Influence of Chemical Treatment and *Fusarium oxysporum* on Velvetleaf (*Abutilon theophrasti*)[1]

ROBERT J. KREMER and LISA K. SCHULTE[2]

Abstract. Germination stimulants were tested for effectiveness on velvetleaf seed imbibition and germination and concurrent microbial attack of seed to deplete weed seed in soil. Several chemicals increased in vitro seed germination and decreased the numbers of hard, viable seed. The proportion of nonviable seed, 5% with *Fusarium oxysporum* (Schlech.), was enhanced to 40% by adding ethephon at 100 μg/ml. Fungal density on seedling roots and imbibed (nonviable seed) was increased by chemical treatment. Seedling emergence was reduced 15% when ethephon or carbofuran was applied to soil with the fungus. Shoot dry weight decreased and root infection increased with all treatments regardless of fungal inoculation. Butylate and carbofuran increased infection of imbibed seed in soil by *F. oxysporum* while ethephon and AC94377 increased infection by other soil fungi. **Nomenclature:** AC94377, 1-(3-chlorophthalimido)-cyclohexane carboximide; butylate, *S*-ethyl bis(2-methylpropyl)carbamothioate; carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate; ethephon, (2-chloroethyl)phosphonic acid; velvetleaf, *Abutilon theophrasti* Medik. #[3] ABUTH.
Additional index words: Pesticide-microorganism interactions, integrated weed management, weed biocontrol, butylate, ethephon, AC94377, ABUTH.

INTRODUCTION

Many annual weeds persist in cultivated fields due to the ability of seed to remain dormant in soil for prolonged periods. The impermeable seed coat property ('hardseededness'), which contributes to dormancy in many weeds, enables persistence, sporadic emergence throughout the growing season, and resistance to microbial attack in soil (12, 13, 16). An approach to decreasing weed seed in soil would be to stimulate consistent imbibition and germination of dormant seed by some chemical means and to destroy the resulting imbibed seed and seedlings mechanically, chemically, or biologically with selective microbial agents.

Soil-applied pesticides and other plant growth regulating chemicals stimulate germination and emergence of dormant seed in vitro and in soil (6, 7, 10, 14, 17). Certain pesticides also enhance root infection of crop plants by either phytopathogenic or saprophytic soil microorganisms and increase the development or severity of root diseases (1, 8, 11, 15, 20). Only limited information exists on interactions of herbicides and soilborne pathogens to control specific weeds (19). Managing weeds in the seed/seedling stages with selected soilborne microorganisms can reduce establishement of competitive weed populations and can be an alternative to application of foliar pathogens.

The objective of this study was to evaluate the effects of several chemical treatments alone and combined with a selected fungus on seed imbibition, germination, and seedling growth and on development of seed/seedling disease on velvetleaf. Velvetleaf was chosen for study because of its economic importance in row crops (18) and because its seed dormancy (10, 16) and deterioration resistance (12, 13) mechanisms have been characterized.

MATERIALS AND METHODS

Mature velvetleaf seed were collected from a plant accession grown at Columbia, MO, in 1985.

[1] Received for publication Sept. 6, 1988, and in revised form Jan. 16, 1989. Contribution of Agric. Res. Serv., U.S. Dep. Agric. and Mo. Agric. Exp. Stn. J. Ser. No. 10656.

[2] Microbiol., U.S. Dep. Agric., Agric. Res. Serv., Crop Syst. Water Qual. Res. Unit, Univ. Mo., Columbia, MO 65211; and Student Res. Asst., Dep. Agron., Univ. Mo., Columbia, MO 65211.

[3] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 W. Clark St., Champaign, IL 61820.

Seed were stored at 4 C for 18 months before use. A fungus previously isolated from necrotic tissue of an infected velvetleaf seedling during routine seed germination assays and classified as *F. oxysporum* was used. The fungus will be referred to as *Fusarium* in this paper. The culture was maintained on potato (*Solanum tuberosum* L.) dextrose agar, PDA[4] (4). A spontaneous antibiotic-resistant mutant of the fungus resistant to 100 μg/ml cycloheximide was developed for the soil studies. Preliminary assays indicated that the pathogenicity of the mutant was not altered.

Technical grade (purity ≥95%) chemicals were assayed at various concentrations based on previous research on germination stimulation of weed seed (3, 6, 10, 14). Potassium nitrate (KNO_3), sodium azide (NaN_3), gibberellic acid (GA_3), ethephon, and AC94377 are germination stimulants; butylate, EPTC (*S*-ethyl dipropyl carbamothioate), and trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl) benzenamide] are herbicides; and carbofuran and cloethocarb [2-(2-chloro-1-methoxyethoxy)phenyl methylcarbamate] are insecticides. Stock solutions of KNO_3, NaN_3, GA_3, ethephon, and AC94377 were made in 500-ml sterile distilled water. Butylate, EPTC, trifluralin, carbofuran, and cloethocarb were dissolved in minimal amounts of acetone before diluting to a final volume of 500 ml with water.

Laboratory studies. Agar (11 g/L distilled water) was autoclaved for 15 min at 120 C and was placed in a 50 C water bath. Aliquots of the stock chemical solutions were filter sterilized (0.2 μm) and were combined with water agar to yield the test concentrations. The agar solutions were poured into disposable petri plates (100 by 15 mm) and were allowed to solidify. Half of the plates in each chemical treatment were inoculated with approximately 10^5 colony forming units per ml consisting of 90% spores and 10% mycelial fragments of *Fusarium*.

Preliminary studies showed that growth of the fungus was not inhibited by any of the chemicals at the concentrations used. Ten velvetleaf seed, surface-sterilized 4 min in 1.25% sodium hypochlorite, 2 min in 70% ethanol, and rinsed in sterile distilled water and blotted, were placed equidistantly in each petri plate. The plates with seed were incubated in the dark for 4 days at 28 C.

After incubation, seed with radicles >2 mm were considered germinated, and radicle lengths were measured; the remaining seed were classed and were recorded as either hard (nonimbibed, viable) or nonviable (imbibed, nongerminating). Hard seed were considered viable because 99.5% of hard seed produced normal seedlings when tested for germination after scarification. Fungal colonization of seed and seedlings was determined visually by rating the density of mycelia and spores as light, medium, or heavy (12). The experimental design was a randomized complete block with a factorial arrangement and four replications. The experiment was conducted four times, and the data were combined for analysis.

Greenhouse studies. A Freeburg silt loam (fine-silty, mixed, mesic, Aquic Hapludalf) surface soil (0 to 10 cm deep) was collected from a field that had no pesticides applied during the previous 5 yr. The soil contained 1.8% organic matter, cation exchange capacity was 8 meq/100 g, and the pH was 6.5. Technical grade chemicals at rates based on the laboratory assays were applied to soil and were incorporated by mixing soil and chemical in a plastic bag. Treated soils were dispensed in 5.0 kg amounts into 50 by 25 by 6-cm flats.

Fungal inoculant, consisting of *Fusarium* cultured on shredded wheat medium (5) for 10 days, was incorporated in soils of half the flats at 140 g/flat. The fungal inoculant contained approximately 10^6 colony forming units/ml. Fifty velvetleaf seed were planted 1 cm deep and 5 cm apart in each flat. Soil moisture was maintained near field capacity (60% water-holding capacity) by surface irrigation. Plants were grown for 4 weeks after planting with a greenhouse temperature of 28 ± 5 C. Seedling emergence was determined, and nongerminated seed were recovered from soil and were classified as hard or nonviable.

Seed were placed on selective PDA medium supplemented with 100 μg/ml cycloheximide which suppressed indigenous soil fungi and allowed detection of *Fusarium* from the inoculum. Roots were separated from harvested plants, were rated for injury, and were imprinted on selective PDA medium to assess fungal infection. Ratings of injury and fungal infection were based on indices sug-

[4] Abbreviations: PDA, Potato dextrose agar.

gested by Curl and Rodriguez-Kabana (4). Shoots were dried at 80 C for 48 h before dry weight measurement. The experimental design was a randomized complete block using a split-plot treatment arrangement with three replications. Main plots were chemicals, and sub-plots were two fungal treatments randomized within each main plot. The experiment was conducted three times, and the data were combined for analysis.

All data from the laboratory and greenhouse studies were subjected to appropriate analysis of variance. Where F-values were significant at the $P \leq 0.05$ level, means were compared using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Laboratory studies. Velvetleaf germination was enhanced, and hard seed numbers were decreased by all test chemicals except KNO_3, butylate, and trifluralin (Table 1). Nonviable seed were increased significantly only with trifluralin. The proportion of hard seed was decreased only when *Fusarium* was combined with butylate. However, the proportion of germinated seed significantly decreased when *Fusarium* was combined with KNO_3, ethephon AC94377, EPTC, or cloethocarb. The resulting increases in nonviable seed associated with KNO_3, ethephon, and AC94377 was likely due to detrimental effects of *Fusarium* on the imbibible (germinable) fraction of seed, which were enhanced by treatment of seed with these chemicals.

In the absence of *Fusarium*, radicle growth of velvetleaf seedlings was stimulated by GA_3 and was inhibited by ethephon, AC93477, EPTC, KNO_3, and NaN_3 while butylate, carbofuran, and cloethocarb had no effect (Table 1). In *Fusarium* combinations with chemicals, root growth was inhibited further in all treatments except KNO_3, GA_3, and EPTC. Seedlings developing in medium amended with KNO_3, NaN_3, and trifluralin exhibited necrosis and abnormal growth including inhibited lateral root growth.

Ethephon alone enhanced lateral root development. *Fusarium* caused moderate necrosis on control seedlings; however, root necrosis was increased by KNO_3, GA_3, butylate, EPTC, carbofuran, and cloethocarb. Ethephon-treated seedlings with *Fusarium* exhibited heavy fungal infection of the hypocotyl and radicle and decreased lateral root development.

Overall growth of *Fusarium* and coincident infection of seedlings was enhanced greatly with all chemicals except NaN_3 and trifluralin (Table 2). Nonviable seed were heavily colonized in KNO_3, ethephon, and AC94377. Hard seed were lightly colonized, reflecting the antifungal properties of these intact seed (12).

Previous laboratory studies indicated inconsistencies in the effects of the thiocarbamate herbicides,

Table 1. Effects of chemical treatments and *Fusarium* on in vitro seed viability and seedling growth of velvetleaf.

Treatment	Rate	Germinated seeds		Radicle length		Hard seed		Nonviable seed	
		Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*
	(μg ai/ml)	(%)		(mm)		(%)			
None		35	35	38	33	62	60	2	5
KNO_3	100	42	20	20	18	52	55	5	25
NaN_3	15	45	44	21	14	50	52	5	4
GA_3	100	54	55	50	58	44	45	2	0
Ethephon	100	50	5	27	22	45	55	5	40
AC94377	250	48	36	33	18	48	42	4	22
Butylate	20	42	55	38	33	58	40	0	5
EPTC	20	56	48	20	19	42	48	2	4
Trifluralin	6	38	39	39	30	53	51	9	10
Carbofuran	20	58	62	41	31	42	38	0	0
Cloethocarb	20	55	45	40	33	45	55	0	0
LSD (0.05)[a]		10	16	5	6	12	20	4	12
LSD (0.05)[b]		6		5		10		4	

[a]LSD (0.05) between seed viability parameter within an inoculation treatment.

[b]LSD (0.05) between inoculation treatments within a seed viability parameter.

Table 2. Growth of *Fusarium* (shown as density of mycelia) on seedlings and hard and nonviable seeds of velvetleaf exposed to chemical treatments after 4 days of incubation[a].

Treatment	Rate	Seedlings	Hard seed	Nonviable seed
	(μg ai/ml)			
Control		0.6	0.4	0.8
KNO_3	100	2.2	1.4	2.8
NaN_3	15	1.0	0.8	1.2
GA_3	100	1.5	0.8	...[b]
Ethephon	100	2.2	0.8	2.9
AC94377	250	2.2	1.0	2.9
Butylate	20	2.1	0.8	1.8
EPTC	20	1.4	0.7	1.5
Trifluralin	6	0.8	0.5	2.0
Carbofuran	20	2.3	0.9	...[b]
Cloethocarb	20	0.9	0.5	...[b]
LSD (0.05)		0.5	0.6	0.6

[a]Density rating: 1 = light, 2 = medium, 3 = heavy.

[b]Nonviable seed not obtained from these treatments.

butylate and EPTC, on the germination and hardseededness of velvetleaf. In the present study, butylate and EPTC and the carbamate insecticides, carbofuran and cloethocarb, stimulated germination of velvetleaf, which agrees with Fawcett and Slife (7) who reported enhanced germination with butylate and EPTC. However, other researchers reported this herbicide class did not increase germination of hardseeded velvetleaf (10). Velvetleaf was not stimulated to germinate with KNO_3 nor NaN_3, likely due to lack of imbibition by hard seed (Table 1). Similarly, Fawcett and Slife (8) reported that exogenous nitrate failed to stimulate velvetleaf germination. Promoting velvetleaf seed germination by GA_3, ethephon, and AC94377 generally agrees with other reports of germination stimulation of weed species by these compounds (3, 14, 17).

The increases in nonviable seed and inhibition of radicle growth (Table 1) observed when *Fusarium* was added to certain treatments may have resulted from chemically induced susceptibility of the velvetleaf seed and seedling tissues. Microbial invasion of plant tissue not treated with chemicals usually is restricted to the epidermis and naturally disrupted area such as points of lateral root emergence (9). However, when plants are treated with certain chemicals, like mecoprop [(±)-2-(4-chloro-2-methylphenoxy)propanoic acid] on wheat (*Triticum aestivum* L.) seedlings (9), microbial colonization is more extensive within plant tissues other than those physically disrupted by radicle and lateral root emergence. This was reflected in the present study where fungal colonization of seedlings and imbibed seed was considerably heavier with most chemicals than without chemicals (Table 2).

Katan and Eshel (11) proposed several mechanisms for increased host susceptibility to pathogens induced by chemicals. These include alteration of growth patterns and tissue composition of plant, which enables less impeded penetration, colonization and toxin production by the pathogen; increased production of root exudates, which stimulates soil-borne pathogens; and, alteration of host chemical defense mechanisms against pathogens.

Greenhouse studies. In the absence of *Fusarium*, no treatment effectively reduced velvetleaf emergence (Table 3). However, when combined with *Fusarium*, ethephon and carbofuran significantly reduced emergence by 11 and 12%, respectively. Emergence may underestimate the efficiency of soil-applied chemicals (6); thus, nongerminated seed recovered from soil showed additional effects of chemical treatments.

Highest numbers of nonviable seed found in soil resulted from ethephon treatment with or without *Fusarium* (Table 3). All chemicals except NaN_3 increased the number of nonviable seed with *Fusarium*. Nonviable seed recovered from soil treated with ethephon, AC94377, butylate, and carbofuran plus *Fusarium* were heavily infected with mycelia. Increases in nonviable seed and decreases in seedling emergence may be due to effects of chemical-fungus interactions on the imbibible fraction of the seed in soil since hard seed content was not reduced significantly by any treatment combination. Other studies similarly reported few effects of chemical treatments on hardseeded velvetleaf in soil (8, 10).

Butylate and carbofuran injured roots moderately, which likely contributed to the moderate to heavy fungal infection of roots in soil inoculated with *Fusarium* (Table 3). Fungal infection of seedling roots also increased with ethephon and AC94377 regardless of inoculation, indicating that soil fungi other than the *Fusarium* inoculum were induced to attack. Others also have reported that pesticide-related injury of roots may interact with fungal

Table 3. Seedling emergence, seed class, root damage, and shoot dry weight of greenhouse-grown velvetleaf as influenced by soil-applied chemicals with and without *Fusarium*.

Treatment	Rate	Emergence		Hard seed		Nonviable seed		Root injury[a]		Root infection[b]		Shoot dry wt	
		Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*	Control	*Fusarium*
	(mg/kg)	(%)										(mg/plant)	
None		54	51	39	34	6	14	0.2	1.0	0.1	1.8	160	110
KNO_3	100	52	50	33	28	15	21	0.6	2.0	1.0	1.8	140	90
NaN_3	75	56	52	36	36	8	12	0.8	1.0	1.1	1.5	130	100
Ethephon	100	50	40	24	36	26	29[c]	1.2	1.4	3.2	3.0	110	80
AC94377	250	49	46	38	36	12[c]	18[c]	1.0	1.9	2.2	2.4	90	80
Butylate	20	63	50	29	32	8	18[c]	2.2	2.5	2.8	3.2	100	80
Carbofuran	20	54	39	38	38	9	23[c]	2.2	2.6	1.2	2.3	70	70
LSD (0.05)[d]		10	11	NS	NS	10	4	0.8	0.6	1.0	1.1	25	30
LSD (0.05)[e]		8		NS		12		0.6		1.0		20	

[a]Injury rating: 0 = no injury, 5 = severe injury (tap root stunted, poor lateral root developments).

[b]Fungal infection rating: 0 = no injury, 5 = heavy fungal covering on tap and lateral roots.

[c]Infected with fungi.

[d]LSD (0.05) between seed/seedling parameters within an inoculation treatment.

[e]LSD (0.05) between inoculation treatments within a seed/seedling parameter.

pathogens and may increase the severity of root diseases (11, 15, 19, 20).

Ethephon and AC94377 applied to soil slightly injured roots yet increased fungal infection significantly compared to nontreated controls (Table 3). Effects of ethephon and AC94377 on roots may be more subtle than those observed with butylate and carbofuran. Assays of exudates from roots treated with ethephon and AC94377 showed increased contents of soluble carbohydrates and amino acids (data not shown), which can stimulate fungal growth rates and colonization of roots leading to increased infection (1, 11).

Certain herbicides can increase root exudation by other plant species, increasing seedling diseases (1, 2, 9). Chemical-enhanced fungal infection of seedling roots also likely contributed to the significant reductions in shoot dry weights shown for ethephon, AC94377, butylate, carbofuran, and cloethocarb (Table 3).

In summary, certain available chemicals may effectively promote fungal attack of imbibing and germinating velvetleaf seed. An integrated approach of combining low rates of soil-applied chemicals and selected microorganisms might be useful in rapidly depleting the weed seed bank and decreasing seedling emergence and vigor. The potential beneficial impact of microbiological control of weeds by exploiting plant-chemical-microorganism interactions includes reductions in future weed infestations, herbicide use, and environmental damage. However, to be useful, such approaches must meet several criteria (14), including persistence of the agents in soil, economic soundness, and effectiveness against a range of weed species.

ACKNOWLEDGMENTS

Don Gates and Byron McVay of American Cyanamid Company provided the AC94377; Russ Nash of Rhone-Poulenc, the ethephon; Barbara Hook of ICI Americas, the butylate and EPTC; Scott Gibson of BASF, the cloethocarb; and Bob Kinsinger of FMC, the carbofuran. Diana Call, Darline Koch, and Lynn Stanley provided technical assistance. Schulte was partially funded through a College of Agriculture Student Internship.

LITERATURE CITED

1. Altman, J., and C. L. Campbell. 1977. Effect of herbicides on plant diseases. Ann. Rev. Phytopathol. 15:361–385.
2. Altman, J., and C. L. Campbell. 1977. Pesticide-plant disease interactions: effect of cycloate on sugar beet damping-off induced by *Rhizoctonia solani*. Phytopathology 67:1163–1165.
3. Bradow, J. M. 1986. Germination promotion in dormant shepherdspurse (*Capsella bursa-pastoris*) seeds by strigol analogs and other stimulants. Weed Sci. 34:1–7.
4. Curl, E. A., and R. Rodriguez-Kabana. 1986. Herbicide-plant disease relationships. p. 429–455 *in* N. D. Camper, ed. Research Methods in Weed Science. South. Weed Sci. Soc., Champaign, IL.
5. Cutler, H. G. 1986. Isolating, characterizing, and screening mycotoxins for herbicidal activity. p. 147–170 *in* A. R. Putnam and C. Tang, ed. The Science of Allelopathy. John Wiley & Sons, New York.
6. Donald, W. W., and R. A. Hoerauf. 1985. Enhanced germination and emergence of dormant wild mustard seed by two substituted phthalimides. Weed Sci. 33:894–902.
7. Fawcett, R. S., and F. W. Slife. 1975. Germination stimulation

properties of carbamate herbicides. Weed Sci. 23:419–424.
8. Fawcett, R. S., and F. W. Slife. 1978. Effects of field application of nitrate on weed seed germination and dormancy. Weed Sci. 26:594–596.
9. Greaves, M. P., and J. A. Sargent. 1986. Herbicide-induced microbial invasion of plant roots. Weed Sci. 34(Suppl. 1):50–53.
10. Horowitz, M., and R. B. Taylorson. 1985. Behavior of hard and permeable seeds of *Abutilon theophrasti* Medik. (velvetleaf). Weed Res. 25:363–372.
11. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145–177.
12. Kremer, R. J. 1986. Antimicrobial activity of velvetleaf seeds. Weed Sci. 34:617–622.
13. Kremer, R. J., L. B. Hughes, Jr., and R. J. Aldrich. 1984. Examination of microorganisms and deterioration resistance mechanisms associated with velvetleaf seed. Agron. J. 76:745–749.
14. Metzger, J. D. 1983. Promotion of germination of dormant weed seeds by substituted phthalimides and gibberelic acid. Weed Sci. 31:285–289.
15. Nilsson, H. E. 1977. Influence of herbicides on infection by *Verticillium dahliae* on oil seed rape, *Brassica napus* var. *oleifera*. Phytopathol. Z. 90:361–363.
16. Rolston, M. P. 1978. Water impermeable seed dormancy. Bot. Rev. 44:365–396.
17. Saini, H. S., P. K. Bassi, and M. S. Spencer. 1986. Use of ethylene and nitrate to break seed dormancy of common lambsquarters (*Chenopodium album*). Weed Sci. 34:502–506.
18. Spencer, N. R. 1984. Velvetleaf, *Abutilon theophrasti* (Malvaceae), history and economic impact in the United States. Econ. Bot. 38:407–416.
19. Weidemann, G. J., and G. E. Templeton. 1988. Control of Texas gourd, *Cucurbita texana*, with *Fusarium solani* f. sp. *cucurbitae*. Weed Technol. 2:271–274.
20. Wyse, D. L., W. F. Meggitt, and D. Penner. 1976. Effect of herbicides on the development of root rot on navy bean. Weed Sci. 24:11–15.