0971-4693/94 US $ 5.00
Allelopathy Journal 27 (2): 237-244 (2011) *International Allelopathy Foundation* 2011
Tables: 2, Figs : 3

Hairy vetch (*Vicia villosa*) seed size affects germination response to coumarin

R.D. WILLIAMS* and P.W. BARTHOLOMEW

USDA-ARS-GRL, Research and Extension, Langston University
P.O. Box 1730, Langston, OK 73050 USA.
E. Mail: Robert.Williams@ars.usda.gov

(Received in revised form: January 12, 2011)

ABSTRACT

The inhibition of seed germination by an allelochemical is generally greater in small seeds than in large seeds. This response may have significant impact on weed control by allelopathic cover crops, where the small-seeded weeds would be more effectively controlled than large-seeded species. The studies reporting these results used a large number of plant species that varied in seed size, which might have introduced differences in germination characteristics or various parameters required for growth and emergence. One approach to avoid factors associated with the use of a mixed seed size population is to separate a large population of seeds from a single species into various size classes. This allows the analysis of the effects of a given allelochemical directly as a function of seed size. Here, hairy vetch (*Vicia villosa* Roth.) seeds were separated into three classes based on their seed weight (large, 54 mg; medium, 40 mg; and small, 26 mg) and compared their germination response to coumarin at 10^{-3} and 10^{-5} M. There was no significant difference in germination to 10^{-5} M coumarin with respect to seed size. However, germination was reduced and delayed in seeds exposed to 10^{-3} M coumarin, and this effect was more noticeable in the small seed class. Expressed as a percent of control, germination for large, medium and small seeds was 72, 76, and 55% respectively and germination of small seeds was also delayed by two days. These results confirm that small seeds are more sensitive to an allelochemical and this sensitivity is selective within a species with respect to small versus large seed. If expressed by a cover crop, this selection pressure could have significant impact on the weed community over time.

Key words: Allelochemical, germination inhibition, hairy vetch, seed size, seed weight, small seed sensitivity, *Vicia villosa*.

INTRODUCTION

Smaller seeds appear to be more sensitive to allelochemicals than larger seeds (10). These authors showed that as the concentration of methyl-isothiocyanates solutions increased, germination decreased in regards to seed size; i.e., small seed germination was inhibited at a lower concentration than larger seeds. For example, at 5 mg L^{-1} concentration the germination of small seeded smooth pigweed was 48%, while that of larger seeded wheat was 95% (10). Similar results were reported by Liebman and Sundberg (8) who observed a general decline in germination and radicle inhibition concomitant with a decrease in seed size of a large number of species. Although the

*Correspondence author

difference noted in small versus large seed response to allelochemicals is generally accepted in the allelopathy literature, there is still the problem as to amount of solution used in the bioassay in these types of studies.

As the seed size changes, e.g. from the relatively large [Corn (*Zea mays* L.) or soybean (*Glycine max* (L.) Merr.) seeds to the smaller smooth pigweed (*Amaranthus hybridus* L.)] seeds, the amount of solution available for the seed to imbibe during the bioassay varies. As the seed size increases the amount of solution per unit volume of seed decreases, reducing the amount of allelochemicals available to the individual seed (11). Most researchers avoid this problem by increasing the amount of solution used in the bioassay as the seed size increases, or reducing the number of large seeds in the bioassay than number of small seeds used (4).

The variation in seed size also poses a problem. To obtain a range in seed sizes, many species are used in the study with differences in the seed weights over several orders of magnitude. For example, the mean seed weight for soybean, velvetleaf (*Abutilon theophrasti* Medik.), johnsongrass (*Sorghum halepense* (L.) Pers.) and smooth pigweed are 114, 9, 4, and 0.3 mg, respectively, which is nearly a 400-fold difference in seed size. When we use several species, we may be including other factors that may or may not be related to seed size; seed quality or rate of germination for example. Also in setting up a germination bioassay to study the effects of an allelochemicals, or an extract of a suspected allelopathic plant, the experimental conditions used should be optimum for the test species, which is difficult when numerous test species are used (4).

One approach to see if seed weight does effect the germination response would be to separate the seed within a species by size. This would eliminate part of the problems identified above, especially the use of several different species to obtain a range in seed weights. However, the range in seed weight will be narrower than that obtained in the studies cited above. Also when separating a seed population into class sizes the germination characteristics of the subpopulations may be different from the parent population. It is common for the larger seed to have better seed quality and more vigorous seedlings than smaller seed (3,5).

Here, a hairy vetch seed sample was separated into three different seed size/weight classes. The effect of coumarin on the germination of these different size hairy vetch seed subsamples was tested to determine if there is an interaction between the seed size and the effectiveness of an allelochemical. Coumarin was selected as the test compound because it is a very good germination inhibitor (12) and has been implicated as the active compound in allelopathy studies (1,2).

MATERIALS AND METHODS

Hairy vetch seed were obtained from a commercial source.[1,2] One-thousand seed weight (TSW), moisture content and mean diameter were determined for the bulk seed. TSW was determined by weighing three separate samples of 50-seeds each. After determining the fresh weight of the seed lots, the seed were dried (50 °C for 72 h) and

[1] Ernst Conservation Seeds LLP, Meadville, PA, USA.
[2] Mention of trade names or commercial products in the publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

weighed. Percent moisture was calculated by ((fresh weight – dry weight)/fresh weight) x 100). For the bulk seed, mean seed diameter was determined by measuring 100 randomly selected seed to the nearest 0.1 mm with a digital caliper.[3] Based on preliminary measurements of the bulk seed and earlier separation trials, it was determined that the bulk seed could be separated into three class sizes by carefully screening the seed using standard sieves.[4] Damaged and deformed seeds were removed during the screening process. TSW of each class size was determined as above. For seed classes the arithmetic mean diameter, geometric mean diameter, and sphericity were determined by measuring 50 randomly selected seed from each class size. Maximum seed length and width were measured across two horizontal axes at right angles to each other, while seed depth was measured along a vertical axis using the depth gauge on the digital caliper. Each measurement was made to the nearest 0.1 mm. By definition, length (a), width (b) and depth (c) were ranked from the largest to smallest dimension of the seed, (i.e. 'a' was always the largest dimension of the seed) (9).

Arithmetic mean diameter, geometric mean diameter and sphericity were calculated according to Mohsenin (9) where arithmetic mean diameter (Da), Geometric mean and Sphericity were calculated as under:

Arithmetic mean $$Da = (a + b + c) / 3 \quad [i]$$

Geometric mean $$Dg = (abc)^{1/3} \quad [ii]$$

Sphericity $$\varphi = [(abc)^{1/3}] / a \quad [iii]$$

All germination tests were done in dark at 20 ˚C; seeds were only exposed to light during the time necessary to count and remove germinated seed. For each test, 30 seeds were placed on one sheet of Whatman No. 3 filter paper in a 9-cm glass petri dish wetted with 8 mL distilled water or test solution. Seed germination, defined as radicle extension of 2 mm or greater, was recorded daily for 5 days. Germinated seeds were removed from the petri dish. Germination tests were conducted on both the bulk seed and the seed size classes to determine percent and rate of germination. A 10^{-3} M coumarin[5] stock solution was prepared with distilled water with slight warming and gentle stirring to dissolve the chemical. A 10^{-5} M solution was made by appropriate dilution of 10^{-3} M stock. Fresh coumarin solutions were prepared for each experiment.

A preliminary germination test using distilled water as the wetting medium was conducted on the three seed sizes of hairy vetch screened from the bulk seeds. Thirty seeds were placed on one sheet of filter paper in a 9-cm petri dish and wetted with 8 mL distilled water. The dishes were placed in dark at 20 ˚C and checked daily for germination. The experiment was in a randomized block design with three replications per seed size. Analysis of variance was performed using Excel statistical data analysis software.[6]

To test for interaction between the seed size and coumarin, a germination study was conducted using 3-seed-sizes (large, medium and small) and 2 levels of coumarin (10^{-3} M and 10^{-5} M). The germination test was conducted as outlined above. The

[3] Oshlun Inc., Henderson, NV, USA.
[4] U.S.A. Standard Test Sieve, Hogentogler and Co., Inc., Columbia, MD, USA.
[5] Sigma-Aldrich Corp., St. Louis, MO, USA.
[6] Microsoft Corp., Redmond, WA, USA.110

experimental design was randomized block with three replications [three seed sizes x three coumarin levels (distilled water control, 10^{-3} M and 10^{-5} M coumarin) x five daily counts]. The experiment was repeated in time and the data combined for analysis providing six replications per seed size-treatment levels. Data were subjected to ANOVA with repeated measures and mean separation tests were made by Fisher's protected least significant difference test (LSD, p = 0.05) using Genstat 7.[7]

RESULTS AND DISCUSSION

The one-thousand-seed weight (TSW) of the hairy vetch bulk seed was 36.6 g with a moisture content of 10.2 %. Both of these values are well within the range of the TSW and moisture content determined for other seed lots of hairy vetch in this laboratory. Germination tests on the bulk seed indicated that most of the seed germinated within 3 days at 20 °C, with very little germination occurring on days 4 and 5, and a mean germination of 84%. The mean seed diameter ranged from 5.1 mm to 2.3 mm with a mean of 3.9 mm and a standard deviation of 0.1 mm. These results are similar to those reported for common vetch (*Vicia sativa* L. var. Kuba) with regard to seed dimensions and moisture content (13). However, a TSW of 55.4 g was reported for the Kuba cultivar. We found no reports on the physical properties of hairy vetch for comparison.

Careful screening of the bulk seeds provided three weight/size seed classes with regard to TSW and mean seed diameter (Table 1). The differences among seed size classes as to weight or mean diameter were significantly different ($p < 0.05$), although there was some overlap between the distributions of mean diameters among the classes. For example the range for the large seed mean diameters was between 3.6 and 4.9 mm, while the range for the medium was between 3.3 and 4 mm. There was no overlap between the medium and small seed. Even though there was a slight overlap between the medium and large seed classes, the majority of the large seed mean diameters were between 4.7 and 4.2 mm, while those for the medium seed class were between 3.9 and 3.6 mm.

The daily germination of hairy vetch seeds peaked on day 2, with the large seeds having a higher percentage germination (82%) than either the medium (62%) or the small seeds (61%) (Figure 1A). However, this difference in germination was not significant between the seed size classes (p = 0.06). Additionally, there was no difference final germination, 89% for the large seeds and 77% for both medium and small seeds classes. The overall germination for the separated seed was 80%, which was close to 84% for bulk seed (Figure 1B). Some of the small seeds imbibed but did not germinate, while a few seed did not imbibe. Whether the latter seeds were "hard-seed" is unknown. It is common for larger seed to have greater germination potential and seedling vigour than smaller seeds from the same seed lot (3,5,6,7).

There were significant differences as to treatment, time (day counted), time by seed size, and time by treatment (p = 0.001). However there was no significant interaction as to seed size by treatment, or the three-way interaction (time by seed size by treatment). The germination in the small seed class was lower than in previous experiment. Final

[7] VSN International Ltd., Oxford, UK..

Table 1. Seed size, dimensions, and weight of three hairy vetch seed classes

Parameter[a]	Seed-size class		
	Large	Medium	Small
		(mm)[b]	
Da	4.3±0.2	3.7±0.2	3.4±0.2
Dg	4.3±0.2	3.7±0.2	3.3±0.2
Sphericity	0.9±0.04	0.9±0.03	0.9±0.04
		(mm)	
a	4.7±0.4	4.1±0.3	3.7±0.3
b	4.5±0.3	3.8±0.2	3.5±0.3
c	3.7±0.3	3.3±0.2	2.8±0.2
		(mg)	
SW	54.8±3.6	39.8±1.2	26.1±0.7

[a] Da, average diameter; Dg, geometric mean; a, b and c, seed length, width, and depth, respectively; SW, seed weight. [b] Mean ± standard deviation

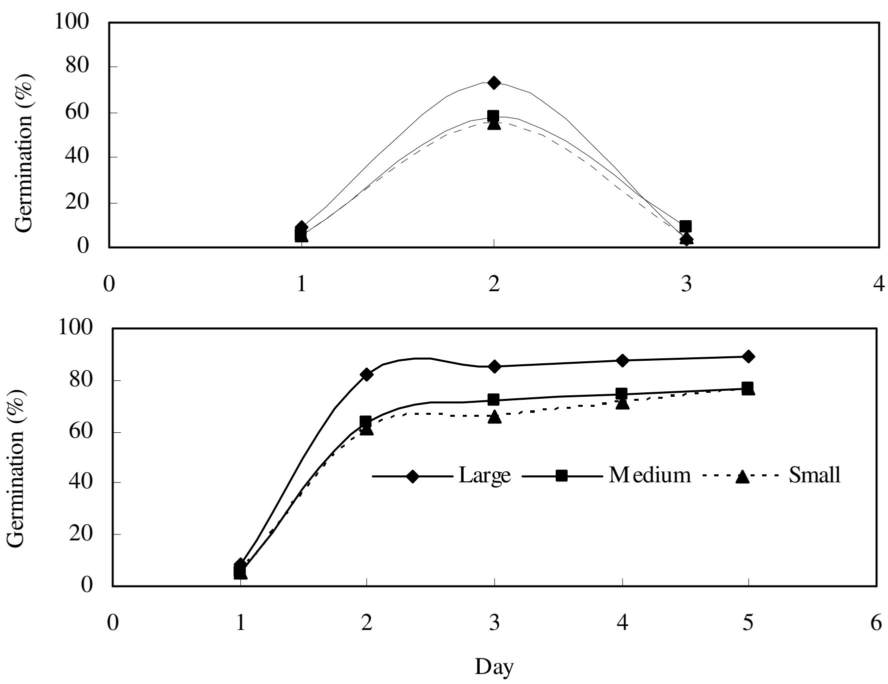

Figure 1. The effects of seed size on hairy vetch seed germination daily (A) and cumulative germination (B).

germination in control treatments was 82, 71 and 56% for large, medium and small seeds, respectively. There was significant difference ($p = 0.001$) as to seed size with the overall germination, regardless of treatment or day counted. There was no difference between the control and the 10^{-5} M coumarin treatment: however, both were significantly different from the 10^{-3} M coumarin treatment (Table 2). Overall the 10^{-3} M coumarin treatment delayed and reduced the seed germination. This effect was more noticeable in small seed class. Treating the seeds with 10^{-3} M coumarin, delayed the germination by 1 day, germination at 2 days was reduced and the reduction in germination commensurate with seed size. The smaller seeds showed a greater delay in germination than other seed sizes (Figure 2). The delay and reduction in germination (as percent of control) was greater in the small seed size than in the large and medium seed classes (Figure 3). However, there was still a delay in the large and medium seed; and a definite trend in a greater reduction in germination at 2 days in the medium seed. Expressed as percent of control in each seed class, germination for the large and medium seed at 10^{-3} M was 72 and 76%, respectively, while it was 55% for small seeds. Based on these results, small seed showed greater sensitivity to the allelochemical.

Table 2. Effects of seed size and coumarin concentration on hairy vetch germination (%)

Seed size	Coumarin concentration			Mean Seed
	10^{-3} M	10^{-5} M	Control	Size[b]
Large	38	51	53	47
Medium	35	49	46	43
Small	18	34	33	28
LSD (0.05)	-------------------(6)-------------------			(5)
Treatment[c]	30	45	44	
LSD (0.05)	-------------------(5)-------------------			

[a] Seed size by treatment means regardless or day counted; [b] Seed size mean regardless of treatment or day counted; [c] Treatment means regardless of seed size or day counted.

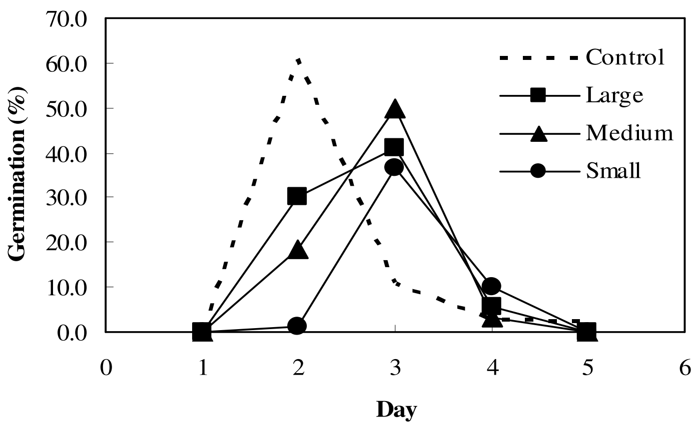

Figure 2. Hairy vetch seed germination by seed size at 10^{-3} M coumarin compared to the control, where the control germination is the pooled germination means regardless of seed size.

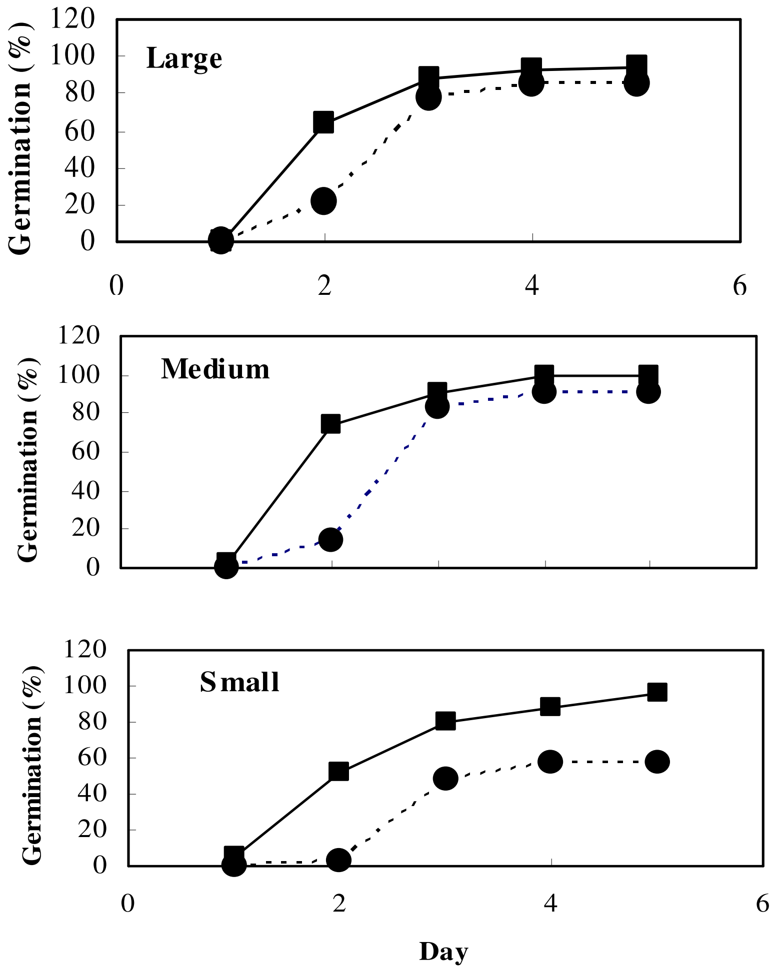

Figure 3. Hairy vetch seed germination by seed size at 10^{-3} M (●) and 10^{-5} M (■) coumarin expressed as percent of control.

The range in seed size in Petersen *et al.* (10) was from 0.2 to 45.3 mg (most were < 0.8 mg) based on six species, while the seed size ranged from 0.2 to 262.0 mg in Liebman and Sundberg (8), based on 62 accessions. These ranges in seed size are rather large compared to the range observed in the present study (26.1 to 54.8 mg). When such large ranges in seed weights exist within a weed population, it is likely that the small seeds (as little as 0.2 mg each) are more strongly affected by an allelochemical than larger seeded species, provided that the availability of compound is equivalent in both cases. Looking at the hairy vetch data presented here with a difference of 33 mg between the large and small seed classes, finding a difference in sensitivity is somewhat remarkable. Nevertheless, it is consistent with the general observation reported in the allelopathy literature that smaller seeds are more sensitive to allelochemicals than larger seeds. As pointed out by Liebman and Sundberg (8), this phenomenon has an implication with regard to weed control using cover crops. But it also raises the following question: assuming that the mode of action of an allelochemical is the same in small and large seeds, why are small seeds more sensitive? When comparing smooth pigweed with velvetleaf and/or soybean, we could attribute the difference in germination response to differences in seed coats, water uptake rates, or more seed reserves to repair the damage and avoid the stress. But these do not explain the sensitivity in the smaller hairy vetch seed presented here. Further research should be conducted to elucidate this phenomenon.

ACKNOWLEDGMENTS

The authors gratefully recognize the valuable technical assistance provided by Justin Cash and Kathie Wynn during the completion of the study.

REFERENCES

1. Aliotta, G., Cafiero, G., Fiorentino, A. and Strumia, S. (1993). Inhibition of radish germination and root growth by coumarin and phenylpropanoids. *Journal of Chemical Ecology* **19**: 175-183.
2. Duke, S. O., Dayan, F. E., Rimando, A. M., Schrader, K. K., Aliotta, G., Oliva, A., and Romagni, J. G. (2002). Chemicals from nature for weed management. *Weed Science* **50**: 138-151.
3. Cideciyan, M.A. and Malloch, A.J.C. (1982). Effects of seed size on the germination, growth and competitive ability of *Rumex crispus* and *Rumex obtusifolius*. *Journal of Ecology* **70**: 227-232.
4. Hoagland, R.E. and Williams, R.D. (2004). Bioassays Useful tools for the study of allelopathy. In: *Allelopathy: Chemistry and Mode of Actions of Allelochemicals* (Eds., F.A. Macìas, *et al*.) p. 315-351. CRC Press, Boca Raton, FL.
5. Kneebone, W.R. and Cremer, C.L. (1955). The relationship of seed size to seedling vigor in some native grass species. *Agronomy Journal* **47**: 472-477.
6. Larsen, S.U. and Andreasen, C. (2004). Light and heavy turfgrass seeds differ in germination percentage and mean germination thermal time. *Crop Science* **44**: 1710-1720.
7. Leffler, H.R. and Williams, R.D. (1983). Seed density classification influences germination and seedling growth of cotton. *Crop Science* **23**: 161-165.
8. Liebman, M. and Sundberg, N.D. (2006). Seed mass affects the susceptibility of weed and crop species to phytotoxins extracted from red clover shoots. *Weed Science* **54**: 340-345.
9. Mohsenin, N.N. (1970). *Physical Properties of Plant and Animal Materials.* Gordon and Breach Science Publishers, New York.
10. Petersen, J., Belz, R., Walker, F. and Hurle, K. (2001). Weed suppression by release of isothiocyanates from turnip-rape mulch. *Agronomy Journal* **93**: 37-43.
11. Weidenhamer, J.D., Morton, T.C. and Romeo, J.T. (1987). Solution volume and seed number: Often overlooked factors in allelopathic bioassays. *Journal of Chemical Ecology* **13**: 1481-1491.
12. Williams, R.D. and Hoagland, R.E. (1982). The effects of naturally occurring phenolic compounds on seed germination. *Weed Science* **30**: 206-212.
13. Yalcin, I. and Ozarslan, C. (2004). Physical properties of vetch seed. *Biosystems Engineering* **88**: 507-512.