Studies in Wild Oat Seed Dormancy

I. THE ROLE OF ETHYLENE IN DORMANCY BREAKAGE AND GERMINATION OF WILD OAT SEEDS (Avena fatua L.)

Received for publication April 4, 1980 and in revised form August 14, 1980

Stephen W. Adkins¹ and James D. Ross

Department of Botany, University of Reading, Whiteknights, Reading, RG2 6AS, United Kingdom

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 C₂H₄ (1, 2, 6, 7, 19, 20) directed us to look at the effect of this gas and the commercially available C₂H₄ 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 C₂H₄ during the germination process (11, 16, 19), several reports have suggested the possibility that production of C₂H₄ during early imbibition may contribute to the breaking of dormancy (9, 19). Takayanagi and Harrington (17) considered that C₂H₄ production in rape seeds acted as a stimulant for germination and embryo growth. Van Staden et al. (21) also suggested that C₂H₄ 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 C₂H₄ acts in dormancy breakage and early germination of this species. Here, evidence is presented which suggests that C₂H₄ 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 x 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 GA₃ 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 N₂. 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 sub-seal 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 CO₂ concentration effects similar to those previously observed (6, 10). Prior to imbibition, the seeds

¹ This work was supported by a Science Research Council-Cooperative Awards in Science and Engineering studentship (to S. W. A.) in cooperation with the Weed Research Organization, Oxford, United Kingdom.
were given a 30-s wash in 1% mercuric chloride followed by 10 washes in distilled H2O before blotting dry. This treatment removed surface microorganisms which had previously been shown to produce low levels of C2H4 (unpublished data). The growth regulator Ethrel was used as another source of C2H4 (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 x 6 mm), was packed with Porapak R (80-100 mesh). The chosen carrier gas (N2) flow rate was 10 cm³ min⁻¹. The column inlet port and detectors were kept at 60°C. Standard C2H4 peaks and retention times were obtained using a dilution series made from gas samples taken from the standard gas mixture of 1.05% C2H4 in N2. At the conclusion of the experiment to test the effect of C2H4 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 C2H4 during the experiment and that injected C2H4 was not adsorbed, were also set up.

Soil-stored Seeds. To ascertain whether a natural increase in the capacity to produce C2H4 could coincide with the loss of dormancy in soil-stored seed, the following experiment was designed. Dormant dewaqued 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 H2O 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 H2O or Ethrel (100 µg g⁻¹). Samples of seed were also set aside for the measurement of the C2H4 evolved after the first 5 h reimmersion 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 GA3 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 C2H4. The optimum germination temperature for the control stock was 15°C; C2H4 was also shown to be most efficient at increasing germination at this temperature (Fig. 3).

C2H4 Evolution. Inasmuch as certain seed batches imbibed under optimum temperature conditions show a marked stimulation of germination by C2H4, the questions arise whether natural metabolic C2H4 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 (non-dormant) were used in an experiment to measure the rate of C2H4 evolution during imbibition. Special interest was directed to the very early stages of imbibition because it could be the C2H4 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 C2H4 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.

Dormancy Breakage and C2H4 Evolution. To establish whether the C2H4 evolved by the nondormant seed was related to the removal of a dormancy block in a field situation, the rate of C2H4 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. C2H4 evolution during early imbibition at 15°C, from seed taken at various times from these two
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 \( \mu g \) 1\(^{-1} \)) 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.
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 C₂H₄ on completely dormant and partly dormant lettuce (1) and mature and immature peanut seeds (19).

Further results with C₂H₄ 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 C₂H₄, 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 C₂H₄ production which could modify the effect of the exogenous C₂H₄ or Ethrel was not investigated.

These results indicate that C₂H₄ may be a more effective dormancy breaking agent than previous reports suggest (12, 18). The inability of earlier tests to demonstrate the effectiveness of C₂H₄ 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 C₂H₄ (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 C₂H₄ 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 C₂H₄ 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 buildup of C₂H₄ 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 C₂H₄ 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 C₂H₄ production. Seed kept at cold temperatures which maintained dormancy had a pattern of C₂H₄ evolution similar to that of the nondormant seed. It is concluded that this early period of C₂H₄ production noted during the imbibition of the dry after-ripened seed is not related to dormancy breakdown.

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 C₂H₄. As the seed ages under conditions that are well above 5°C, the effect of exogenous C₂H₄ or Ethrel (Table I) becomes manifest because a limiting system has been reduced or removed. Naturally produced C₂H₄ 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 C₂H₄ 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 C₂H₄ coupled with endogenous seed C₂H₄ 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 C₂H₄ 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 C₂H₄-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 C₂H₄ (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.

Acknowledgments—We would like to thank Mr. R. J. Chancellor and Dr. N. C. B. Peters, of the Weed Research Organization, Oxford, for collaborating on some of the experiments.

LITERATURE CITED
2. BALLS AK, WS HALE 1940 The effect of ethylene on freshly harvested wheat. Cereal Chem 17: 490-494
3. BERRIE AMM 1979 Possible role of volatile fatty acids and abscisic acid in the dormancy of oats. Plant Physiol 63: 758-764
4. CHANCELLOR RJ, C PARKER, T TEPPERDSON 1979 Stimulation of dormant weed seed germination by 2-chloroethylphosphonic acid. Pestic Sci 2: 35
8. FAY PR 1973 The effect of ethylene stimulators on wild oat (Avena fatua L.) emergence in the field. Proc North Cent Weed Conf 30: 110
12. OLATSOY ST, MA HALL 1972 Interactions of ethylene and light on dormant weed seeds. Proc Easter Sch Agric Sci 19: 2
19. TOOLE VK, WK BAILEY, EH TOOLE 1964 Factors influencing dormancy of
peanut seed. Plant Physiol 39: 822-832