Plant Physiol. (1972) *50*, 293–297

Volatile Metabolites Controlling Germination in Buried Weed Seeds

Received for publication February 9, 1972

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ABSTRACT

Velvetleaf (*Abutilon theophrasti* Medic), morning glory (*Ipomoea purpurea* [L.] Roth), and wild mustard (*Brassica kaber* [D.C.] L. C. Wheeler) seeds exhibited decreased germination with increased planting depth in soil. Flushing the soil for 2 minutes each day with air overcame the inhibition. A sealed *in vitro* system was used to sample the volatile components produced by weed seeds. Inhibition of seed germination was accompanied by decreased O_2 levels and production of volatile metabolites identified as acetaldehyde, ethanol, and acetone. The effectiveness of these compounds in reducing germination was dependent on O_2 levels.

Estimates of large populations of viable buried weed seeds which cause weediness of arable land following cultivation range as high as 158 million seeds per acre (2). Germination of these buried weed seeds occurs when the soil containing the seed is disturbed (9, 13, 14) and causes a continuing problem for agriculturists. The germination of many buried weed seeds is dependent on exposure to light (9, 13, 14), specifically red light involving the phytochrome system (4, 5, 12).

Some species of freshly harvested weed seeds, however, do not require light for germination and others are inhibited by light (5, 15). After burial, the seeds require light for germination. Therefore, an apparent change in light sensitivity takes place while the seeds are buried. Wesson and Wareing (15) conclude that buried seeds produce a gaseous substance which inhibits their germination. Freshly harvested weed seeds buried in the soil germinate much less than when germinated on filter paper in the dark. After several weeks of soil burial, germination becomes more dependent on light. Germination of the buried seeds is similar to that of unburied seeds if compressed air is forced through the soil. Other experiments rule out CO_2 as causing the inhibition and suggest that the volatile inhibitor(s) is produced by the seed and not by microbial action (15).

This investigation was undertaken to identify the volatile inhibitor(s) produced by weed seeds under conditions where freshly harvested seeds develop a light requirement for germination during burial.

MATERIALS AND METHODS

Tall morning glory (*Ipomoea purpurea* [L.] Roth), velvetleaf (*Abutilon theophrasti* Medic), and wild mustard (*Brassica kaber* [D.C.] L. C. Wheeler) seeds were obtained from Roy Swanson, Thorp, Wisconsin. Seeds were cleaned, dried to a 6 to 8% moisture content, and stored at 3 C in 4- × 15-cm metal tubes fitted with 1-cm screw caps.

Weed seeds were planted in greenhouse potting soil at a depth of 7.5 cm in 20 cm diameter foam pots in a 6 mm layer of quartz sand to facilitate later location of the seeds. The pots were placed outdoors in plastic trays containing 5 cm of soil in February and were exposed to the winter, spring, and summer climate of northern Ohio. Seedlings that did emerge were removed by cutting as soon as they penetrated the soil surface.

After 6 months burial, the seeds were dug up in a dark room illuminated with light from green fluorescent tubes filtered through 3-mm green Plexiglas (Rohm and Haas No. 2092) and 3-mm amber Plexiglas (Rohm and Haas No. 2451). Light-sensitive lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds did not germinate under this lighting, which has no transmission below 540 nm or above 585 nm (10). Only the ungerminated seeds were used for experiments. The unearthed seeds were transferred to 100-mm plastic Petri dishes containing 1% water agar. The seeds were germinated for 2 weeks at 23 C in light supplied by a mixture of fluorescent and incandescent light (1400 ft-c) or in the dark at the same temperature.

Laboratory seed burial experiments were run in 70- × 140-cm plastic bottles that had the base removed. The screw cap ends of the bottles were plugged with rubber stoppers fitted with glass tubes and vaccine caps. The bottles were held by clamps with the plugged end down. Cotton was placed over the glass tubes to prevent clogging by soil. Gravel (2.5 cm) was placed over the cotton followed by 3 cm of greenhouse potting soil (screened through a No. 8 mesh sieve). This was followed by 1 mm of white quartz sand, 50 or 100 weed seeds, 1 mm of sand, 2.5, 3.75, or 5 cm of soil. The soil core below and above the seeds was formed by adding 50 g of soil, packing it with a wood disc, moistening it with distilled water, and packing it further by pulling air through the bottle by vacuum. Some bottles had no treatment during the 2-week period (controls). Others had air or N_2 (supplied from a cylinder through a funnel inverted over the bottles) pulled through the soil core by vacuum for 2 min each day. Any water lost from the soil (1–2 ml) was trapped in a filter flask and added back to the soil.

In some experiments, the identified seed volatiles were added to the soil flush. The chemicals were added to 5-liter flasks sealed with rubber corks fitted with glass tubes and vaccine caps. Appropriate amounts of chemicals were added to give 0.1 μl/l acetaldehyde and acetone and 1 μl/l ethanol in the vapor phase (checked by gas chromatography). The glass tube on the flask was then coupled to a funnel inverted over the plastic bottle. A vacuum line was connected to the glass tube on the plugged end of the bottle and two soil volumes (about 2 liters) of volatiles were passed through the soil core. This was done daily during the 2-week burial period.

After this period in the soil core burial experiments, the soil was removed down to the white quartz sand layer and germination (as evidenced by radicle emergence) was recorded. In some experiments the ungerminated seeds were removed under green safelight and transferred to 1% water agar in Petri dishes and placed in the light or dark at 23 C for an additional 2 weeks.

Other germination experiments were carried out in 100-ml beakers containing 30 ml of 1% water agar. Vials (2 × 4 mm) containing filter paper strips were inserted into the agar and enough chemical was added to the filter paper to give 0.01, 0.1, or 1.0 μl/l in the gas phase. Fifty seeds were placed on the agar surface, and the beakers were sealed with Parafilm. The beakers were placed in the dark for 2 weeks at 23 C after which germination was recorded.

Volatile production by weed seeds was observed in 6- × 15-cm bottles containing 55 ml of sterilized white quartz sand which was moistened with distilled water. No bacterial or fungal growth was observed on the seeds. Several experiments were repeated with seeds surface sterilized by rinsing in sodium hypochlorite and yielded the same results. Twenty-five or 50 seeds were placed on the sand surface, and the bottles were sealed with rubber vaccine caps. Some bottles contained 2- × 4-mm vials with filter paper strips soaked in 10% KOH to absorb CO_2. The sealed bottles were placed in the dark for 2 weeks at 23 C before germination was recorded. In some experiments, air samples for gas chromatography were withdrawn from above the seeds by a syringe via a needle inserted through the vaccine cap.

Weed seed volatiles were separated using a Victoreen gas chromatograph on a 0.3- × 1829-cm column packed with Porapak Q. Gas flows were as follows: helium, 35 ml/min; hydrogen, 25 ml/min; and air, 60 ml/min. The column temperature was 150 C, and the injection port temperature was 280 C. The flame ionization detector was used to observe the volatiles which were identified by retention times and co-chromatography with standards. Oxygen was determined using the same column and thermal conductivity detection.

All experiments were run three or more times with three replicates of 50 or 100 seeds per replicate. The results given are the average ± SD from these experiments.

Table I. *Germination Responses of Freshly Harvested and Buried Weed Seeds in the Light and Dark*

Freshly harvested seeds were germinated in Petri dishes at 23 C in the light and dark. Other seeds from the same lot that had been buried in the soil for 6 months at 7.5 cm depth and dug up were germinated in Petri dishes under the same conditions.

Weed Seed	Freshly Harvested Seed		Buried Seed[1]	
	Light	Dark	Light	Dark
	% germination			
Morning glory	34 ± 2[2]	30 ± 3	56 ± 4	6 ± 2
Velvetleaf	36 ± 5	43 ± 4	22 ± 3	0
Wild mustard	29 ± 2	28 ± 1	20 ± 2	0

[1] Values based on number of seeds recovered, not on the initial buried number of seeds.

[2] Values are means ± SD from three experiments each with three replicates of 100 seeds per treatment.

RESULTS AND DISCUSSION

Freshly harvested morning glory, velvetleaf, and wild mustard seeds germinated equally well in the light or dark (Table I). However, after burial in soil outdoors for 6 months, seeds required light for germination. Morning glory germinated at a low rate in the dark, whereas velvetleaf and wild mustard showed no dark germination. The seeds changed their sensitivity toward light during burial as indicated by the light requirement for germination. Similar results were obtained by Wesson and Wareing (15) for seeds buried at 4 cm depth for 50 weeks, after which the soil was disturbed under controlled illumination. In three species (field poppy [*Papaver dubium* L.], buckhorn plantain [*Plantago lanceolata* L.], and corn spurry [*Spergula arvensis* L.]) germination was unaffected by light before burial. After burial, germination took place only in the light.

Freshly harvested weed seeds could be made light-requiring by burial in soil cores under laboratory conditions (Table II). Inhibition of germination was dependent on the depth of burial and seed size. The smaller wild mustard seeds (1.85 mg/seed) were inhibited at a shallower depth than the larger velvetleaf (8.93 mg/seed) and morning glory (20.95 mg/seed). Several investigators have shown poorer weed seed germination at greater planting depths (3, 6). Flushing the soil with air for 2 min per day overcame much of the inhibition due to burial and indicated possible soil oxygen depletion and/or removal of volatile inhibitor(s) or CO_2 in the soil. Nitrogen flushing provided some relief from the inhibition caused by burial.

Table II. *Alteration of Weed Seed Germination at Different Soil Depths with Air or Nitrogen Flushings*

Freshly harvested seeds were germinated on 1% water agar in the dark (Petri dish controls). Other seeds were buried in soil cores prepared in plastic bottles. The soil was left undisturbed (control) or flushed for 2 min per day with air or N_2. Germination was recorded after 2 weeks. Those ungerminated control seeds at the greatest burial depth were transferred to Petri dishes and germinated for an additional 2 weeks in the light or dark.

Seed	Dark Petri Dish Control	Burial Depth							Control Seeds After Burial[1]	
		2.5 cm		3.75 cm		5.0 cm				
		Control	Air	Control	Air	Control	Air	N_2	Light	Dark
		% germination								
Morning glory	32 ± 3[2]	33 ± 2	33 ± 3	28 ± 3	33 ± 2	10 ± 2	28 ± 3	15 ± 1	18 ± 2	2 ± 1
Velvetleaf	43 ± 4	45 ± 4	45 ± 5	23 ± 2	43 ± 5	10 ± 1	43 ± 4	21 ± 1	20 ± 3	0
Wild mustard	28 ± 1	5 ± 1	25 ± 2	0	20 ± 2	—	—	—	15 ± 2	3 ± 1

[1] Values are based on percentage of the initial number of seeds. Morning glory and velvetleaf seeds were recovered from 5.0 cm, wild mustard from 3.75 cm.

[2] Values are means ± SD from three experiments each with three replicates of 50 seeds per treatment.

Table III. *Sealing of Weed Seeds in Bottles and Subsequent Germination in the Light and Dark*

Seeds were sealed in bottles containing moistened sterilized quartz sand kept in the dark. Some bottles had vials containing 10% KOH and filter paper to absorb CO_2. After 2 weeks, the germination was recorded, and the ungerminated control seeds were placed in Petri dishes and germinated in the light or dark for an additional 2 weeks.

Seed	Petri Dish Control		Sealed Bottle Dark		Ungerminated Control Seeds After Sealed Bottle Treatment	
	Light	Dark	Control	KOH	Light	Dark
	% germination					
Morning glory	32 ± 2[1]	28 ± 2	8 ± 1	9 ± 2	12 ± 2	4 ± 1
Velvetleaf	36 ± 5	42 ± 4	5 ± 2	8 ± 2	30 ± 3	8 ± 1
Wild mustard	29 ± 2	28 ± 1	8 ± 2	7 ± 1	16 ± 1	0

[1] Values are means ± SD from three experiments each with three replicates of 50 or 100 seeds per treatment.

Table IV. *Measurement of Oxygen Levels and Volatile Production in Sealed Bottles Containing Weed Seeds*

Seeds were sealed in bottles prepared as described in Table III. Oxygen levels and production of volatile components were monitored daily by gas chromatography.

Seed	Sampling Day							
	1		2		4		6	
	O_2	Volatile	O_2	Volatile	O_2	Volatile	O_2	Volatile
	%	unit[1]	%	unit	%	unit	%	unit
Morning glory	16 ± 2[2]	1 ± 0.1	10 ± 1	3 ± 1	5 ± 1	110 ± 5	2 ± 0.5	182 ± 8
Velvetleaf	12 ± 1	1 ± 0.2	6 ± 1	49 ± 3	3 ± 1	125 ± 10	2 ± 0.5	132 ± 7

[1] Chart units of major volatile.

[2] Values are means ± SD from three experiments each with three replicates of 50 seeds per treatment.

Wesson and Wareing (15) ruled out O_2 depletion as an inhibitory factor by showing that flushing with N_2 aeration for 30 min per day gave similar results as continuous aeration. It may be difficult to make a direct comparison of results because Wesson and Wareing (15) had to use high soil moisture levels (80% and above) to get inhibition by soil burial and had to bubble the gases through the soil. I found this could not be done with tightly packed soil cores so the gases were drawn through the soil by vacuum. This allowed for uniform gas exchange in the soil and kept soil moisture levels between 30 and 40%. Ungerminated seeds removed under green safelight from the control soil cores at the greatest depth (3.75 cm for wild mustard and 5.0 cm for velvetleaf and morning glory) required light for germination. This indicated that light-requiring seeds could be produced in the laboratory as they were outdoors by burial.

To sample weed seed volatiles conveniently, seeds were placed on sand in sealed bottles, some of which contained KOH to trap CO_2. Sealing the seeds in bottles inhibited their germination as compared to Petri dish controls (Table III). The bottles containing KOH did not have higher germination, indicating that CO_2 build-up was not the cause of the inhibition. Wesson and Wareing (15) also ruled out CO_2 build-up as causing the inhibition by showing that 12.5% CO_2 added to periodic flushing with N_2 caused no inhibition of germination. Removal of ungerminated control seeds under green safelight and transfer to germination conditions in the light and dark showed that the seeds required light for germination as did those retrieved from laboratory (Table II) and outdoor (Table I) soil.

The gas phase above the seeds sealed in the bottles was analyzed by gas chromatography (Table IV). Production of large amounts of volatile components occurred only after O_2 levels had declined. After 4 days, the O_2 level had dropped by over 75% and volatile production had increased over a 100-fold.

Table V. *Germination of Morning Glory Seeds Exposed to Volatiles in the Vapor Phase*

Seeds were placed in 100-ml beakers containing 30 ml of 1% water agar and 2- × 4-mm vials containing strips of filter paper. The chemicals were applied to the filter paper in amounts sufficient to obtain the desired concentration in the vapor phase. The beakers were sealed with Parafilm and placed in the dark for 2 weeks.

Volatile Added	Amount of Volatile Added per Beaker (μl/l)			
	0	0.010	0.10	1.0
	% germination			
Ethanol	40 ± 3[1]	40 ± 2	32 ± 2	26 ± 3
Acetone	40 ± 3	34 ± 4	30 ± 1	25 ± 2
Acetaldehyde	40 ± 3	30 ± 2	22 ± 2	20 ± 1
Combination[2]	40 ± 3	28 ± 2	20 ± 1	18 ± 1

[1] Values are means ± SD from three experiments each with three replicates of 50 seeds per treatment.

[2] Ethanol, acetaldehyde, and acetone at the concentration indicated.

Since the seeds were changing from aerobic to anaerobic metabolism during sealing, the three volatile components detected were compared with a number of known anaerobic metabolites and were identified as acetaldehyde, ethanol, and acetone. After 6 days, ethanol was present in the highest concentration (0.1 ± 0.02 μl/l in wild mustard and 1.0 ± 0.1 μl/l in morning glory), while acetaldehyde and acetone were

Table VI. *Effect of Soil Flushing and Volatile Application to Weed Seeds Buried in Soil*

Seeds were buried in soil at 2 depths in plastic bottles for 2 weeks. The soil was not disturbed (control) or it was flushed for 2 min per day with air or air containing the volatile inhibitors (0.1 μl/l acetone and acetaldehyde and 1 μl/l ethanol).

Seed	Dark Control	Shallow Soil[1]			Deep Soil[2]		
		Control	Air	Volatile	Control	Air	Volatile
	% germination						
Morning glory	34 ± 3[3]	30 ± 2	32 ± 3	30 ± 2	5 ± 1	24 ± 2	10 ± 2
Velvetleaf	50 ± 5	48 ± 4	48 ± 5	47 ± 4	6 ± 1	38 ± 3	12 ± 1
Wild mustard	30 ± 2	28 ± 3	30 ± 3	28 ± 2	0	25 ± 4	5 ± 1

[1] For morning glory and velvetleaf, 2.5 cm; for wild mustard, 0.3 cm.
[2] For morning glory and velvetleaf, 6 cm; for wild mustard, 2.5 cm.
[3] Values are means ± SD from three experiments each with three replicates of 50 seeds per treatment.

Table VII. *Effect of Volatiles and Oxygen on Weed Seed Germination in Sealed Bottles*

Weed seeds were sealed in bottles fitted with serum caps as described in Table III. Some bottles were flushed with N_2 and had O_2 added back to give an initial concentration of 2.5%. Other bottles had volatile added, and another set had a combination of 2.5% O_2 and volatile. The bottles were kept in the dark for 2 weeks.

Seed	Petri Dish	Sealed Control	Sealed 2.5% O_2	Sealed Volatile Mixture[1]	Sealed 2.5% O_2 + Volatile Mixture[1]
	% germination				
Morning glory	34 ± 3[2]	12 ± 1	8 ± 2	4 ± 1	0
Wild mustard	30 ± 2	10 ± 2	8 ± 1	6 ± 2	0

[1] One μl/l ethanol + 0.1 μl/l acetone and acetaldehyde.
[2] Values are means ± SD from three experiments each with three replicates of 50 seeds per treatment.

detected at 0.01 ± 0.001 to 0.1 ± 0.02 μl/l, respectively, in both species. Acetaldehyde arises from pyruvate by the action of pyruvate decarboxylase, an enzyme widespread in plants (1). Ethanol arises from acetaldehyde by reduction with NADH and alcohol dehydrogenase (1). I can find no precedent for acetone production by plant tissue in the literature. Acetone can arise from the metabolism of acetoacetate by the action of acetoacetate decarboxylase (7). The volatile inhibitors originated from the metabolism in seeds because experiments carried out with surface-sterilized seeds gave similar results.

The identification of these seed-produced metabolites does not rule out the importance of other compounds which, in these experiments, would not be detected in the atmosphere above the seeds. Sherwin and Simon (11) reported that lactic acid as well as ethanol accumulated in dwarf French bean cotyledons (*Phaseolus vulgaris* var. Belfast New Stringless) when beans were germinated under wet conditions or under N_2. Missen and Wilson (8) used tritiated water to study the metabolism of white mustard (*Sinapis alba*) seeds under anaerobic conditions in water. They found large amounts of tritium in lactic acid which declined on transfer to an oxygen atmosphere. Lactate added to seeds under aerobic conditions inhibited germination, although a relatively high concentration (0.1 M) was used. Thus, lactate could have been formed in weed seeds and contributed to the inhibition of germination observed. Ethylene is known to alter the germination of some seeds. However, in these experiments no ethylene was detected with the chromatographic system used which had a sensitivity of 0.01 μl/l.

All three volatiles added to the vapor phase inhibited the germination of morning glory seeds in beakers (Table V). Acetaldehyde reduced germination more than acetone and ethanol at the three concentrations tested. When added together the volatiles caused the greatest degree of inhibition, but the inhibition was not additive. The reduction observed in the beakers was not as great as that obtained in the soil (Table II) or sealed bottle (Table III) experiments. At each concentration tested, acetaldehyde could account for over 90% of the combined inhibition observed. The oxygen levels in the beakers were checked by gas chromatography and were found to be not less than 18%.

The volatile components were added to the air flush of soil cores containing seeds planted at two depths (Table VI). Seeds planted near the surface were not inhibited by the burial or subsequent exposure to the volatiles. However, when planted in deeper soil, germination of the seeds was reduced by about 80%. This inhibition was largely overcome by a 2 min per day soil flush. Addition of the volatiles to the air flush reimposed most, but not all of the inhibition caused by burial. The inability to reduce germination completely with the volatile flush could have been due to a failure to reduce O_2 levels to what would normally occur at greater soil depths.

To test this possibility, morning glory and wild mustard seeds were sealed in bottles as previously described. However, some bottles were flushed with nitrogen to remove all O_2 before 2.5% O_2 or 2.5% O_2 plus the volatiles were added to the atmosphere above the seeds. Other bottles had the volatiles added in laboratory air. The greatest degree of inhibition was obtained with the initial atmosphere containing the low O_2 and volatiles (Table VII).

Since the volatile compounds were produced only after the seeds had lowered the O_2 levels (Table IV), the low O_2 levels appear necessary not only for volatile production but also for them to exert maximum inhibition. A model for the inhibition of weed seed germination by burial is proposed from these observations. Freshly harvested seeds have some degree of primary dormancy which varies with the species. In this study, only 30 to 40% of the freshly harvested seeds germinated. After burial, the seeds converted from aerobic to anaerobic metabolism. The degree of conversion depends on burial depth, soil moisture, soil type, and various environmental factors. When O_2 depletion in the soil atmosphere surrounding the seeds reaches a low level (about 5%), anaerobic metabolites (acetaldehyde, acetone, and ethanol) inhibitory to seed germination are produced. These factors are involved in conversion from primary to secondary dormancy and the acquisition of a light requirement for germination.

The phytochrome pigment system is involved in initiating

the events leading to germination after light exposure (4, 5, 12). Holm and Miller (5) showed the germination of light-requiring weed seeds could be enhanced in the dark by acetylcholine, GA, and adenosine 3′,5′-monophosphate (cyclic AMP). They proposed that these compounds acted in the order indicated to trigger germination after exposure of seeds to light.

This model for the control of weed seed germination leaves a lot of questions unanswered, but serves to explain a number of previous observations and helps to direct future research efforts.

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