Interaction of Insects, Fungi, and Burial on Velvetleaf (*Abutilon theophrasti*) Seed Viability[1]

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Abstract. A scentless plant bug feeds on velvetleaf seeds. Fungi, dominated by the genera *Fusarium* and *Alternaria*, were isolated from insect-attacked seeds at levels related to insect density on the plants. The combined effects of insect feeding and fungal infection decreased seed germination. Burial of insect-attacked seeds in soil for 24 months reduced seed survival and increased *Fusarium* infection. Decreases in velvetleaf seed viability and survival in soil caused by a seed-feeding insect and associated seed fungi suggests that subsequent infestations by velvetleaf can be decreased through integrated use of the two biological control agents. Nomenclature: Scentless plant bug, *Niesthrea louisianica* Sailer; velvetleaf, *Abutilon theophrasti* Medik. #[3] ABUTH.

Additional index words: Biocontrol, seedborne microorganisms, buried seed, seed longevity, ABUTH.

INTRODUCTION

Biological control of weeds may be most successful when targeted at eliminating viable seed production by integrating both selective seed-attacking insects and seedborne pathogenic microorganisms. Organisms that attack propagules generally are highly effective for biological control of annual weeds (7). *Niesthrea louisianica*, a native scentless plant bug, extensively decreases seed production of the economically important weed, velvetleaf.

Only a limited number of other malvaceous species are known hosts of the scentless plant bug, and development of the insect on these plants is incomplete or considerably slower than on velvetleaf (8). Thus, crop plants in the same field with velvetleaf are not damaged by this insect. The insect, which feeds on immature and developing seeds by probing with a flexible hollow stylet, can decrease seed production and viability of velvetleaf by 74 and 60%, respectively (15). Often, insect-damaged seeds are infected with numerous fungal pathogens, which apparently contribute to decreases in seed viability (10). Similarly, insect-feeding on crops has been implicated in the transmission of numerous seedborne fungal pathogens (1).

Many weeds persist in crop production fields because of their prolific production of viable seeds that survive in soil for many years (2, 6). For example, the predicted burial time in soil to reduce velvetleaf seed germination to 1% was 12 yr (6). Although cultivation and herbicides are used to control weeds in crops, some weeds escape these control measures to mature and to produce viable seeds.

Weeds escaping control can be managed by herbicides applied postemergence. However, the additional expenses involved in chemicals and their application and potential crop injury often do not justify these measures (3). Therefore, biocontrol methods for decreasing seed viability or for eliminating seed production by weeds growing in crop fields are attractive and economic alternatives to conventional methods.

The objective of the overall research program is to develop management systems using inexpensive biological control practices for weeds that escape conventional control. Specific objectives of the study presented here were to determine the interactions between two different organisms, the scentless plant bug and seedborne fungi, in decreasing seed viability of velvetleaf and to examine the longevity and fungal infection of insect-attacked seeds during burial in soil.

MATERIALS AND METHODS

Velvetleaf plants were established in the field at Stoneville, MS, and Columbia, MO, in the spring of 1985. Scentless plant bugs were reared on moistened velvetleaf seeds in the laboratory at Stoneville, MS (8). Screen cages 90 by 90 by 180 cm were

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[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.

placed over velvetleaf plants in monoculture at the Mississippi site June 30, 1985. The cages were infested with 0, 25, or 50 winged scentless plant bug adults and late instar nymphs per plant when the plants were beginning to produce seeds. Each treatment was replicated six times with each cage containing a single plant. At the Missouri site, screen cages 180 by 180 by 180 cm were placed over flowering velvetleaf established in soybean [*Glycine max* (L.)Merr.] plots. The cages were infested with 0 or 10 winged scentless plant bug adults and late instar nymphs per plant July 12, 1985. Each treatment was replicated three times with each cage containing 20 plants.

Seeds were harvested from plants as they matured during the growing season and were assayed for viability (germination) and fungal infection. Triplicate samples of 200 seeds from each treatment were divided into 10 lots of 20 seeds each and were surface sterilized (1 min immersion in 70% ethanol followed by sterile water rinses). Each seed lot was placed on autoclaved filter paper (Whatman No. 3) moistened with 6 ml of sterile malt-salt solution (7.5 g sodium chloride and 15 g malt extract in 1 L distilled water, pH 6.8) in glass petri dishes. This technique allowed for germination of all viable velvetleaf seeds and coincident development of natural groupings of fungi most likely involved in seed infection.

After incubation at 28 ± 1 C for 6 days, seed viability was recorded to include germinated seeds (radicle protrusion >1 mm) plus hard (impermeable) seeds. Hard seeds were considered viable because previous trials showed that essentially all hard seed produced normal seedlings when tested for germination after seed coats were scarified. Fungi developing on seeds, as indicated by the presence of mycelia covering the seed surfaces, were examined using a stereomicroscope with magnifications up to 50X and were identified using procedures outlined previously (11).

Additional seeds were selected at random from each treatment for examination under SEM[4]. These specimens were dried by the critical point method in liquid carbon dioxide, were mounted on SEM stubs, and were sputter-coated with Au:Pd alloy. They were examined and were photographed with a JEOL 35 U scanning electron microscope[5] operating at 20 kV.

To explore the effects of insect feeding and fungal infection on seed survival, seed samples were buried in soil in the field. This simulates what occurs in the field when mature weed seeds, dispersed from parent plants, become incorporated into soil by natural means and cultural practices. A random selection of 900 seeds from each treatment from both the Mississippi and Missouri sites was divided into nine lots of 100 seeds each. Each seed lot was mixed with sieved soil taken from the upper 10 cm of a Mexico silt loam (fine, montmorillonitic, mesic Udollic Ochraqualf). This mixture was placed in polypropylene mesh bags. The 6- by 10-cm bags were prepared from fiberglass window screen and nylon thread.

The bags were buried 10 cm deep in February, 1986, at the University of Missouri Agronomy Research Center near Columbia. The study contained three replications arranged as a randomized complete block with sufficient seed bags for three recovery dates per seed infestation level. The soil at the burial site was a Mexico silt loam of pH 5.5 and 1.2% organic matter. A tall fescue (*Festuca arundinacea* Schreb. # FESAR) sod was maintained at the study site. Bags containing the seeds were exhumed at 6, 12, and 24 months after burial and were examined for viability and fungal infection as described above.

Analysis of variance was conducted on all data. Where F-values were significant at $P \leqslant 0.05$ levels, means were compared using least significant difference (LSD) tests.

RESULTS AND DISCUSSION

After introduction onto the plants, the insects fed intensively on flower buds, open flowers, and immature capsules containing developing seeds. Several generations of the insects developed on velvetleaf during the experiment; thus, an insect population (≤100 nymphs and adults per plant) higher than the original infestation was achieved as the season progressed. In general, viability of seeds from all infested plants at harvest from both sites was decreased significantly ($P = 0.05$) compared to viability of seeds from plants not infested

[4] Abbreviations: SEM, scanning electron microscopy.

[5] JEOL U.S.A., Inc., 11 Dearborn Rd., Peabody, MA 01960.

with insects (data not shown). These findings agree with other reports (8, 14, 16), indicating that high densities of the scentless plant bug can effectively reduce viability of seeds produced on velvetleaf plants.

The 10 insects per plant level at Missouri and 50 insects per plant level at Mississippi reduced velvetleaf seed viability by 36 and 24%, respectively, before burial in soil (Figure 1). The lower initial insect populations at Missouri more effectively reduced seed viability than the higher initial populations at Mississippi. This anomaly may be due to the release of insects on flowering plants before seed formation at Missouri while at Mississippi, insects were released on plants actively forming seed capsules.

Previous research with scentless plant bugs on caged velvetleaf indicated that during periods of active seed production, the ability of velvetleaf to produce new capsules was greater than the increase in insect numbers necessary to retard seed viability (15). Thus, as suggested in other studies (10, 16), the timing of insect release with the reproductive stage of velvetleaf development is critical in reducing total viable seeds produced during the season.

The detrimental effects of the scentless plant bug on longevity of velvetleaf seeds were apparent when insect-attacked seeds were buried in soil. Viability of seeds collected in Mississippi infested with 25 and 50 insects decreased significantly (P = 0.05) from 68 and 56%, respectively, at the time of burial to 25 and 10% after 24 months in soil (Figure 1). Similar decreases in viability during seed burial were observed for insect-attacked seeds from the Missouri site, which declined from 52 to 2% after 24 months. Viability of seeds from noninfested plants collected at both sites did not deviate significantly from the 80 to 90% level during the 24-month period. The ability of noninfested velvetleaf seeds to maintain viability in soil at high levels over this length of time has been reported previously (5).

Velvetleaf seeds persist in soil for long periods due largely to dormancy imposed by a hard, impermeable seed coat (12, 13). Initial viability of subsamples of seeds from all treatments stored at 0 C did not decrease significantly over the 24-month period indicating that soil biotic and environmental factors influenced seed decline during burial (data not shown). Also, Egley and Chandler (5) indicated that dormancy of velvetleaf seeds may be reduced during burial, which also could contribute to decreases in viability.

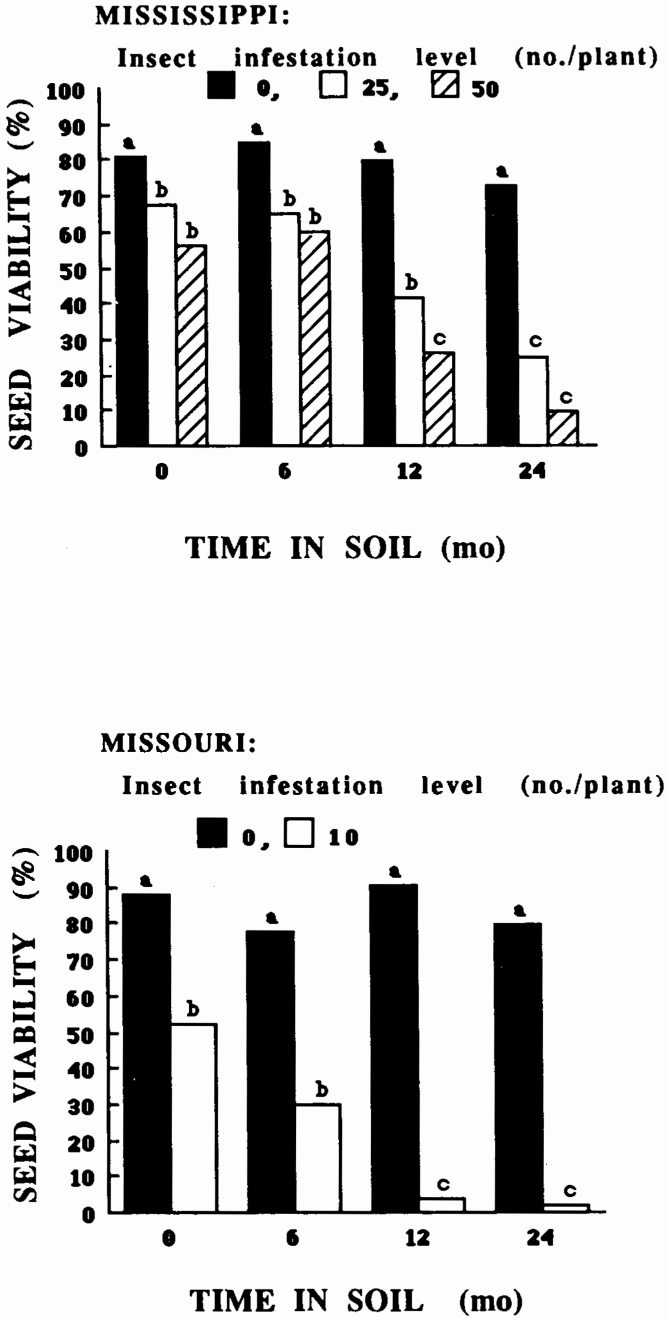
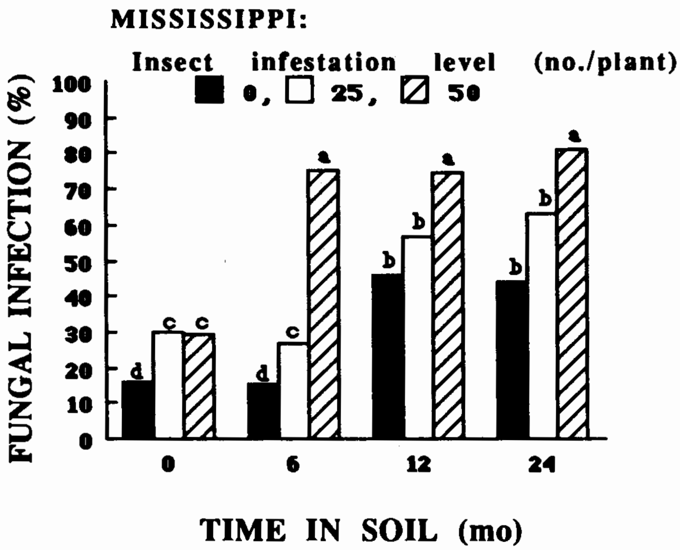


Figure 1. Effects of level of scentless plant bug infestation and location on viability of velvetleaf seeds before and after burial in soil. Bars within a location with the same letter do not differ at the 5% probability level.

Despite the apparent random occurrence of naturally occurring, epiphytic fungi on velvetleaf seeds in the absence or presence of scentless plant bugs, seeds of insect-infested plants were more subject to fungal infection than noninfested plants (Figure 2). Infection by fungi at harvest ranged from about 30% for both infestation levels at Mississippi to 65% for seeds infested with 10 insects per plant at Missouri. Fungal infection of seeds from control (noninfested) plants was no more than 17% at either location, which approximates infection levels in field-collected velvetleaf seeds (11).



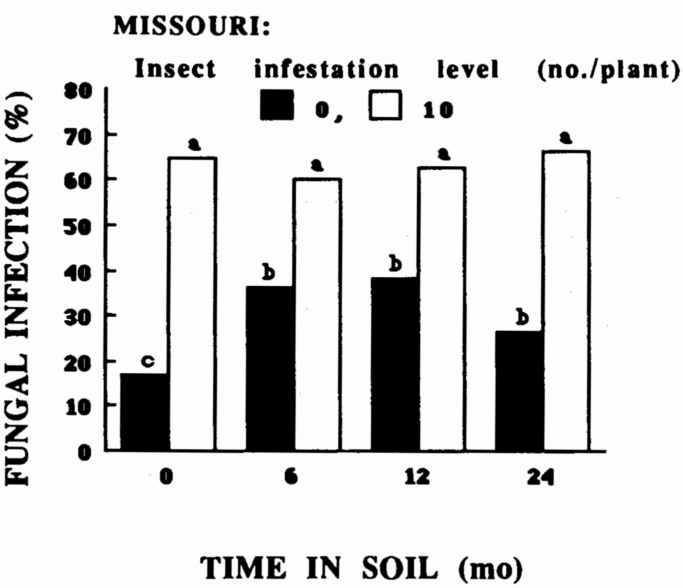


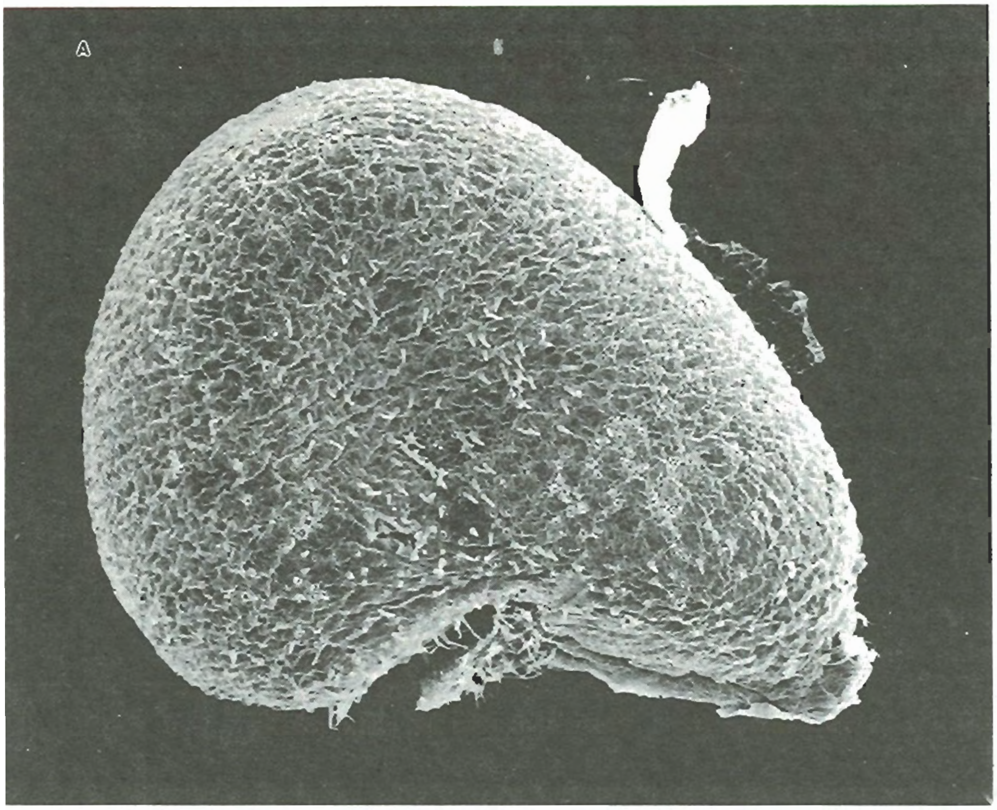
Figure 2. Fungal infection levels of velvetleaf seeds as influenced by infestation levels of scentless plant bug and location before and after burial in soil. Bars within a location with the same letter do not differ at the 5% probability level.

Fusarium, Alternaria, and *Cladosporium* were the most prevalent fungal genera, in that order, associated with insect-attacked seeds (data not shown). The higher incidence of infection also may be attributed to higher numbers of *Fusarium* on insect-infested velvetleaf. The incidence of *Fusarim* increased from 10% in nonattacked seeds to 35% in insect-attacked seeds. Previous studies have shown that *Fusarium* is involved most frequently in attack on weed seeds and often causes the greatest seed deterioration (9, 11).

Fungal infection of seeds also increased significantly during seed burial (Figure 2). This was most evident for the occurrence of *Fusarium* and *Alternaria* in insect-attacked seeds from Mississippi, which increased to 50 and 10%, respectively, for the 25 insects per plant infestation level and to 60 and 20%, respectively, for the 50 insects per plant level after 24 months of burial (data not shown).

Seeds attacked by the scentless plant bug were lighter in color, smaller in size, shriveled, or had sunken areas compared to nonattacked seeds. Seed coats from velvetleaf not infested with the scentless plant bug examined by SEM revealed a smooth, uniform appearance with few microorganisms present (Figure 3A). Seeds attacked by the insects revealed punctured seed coats and development of dense fungal mycelia associated with the damaged seeds (Figures 3B and 3C). These SEM findings strongly suggest that feeding on velvetleaf seeds by the scentless plant bug fractured the seed coats causing loss in viability, increased fungal infection, and contributed to decreased longevity of seed in soil.

Under field conditions and with no further input of seed to soil, an estimated 12 yr are required to deplete the viable velvetleaf seed content in soil to 1% (6). In the present study, viable velvetleaf seed content originating from plants infested with scentless plant bugs could be reduced as low as 2% after 24 months in soil (Figure 2). The simultaneous increased infection of insect-punctured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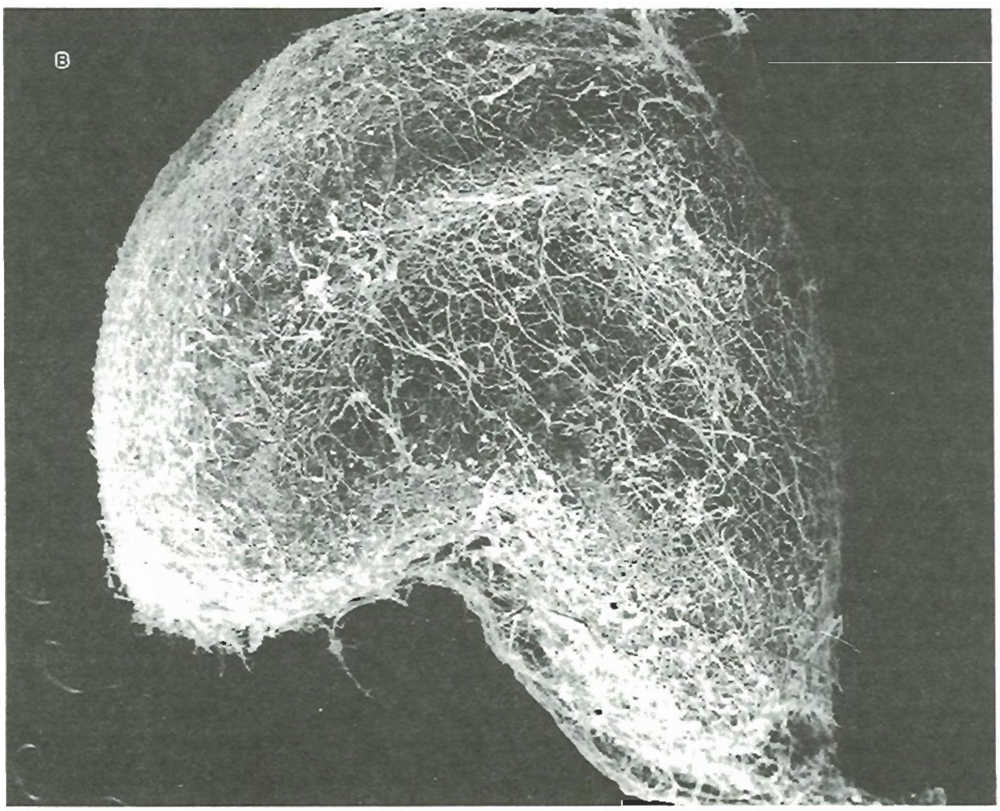
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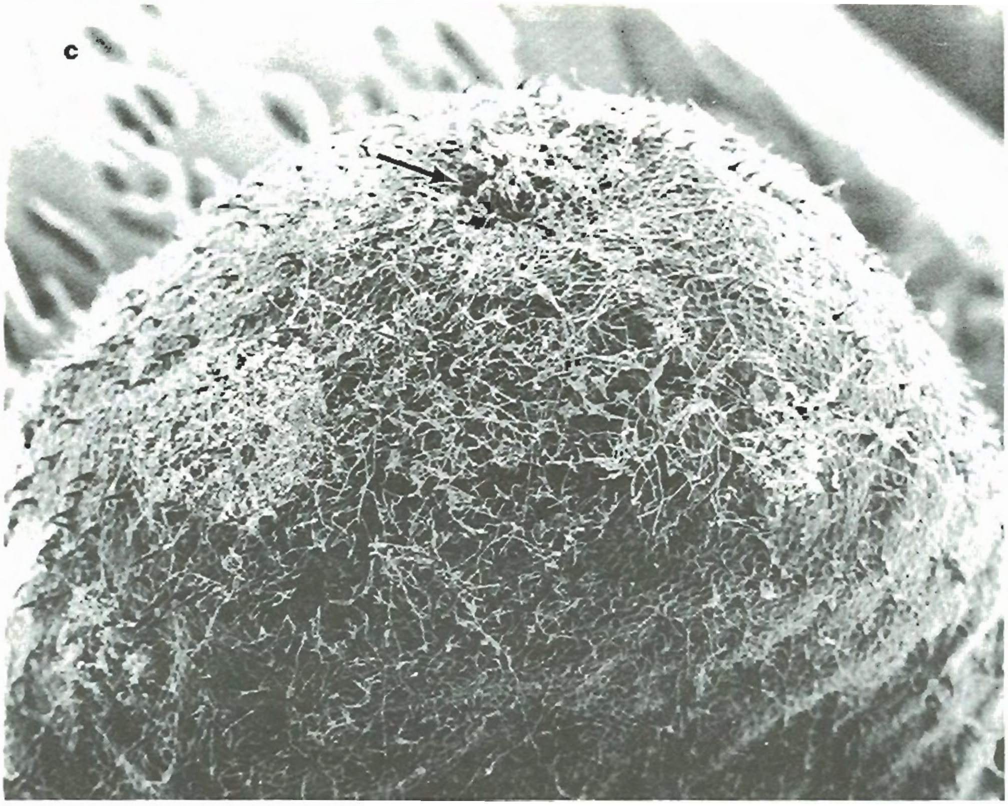

Figure 3. Scanning electron micrographs of mature velvetleaf seeds (A) not attacked by scentless plant bug or fungi, (B) attacked by scentless plant bug and covered with fungal mycelia, and (C) attacked by scentless plant bug showing fungal mycelia associated with seed coat puncture (arrow) resulting from feeding by the insect. Magnification, X68 (A); X75 (B); X100 (C).

seeds with fungi also increased decomposition of seeds in soil. Under field conditions, this could lead to elimination of a high proportion of viable seeds in soil, requiring less time than 'natural' depletion and to enhanced depletion of subsequent weed infestations.

Eliminating weed seeds in the seedbank using microbial agents has been suggested as a desirable method of weed control (11). In situations where seed production by velvetleaf cannot be controlled successfully using conventional methods, the viability and subsequent longevity of these seeds in soil may be decreased considerably using the scentless plant bug in the presence of fungi.

The scentless plant bug possesses features of classical biological control agents (17) including the probability of high success because it attacks and damages seeds (7). It also allows entry of additional agents (fungi), which can contribute to the overall decrease in seed viability and decomposition of seeds in soil. A previous study showed that foliage of the aquatic weed waterhyacinth [*Eichornia crassipes* (Mart.)Solms] was infected more frequently with bacteria and fungi in the presence of two selected arthropods as the primary biocontrol agents and improved control of the weed (4).

An integrated approach of combining potential plant pathogens with selective insects would improve prospects for weed control over the use of only one biocontrol agent. Selecting and/or inducing the occurrence of pathogenic fungi in nature and combining with the scentless plant bug for attack on velvetleaf seed would increase the value of the insect as a potential biological control agent.

The scentless plant bug may be susceptible to insecticides that control insect pests in field crops; and, therefore, the use of this insect may be most feasible in production regions, such as the Midwestern U.S. soybean producing region, where the risk of economic loss due to insect pests is low and where insecticide application is not a common practice (18). This study emphasizes the importance of combined effects of two different organisms for biological control of weeds and has implications for integrated biological control of other important agricultural pests.

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