AC-94,377 For Breaking Dormancy of Wild Mustard Seed in Soil

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Annual weed species recur on agricultural land because of their persistent seed reserve or "bank" in the soil (14, 18). This seed bank results, in part, from the unique seed dormancy characteristics of individual weed species. Seed dormancy and persistence vary among different weeds and with location, micro-climate, burial depth, and soil disturbance (18).

One approach to eradicating weeds on farmland might be to speed the loss of weed seed from the soil bank by using chemicals to force germination of dormant seed. Then, weed seedlings could be killed by tillage, herbicides, or unfavorable weather.

Few chemicals have been reported to promote germination of dormant seeds in the field. Shallowly incorporated applicaitons of butylate (Sutan®), EPTC (Eptam®), vernolate (Vernam®), diallate (Avadex®), CDEC (Vegedex®), and chlorpropham (Furloe® , Chloro-IPC, CIPC®) at 0.1 pound per acre increased velvetleaf (*Abutilon theophrasti* Medic.) emergence in Illinois (9). Incorporated butylate at 1 pound per acre also enhanced emergence of common lambsquarters (*Chenopodium album* L.) (9). Ammonium nitrate and sodium azide stimulated emergence of dormant wild oat (*Avena fatua* L.) after incorporation in the field in North Dakota (11, 12, 19). However, field applications of ammonium nitrate did not enhance emergence of common lambsquarters, giant foxtail (*Setaria faberi* Herrm.), velvetleaf, jimsonweed (*Datura stramonium* L.), or redroot pigweed (*Amaranthuse retroflexus* L.) (10). In the Carolinas, injection of ethylene gas, a natural plant growth regulator, into the soil has been used to promote germination of witchweed (*Striga lutea* Lour.), a parasitic weed of monocot crops and weeds (5, 6, 7). Recently, the substituted phthalimides plant growth regulators AC-94,377 and AC-99,524 [1 = tetrahydrophthalimidecyclohexanecarboximide] were used to stimulate germination of dormant weed seed in the laboratory (17). Wild mustard, wild oat, field pennycress (*Thlapsi arvense* L.), and curly dock (*Rumex crispus* L.) were highly responsive to both substituted phthalimides and gibberellic acid, a natural growth regulator.

The overall objective of our greenhouse research was to learn how AC-94,377 behaves in soil before evaluating it in the field. The emergence response of dormant wild mustard seed was used to study factors limiting the activity of this substituted phthalimide plant growth regulator. In fact, stimulation of emergence was due to enhanced germination of dormant seed. Generally, emergence has been used to measure the efficiacy of germination stimulators in the field rather than germination or changes in total numbers of weed seed. However, emergence may underestimate the efficacy of germination stimulators in the field.

Wild mustard was chosen for study because it is an important weed in cereals and sunflower (*Helianthus annuus* L.) in North Dakota (5, 14). Also, wild mustard seed dormancy mechanisms have been studied (1, 2, 3, 8, 13, 15, 21, 22). In fact, progressively deeper burial of wild mustard seed in soil reduced germination and imposed dormancy by inducing a light requirement for germination which could be overcome by applications of gibberellic acid (15). Wild mustard also was one of the most susceptible species to AC-94,377 to be tested in the laboratory (21). In addition, wild mustard is representative of most other annual weeds of cereals which emerge over an extended period from shallow depths in the soil profile (4, 15), usually less than 2 inches.

MATERIALS AND METHODS

Experiment 1. The effects of soil type and surface-applied AC-94,377 at 0, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 pounds per acre on emergence of dormant wild mustard seed were studied. The soils were a greenhouse potting mixture and samples collected from the soil surface on the North Dakota experiment stations at Carrington, Casselton, Dickinson, Fargo, Langdon, Mandan, Minot, and Williston (Table 1). Soils were air dried and pulverized prior to use. Ripe, dormant wild mustard seed were collected near Fargo, air dried, cleaned and stored in a freezer in darkness before use. Storage in a freezer maintains seed dormancy for several months. At harvest, seed were only 5 percent to 7 percent germinable in darkness. One hundred

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Table 1. Characteristics of field soils used in experiments.

Source	Texture	Sand	Silt	Clay	O.M.	pH
		(%)				
Carrington[a]	Loam	49	40	11	3.6	7.9
Casselton[b]	Silty clay loam	14	55	31	3.0	7.7
Dickinson[c]	Loam	49	30	21	1.7	5.9
Fargo[d]	Silty clay	3	48	49	3.0	8.2
Langdon[e]	Loam	29	50	21	5.0	5.9
Mandan[f]	Loam	48	39	13	3.8	7.9
Minot[g]	Loam	46	38	16	2.7	6.0
Williston[h]	Loam	51	35	14	1.5	6.5

[a] Helmdal loam, coarse-loamy, mixed, Udic Haploborolls.
[b] Bearden silty clay, fine silty, frigid Aeric calcaiquils.
[c] Arnegard loam, fine loamy, mixed, Pachic Haploborolls.
[d] Fargo silty clay, fine Montmorillonitic, frigid, Vertic Haplaquolls.
[e] Svea fine loam, mixed, Pachic Udic Haploboroll.
[f] Williams loam, fine loamy, Typic Agriboroll.
[g] Williams loam, fine loamy, Typic Agriboroll.
[h] Max loam, fine loamy, mixed, Typic Haploborolls.

dormant wild mustard seed were planted 0.25 inch deep in pots which were 4 inches high and 4 inches in diameter. The 50 percent wettable powder formulation was applied at various rates with a greenhouse hood-sprayer. Percent emergence was determined three times each week for 21 days after placing the treated pots in the greenhouse. Sunlight was supplemented with fluorescent lighting in the greenhouse to give a spring daylength of at least 14 hours. The day temperature in the greenhouse ranged between 64 and 82 F and the night temperature ranged between 59 and 72 F throughout the winter, when most experiments were conducted. Pots were watered daily so that the soil surface was moist throughout each experiment. A completely randomized design was used with four pots for each individual combination of soil type and rate of AC-94,377. Data were subjected to analysis of variance (20) and regression analysis (16).

Experiment 2. This experiment was set up very much like the first one. However, AC-94,377 was applied to the soil surface at 0, 1.5, and 2.5 pound per acre after dormant wild mustard seed were planted 0, 0.5, 1, and 1.5 inches deep. Percent emergence was determined six weeks after seeding.

RESULTS AND DISCUSSION

Application of AC-94,377 to the soil surface in Experiment 1 stimulated the germination of dormant wild mustard seed buried 0.25 inch deep, as estimated by emergence (Figure 1 and Table 2). This substituted phthalimide plant growth regulator is nonvolatile and probably leached from the soil surface to the burial depth of the dormant wild mustard seed to stimulate germination and emergence. Emergence was chosen to measure the relative effectiveness of AC-94-377 as a germination stimulator of dormant wild mustard seed. Emergence is conveniently measured and is unlikely to seriously underestimate percent germination because of the shallow depth of burial that was used in this experiment. Wild mustard normally emerges from shallow depths in the

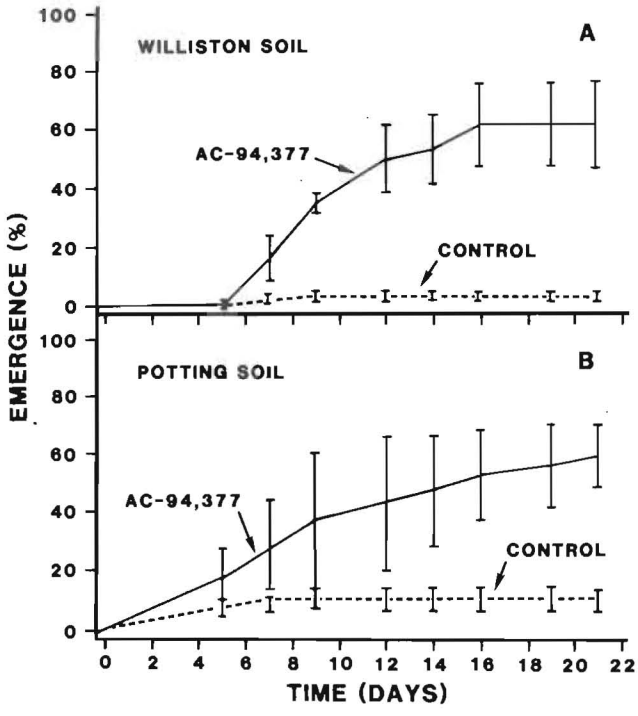

Figure 1. The effect of various rates of surface-applied AC-94,377 on percent emergence of dormant wild mustard three weeks after treatment in various soils (P = 0.0001). Seed were planted 0.25 inch deep.

Table 2. The best regression equation for Figure 1 relating percent emergence (Y) of dormant wild mustard seed to rate (lb/A) of surface-applied AC-94,377 (X) three weeks after treatment in various soils (P = 0.0001). Seed were planted 0.25 inch deep. The slopes or intercepts were either not significantly different from zero (ns) or were different from zero at P = 0.05 (*), P = 0.01 (**), or P = 0.0001 (***).

Soil location source	Best model equation	Coefficient of determination
Langdon	$Y = \underset{*}{6.28} + \underset{***}{17.22}X$	$r^2 = 0.86$
Potting soil	$Y = \underset{***}{11.64} + \underset{***}{14.46}X$	$r^2 = 0.65$
Williston	$Y = \underset{ns}{6.26} + \underset{***}{13.69}X$	$r^2 = 0.57$
Carrington	$Y = \underset{***}{11.66} + \underset{***}{8.79}X$	$r^2 = 0.65$
Fargo	$Y = \underset{***}{13.21} + \underset{***}{9.97}X$	$r^2 = 0.70$
Mandan	$Y = \underset{***}{13.73} + \underset{***}{7.46}X$	$r^2 = 0.60$
Casselton	$Y = \underset{***}{12.07} + \underset{***}{12.36}X - \underset{*}{1.81}X^2$	$r^2 = 0.59$
Minot	$Y = \underset{ns}{4.74} + \underset{***}{29.24}X - \underset{***}{6.52}X^2$	$r^2 = 0.42$
Dickinson	$Y = \underset{***}{14.79} + \underset{**}{16.66}X - \underset{*}{3.04}X^2$	$r^2 = 0.35$

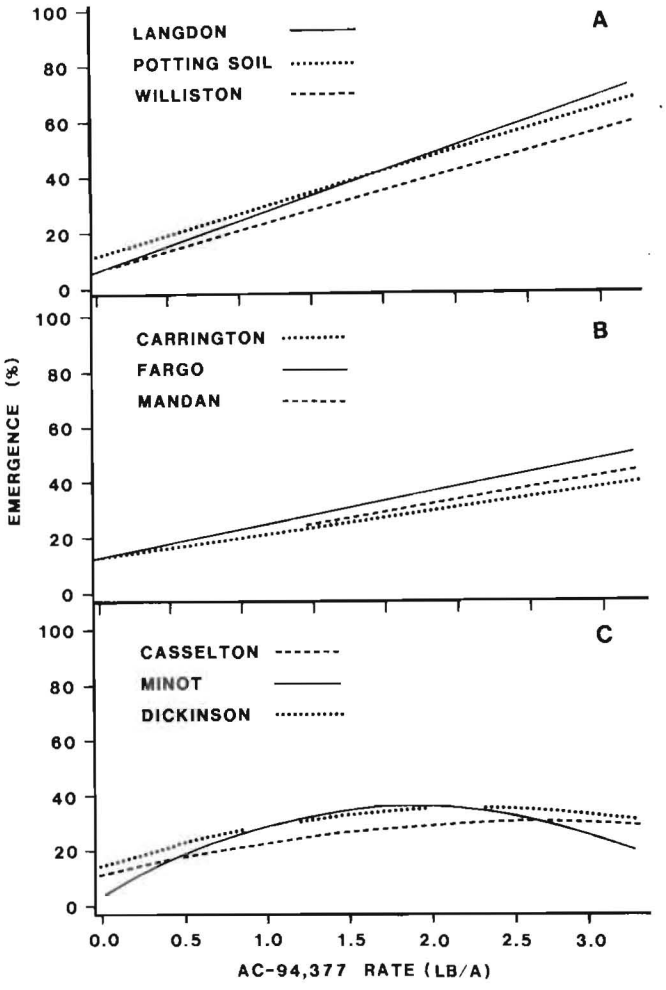

Figure 2. The emergence behavior of dormant wild mustard over time in either the Williston soil or the potting soil and in either the presence or absence of surface-applied AC-94,377 at 3.0 lb/A. Means and standard deviations are presented.

field. In addition, burial of germinable, nondormant wild mustard seed induced dormancy (15). In our experiments, burial of dormant, untreated wild mustard seed reduced rather than stimulated germination and emergence. Emergence decreased as seed were buried progressively deeper.

Soil type modified the activity of AC-94,377 in stimulating emergence of dormant wild mustard seed (Figure 1 and Table 1). In six of the nine North Dakota soils, emergence increased linearly in direct proportion to increasing AC-94,377 dose. Depending upon soil, emergence increased 10 percent to 60 percent above that of the controls when AC-94,377 was applied at 3.0 pounds per acre. In the Casselton, Minot, and Dickinson soils, the response to increasing dose of AC-94,377 was curvilinear (Figure 1C). The reasons why higher rates were relatively less effective than expected in these soils compared to the other soils (Figures 1A and 1B) were not investigated. Emergence response to AC-94,377 was not correlated with percent silt, percent clay, percent organic matter, pH, cation exchange capacity (CEC), nitrate level, phosphorus level, or potassium level of the soils. Environmental conditions in the greenhouse and the seed source were controlled within broad limits, but differences in soil microbial activity, soil water retention, or soil mechanical properties might be responsible for differences in the activity of AC-94,377 in various types of soil.

AC-94,377 at 3.0 pounds per acre increased both the rate and duration of emergence in the potting soil and Williston soil (Figure 2). Emergence was completed by at least 21 days after treatment, although an exact day could not be determined. Other greenhouse experiments using potting soil demonstrated slight, additional emergence up to six weeks following spray treatment, but not in controls. This period of biological activity may reflect the length of time that an effective concentration of the germination stimulator either persists in the soil before being degraded or remains near seed before being washed down in the soil. These alternative hypoteses remain to be tested.

Soil surface application of AC-94,377 enhanced germination and emergence of dormant wild mustard seed from all planting depths that were tested in Experiment 2, including 1.5 inches (Table 3). Emergence of untreated wild mustard seed decreased as seeding depth increased. Increasing rates of AC-94,377 applied to the soil surface enhanced emergence from each planting depth, although efficacy decreased slightly as seeding depth increased (Table 3). The simplest explanation for this is that AC-94,377 must have moved from the soil surface to the 1.5 inch planting depth to act on the dormant seed. Progressively greater depths of burial imposes dormancy on wild mustard seed and induces a light requirement for germination (15, 17). Holm (15) observed limited germination from 1 to 1.5 inches and none from 2 inches.

Soil surface treatment with the substituted phthalimide germination stimulator AC-94,377 enhanced the emergence of dormant wild mustard seed at doses from 1.5

Table 3. The effect of rate of surface-applied AC-94,377 and depth of planting of dormant wild mustard seed on percent emergence six weeks after treatment. Means followed by the same letters were not different by Duncan's multiple range test at P'0.05.

AC-94,377 Rate	Planting Depth	Emergence
(lb/A)	(inches)	(%)
0	0	18 c
0	0.5	17 c
0	1.0	21 c
0	1.5	20 c
1.5	0	59 a
1.5	0.5	44 b
1.5	1.0	40 b
1.5	1.5	38 b
2.5	0	67 a
2.5	0.5	57 a
2.5	1.0	43 b
2.5	1.5	37 b

to 3 pounds per acre in several soil types (Figure 1). AC-94,377 was most active on shallowly buried seed or seed lying on the soil surface, but AC-94,377 also moved into the soil profile at least 1.5 inches to stimulate some emergence of dormant wild mustard seed planted 1.5 inches deep (Table 3). The extent to which AC-94,377 will move into the soil profile in the field may differ with soil type and texture and may depend upon seasonal rainfall and soil characteristics. Because a higher proportion of the seed bank is more shallowly buried in conservation tillage cropping systems, the soil surface treatment with the substituted phthalimides may be most effective in these cropping systems, rather than in soils which are moldboard plowed. AC-94,377 was active for at least two to three weeks in the soil under a relatively heavy greenhouse watering regime, as judged by the period over which surface applicaiton enhanced germination and emergence of dormant wild mustard seed from 0.25 inch deep (Figure 2).

The effective range of rates, leaching characteristics, and soil persistence of AC-94,377 in the greenhouse make it a desirable potential candidate as a germination stimulator in the field. AC-94,377 has shown activity in the field in two years of field testing at Fargo (unpublished results). The substituted phthalimide germination stimulators may act on dormant seed of a wide range of weed species, especially those whose dormancy is broken by gibberellins, as pointed out by Metzger (17). The range of weed species which respond is under investigation. The next step is to determine how to integrate such chemicals into current farming practice for long-term eradication of weeds. An integrated approach to preventing seed input into the soil reserve would be a prerequisite to using germination stimulators to totally deplete the soil weed seed bank of certain weeds.

LITERATURE CITED

1. Andersen, R.N. 1968. Germination and Establishment of Weeds for Experimental Purposes. W.F. Humphrey Press, NY. 236 pp.
2. Chakrabarti, A.G. 1976. Effect of selected growth retardants on the germinability of weed seeds. J. Seed Technol. 1:60-63.
3. Edwards, M.M. 1969. Dormancy in seeds of charlock. IV. Interrelationships of growth, oxygen supply and concentration of inhibitor. J. Exp. Bot. 20:876-894.
4. Edwards, M. 1980. Aspects of the population ecology of charlock. J. Appl. Ecol. 17:151-171.
5. Egley, G.H. and J.E. Dale. 1970. Ethylene, 2-chloroethylphosphonic acid, and witchweed germination. Weed Sci. 18:586-589.
6. Eplee, R.E. 1975. Ethylene: a witchweed seed germination stimulant. Weed Sci. 23:433-436.
7. Eplee, R.E. and M.A. Langstron. 1976. Developments in the control of *Striga* in the USA. PANS 22:61-64.
8. Everson, L. 1949. Preliminary studies to establish laboratory methods for the germination of weed seed. Proc. Assoc. Off. Seed Anal. 39:84-89.
9. Fawcett, R.S. and F.W. Slife. 1975. Germination stimulation properties of carbamate herbicides. Weed Sci. 23:419-424.
10. Fawcett, R.S. and R.W. Slife. 1978. Effects of field applications of nitrate on weed seed germination and dormancy. Weed Sci. 26:594-596.
11. Fay, P.K. and R.S. Gorecki. 1978. Stimulating germination of dormant wild oat (*Avena fatua*) seed with sodium azide. Weed Sci. 26:323-326.
12. Fay, P.K., R.S. Gorecki, and P.M. Fuerst. 1980. Coating sodium azide granules to enhance seed germination. Weed Sci. 28:674-677.
13. Fogg, G.E. 1950. Biological flora of the British Isles No. 146. *Sinapis arvensis* L. J. Ecol. 38:415-429.
14. Harper, J.L. 1977. Population Biology of Plants. Academic Press, NY. 892 pp.
15. Holm, R.E. 1972. Volatile metabolites controlling germination in buried weed seeds. Plant Physiol. 50:293-297.
16. Kleinbaum, D.G. and L.L. Kuper. 1978. Applied Regression Analysis and Other Multivariate Methods. Durbury Press, North Scituate, MA. 556 pp.
17. Metzger, J.D. 1983. Promotion of germination of dormant weed seeds by substituted phthalimides and gibberellic acid. Weed Sci. 31:285-289.
18. Roberts, H.A. 1983. Seed banks in soils. Adv. Applied Biology 6:1-55.
19. Sexsmith, J.J. and U.J. Pittman. 1963. Effect of nitrogen fertilizers on germination and stand of wild oats. Weeds 11:99-101.
20. Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W.H. Freeman and Co., San Francisco, CA. 776 pp.
21. Warnes, D.D. and R.N. Andersen. 1984. Decline of wild mustard (*Brassica kaber*) seeds in soil under various cultural and chemical practices. Weed Sci. 32:214-217.
22. Witcombe, J.R. and W.J. Whittington. 1972. The effects of selection for reduced dormancy in charlock (*Sinapis arvensis*). Heredity 29:37-49.