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