

Impact of a Seed-Feeding Insect and Microorganisms on Velvetleaf (*Abutilon theophrasti*) Seed Viability¹

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Abstract. Field infestations of a seed-feeding insect developed from overwintered populations reduced viability of velvetleaf seed to 17.5 and 15.5% at two locations in central Missouri, compared to 95.5 and 87.5% at insect-free sites. Insect feeding enhanced the proportion of seed-borne microorganisms in seed up to 98% compared to the average fungal infection of 8% for seed not exposed to the insect. There was a strong negative correlation between fungal infection associated with insect feeding and percent velvetleaf seed viability. The insect transmits microorganisms externally just as pollen is carried by various other insect species and not by ingestion and regurgitation. The effectiveness of the insect on reducing seed viability and seed production in central Missouri is mainly limited by the time required to build up populations capable of significantly affecting early-season velvetleaf seed production. Nomenclature: Scentless plant bug, *Niesthrea louisianica* Sailer; velvetleaf, *Abutilon theophrasti* Medic. ≡³ ABUTH.

Additional index words. Biocontrol, microbial ecology, seedborne microorganisms, seed viability, ABUTH.

INTRODUCTION

Velvetleaf is one of the most troublesome and economically important weeds in row crops throughout the north-central and in most of the southern United States (12, 23). The characteristic of staggered maturation of velvetleaf (10) accounts for the production of high numbers of viable seed during several weeks in the growing season. A high proportion of the seed is dormant (possessing hard and impermeable seed coats) and may remain viable in soils 50 yr or more during which germination occurs gradually (6, 18). The hardseeded attribute is a major factor for the occurrence of velvetleaf as a serious weed of row crops (17).

Velvetleaf often escapes early-season weed control, especially when soil-applied herbicides fail to perform satisfactorily (13). Velvetleaf often emerges late during the growing season and, if not controlled, will not only compete with crop plants for light, moisture, and nutrients but also produce viable seed for addition to the soil seed bank. Methods are needed to reduce the annual accumulation of seed in soil by preventing seed production and reducing seed viability.

Selective postemergence herbicides can be used to eliminate velvetleaf escaping early-season control; however, the expense of the chemicals and application technology required and potential injury to the crop may not be justified because the escapes often do not significantly reduce crop yields (2). An alternative strategy would be to use biological control agents that effectively prevent seed production and/or reduce seed viability.

Microorganisms are potential biological control agents for reducing the input of viable weed seed in soil. Microorganisms able to infect crop seed rapidly establish and metabolize seed contents resulting in decreased seed viability and seedling vigor (8). However, many weed species, including velvetleaf, exhibit very low microbial infection under field conditions because penetration of the seed by surface microorganisms is limited by physical barriers and antimicrobial compounds localized within the seed coats (7, 14, 16). When these barriers in velvetleaf seed were surmounted by rupturing the seed coat, seed surface microorganisms readily infected and decomposed the seed contents (16). Therefore, methods of weakening the seed coat of developing velvetleaf seed in the field could allow microbial penetration and attack of seed before dispersal to the soil.

Recent studies on the native scentless plant bug revealed that this seed-feeding insect reduces velvetleaf seed numbers and viability in controlled greenhouse/environment chambers and on caged plants in the field (11, 21, 24). The insect feeds by penetrating immature capsule walls with a flexible, hollow stylet and imbibes seed contents, probing the developing seed from which it obtains nutrients for growth. Plants of other malvaceous species are the only other known hosts of the scentless plant bug and development of the insect on these plants is incomplete or considerably slower than that on velvetleaf (11).

Wounding and penetration of the velvetleaf seed coat by the scentless plant bug may promote microbial infection by providing ingress points for seed-associated microorganisms. Although several insect-microorganism associations involved in seed deterioration have been reported for field crops (1, 9, 19), no published information exists for such associations on seed of weed plants. Thus, the potential exists for developing a biological control strategy based on integration of the scentless plant bug and selected microorganisms for enhanced control of velvetleaf seed production.

The present study is a portion of a project examining the effects of the scentless plant bug on reproduction of velvetleaf in central Missouri. Although the insect is native from Arizona to Florida north to New York and west to Iowa in the Mississippi Valley, its greatest impact on velvetleaf is limited to the southern United States and it occurs only in sporadic numbers in the Midwest so that no effect

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

on velvetleaf is evident (22). Insects for this study were mass released in July 1985 and subsequently overwintered at release sites where infestations on velvetleaf stands developed during the 1986 and 1987 growing seasons. Consequently, the objectives of the present study were to: a) assess reinfestations of the scentless plant bug developed from overwintered populations at velvetleaf-infested sites in Missouri for effects on seed production; b) determine the relationship between insect feeding and microbial infection of velvetleaf seed; and c) demonstrate the influence of the insect-microorganism relationship on velvetleaf seed production and viability.

MATERIALS AND METHODS

Six velvetleaf-infested sites in soybean fields at each of two locations in central Missouri (Boone and Osage counties) were sampled. The sample sites were established in 1985 near release sites of laboratory-reared scentless plant bugs at each location. The insects were reared at Stoneville, MS, on moistened velvetleaf seed (11). In July 1985, 9000 to 10000 scentless plant bug adults were released on velvetleaf plants growing within a 10-m² area at both locations. A minimum of one insect-free site at each location was established approximately 1 km from the infested sites. In 1985, 20 randomly selected velvetleaf plants were tagged and all seed produced/plant during the season were collected as one sample. In 1986 and 1987, the same sampling scheme was followed in addition to collecting four samples of 15 random mature (brown) seed capsules from other plants at each site every 10 to 14 days beginning in July and continuing over 8 to 10 sampling dates into October. In 1987, the influence of staggered maturity characteristics of velvetleaf (10) on the effectiveness of the scentless plant bug on seed viability and microbial infection was determined by sampling mature seed capsules according to position on eight selected plants for comparison with randomly collected capsules. Also in 1986 and 1987, overwintering populations of the scentless plant bug were monitored for initiation and rate of infestation by transecting in several directions from the 1985 release sites. At each collection date, seed capsule samples from each site were placed in bags, transported to the laboratory, and stored at 0 C until assays were performed. Seed and any scentless plant bugs (nymphs and adults) were separated from the capsules and counted.

Microbial infection and seed viability were determined for all samples. A random selection of 200 seed from each sample was divided into 10 lots of 20 seed each. Each seed lot was placed on autoclaved Whatman No. 3 filter paper moistened with 6 ml of malt-salt solution, MSS⁴, in glass petri dishes (100 × 15 mm). The MSS was modified from Mills et al. (20) and contained 15 g malt extract⁵ and 7.5 g

sodium chloride in 1-L distilled water adjusted to pH 6.8 before autoclaving. This technique favored the germination of all viable velvetleaf seed and coincided with the development of natural groupings of different microorganisms most likely involved in seed deterioration. The petri dishes were placed in a dark germination chamber for 6 days at 28 ± 1 C. After incubation, seed viability was recorded to include germinated seed (radicle protrusion >1 mm) plus hard (impermeable) seed. Hard seed were considered viable because previous trials showed that 99.5% of hard seed produced normal seedlings when tested for germination after scarification. Imbibed seed that failed to germinate were recorded as nonviable.

Fungi developing on seed were examined using a stereomicroscope with magnifications up to 50× and identified to genus using appropriate keys (3, 5). Bacteria developing on seed were subcultured on nutrient agar⁵. The bacteria were characterized as previously reported (15). The frequency of occurrence of all microorganisms on both the viable and nonviable seeds was determined.

Insects (dead after frozen storage) collected with seed capsules were randomly selected and assayed for the presence of microbial propagules associated with the exoskeleton based on procedures modified from Stephenson and Russell (25). Eight to 10 nonsterile or surface-sterilized [1 min immersion in 70% (v/v) ethanol followed by sterile water rinses] insects were transferred to culture plates containing nutrient agar or potato dextrose agar, PDA. Insects were removed from the plates after 15 min and culture plates incubated. Surface-sterilized insects were also assayed for internal microorganisms by aseptically dissecting insects (adults and late-instar nymphs) to remove internal organs, homogenizing the organs in sterile distilled water, and plating on nutrient agar and PDA. All plates were incubated in the dark at 28 ± 1 C for 5 days.

Analysis of variance was conducted on all data for each or combined years. Where F-values were significant at P ≤ 0.05 level, LSD's were calculated for mean separations. When appropriate, linear regression procedures were used to describe relationships between seed viability and microbial parameters. The interaction of sampling date and years (1986 and 1987) within location was nonsignificant for total seed/sample, seed viability, total viable seed, fungal and bacterial infection, or frequency of microorganisms within seed classes; thus, data on these parameters for the 2 yr were combined.

RESULTS AND DISCUSSION

After release of scentless plant bugs in 1985 average populations of 20 to 25 nymphs and adults/plant developed within 4 weeks. The plants were approximately at the R5 to R6 stages of development (10). Feeding insects completely covered the immature capsules. Insects dispersed in all directions from the release center to establish high populations (over 100 nymphs and adults/plant) on velvetleaf within a 10- to 15-ha area. These high populations and feeding intensities (20 to 30 insects/capsule) persisted as the growing

⁴Abbreviations. MSS, malt-salt solution; PDA, potato dextrose agar.

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Table 1. Effects of the scentless plant bug on mean viability and fungal infection of total velvetleaf seed produced during the growing season at two release sites in Missouri during 1985, 1986, and 1987^a.

Location	Year	Control		Infested ^b	
		Viable seed	Fungi	Viable seed	Fungi
(%)					
Boone	1985	87	7	52	56
	1986	81	7	38	77
	1987	88	8	42	57
Osage	1985	91	6	56	54
	1986	84	9	44	60
	1987	83	12	47	60
LSD (0.05)		6.0	5.0	5.1	8.6

^aLSD (0.05) between control and infested treatments for % viable seed and % fungi are 12.1 and 14.2, respectively.

^bInsects were introduced at both sites on velvetleaf plants at the R3 to R4 developmental stage (ref. 10) in 1985; infestations in 1986 and 1987 developed from overwintering insect populations.

season progressed into early October before the first frost occurred. In 1986 and 1987, overwintered adults were first observed on young velvetleaf plants in late June at both locations. Insect populations increased slowly from two or three adults to >10 adults/plant and the range of infestation on velvetleaf expanded to only about 1500 m² in 30 days. After 60 days, the area of infestation had expanded to 15 to 20 ha, and all stages of the scentless plant bug were found on velvetleaf within this area. These observations are similar to those of Wheeler (26) who described a characteristic late summer buildup of scentless plant bug numbers on Rose-of-Sharon (*Hibiscus syriacus* L.) in North Carolina. Also, surveys of the scentless plant bug in Mississippi revealed dense populations of "several hundred nymphs on individual velvetleaf plants" from July through October (11).

The mean viability of velvetleaf seed samples comprising all mature seed produced/plant harvested at the R6 stage in mid-September in 1985, 1986, and 1987 was significantly reduced by the scentless plant bug (Table 1). Seed viability from infested sites ranged from 38% at the Boone location in 1986 to 56% at the Osage location in 1985. Seed viability from control sites was greater than 80% over the 3-yr period. Although no other field studies exist for comparison, the trend in significant reductions in seed viability by the scentless plant bug was similar to those reported in previous controlled studies (11, 21, 24). However, the magnitude of decreases in seed viability was not as high as the previous reports because the insects were not confined to plants from the onset of seed production. Thus, assays on seed accumulated from field-grown plants during the growing season may underestimate the impact of insect feeding on viability.

Fungal infection was considerably higher in seed attacked by the scentless plant bug and ranged from 54% at the Osage location in 1985 to 77% at the Boone location in 1986 (Table 1). Fungal infection of seed from control sites was no more

than 12%, which approximates the infection level in field-collected velvetleaf seed reported previously (16). The enhanced fungal infection of velvetleaf seed by the scentless plant bug is similar to that for insect-fungus relationships implicated in infection promotion and disease development in many crop seeds (1, 19, 25). The present report is the first account of an insect-fungus relationship on seed deterioration of an economically important weed species.

When velvetleaf seed were assayed by maturity date, viability of the seed from infested plants decreased as the season progressed (Table 2). Feeding by the scentless plant bug did not consistently reduce total seed produced/sample; however, total viable seed produced was significantly reduced throughout the season with the greatest reductions occurring at mid- to late-season harvests. Thus, as insect populations increased, newly produced seed capsules were readily attacked resulting in subsequent reduced seed viability and increased microbial infection of seed developing at that time. This is also reflected in the relative increase in numbers of feeding insects/capsule observed as the season progressed (Table 2).

Scentless plant bugs fed intensively and completed development only on available immature capsules and did not affect mature seed even when present on the same plant, a characteristic feeding pattern previously observed in greenhouse studies (11). Selected plants from which capsules at different positions were assayed became infested with scentless plant bugs (early to mid-August) as the range of the insect expanded to include these plants. Capsules from lower positions of the plants (nodes 1 through 6) in early September contained mature seed with high viability and low fungal infection compared to low viability and high infection of seed in capsules from upper positions of the plant (node 7 and above) (Table 3). Insect presence and activity was most intense on developing capsules on upper positions of the plants. Assays of randomly collected capsules yielded seed viability and fungal infection levels approximating the average of those for the lower plus upper samples. Even though the scentless plant bug was effective in limiting viability and promoting fungal infection of immature seed developing in capsules, viable seed produced before insect infestation escaped attack and were dispersed at maturity. These results suggest that reduction of seed viability in the early stages of velvetleaf infestation is critical in reducing total viable seed produced during an entire season.

Coincident with the progressive decline in seed viability during the course of the season was the increase in fungal and bacterial infections of seed (Table 2). Indeed, examination of data from 3 yr of sampling revealed a strong negative correlation between frequency of fungal infection and percent seed viability (Figure 1). These data suggest that seed viability decreased with increasing microbial infection as a result of feeding by an increasing insect population as the season progressed. This relationship is similar to those reported for effects of fungal infection on seed viability in studies of deterioration of crop seed (8).

The majority of seedborne microorganisms was isolated from nonviable velvetleaf from both control and infested

Table 2. Effects of the scentless plant bug on production, viability, and microbial infection of seed of field-grown velvetleaf sampled at various dates during the 1986 and 1987 growing seasons^{ab}.

Date	Location	Total seed		Seed viability		Total viable seed		Fungal infection		Bacterial infection		Insects/capsule
		Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Infested
		(no.)		(%)		(no.)		(%)				(no.)
7/9	Boone	550	...	88	...	480	...	3	...	0
7/21	Boone	580	530	95	62	550	330	6	48	4	22	2
8/5	Boone	640	520	96	55	610	290	11	40	1	20	2
8/8	Osage	650	520	86	78	560	410	9	12	1	4	1
8/19	Boone	620	550	94	49	550	270	14	47	4	39	2
8/20	Osage	560	620	81	53	460	320	8	46	1	25	3
9/1	Boone	580	480	86	49	500	240	12	49	13	41	4
9/10	Boone	560	460	86	42	480	190	14	58	14	26	10
	Osage	640	590	88	22	560	130	6	57	4	32	11
9/22	Boone	470	470	80	27	380	120	16	69	8	27	13
	Osage	470	880	85	20	400	80	16	86	8	25	15
10/5	Boone	520	480	76	17	400	80	19	78	18	32	13
10/8	Osage	550	445	78	15	430	70	17	98	12	36	16
LSD (0.05)		58	29	5	6	65	50	7	11	9	11	...

^aValues are means of four replicate samples of 15 capsules each from two sites at each location.

^bLSD (0.05) between control and infested treatments for total seed = 52; seed viability = 14.3, total viable seed = 71.2, fungal infection = 10.5 and bacterial infection = 12.0.

^cQualitative data included to illustrate relative trends in insect infestation.

sites (Table 4). The highest incidence of microorganisms was associated with nonviable seed from infested sites where up to 98% of seed collected on certain sampling dates was infected with fungi and bacteria. Three main fungal genera were consistently isolated from nonviable seed and were up to seven times more prevalent in seed attacked by the scentless plant bug than in seed from control sites (Table 4). *Alternaria*, *Cladosporium*, and *Fusarium* were also the most prevalent genera associated with external surfaces of velvetleaf capsules (unpublished data) and mature seed (16) on field-grown plants. Bacterial infection, which occurs naturally at low levels in field-collected seed (15, 16), increased over twofold in insect-attacked seed. Major bacterial genera isolated included *Pseudomonas*, *Erwinia*, and *Flavobacterium*, which are similarly found in field-grown velvetleaf seed (15).

Seed attacked by the scentless plant bug were lighter in color, smaller in size, and had sunken areas compared

to nonattacked seed. Microscopic examination of seed from infested plants revealed depressions in and/or puncture holes through seed coats. Reddish to orange-red calli or exudates from feeding punctures were often observed. These types of feeding injury are typical of attack on seed by hemipterous insects (1, 4). Also, fungal mycelia covered the surfaces of over 78% of insect-attacked seed. Light and scanning electron microscopic studies revealed fungal hyphae and bacterial cells intimately associated with feeding punc-

Table 3. Relationship of capsule position on velvetleaf plants to feeding pattern of the scentless plant bug and viability and fungal infection of seed.

Capsule position	Seed viability	Fungal infection	Insect presence ^a
	(%)	(%)	(no.)
Lower	82	8	<1
Upper	34	62	>25
Random	53	25	2 - 25
LSD (0.05)	17.5	18.0	

^aApproximate number of insects (nymphs and adults)/capsule.

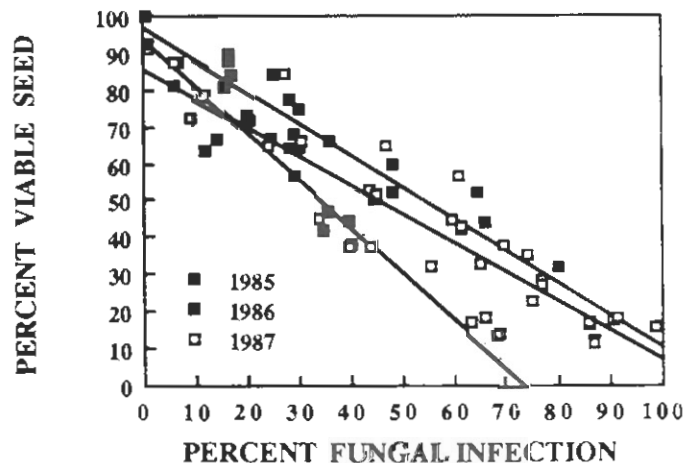


Figure 1. Relationship between velvetleaf seed viability and fungal infection at sites infested and noninfested with *N. louisianica* in 1985, 1986, and 1987. The regression equation for 1985 is $Y = 96.88 - 0.86X$ ($r^2 = 0.88$); for 1986 is $Y = 93.62 - 1.26X$ ($r^2 = 0.85$); for 1987 is $Y = 85.47 - 0.78X$ ($r^2 = 0.80$).

Table 4. Frequency of microorganisms within seed classes influenced by insect feeding during 1986 and 1987^a.

Microorganism	Location	Viable seed		Nonviable seed	
		Control	Infested	Control	Infested
—————(%)—————					
<i>Alternaria</i> spp.	Boone	2	10	8	43
	Osage	3	8	10	36
<i>Cladosporium</i> spp.	Boone	1	10	5	32
	Osage	3	9	10	26
<i>Fusarium</i> spp.	Boone	2	10	10	40
	Osage	4	9	5	40
Other fungi	Boone	0	1	1	2
	Osage	0	0	0	2
Bacteria	Boone	0	6	18	42
	Osage	1	2	22	52
LSD (0.05)		2	5	9	9

^aLSD (0.05) between control and infested treatments for viable seed and nonviable seed is 5.2 and 9.5, respectively.

tures and present within seed coat and endosperm tissues of insect-attacked seed (unpublished results). Seed exhibiting this level of infection were always nonviable.

Predominant microorganisms infecting seed from infested plants (Table 4) were also isolated from over 90% of the exoskeletons of scentless plant bugs assayed. However, these microorganisms were not isolated from internal contents of surface-sterilized insects indicating that the microorganisms were not harbored internally. Transmission of microorganisms to seed would appear, therefore, to be mechanical much like pollen is transmitted externally by bees (Apoidea). Microorganisms occurring epiphytically on velvetleaf capsules may adhere to, and be transported by, the insect for introduction into developing seed during or shortly after insect stylet penetration. Thus, the insect provides a natural means for overcoming velvetleaf seed coat barriers to microbial infection. Microbial invasion through injuries created by feeding-wounding insects is a common means of insect-mediated transmission of many bacterial (9) and fungal (1, 19) plant pathogens. The ecological relationships among the scentless plant bug, epiphytic microorganisms, and velvetleaf seed are comparable to those described for the stinkbugs, *Acrosternum bilarum* Say and *Euschistus* spp., the yeast, *Nematospora coryli* Peg., and soybean seed, which contribute to the development of yeast spot disease in soybean (4, 19).

The results of this study show that under field conditions the scentless plant bug can reduce production of viable velvetleaf seed directly through feeding and promoting infection of seed with detrimental microorganisms. In practice, the scentless plant bug may be reared economically on velvetleaf seed and used for early inundation of areas in the Midwest, where the insect occurs only sporadically in late summer, with no effect, to control velvetleaf. Selection of microorganisms more effective in seed deterioration may further improve control of velvetleaf seed production with the

scentless plant bug and lead to an integrated biocontrol approach. If this can be achieved, the reliance on post-emergent chemical control of velvetleaf might be reduced considerably.

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