

# Isolation and Identification of a Slug-Specific Molluscicide from Quackgrass (*Agropyron repens*, L. Beauv.)<sup>†</sup>

Roger D. Hagin\* and Suzanne J. Bobnick

USDA-ARS-NER-NAA, Agronomy Department, 624 Bradfield Hall, Cornell University, Ithaca, New York 14853-0144

A quackgrass (*Agropyron repens*, L. Beauv.) extract fraction containing "phenolic glycosides" showed both dermal and gastrointestinal toxicity toward the two slug species *Deroceras reticulatum* (Müller) and *Deroceras laeve* (Müller). These same phenolic glycosides were tested, with no apparent effect, against three freshwater snail species and a garden soil worm. The active compound in the phenolic glycoside fraction, 6-hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid (6-HT $\beta$ C-3-COOH), was identified by use of TLC, CIMS, UV, and IR spectrophotometry, and comparison of the isolate to authentic compounds. The LD<sub>50</sub> for the more resistant slug, *D. laeve*, tested against 6-HT $\beta$ C-3-COOH, was estimated at approximately 5 mg/kg. A bait, containing 6-HT $\beta$ C-3-COOH as the active ingredient, was highly active when field tested against the slug *Arion subfuscus* (Draparnaud). A survey of the literature indicated that 6-HT $\beta$ C-3-COOH possesses little or no mammalian toxicity.

Minimum or no-tillage methods of crop production have gained increasing acceptance since the early 1950s. These methods improve timeliness of operations and reduce soil erosion—both important objectives from a farmer's point of view. No-tillage methods, in which weed cover is killed by chemicals and the crop planted directly thereafter, represent desirable means to achieve these objectives. Problems such as perennial weeds, insects, pathogens, nematodes, slugs and snails, and possible allelopathic compounds released from killed weeds are impediments to fuller utilization of no-tillage procedures. These problems are particularly severe in the temperate, humid, northeastern region of the United States. All and Musick (1986) considered that the most serious minimum tillage problem was the attack by slugs on crop seed and seedlings, particularly in killed sods that had not been tilled for a number of years. In New York, Ramsey (1984) reported that slugs were a major problem in continuous no-till corn (*Zea mays*); Dowling and Linscott (1985) reported that slugs were a primary limitation to the establishment of sod-seeded lucerne (alfalfa, *Medicago sativa* L.).

A relatively large number of chemicals are available to deal with weeds, insects, plant pathogens, and other pests. Only one, metaldehyde, is currently registered for control of slugs and snails. Metaldehyde is relatively expensive for agronomic usage and is somewhat toxic to mammals and/or birds. Metaldehyde has been reported, frequently, as the cause of poisoning of dogs, cats, sheep, and poultry (Homeida and Cooke, 1982; Osweiler et al., 1985). In the state of Delaware, according to press reports, the most effective treatment available for slugs in no-till corn was liquid nitrogen fertilizer applied to early stage corn at night after slugs had emerged onto crop plants (*Lancaster Farming*, 1989). The fertilizer presumably kills slugs by salt effects, causing them to emit copious amounts of slime which leads to dehydration of the animals. The method is limited by the requirement to spray the material directly on the slugs. Clearly, additional molluscicides are needed

that are highly selective and toxic for slugs and/or snails with minimal effect on other species.

In 1980, in areas of a no-till corn field, Hagin noted that where quackgrass had been killed there were few slugs and little effect of their feeding on corn. Conversely, corn seedlings in the remainder of the field were decimated by slug attack. At the time he was working to identify compounds responsible for allelopathic effects of killed quackgrass (*Agropyron repens*, L. Beauv.) on minimum tillage planted forage legume seedlings (Hagin, 1989) and deduced that there might be a connection between the two observed effects. Hagin and Bobnick soon established that a dried quackgrass root extract fraction (labeled "phenolic glycosides"), responsible for the allelopathic effects on seedling forage legumes, also showed molluscicidal effects on two slug species. The fraction showed both dermal and gastrointestinal toxicity to the two slug species *Deroceras reticulatum* (Müller) and *Deroceras laeve* (Müller) (Hagin and Bobnick, 1985).

The objectives of the work reported herein were to identify the compound or compounds responsible for the observed molluscicidal effects, isolate or synthesize enough of the material to determine an approximate LD<sub>50</sub> for the pure material on slugs, conduct a initial field test of the material as a molluscicide, and provide some evidence of specificity of the phenolic glycoside fraction beyond slugs, namely snails and earthworms.

## EXPERIMENTAL PROCEDURES

**Reagents and Standards.** All reagents, solvents, and standards were of analytical grade. The following standards and reagents were purchased from Sigma Chemical Co.: 5-hydroxy-indoleacetic acid, (5-HIAA), 98-100%; L-tryptophan, Sigma grade; 5-hydroxy-L-tryptophan (5-HTP), 99%.

Synthesis of 1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid (T $\beta$ C-3-COOH) was accomplished by the method of Brossi and Focella (1973). Reaction between L-TP and formaldehyde by Pictet-Spengler condensation produced the final product in 40% yield. Synthesis of 6-hydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline-3-carboxylic acid (6-HT $\beta$ C-3-COOH) was accomplished by condensation between 5-HTP and formaldehyde (Brossi and Focella, 1973). The pure product was isolated with a yield of 81%. All solvent operations, derivatizations, and reactions were carried out in a fume hood.

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**Materials.** Quackgrass roots and rhizomes (collectively called roots) were harvested from an established quackgrass sod in Tompkins County, New York, July, 1985. The plants had just completed pollination at the time of harvest. Roots were washed several times with tap water, rinsed with distilled water, and blotted dry with absorbent paper. The roots were oven dried at 60–70 °C, ground in a Wiley mill to pass through a 40-mesh screen, and stored in glass bottles in a freezer at –20 °C until extraction.

**Sample Extraction and Purification.** Plant material was extracted by a two-stage aqueous alcohol system employing methanol or 2-propanol as the organic phase. The extract was taken to dryness by using a rotary vacuum evaporator. The dried residue labeled "total extract" was dissolved in distilled water and subjected to hexane extraction followed by ether extraction of the acidified aqueous phase (adjusted to pH 2 with HCl) to produce two fractions labeled "lipids" and "organic acids", respectively. The remaining water solution was adjusted to pH 7.5 and passed through a glass column containing Amberlite XAD-2 resin. The material that passed through the column in addition to a distilled water rinse of the column was concentrated on a rotary vacuum evaporator. This fraction was labeled "water soluble". Material retained on the XAD-2 column was eluted with 70% methanol/30% H<sub>2</sub>O (v/v). The eluate was concentrated to dryness on a rotary vacuum evaporator. This fraction was labeled "phenolic glycosides".

Phenolic glycosides were fractionated by molecular size. This was accomplished by passing a concentrated water solution of the glycosides through a glass column packed with Sephadex G-25. The column was eluted with distilled water to produce five column fractions, labeled 1–5, on the basis of visible color or UV absorption. More details of the foregoing operations can be found in the paper by Hagin (1989).

**TLC of Standards and Isolated Aglycons.** The TLC system used primarily employed silica gel G plates (0.25 mm thick layer) and a solvent mixture of 1-butanol/acetic acid/H<sub>2</sub>O (4/1/1 by volume). Under this system 5-HTP, T $\beta$ C-3-COOH and 6-HT $\beta$ C-3-COOH had  $R_f \times 100$  values of 51, 56, and 27 respectively.

**UV, IR, and Mass Spectral Analyses.** Details of these analyses and further detail on TLC are covered in the paper by Hagin (1989).

**Animal Test Species.** The slug species *D. reticulatum* (Müller) and *D. laevis* (Müller), used in laboratory tests, were harvested by using trapping methods as outlined by Rollo and Ellis (1974). Slugs were kept in polycarbonate animal cages 35 cm long  $\times$  30 cm wide  $\times$  16 cm high. The bottom and sides of the cages were lined with paper towels moistened with distilled H<sub>2</sub>O, and the top was covered with a glass plate to retain the slugs. A maximum of 40 slugs per cage was maintained to prevent cannibalism. Slugs were fed a pelleted food placed on a glass plate in the bottom of the cage. They were kept in an incubator set at 20 °C in the dark prior to testing. For feeding trials, the food source was removed 24 h prior to testing. The slug species *Arion subfuscus* (Draparnaud) was the principal slug found in the field test. Slugs were identified according to the method of Chichester and Getz (1973).

Three freshwater snail species were tested: *Helisoma trivolvis* (Say), *Lymnaea humulus* (Say), and *Physa gyrina* (Say). The first two were harvested from a pond and stream near Ithaca, NY, in early spring. *P. gyrina* was purchased from Wards Natural Science Establishment, Inc., 5100 West Henrietta Road, Rochester, NY. Identifications were confirmed according to the method of Harman and Berg (1971). Snails were tested for sensitivity to quackgrass isolates (after acclimatization for 24 h in 1-L beakers of distilled H<sub>2</sub>O) in a growth chamber maintained at 20 °C with a 14-h day length.

The pink soil worm *Eisenia rosea* (Savigny) was harvested in late fall from a local garden. Identification was confirmed by using the key of Reynolds (1977). This earthworm was tested directly on transfer to the lab.

**Slug Food and Bait.** Original food testing methods used by Hagin and Bobnick (1985) required an artificial medium developed by Wehlan (1982). Calcium alginate gel, the artificial medium, was the control food base to which lettuce leaf extract was added to provide a control food. Other plant sources or pure

chemical could replace the lettuce extract for purposes of testing. Lettuce extract controls, in our hands, had too many problems with mold growth, and we altered our basic control food, replacing lettuce extract with red clover (*Trifolium repens* L.) extract. This proved to be more acceptable to slugs than lettuce-based alginate gels and had fewer problems with molds.

To overcome the necessity to continually make gels for feeding slugs, we made up a pelleted food using powdered red clover, powdered bran, powdered wheat germ, and dried milk in proportions of 2:1:1:1. This served as a regular laboratory food for slugs. For chemical tests the appropriate level of chemical was added in solution, the material was blended, and additional water was added, as needed, to form a thick paste. The paste was forced through 4-mm round holes in a food grinder to form pellets. The pellets were baked at 60 °C and stored in plastic bottles prior to use.

Bait for the field trial of 6-HT $\beta$ C-3-COOH was made up of ingredients in the following proportions: 250 g of dry corn bran, 20 g of dry powdered milk, and 20 g of amylose. This was blended with a food mixer. While blending, 400 mL of a yeasty tasting draft beer (highly attractive to slugs) containing 350 mg of 6-HT $\beta$ C-3-COOH was added. Additional beer was added as needed to make a thick paste. Pellets were formed and dried as noted above. The level of chemical in the bait was set at 1000 ppm on a dry weight basis.

**Forced Contact Methods.** For forced contact with chemicals with either slugs or earthworms, single animals were placed inside 35  $\times$  80 mm OD Soxhlet extraction thimbles. The open end was folded over and stapled shut. The sealed tubes were placed in individual compartments of 12-section plastic boxes. Each section was 4.5 cm wide  $\times$  8 cm long  $\times$  4 cm high. Four milliliters of distilled water (control) or 4 mL of 1000 ppm test solution was applied to the thimbles. The boxes were closed, and the test animals were kept in a dark incubator set at 20 °C for 24 h. Surviving animals were transferred from the sealed thimbles to a second plastic box (each section of which was lined with water-moistened filter paper), a lettuce leaf was added, and the closed boxes were placed in the incubator. Observations were made for up to 4 days. Ten animals were used per treatment, and the treatments were replicated four times.

**Snail Test Methods.** Chemicals were tested on snails according to standardized methods (Webbe and Sturrock, 1964; Malek and Cheng, 1974). Each species was exposed to a distilled water control: 5, 50, or 500 ppm of phenolic glycosides for 24 h. Ten animals were used for each treatment level. After 24 h, the animals were washed with distilled water and transferred to distilled water for observation. Pieces of seaweed were added to the beakers as a food source, and the snails were observed for up to 4 days.

**Laboratory Slug Test Methods Employing Treated Food.** Two 9 cm diameter, Whatman No. 1 filter papers were placed in the bottom of a 9 cm diameter Petri dish and moistened with water. A 12 mm long food (control) or chemical test pellet was placed on a 18 mm diameter cover slip and moistened with distilled water. Twelve millimeter pellets averaged 92 mg dry weight each. After weighing, the pellet and cover slip was placed in the center of the filter paper. One slug of the test species was placed in the Petri dish, the cover was placed on the Petri dish, and the Petri dish was placed in a dark incubator set at 20 °C. Ten animals were used for each control or chemical treatment. Food consumption and activity were recorded daily, and observations were made for 7 days. Tests in which more than two of the control slugs died were discarded on the basis that the population was unhealthy. Feeding tests were conducted in this fashion up through feeding trial I. For feeding trial II, chemicals in water solution were applied to fresh control pellets 12 mm long. 5-HIAA was added at a level of 50  $\mu$ g/pellet. Other chemicals were added at a level of 12.5  $\mu$ g/pellet. Moistened pellets and cover slips were weighed as noted earlier. At the end of the test evaluation period all slugs either dead or inactive were considered to be dead (Malek and Cheng, 1974).

**Feeding Trials I and II.** Feeding trials I and II were designed to test the molluscicidal effectiveness of 5-HIAA, T $\beta$ C-3-COOH, and 6-HT $\beta$ C-3-COOH and a combination of 5-HIAA with each of the latter two. Concentrations used were noted above. Feeding trial II was designed to test the possibility of

compound alteration or breakdown during drying of pellets. Slugs averaged 0.2 g each in feeding trials I and II.

**Molluscicidal Field Test—1987.** The experimental site was established in Lansing, NY, May 30, 1987, adjacent and parallel in the long dimension (north-south) to a field of crownvetch (*Coronilla varia* L.). The site was located at the north-west corner of the crownvetch field—both the experimental area and the field were bordered on the north by a highway. The south and west sides of the site were bordered by bluegrass sod. The land was conventionally tilled and laid out in 12 north-south rows 0.75 m wide. The two east rows were planted to sweetcorn (*Z. mays* L., var. Pennfresh ADX). The next two rows to the west were planted to snapbeans (*Phaseolus vulgaris* L., var. Long Tendergreen). A variety of vegetables were planted in the remaining eight rows to the west.

In mid-June rains (as periodic light showers) came as a near daily occurrence. Temperatures reached daily highs of up to 30 °C and lows of around 20 °C. The humidity averaged near 100%. Conditions were ideal for slug growth and reproduction (Godan, 1983). Rainfall (in centimeters) for the period was as follows: June 21, 0.5; June 22, 0.5; June 23, 3.8; June 25–29, 1.9; June 30, 0.5; July 1, 0.25; July 2, 0.25; and July 3, 1.27.

On the evening of June 25 a large population of the slug *A. subfuscus* moved from the crownvetch field, passing through the two corn rows, and heavily attacked the beans. Some sections of the bean plants, which should have been 10–15 cm tall, were completely defoliated. An area enclosing the corn and bean rows, 3.9 m wide × 23 m long, was marked out and 250 g of molluscicide bait (containing 1000 ppm of 6-HT $\beta$ C-3-COOH) was uniformly spread around the perimeter. The bait was spread in a band 38 cm wide centering outside the bean rows on the west and outside the corn rows on the east. The bait crossed the bean and corn rows on the north and south. The bait was applied at 8:00 p.m. initially. As the bait was exhausted it was replaced at 125 g/treatment around the perimeter. It was reapplied July 1, 3, and 5, 1987.

Four randomized sections, 38 cm × 91 cm, were marked out between the corn rows and the crownvetch as well as within the bean rows. Slugs were counted before the experiment began and periodically during it. Pictures were taken initially as well as periodically through bean harvest.

## RESULTS AND DISCUSSION

Hagin (1989) reported that a quackgrass extract fraction previously thought by Hagin and Bobnick (1985) to be a single compound was, in fact, a series of glucosides ranging up to 4159 *M*, or greater. On the basis of colorimetric tests he labeled the quackgrass extract fraction "phenolic glucosides". Earlier exploratory work by Hagin and Bobnick (1985) indicated that a compound (phenolic glucosides) from extracts of dried quackgrass roots was both dermally and gastrointestinally toxic to the slugs *D. reticulatum* and *D. laevis*. They indicated that the compound was also allelopathic to seedling alfalfa, birdsfoot trefoil, red clover, and red kidney bean.

In the current study, forced contact tests of phenolic glucosides at 1000 ppm had no effect on the earthworm *E. rosea* (data not shown). The material tested at 1000 ppm against the freshwater snails *H. trivolvis*, *L. humilis*, and *P. gyrina* also was without effect (data not shown).

Hagin (1989) split the phenolic glucoside fraction into five fractions labeled 1–5, decreasing in molecular weight from  $\geq 4159$  for fraction 1 to  $\approx 150$  for fraction 5. He found the glucosides to be composed solely of glucose and a number of aglycons. As he previously reported, the principal allelopathic activity toward the seedlings of a number of plant species existed in fractions 4 and 5. The principal aglycons in fractions 4 and 5 were identified as 5-HIAA, 5-HTP, and TP. Smaller amounts of these aglycons existed in fractions 1–3. The principal allelopathic aglycons in quackgrass, toward a number of plant species seedlings, proved to be 5-HIAA and 5-HTP.

**Table I. Feeding Trial I: Compounds Potentially Molluscicidal to the Slug *D. laevis***

compd <sup>a</sup>	mg of bait consumed/ slug	% slugs dead	$\mu$ g of chemical/ slug	$\mu$ g of chemical/ g of slugs
control	36 $\pm$ 20	10	0	0
5-HIAA	28 $\pm$ 17	100	28	140
6-HT $\beta$ C-3-COOH	20 $\pm$ 16	100	5	25
T $\beta$ C-3-COOH	25 $\pm$ 20	0	6.2	31
5-HIAA	10 $\pm$ 6	90	10	100
+6-HT $\beta$ C-3-COOH			2.5	12
5-HIAA	31 $\pm$ 33	0	31	156
+T $\beta$ C-3-COOH			7.7	38

<sup>a</sup> 5-HIAA was supplied at 1000 ppm, and the other compounds were supplied at 250 ppm (dry weight basis).

**Table II. Feeding Trial II: Compounds Potentially Molluscicidal to the Slug *D. laevis***

compd <sup>a</sup>	mg of bait consumed/ slug	% slugs dead	$\mu$ g of chemical/ slug	$\mu$ g of chemical/ g of slugs
control	70 $\pm$ 33	20	0	0
5-HIAA	29 $\pm$ 35	10	16	80
6-HT $\beta$ C-3-COOH	10 $\pm$ 4	80	1.4	7
T $\beta$ C-3-COOH	35 $\pm$ 31	70	4.9	24
5-HIAA	34 $\pm$ 35	60	18	90
+HT $\beta$ C-3-COOH			4.8	24
5-HIAA	32 $\pm$ 23	40	17	85
+T $\beta$ C-3-COOH			4.5	22

<sup>a</sup> Chemicals were applied to fresh control bait pellets in solution. 5-HIAA was added at a level of 50  $\mu$ g/pellet. Other chemicals were added at a level of 12.5  $\mu$ g/pellet.

In the current work we found that the major fraction showing toxicity toward *D. reticulatum* and *D. laevis* was fraction 3, with lesser effects in fractions 1 and 2. Fractions 4 and 5 had no effect on the two slug species.

Chemical ionization mass spectrometry of fraction 3 indicated a principal mass peak (50% of base peak produced at a probe temperature of 460 °C) at *m/z* 232. A secondary peak was produced as the probe was heated through 300–400 °C at *m/z* 233. Assuming that these fragments represented *M* + 1 peaks, it was deduced that the compound had a mass of 232 and amu 231 was a *M* – 1 peak. The *M* – 1 peak was presumed to have been produced from the aglycon split from glucose. 5-HTP possesses amu 220 and a formula of C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>. The calculated formula of amu 232 is C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>. The compound was tentatively identified as 6-HT $\beta$ C-3-COOH. This compound is known to be produced from 5-HTP in mammalian tissues along with T $\beta$ C-3-COOH produced from TP (Brossi et al., 1973). Both 5-HTP and 6-HT $\beta$ C-3-COOH were synthesized according to the methods of Brossi et al. 6-HT $\beta$ C-3-COOH proved to be identical with the principal material isolated from aglycons in fraction 3 (comparison by TLC, UV, and IR methods).

The two compounds along with 5-HIAA were tested against the slugs *D. reticulatum* and *D. laevis*. The results of the test against *D. reticulatum* were discarded due to general poor health of the test animals. The tests on *D. laevis*, a smaller species of slug than *D. reticulatum* with greater resistance to molluscicides (Godan, 1983, Chapter 3, p 189), are shown in Tables I and II.

In both trials 6-HT $\beta$ C-3-COOH proved to be the most effective molluscicidal compound, while T $\beta$ C-3-COOH showed variable activity. When in combination, 5-HIAA appeared to exhibit some antagonistic activity toward 6-HT $\beta$ C-3-COOH. Extrapolating backward from the 100% kill for 6-HT $\beta$ C-3-COOH in Table I and the 80% kill for

the same compound in Table II, it was estimated that the LD<sub>50</sub> for slugs relative to the compound approximated 5 mg/kg of slugs. On this basis 6-HT $\beta$ C-3-COOH would be rated as a highly toxic compound toward slugs (Osweiler et al., 1985). Brossi et al. (1973) concluded that this compound exhibits little mammalian toxicity. On the basis of this information and the lack of activity toward earthworms or snails when tested against the quack-grass phenolic glycosides, it was concluded that 6HT $\beta$ C-3-COOH is likely to be highly specific toward slugs, with little toxicity toward other species, particularly mammals.

Feeding trial I utilized chemicals added before baking, and feeding trial II utilized chemicals added after baking. From a comparison of the results of Tables I and II relative to the method of adding chemicals before or after the bait was baked, two conclusions can be drawn. First, baking may alter 5-HIAA in some way to make it more toxic to slugs. Second, 6-HT $\beta$ C-3-COOH loses some activity due to the baking process. From these conclusions it appears that a method should be found to add 6-HT $\beta$ C-3-COOH to the food mixture and pellets formed (in some way) without heat. Alternatively, a method should be found to add the compound uniformly to prebaked pellets.

Buckholtz (1980) indicated that tetrahydro- $\beta$ -carboline compounds act as nerve poisons in mammalian systems and may specifically act by inhibiting monoamine oxidases in the liver. Homeida and Cooke (1982) found met-aldehyde to act in a similar manner in mice. Singh and Agarwal (1984) found the latex of *Euphorbia royleana* to act as a nerve poison in the snail *Lymnaea acuminata* by altering biogenic amine levels. In this study three species of slugs were killed by 6-HT $\beta$ C-3-COOH, while three species of freshwater snails were unaffected by it. These results indicate that slugs and snails possess either different specificity or different detoxification mechanisms for the compound. The compound 6-HT $\beta$ C-3-COOH could be used to elucidate these species differences, and other species-specific molluscicides might result from such research.

The molluscicide field test data are listed in Table III. *A. subfuscus* is a robust slug primarily inhabiting woodlands. Beyer and Saari (1978) discussed the ecology and feeding habits as well as the distribution of this slug particularly in the Ithaca, NY, area. Chichester and Getz (1969) noted that this slug has the potential to become the most serious slug pest in North America. It was therefore noteworthy that the formulated bait controlled this slug under intense feeding pressure. In Table III we note that two waves of slugs from the crownvetch field attacked the plants in the experimental area. The first wave of adult slugs, occurring at the beginning of the experiment on June 25, 1987, was essentially eliminated by the bait treatment by June 30, 1987. At this time the original bait was gone. Three additions of bait were made July 1, 3, and 5, 1987. The second wave of slugs began arriving on July 1, 1987, and was controlled in the experimental area by July 5, 1987. Further applications of molluscicide were unnecessary, and the bean crop proceeded to maturity and produced a normal crop in areas that had not originally been defoliated.

The development of an effective and attractive bait was almost as important as the identification and testing of the molluscicide itself. The treated bait was so attractive that within 30 min after application of the first bait all of the slugs in the bean rows left the beans and began devouring the bait. This occurred for as long as the bait was available—over a week after the last application. The treated bait therefore has considerable toxicity for a reasonably long time under conditions of high humidity.

Table III. Slug Numbers/Meter<sup>2</sup> of Treated Perimeter Rows or Enclosed Bean Rows

date, time	east side <sup>a</sup>	bean rows	west side	presence of bait
6/25/87 <sup>b</sup>				
7:45 p.m.	29 ± 9	78 ± 34	0	-
8:00 p.m.	bait applied			
8:15 p.m.	26 ± 8	1 ± 3		+
8:30 p.m.	32 ± 6	0		+
6/29/87				
10:00 a.m.	1 ± 1	1 ± 1	0	+
6/30/87				
11:00 a.m.	1 ± 1	0	1	+
7/1/87 <sup>c</sup>				
8:00 a.m.	0	17 ± 14	0	-
8:15 a.m.	bait applied			+
7/2/87				
9:00 a.m.	12 ± 14	1 ± 1	11	-
7/3/87				
9:00 a.m.	19 ± 6	26 ± 12	14	-
9:30 a.m.	bait applied			+
10:00 a.m.	55 ± 14	9 ± 6	319	+
7/4/87				
9:00 a.m.	0	1 ± 1	2	+
7/5/87				
11:00 a.m.	1 ± 3	0	0	-
11:30 a.m.	bait applied			+
12:00 p.m.	12 ± 9	0	12	+
7/6/87				
8:30 a.m.	1 ± 1	0	8	-

<sup>a</sup> Means ± standard deviation for four replicates; entire west side was counted and adjusted to match count areas for east side and bean rows. <sup>b</sup> Mature slugs, average weight 0.8 g each. <sup>c</sup> Immature slugs that were approximately half the size of mature slugs.

Godan (1983, Chapter 3) discussed the problems related to the control of pest gastropods. In addition to problems associated with finding additional effective and specific molluscicides, she stated: "It is clear that the problem of finding a substance as carrier base of the molluscicide and which is really attractive to most, if not all, the gastropods in an infested area has not been solved." (Godan, 1983, Chapter 3, p 189). The bait formulated as a molluscicide carrier in this study should effectively address the problem as she stated it, at least for slugs.

Further testing is required to determine (1) whether the bait can be used effectively under minimum tillage legume establishment conditions, (2) whether seed can be directly treated to afford protection against slugs, (3) whether drill rows can be treated in minimum tillage situations to control slugs in the treated band, and (4) whether 6-HT $\beta$ C-3-COOH could be used systemically to protect plants from slug predation.

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