

MANAGEMENT OF WEED SEED BANKS WITH MICROORGANISMS¹

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Abstract. Successful weed management in agroecosystems centers on manipulating the weed seed bank in soil, the source of annual weed infestations. Despite advances in above-ground weed control and decreases in the production of new seeds, weed infestations continue to be generated from a small portion of the seed bank that persists as a result of dormancy and resistance to decay. Depletion of the persistent seeds using soil-applied chemicals to stimulate germination has received much attention while the search for microorganisms selective for seed decay has been largely overlooked. This paper provides an overview of the effects of microorganisms on weed seed viability relative to seed bank depletion, and how this information can be applied to weed management. Limited studies indicate that microorganisms associated with weed seeds can contribute to seed bank depletion through attraction to seeds by chemotaxis, rapid colonization of the spermosphere and production of enzymes and/or phytotoxins to kill seeds prior to germination. It is recognized, however, that the best opportunity for success will be through integration of selected microorganisms or microbial products with other approaches including germination stimulation, application of low rates of herbicides, manipulation of the soil environment (e.g., solarization), and biological control agents for effectively eliminating dormant, persistent seeds from soil. To achieve success, more in-depth research on microbial factors affecting weed seed banks is required.

Key words: *biological control; deterioration resistance mechanisms; integrated weed management; microbial ecology; seed dormancy; soil environment; weed seed bank.*

INTRODUCTION: THE WEED SEED BANK PROBLEM

Weed management involves systems in which all available strategies are used to reduce weed seeds residing in soil (the seed bank), prevent weed emergence from the seed bank, and minimize competition from weeds growing with crops (Ennis 1977, Aldrich 1984). It is readily apparent that successful, long-term weed management emphasizes control of seeds arising from the soil. Cultivated soils contain large numbers of seeds, primarily from annual weed species, that under favorable conditions germinate and interfere with crop production. The weed seed bank is widely considered the major source of weed infestations in arable land (Cavers and Benoit 1989). The intent of seed bank research is to improve weed management strategies by manipulating weed seed behavior in soil.

Depletion of the seed bank is critically important in overcoming yearly weed infestations (Ennis 1977, Aldrich 1984). Significant reductions in weed seed banks over several years have been reported when seed replenishment was prevented. The following selected examples illustrate the magnitudes by which seed banks can be depleted. After a 4-yr fallow with intensive tillage, only 10% of the original population of velvetleaf

(*Abutilon theophrasti* Medic.) seeds remained in soil (Lueschen and Andersen 1980). A similar study showed that 6 yr of fallow and multiple tillage reduced wild mustard [*Brassica kaber* (DC.) L.C.] seed in soil to < 3% of the original population (Warnes and Andersen 1984). However, over 2×10^6 seeds/ha still remained in the plow layer, and it was suggested these eradication measures were impractical. Under irrigated corn (*Zea mays* L.) receiving annual herbicide applications for weed control, the overall decline of the dominant weed species, redroot pigweed (*Amaranthus retroflexus* L.) and common lambsquarters (*Chenopodium album* L.), after six cropping seasons was 99 and 94%, respectively (Schweizer and Zimdahl 1984). Burnside et al. (1986) reported that the average decline of viable weed seeds in soil at five sites in Nebraska was 95% after 5 yr where herbicides were used for complete weed control in corn.

A consistent conclusion made in these and many other studies was that despite the achievement of "good" weed control (> 80%) over several years with timely use of available technology, weed infestations in succeeding seasons still occurred if intensive weed management was discontinued. Chancellor (1981) similarly concluded that even if a system of weed control was developed that completely controlled above-ground growth and was continued for a long enough time, complete elimination of weeds would not be achieved. Weeds emerge in high numbers even though

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seed banks of some species are composed largely of nonviable seeds (Forcella 1992). Thus, subsequent infestations are due to a small but highly persistent portion of the seed bank that is not affected by conventional weed control practices. It has been noted repeatedly that the key to weed management progress is elimination of the persistent portion of seed banks (Ennis 1977, Chancellor 1981).

The persistent portion of the seed bank is composed of weed seeds that enter and survive in soil for > 1 yr (Thompson and Grime 1979). Germination of persistent seeds does not readily occur under favorable environmental conditions due to dormancy traits (Baskin and Baskin 1989). Dormancy can be imposed physically by impermeable seed coats (hard seeds), which prevent moisture uptake and gas exchange necessary for germination, or physiologically through mechanisms inhibiting germination in the embryo. Seeds in the seed bank can exhibit annual dormancy/nondormancy cycles in which dormant seeds can become nondormant and germinate if environmental factors are nonlimiting (Baskin and Baskin 1989). Seeds failing to germinate can re-enter dormancy and become nondormant for potential germination the following season. It is this cycling that contributes to continuing weed infestations.

The unpredictable emergence of weed seedlings from dormant seeds in soil is the major cause of long-lasting weed problems and recent weed research programs have focused on elimination of this portion of the seed bank. Ennis (1977) suggested that if 98% of the dormant seeds were induced to germinate annually under typical field conditions, the seed bank could be depleted within 6 yr. Cultivation and herbicides are effective only in eliminating persistent seeds that become nondormant at one point in time; thus, methods of inducing the remaining dormant seeds to germinate are necessary. The most satisfactory approach involves chemical means of breaking seed dormancy (Chancellor 1981). Application of chemical stimulants to manipulate seed germination has had limited success; however, more information on mechanisms of seed dormancy and germination is required to fully develop this approach as a practical method for weed control (Egley 1986).

Seed bank processes are important components of predictive models developed to advance new weed management systems. The models generally include a persistent component composed of seeds under different types of dormancy, and a nonpersistent component composed of germinating seeds and seeds losing viability prior to germination. The goal of predictive seed bank models is to understand the phenomena of persistence and depletion among seed bank populations and then use this information to develop strategies to destroy buried seed populations through germination stimulation or by direct action of toxicants (Schafer and Chilcote 1969). Information for input into the models is derived from seed burial and seedling pattern

studies carefully designed to account for depletion through germination and mortality.

Little quantitative information on seed bank mortality caused by microorganisms is available. Results of many buried seed studies have acknowledged indirectly the influence of microbial activity on seed mortality. Indeed, recent weed management models stress the importance of seed mortality caused by microorganisms for accurate prediction of weed populations and for subsequent development of control measures (King et al. 1986). Zorner et al. (1988) applied the seed bank depletion model of Shafer and Chilcote (1969) to their studies on wild oat (*Avena fatua* L.) and concluded that the ideal method of depletion would involve a tillage scheme to enhance seed decay while excluding seedling emergence. Aldrich (1984) first suggested that manipulation of microorganisms to strip seeds of their apparent protection against decay merited examination as an approach for reducing weed seed numbers in soil.

A comprehensive account of microbial effects on viability and germination of seeds relative to potential depletion of weed seed banks is not available. This is an area that deserves more attention, particularly with weed seeds, since they remain in contact with soil much longer than crop seeds. The objective of this paper is to provide an overview of what is known about the ecology of microorganism-weed seed relationships and how this information can be applied to weed management systems.

PRESUMPTIVE EVIDENCE OF MICROORGANISMS AS FACTORS IN SEED BANK DEPLETION

Seed banks depicted by diagrams, flow charts, and models typically include a component for seeds losing viability prior to germination, with microbial activity as a factor contributing to nonviability (Harper 1977, Egley and Duke 1985). Microbial activity has been implicated as a factor in seed depletion in many studies, yet the microorganisms or those conditions causing the observed depletion due to nongermination were rarely examined further. Despite the apparent importance of microorganisms, suggestions that they might be exploited to hasten seed bank depletion have been limited (Aldrich 1984). Some studies have indicated the possible role of microorganisms in "unexplained" losses of seeds in soil and thus serve as presumptive evidence of microbial involvement.

Roberts and Feast (1972) periodically assessed viability of seeds buried in tilled and undisturbed soils for 5 yr and consistently found a proportion of the original population could not be accounted for either as seedlings or as surviving viable seeds. They suggested that, since the seeds were initially viable, their absence must have been due to attack by pathogens resulting in nongerminated, decayed seeds or to seedling mortality before emergence. The rate of seed depletion in other

tillage studies could not be explained fully due to germination alone; thus, losses from "post-germination mortality" were significant (Froud-Williams et al. 1983). The viability of johnsongrass [*Sorghum halepense* (L.) Pers.] seeds at all depths of burial in soil declined from 85 to <2% in 52 mo and was attributed partly to seed decay (Leguizamon 1986). In a study of itchgrass [*Rottboellia cochinchinensis* (Lour.) W. Clayton], a troublesome weed in tropical crops, mortality of 55% of the original seed population in soil was attributed to decay (Bridgemohan et al. 1991). Fungal mycelia observed on rotted seeds indicated that soil fungal attack was a major cause of seed decay. In a study to determine viable seed losses of wild oat, germinated (nonemerged) and dead seeds recovered from soil appeared to be degraded by soil microorganisms (Zorner et al. 1984). An earlier study concluded that the rapid decline in wild oat seed numbers on the soil surface was due to deterioration agents, since the extensive seed depletion could not be explained by germination alone (Banting 1966). Depletion of seeds of proso millet biotypes (*Panicum miliaceum* L.) buried in soil was explained by mortality factors, including decay by seedborne and soilborne microorganisms (Colosi et al. 1988).

Previous observations of fungal growth on decayed seeds suggest that soil microorganisms may attack weed seeds that are already physiologically dead. There are no studies that clearly distinguish between microbial attack causing weed seed death and that occurring after seeds die, even though such a distinction would provide insight into the overall importance of microorganisms in seed mortality. It is possible, however, that soil microorganisms preferentially colonize and decay weed seeds that are inferior in quality due to increased nutrient exudation from damaged membranes and reduced enzymatic activity within seeds, and consequently inhibit germination (Harman and Stasz 1986).

MICROBIOLOGY OF WEED SEEDS

The previous seed bank studies allude to microbial relationships with weed seeds and suggest that microorganisms are partly responsible for seed mortality. However, the studies were conducted in such a way that isolation and characterization of microorganisms associated with buried seeds were not possible. Thorough investigations of microbial associations are necessary to fully understand ecological relationships between weeds, crops, and soil environments and thereby increase our effectiveness in dealing with weeds. Considerable knowledge has been attained regarding shifts in weed populations resulting from different cultural practices and herbicide use. In contrast, very little is known about how those same practices affect the impact of microorganisms on weeds. Perhaps some factor imposed in a seed bank study caused a segment of the microbial population to attack seeds of one or more weed species that resulted in observed seed depletion

simply described as "mortality." Seed bank studies should be designed to investigate *all* aspects of seed depletion to gain a full understanding of factors involved and to assess microorganism-chemical-seed interactions as possible bases for alternative and augmentative weed management.

Characterization of seedborne microorganisms can yield valuable information on unique properties that may affect persistence and deterioration resistance of weed seeds in soil. A collection of such microorganisms can be developed as a source of agents useful for indirect attack of seeds under certain cultural practices or for direct attack as biological control agents applied to soil. For example, Kirkpatrick and Bazzaz (1979) quantitatively determined the seedborne fungi of four annual weed species and found that several strains affected seed germination and seedling development. Kremer et al. (1984) reported that velvetleaf seeds consistently possessed an association of sporulating fungi composed of *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum purpurascens* and *Fusarium* spp., which had little effect on seed viability. A study carried out in India found that fungi associated with weed seeds may cause infection in a manner similar to that for crop seeds (Mishra and Srivastava 1977). Several fungi occurring in field-grown sunflower (*Helianthus annuus* L.) seeds possessed mycotoxic and seed-deteriorative properties (Roberts et al. 1986).

Seedborne bacteria of five species of annual weeds were found to possess an array of diverse properties (Kremer 1987). Prevalence of bacteria in seeds ranged from 0.8% for Pennsylvania smartweed (*Polygonum pennsylvanicum* L.) to 44% for jimsonweed (*Datura stramonium* L.). Distribution of bacterial types varied among the weeds, with 21 species associated with velvetleaf and only one species with Pennsylvania smartweed (*Bacillus* sp.) and cocklebur (*Xanthium strumarium* L.) (*Erwinia herbicola*). Approximately 15% of all bacterial isolates exhibited potential phytopathogenicity, suggesting that germination and initial seedling growth might be detrimentally affected by seedborne bacteria. These cultures are currently under investigation for potential biological control of weed seeds and seedlings.

Very few studies have been conducted of microorganisms directly associated with weed seeds in the soil environment. Seedborne fungi of green foxtail [*Setaria viridis* (L.) Beauv.] and giant foxtail (*S. faberii* Herrm.) were able to persist as colonists of seeds after incorporation into soil in the field (Pitty et al. 1987). In addition, seeds were colonized by two types of fungi occurring only in soil. One isolate completely inhibited germination and deteriorated the seeds, indicating a potential for biological control. Fungal infestations of wild oat seeds included several species that were not consistently pathogenic toward wild oat in seed bioassays (Mortensen and Hsiao 1987). It was concluded that reduced seed viability previously observed in the

field could not be explained entirely by seedborne fungi. In another study with wild oat, depression of seed germination by both soil and seedborne microorganisms was enhanced in soils of different textures at soil water capacities of 50% or more (Kicwnick 1964). Soil and seedborne microorganisms also affected germination of bull thistle [*Cirsium vulgare* (Savi) Tenore] seeds (van Leeuwen 1981).

The fungi association previously described for velvetleaf (Kremer et al. 1984) persists on seeds in contact with soil during the first 32 d after dispersal from the plant (Kremer 1986a). Soilborne microorganisms including potential seed pathogens were unable to colonize the seeds, primarily due to antagonistic activity of seedborne fungi and bacteria. It was concluded that microorganisms inhabiting velvetleaf seeds and soil are restricted to their respective microenvironments, and limited seed decay is initiated by specific seedborne microorganisms.

Although limited to only a few weed species, the foregoing reports indicate that microorganisms associated with seeds prior to and upon entry into soil may contribute to depletion of at least a portion of the seed bank. However, a significant portion of viable and dormant seeds continue to persist and periodically germinate and thus sustain annual weed infestations. Reliable and effective approaches for managing weed seed persistence with microorganisms can be developed only as deterioration resistance mechanisms of weed seeds in soil are characterized.

DETERIORATION RESISTANCE OF WEED SEEDS IN SOIL

Only a small portion of weed seeds in soil succumb to microbial attack. A greater number persists for many years in the presence of millions of soil microorganisms that apparently are unable to exploit the potentially rich nutrient sources within the seeds. The most important component in resistance of seeds of many crop species to deterioration by pathogens is the seed coat (Hallowin 1983). Although resistance in weed seeds is thought to occur in a similar manner, very little quantitative information exists, especially for seed banks in soil. Seeds of weed species apparently rely on complex deterioration resistance mechanisms composed of several factors. Velvetleaf, a representative species possessing the hard-seeded trait, serves as an example. The physical structure of the seed coat consists of a continuous layer of densely packed palisade cells, which resists direct penetration by soil fungi (Kremer et al. 1984). Histological and chemical analyses of the seed coat revealed phenolic compounds present in palisade layers and in the chalazal end of the seeds. Subsequent assays demonstrated that these compounds diffused from intact seeds to inhibit 58% of the bacteria and all fungi tested (Kremer 1986b). The inhibitory activity was greatest with hard, intact seeds, suggesting that

diffusion of antimicrobial substances from these seeds into microhabitats in soil might limit or inhibit potential seed decomposers. The inhibitory substances were later shown to include flavonoids highly active against microbial growth (Paszowski and Kremer 1988). The presence of phenolic compounds in seed coats as a general means of protection is not surprising, since these chemicals are broad-spectrum toxins to microbes (McKey 1979). An additional factor involved in deterioration resistance was the presence of antagonistic, nonpathogenic bacteria located within the velvetleaf seed. Many of these bacteria were found in the chalazal area of the seed, which is the only discontinuity in the palisade layer of the seed coat, and they were capable of releasing antifungal substances presumably inhibiting attack by seed pathogens (Kremer et al. 1984).

Very little information on deterioration resistance mechanisms of other weed species in seed banks is available. Literature on microbial relationships with crop species must be relied upon to explain similar occurrences with weeds. Seeds of some cultivars of oat (*Avena sativa*, L.) and barley (*Hordeum vulgare*, L.) are highly resistant to certain fungi that associate with them. Two types of inhibiting factors were found: (a) the outer seed covering (hull), which provided a mechanical barrier to direct fungal penetration; and (b) several phenolic acids that possessed antifungal activity (Picman et al. 1984). It is possible that similar deterioration resistance in other grass species may function in seed longevity in soil. The recovery of intact and apparently decay-resistant yellow foxtail [*Setaria glauca* (L.) Beauv.] seeds after 4 yr of burial in soil might be explained by these mechanisms (Dawson and Bruns 1975). Lectins, highly specific sugar-binding proteins and glycoproteins common to many seeds, have been implicated in resistance of seeds to microbial invasion and decomposition (Brambl and Gade 1985). In vitro assays showed that purified seed lectins specifically bound to germinating spores of three fungal species and subsequently disrupted cellular growth. Based on these results, Brambl and Gade (1985) suggested that lectins in seeds may provide an additive or more diversified defense against invading microorganisms in soil.

It is not fully understood how physiologically dormant seeds resist microbial decay. Hallowin (1983) cites some examples where dormant, imbibed seeds might be protected through production of anoxic microenvironments or phytoalexin synthesis in the spermosphere.

Although our understanding of deterioration resistance mechanisms of weed seeds in the seed bank is limited, enough information exists to begin devising strategies to overcome barriers to microbial attack and determine the feasibility of the strategies as components of weed management systems. Several strategies must be tested since it is unlikely that one remedy will predispose all weed species in a given seed bank to microbial attack.

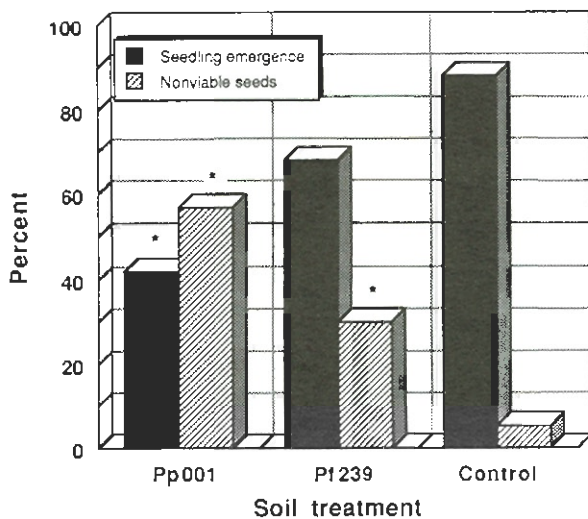


FIG. 1. Effect of soil inoculation with two bacterial isolates on seedling emergence and seed viability of velvetleaf. *Indicates significant difference ($P \leq .05$) from control, based on LSD. Data from Begonia (1989).

APPROACHES TO WEED SEED BANK MANAGEMENT WITH MICROORGANISMS

Stimulation of dormant seeds to germinate as a method to eliminate the persistent portion of the seed bank has received the most attention in modeling and planning new weed management strategies. Breaking of dormancy and stimulation of germination are two different processes, which are rarely manipulated by the same chemical treatment (Egley 1986). In nature, soil microorganisms may not be a significant factor in breaking seed dormancy, primarily due to deterioration resistance mechanisms described previously (Baskin and Baskin 1989). However, dormancy in some species may be broken in the presence of selected fungi that secrete enzymes to soften the impermeable seed coat (Gogue and Emino 1979). Kremer et al. (1984) observed that when the hard seed coat was punctured and velvetleaf allowed to germinate in the presence of seedborne fungi, >60% of the seeds decayed and >50% of the developing seedlings were attacked by the fungi. Fractures of velvetleaf seed coat that occasionally occur under field conditions (LaCroix and Staniforth 1964) may present infection courts for soilborne and seed surface microorganisms. The ability of microorganisms to invade and attack seeds via seed coat openings demonstrates the potential for reduction of the seed bank by microbial decay if a feasible method for surmounting the seed coat barrier can be developed.

Examples of some approaches under investigation that use microorganisms for eliminating persistent seeds are described below. Together, the data from these studies offer evidence for potential depletion of the seed bank by incorporating microorganisms in weed management strategies.

Application of selected microorganisms

Begonia (1989) used inocula prepared with bacteria isolated from velvetleaf rhizospheres to reduce velvetleaf emergence in greenhouse and field studies. The bacterial isolates, Pp001 and Pf239, significantly ($P \leq .05$) reduced velvetleaf seedling emergence (<55%), decreased seedling vigor, and increased the nonviable portion of seeds in soil compared to the noninoculated treatment (Fig. 1). Thus, inocula applied to soil prior to seedling emergence were effective in attacking weed seeds and could be used to hasten seed bank depletion. In addition, isolate Pp001 consistently inhibited root development of those seedlings that did emerge. Both bacteria attained high populations in the spermosphere, thereby sustaining antagonism of velvetleaf seed activity. The deleterious effects on seed viability and seedling growth are consistent with phytotoxin activity of selected bacteria on various weed seedlings (Kremer et al. 1990). It should be noted that a bacterial isolate originally developed to inhibit seedling growth of downy brome (*Bromus tectorum* L.) in winter wheat also decreased viability of seeds in soil, indicating a potential for depleting the seed bank of this weed (Kennedy et al. 1991).

In contrast to direct penetration of seeds, bacteria and fungi can produce an array of metabolites that are toxic to seeds and which can be absorbed by the seed embryo without invasion by the microorganisms (Harman 1983). Use of microbially produced toxins may be an alternative means of indirectly attacking weed seeds possessing impermeable seed coats. Selected microorganisms applied to developing seeds on plants in the field can readily infect them and form toxins prior to development of seed coat impermeability during seed ripening (Hallowin et al. 1991). Toxins produced by selected soil actinomycetes inhibit *in vitro* germination of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.]; however, toxin effectiveness in soil has not been demonstrated (DeFrank and Putnam 1985). The potential of this approach in depleting the seed bank will be realized only after factors affecting microbial toxins and weed seed viability in soil are fully elucidated.

Selection of seed-attacking microorganisms possessing certain key properties should further improve their effectiveness in soil. An ideal combination would be selective attraction of microorganisms (chemotaxis) to weed seeds by exudates diffused from intact seeds or released during germination, rapid seed colonizing ability, and toxin production. In a study investigating potential chemotaxis of selected bacteria to velvetleaf seeds in soil (Begonia 1989), isolate Pp001 actively migrated from an inoculation site in soil to the seed placement site (distance = 2 cm) within 24 h (Fig. 2). The bacterium was not detected at the same distance from the inoculation site in soil without seeds until 72 h post-inoculation. Selective attraction to seeds via

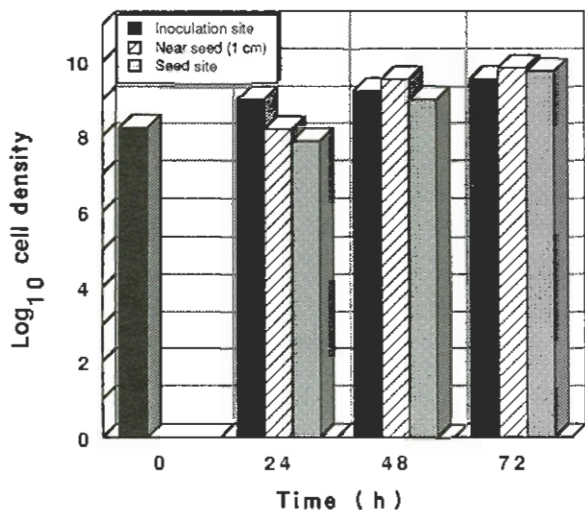


FIG. 2. Chemotaxis of bacterial isolate Pp001 toward velvetleaf seeds in soil (density measured as cells per unit soil dry mass). Bacterial inoculum was dispensed in soil (contained in a culture dish) 2 cm from seed placement. Soil was sampled daily and bacterial populations determined to monitor movement toward seeds. Data from Begonia (1989).

chemotaxis may not only be a competitive advantage in the soil environment but may also allow introduced microorganisms to establish in the spermosphere quickly and invade the target seed.

Integration of microorganisms with chemicals

Kremer and Schulte (1989) reviewed several studies that described the effects of soil-applied pesticides and other plant-growth-regulating chemicals in stimulating germination and emergence of dormant seeds in vitro and in soil. It was also noted that certain chemicals also enhanced seed and seedling root infection of crop plants by either phytopathogenic or saprophytic soil microorganisms. Based on this information, several chemicals were tested alone or combined with a selected fungus (*Fusarium oxysporum*) for effects on seed imbibition, germination, and seedling growth of velvetleaf in soil. Seedling emergence was reduced 15% compared to nontreated controls when ethephon, a germination stimulant, or carbofuran, an insecticide, was applied to soil with the fungus (Fig. 3). This was accompanied by 23 and 17% increases in nonviable seeds caused by ethephon and carbofuran, respectively. Butylate, a herbicide, and "AC94377," a substituted phthalimide and germination stimulant, also significantly ($P \leq .05$) increased nonviable seed content. Nonviable seed numbers were significantly increased with addition of the fungus alone and were increased even more when the fungus and chemical treatments were combined. Nonviable seeds recovered from soil treated with chemicals plus the fungus were heavily infected with mycelia. Increases in nonviable seed and decreases in seedling emergence were apparently due

to effects of chemical-fungus interactions on imbibing seeds, since the hard seed content of the soil was not significantly reduced by any treatment combination (Fig. 3). The chemicals used in this study apparently promoted fungal attack of imbibing and germinating velvetleaf seeds by stimulating nutrient exudation from seeds (Kremer and Schulte 1989). This study demonstrated the potential of an integrated approach of com-

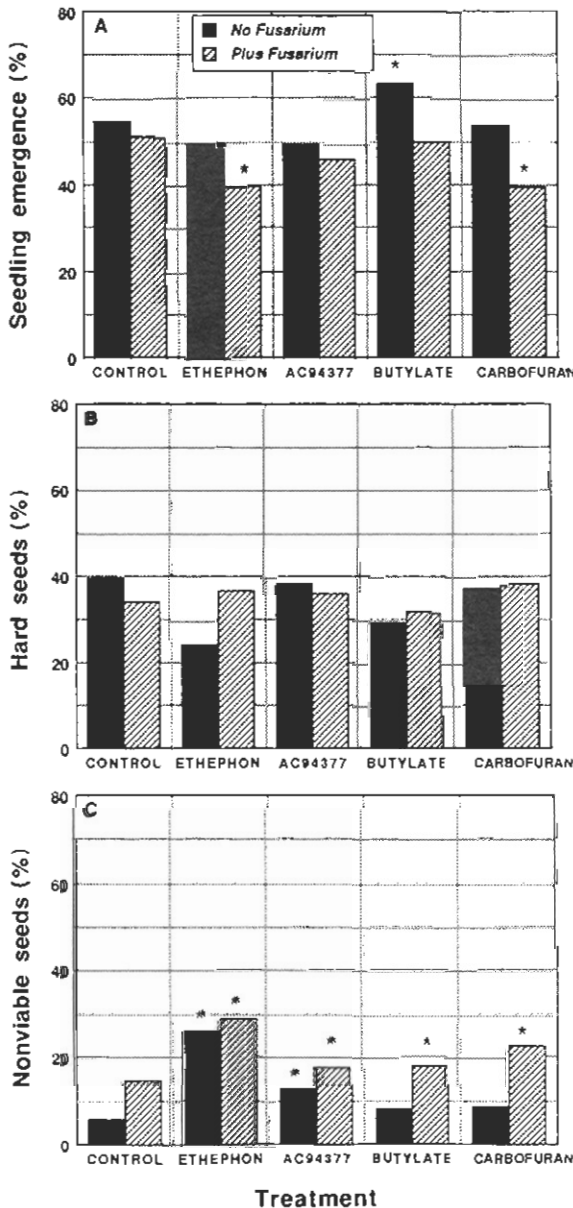


FIG. 3. Seedling emergence (A) and prevalence of hard seed (seeds having coats impermeable to water and to gases) (B) and nonviable seed (C) components of greenhouse-grown velvetleaf as influenced by soil-applied chemicals with and without *Fusarium*. *Indicates significant difference ($P \leq .05$) between treatment combination and comparable control. Modified from Kremer and Schulte (1989).

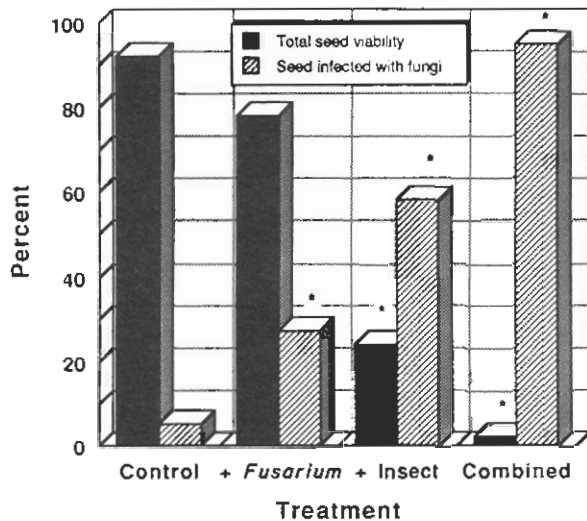


FIG. 4. Effects of *Niesthrea louisianica* (Hemiptera) infestation and *Fusarium* application alone and combined on viability and fungal infection of velvetleaf seeds produced during the 1990 growing season. *Indicates significant difference ($P \leq .05$) between treatment combination and comparable control. R. J. Kremer, unpublished data.

binning low rates of soil-applied chemicals and selected microorganisms for depletion of seed banks.

Integration of insects with microorganisms

Cultivation and herbicide use are bases for current weed management in crop production, yet some weeds continue to escape control measures. Escapes are likely the most difficult problem confronted in weed management, since these weeds contribute significant numbers of viable seeds to the seed bank but do not pose enough of a threat to the growing crop to warrant control (Aldrich 1984). Reduction of seed viability on the plant before dispersal to soil might be accomplished using biological agents. There are few examples of weed seed control using this approach. The potential usefulness of this method was illustrated in a study of the fate in soil of puncturevine (*Tribulus terrestris* L.) seeds that had been attacked by a selective seed-feeding weevil (Goeden and Ricker 1973). Infection by soil microorganisms killed the majority of weevil-damaged seeds, even though the loss of intact, undamaged seeds was low in all soils and at all depths studied. There are limited reports of predation of seeds on the soil surface by insects and subsequent reduction of germinating seeds (Brust and House 1988), but possible contributions of microorganisms to seed deterioration in this situation are not known.

A selective seed-feeding insect (*Niesthrea louisianica*; Hemiptera; Rhopalidae) that attacks immature velvetleaf seeds on the plant has been extensively investigated as a means of reducing viable seed production (Kremer and Spencer 1989a, b). Insect feeding reduced velvetleaf seed viability to 15.5% compared to 95.5%

for insect-free seeds. The insects also enhanced microbial infection in seeds up to 98% compared to 8% infection for nonattacked seeds. When seeds were buried in soil, seed viability continued to decrease to $\approx 2\%$ after 24 mo. These results prompted a subsequent study examining the integrated use of the seed-feeding insect and fungi selected for high pathogenic activity toward velvetleaf seeds. Developing seed capsules on velvetleaf plants in the field were sprayed with suspensions of selected fungi (*Fusarium* spp.). After application of fungi, insects were released onto half of the plants (25–50 adults and nymphs/plant) contained in cages and allowed to feed throughout the growing season. *Fusarium* spp. applied to seeds alone had little impact on viability. Insects attacking seeds without *Fusarium* spp. reduced viability to 25%, and increased infection with naturally occurring fungi to 58% (Fig. 4). Combined fungal application plus insect feeding further reduced seed viability to $< 2\%$, and infection with the selected fungus increased to 98%. These preliminary results indicate that integration of compatible biological agents may be an effective method for reducing seed viability before entry into the seed bank. Thus, pre-dispersal seed mortality, an important process in seed bank dynamics of natural ecosystems (Janzen 1971), might be exploited as an effective method for manipulating the seed bank in agricultural systems.

Manipulation of soil environment to favor microbial attack of weed seeds

Several soil environmental factors affect weed seed germination in the field (Egley 1986). Reduction of weed seed populations by manipulating these factors to enhance germination has received considerable attention due to the nonchemical nature of the approach. For example, soil solarization, a method by which soil temperatures of 40–70°C are attained near the soil surface by covering the soil with plastic, can reduce weed emergence and seed viability (Egley 1983). Viable seed reduction of several weed species by thermal death was most effective in moist soil at 70°C (Egley 1990). Even though solarization reduced seed numbers, it was evident that the seed bank could not be completely eliminated, since sustained temperatures of up to 70°C were not practical under field conditions and seeds of some species exhibited heat tolerance. Most of the observed losses in viability were attributed to seeds stimulated to germinate that died prior to emergence. However, decay of a portion of these seeds by soil- or seedborne microorganisms could not be ruled out (Egley 1983).

Certain soil- and seedborne microorganisms are metabolically active at high temperatures. Under these conditions, growth and enzymatic activities (i.e., pectinase, amylase) of bacteria inhabiting seeds can be enhanced. Soybean seeds were induced to imbibe at high temperatures with simultaneous release of nutrients allowing *Bacillus* spp. to readily attack seed tissues, which resulted in seed decay (Schiller et al. 1977).

No similar work on temperature-microorganism effects on weed seed viability has been conducted. These relationships might be exploited for reducing seed banks where soil temperatures can be modified using methods such as solarization.

In a preliminary investigation of these relationships, seeds of three weed species, velvetleaf, morning-glory, and jimsonweed, were incubated at different temperatures on water agar for 4 d. Seed germination was greatly inhibited at 41°C for all species and also at 34°C for jimsonweed (Fig. 5). The proportion of seeds with bacterial infection increased with increasing temperature to 34°C, and then declined at 41°C. As temperature increased, the incidence of *Bacillus* spp. increased and a concomitant decrease in total viable (germinating + hard) seeds occurred. Heat-induced physical and physiological changes in hard and germinating seeds may have predisposed seeds to attack by rapidly growing bacteria. Selection of heat-tolerant bacteria with certain properties (i.e., enzyme and seed toxin production) combined with solarization might enhance seed-killing effectiveness. Solarized soils are readily colonized with microorganisms applied after treatment, as demonstrated for selected biocontrol agents of plant pathogens, due to reduced competition from indigenous soil organisms (DeVay and Katan 1991). Weed seeds surviving solarization may be weakened and more susceptible to microbial attack. Microorganisms applied immediately after solarization might therefore enhance reductions of viable seeds remaining in soil. That seed viability was reduced by bacteria at temperatures lower than those of effective solarization (Fig. 5) indicates that selected bacteria might also be applied below surface plant residue mulches that only moderately increase soil temperatures.

Little work has been conducted on the relationship of other soil factors affecting weed seed deterioration in soil. The complex nature of soil properties influences the size and composition of microbial populations. Factors having the greatest effect on microbial growth and activity also have greatest impacts on decomposition in soil (Parr and Papendick 1978). As mentioned previously, microorganisms are associated with seeds prior to dispersal from the parent plant. Very little is known of the influence of resident microorganisms on seed decomposition at the soil surface and their interactions with soil microorganisms after incorporation into soil. Investigation of how soil environmental factors interact with potential weed seed decomposers is critical to an understanding of seed decomposition dynamics and to development of strategies for manipulating soil-seed-microorganism relationships for maximum seed depletion. For example, soil moisture and organic matter were implicated as major factors affecting deterioration of seeds of various weed species during 20 yr of burial in undisturbed soils (Lewis 1973). Recent work suggests that organic and inorganic substances accumulated in the soil surface layer in long-

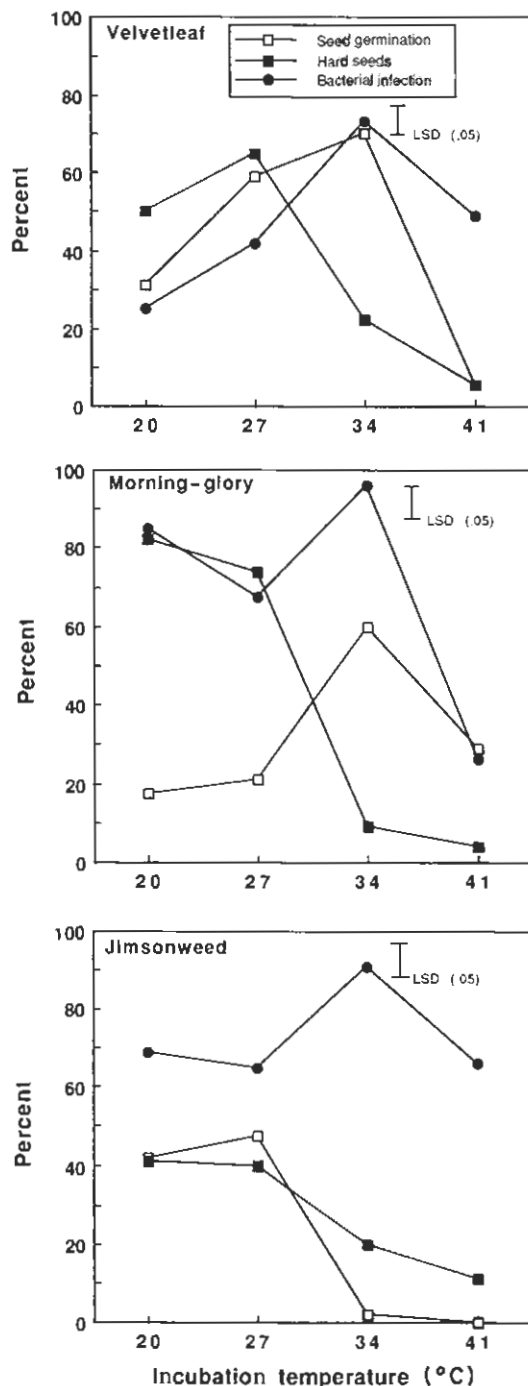


FIG. 5. Seed germination, hard (impermeable) seed component, and bacterial infection of seeds of three annual weed species as affected by temperature. R. J. Kremer, unpublished data.

term no-till fields create unique environments high in biological activity ideal for proliferation of weed seed predators and pathogens, which can cause shifts in weed composition and population dynamics (Cardina et al. 1991). The consideration of methods for manipulating soil and cultural factors to enhance weed seed decom-

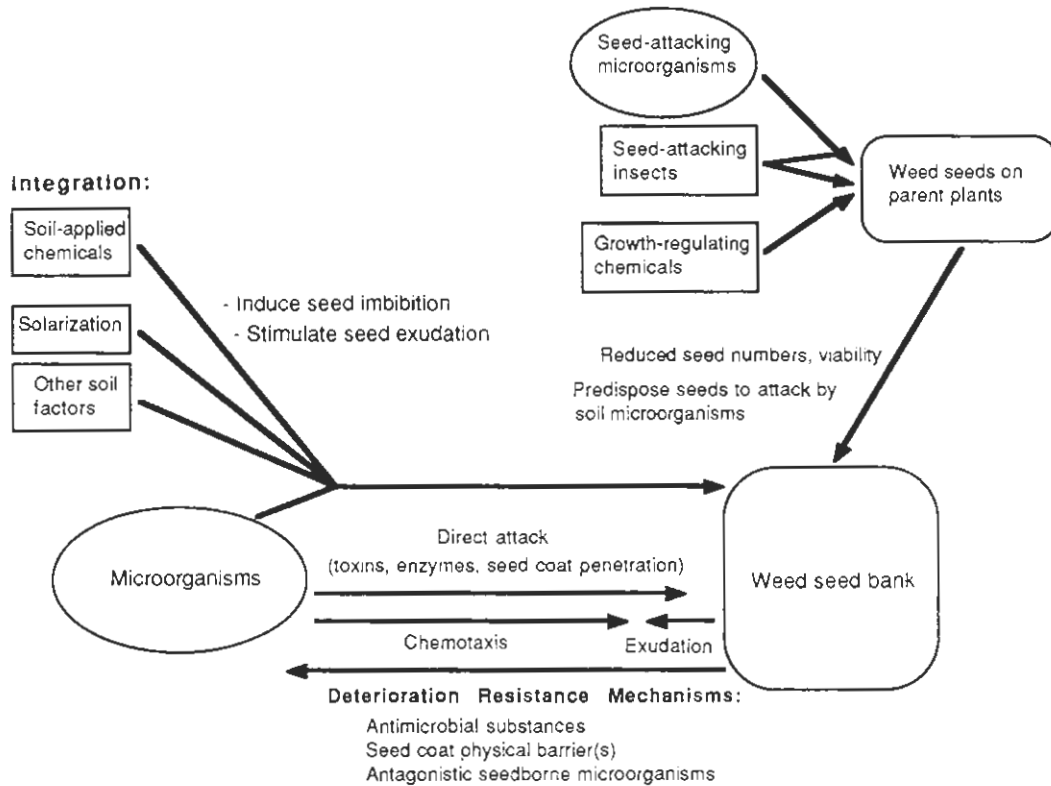


FIG. 6. Relationships of microorganisms and weed seeds in the soil environment. Several methods for depleting the weed seed bank with potential for integration with an approach including microorganisms are indicated.

position is intended to stimulate the development of novel strategies for weed management and not to imply that every approach will be successful. However, all possibilities should be pursued.

SUMMARY: OUTLOOK FOR THE FUTURE

Studies have shown that a small proportion of highly resistant seeds persist in soils despite eradication efforts. These persistent seeds are the source of continuing weed problems and their elimination will be a difficult task. Research to solve the weed seed problem has concentrated on physical and chemical means of breaking seed dormancy to stimulate germination and has largely ignored the use of microorganisms to increase seed mortality.

It is unlikely that a single microorganism will be able to selectively seek out weed seeds in the soil, overcome their protective mechanisms, and kill them before emergence. However, there are several approaches by which microorganisms can likely be integrated into weed management strategies (Fig. 6):

- 1) Application of selected microorganisms or their metabolites (i.e., phytotoxins) to soil or to weeds prior to seed dispersal to enhance current weed management practices;

- 2) Application of low rates of herbicides or growth-regulating chemicals to predispose seeds and seedlings

to attack by applied and/or soilborne microorganisms (Kremer and Schulte 1989);

- 3) Integration of microorganisms with novel seed bank depletion methods including chemical germination stimulants and soil solarization (Egley and Duke 1985, Egley 1986, DeVay and Katan 1991);

- 4) Modification of weed management practices to increase the effectiveness of naturally occurring biological agents including seedborne fungi (Roberts et al. 1986) and seed-feeding insects (Goeden and Ricker 1973, Kremer and Spencer 1989a, b).

It is apparent that research on microbial factors affecting seed banks is in its infancy, with many approaches described only in hypotheses. Exploitation of ecological aspects of weed seed-microorganism-soil environment relationships may lead to real progress toward effective weed management systems. Weed biologists, ecologists, and microbiologists need to pool their efforts in devising integrated strategies to enhance the possibility of success in managing weed seed banks.

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