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EVALUATION OF TWO SYSTEMS USED TO EXTRACT ALFALFA WEEVIL LARVAE (COLEOPTERA: CURCULIONIDAE) FROM ALFALFA SAMPLES[1]

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ABSTRACT

A modified Berlese funnel system was developed to extract alfalfa weevil larvae *(Hypera postica)* from quadrats 30.5 cm on a side. Data from this system were compared with simultaneous data from a hand sorting extraction system. In most instances, the modified Berlese system was as efficient as the hand sorting method and the number of man hours required to process samples by hand was far greater than that required by the Berlese system.

Intensive sampling programs designed to obtain data for constructing life tables, studying population dynamics, and supporting modeling efforts associated with the alfalfa weevil, *Hypera postica* (Gyllenhal), generate samples which often contain several hundred larvae each. Extraction of larvae from large quantities of plant material by conventional systems, such as the one used by Wilson and Armbrust (1970), is inadequate in terms of time involved for processing and efficiency in retrieving larvae. Reported here are results of research implemented to evaluate a modified Berlese funnel system versus hand sorting for speed and efficiency in extracting weevil larvae from alfalfa samples.

MATERIALS AND METHODS

Figure 1 shows the basic design of the Berlese unit. Upper and lower funnels were constructed of galvanized sheet metal and machined as near replicas. Light bulbs (40W) provided the drying temperatures in the funnels. Hardware cloth (6 mm) was fitted inside the bottom funnel to support the sample. Gaskets (top and bottom) were made by glueing split rubber tubing to the funnel rims. Rubber bands were stretched over two sets of bolts to secure the top and bottom halves and prevent escape of larvae. Individual units could be utilized as shown in Figure 2 or in a series (Fig. 3).

Three alfalfa fields were sampled on a degree day basis in Washington County, Illinois during the 1974 growing season. Initial samples were taken on 11 April and sampling continued until first harvest (24-29 May) for a total of 10 sampling dates in field IL-1, eight in IL-3, and nine in IL-6. When fields were sampled, 20 quadrats of alfalfa, 30.5 cm on a side, were cut just above the ground for each field sampled. Alfalfa stems and litter were placed in paper grocery bags. Soil surface was examined for any larvae remaining in the quadrat. After returning the samples to the laboratory, 10 were processed through the Berlese units which extracted larvae into alcohol in the attached canning jars. The contents of the 10 remaining samples (insects, plants, and litter) were placed in a modified Carnoy's fixative (Humason, 1967). These preserved samples were later washed through two Bureau of Standards screens (sieve openings of 12.7 mm and 0.177 mm) to recover

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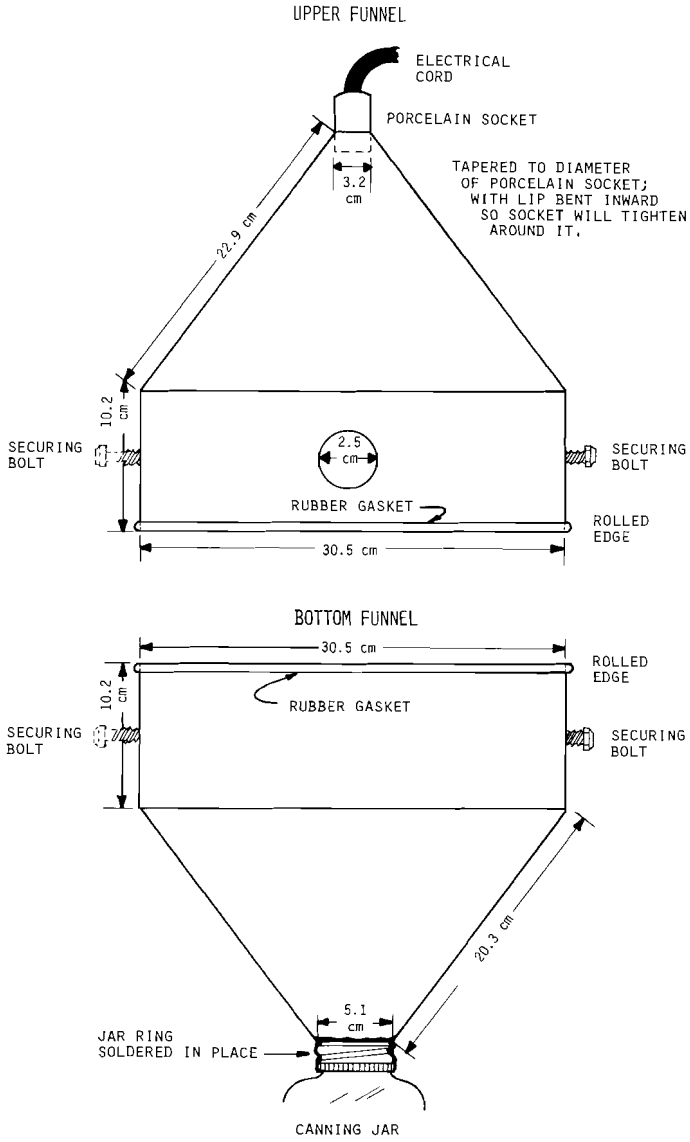

Fig. 1. Berlese unit design depicting the upper and lower funnels, respectively.

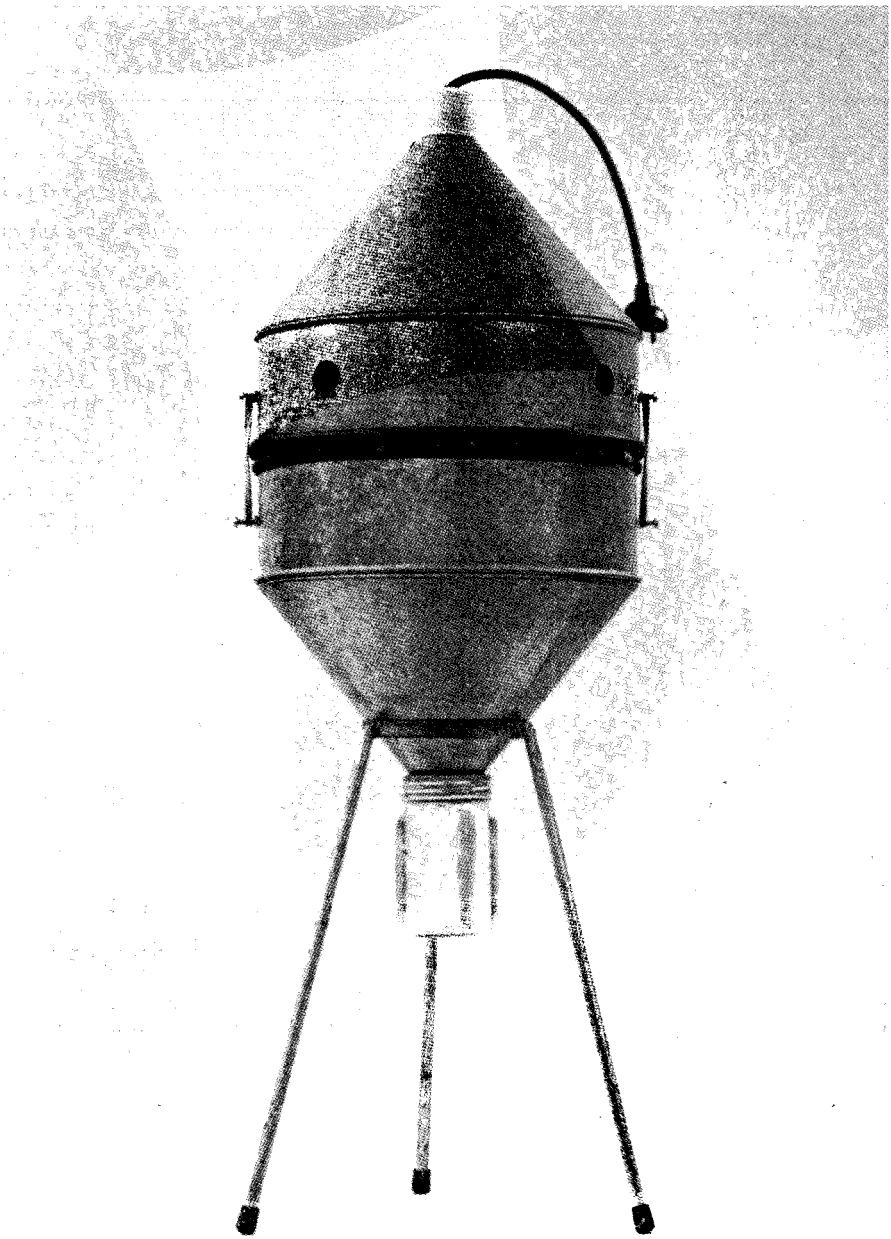

Fig. 2. The Berlese funnel used as an individual unit.

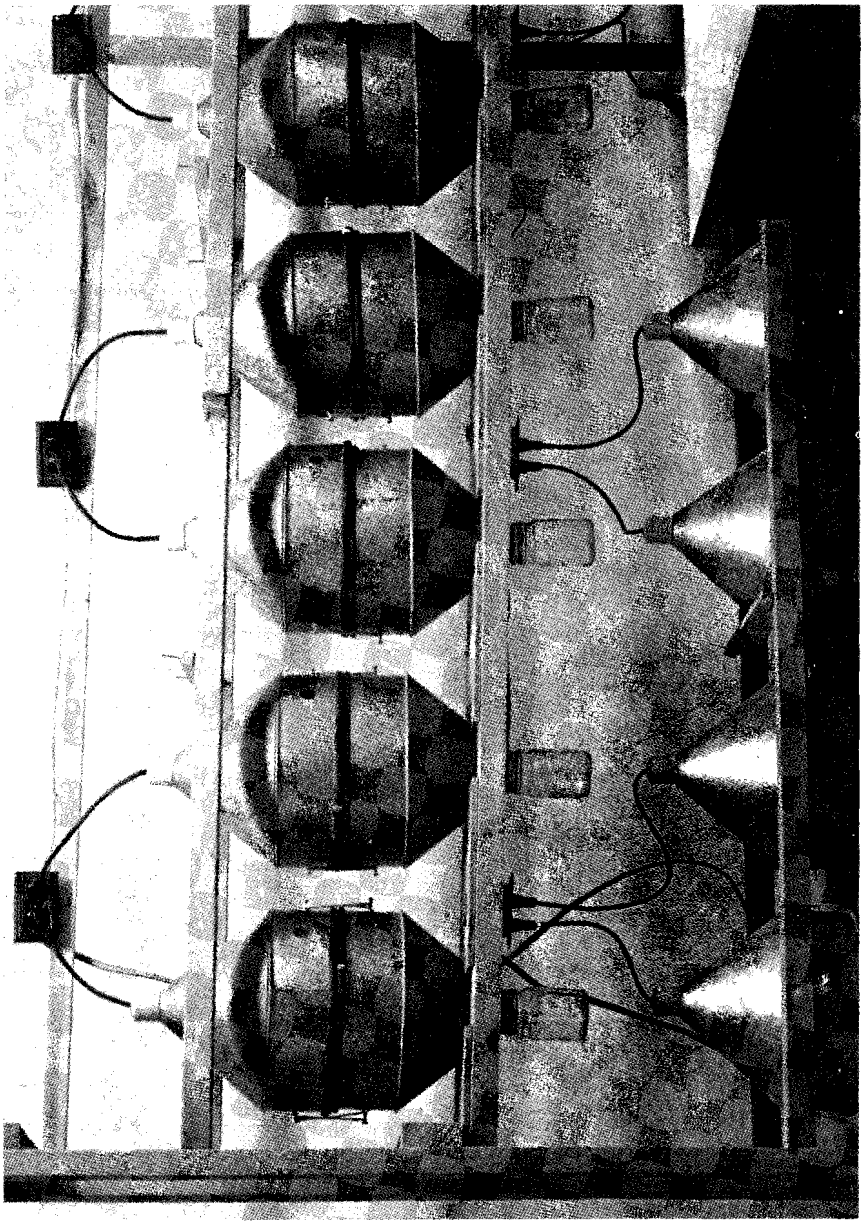

Fig. 3. Berlese units used in series on a double rack frame.

larvae that were free in the sample. The plants were then dissected to extract 1st and 2nd instars lodged in the terminals and leaf axils. The preserve-wash system was considered a measure of absolute density, in that great care was taken to search every plant for all larvae present. Larvae from each extraction system were categorized to instar with a head capsule caliper (Bartell and Roberts, 1974). Although the total extraction time for samples in the Berlese units varied from 36-72 hr, the time involved for hand labor to load and remove 10 samples was ½ hr. In contrast, hand labor for the preserve-wash required 2-3 hr for processing 10 samples. The time required for classifying larvae to instar and counting was independent of both systems.

These sampling and processing procedures yielded 27 data sets × four instars equaling 108 paired means (540 individual sample observations) which represented three different fields, differences in numbers of individuals per life stage during the season, a wide range of sampling conditions (wet, dry, etc.), and different sample sizes (by volume of plant matter) as the crop matured. Data for the four instars recovered by the two systems were compared by using a 2 × 4 factorial design and analysis for each date. The two factors were methods (2 levels) and instars (4 levels) thus making a 2 × 4 factorial.

RESULTS AND DISCUSSION

The F tests of the 108 paired means are essentially LSD comparisons, but were made only in those instances after the significant interactions of extraction systems used, and number of instars recovered showed evidence of real differences attributable to the four instars extracted and evidence of the nature of these differences. These differences were included in a separate and combined analysis of the data from the three fields. From the 108 comparisons, there were 22 and 3 in the separate and combined analysis respectively, with significant differences between the method used and the four instars extracted. Conversely, for 80% of the separate and 97% of the combined analysis there were no significant differences. Table 1 shows by field, date, and instar the 25 paired means that were significantly different. Twelve of these differences were attributed to higher numbers in the funnels, while 13 resulted from higher numbers in the preserve-wash system.

Peak density in the three fields was indicated by both extraction systems between 19 and 24 April. All fields had insecticide applications for larvae after 19 April but before 24 April. In field IL-6, 28 April, and 24 April of the combined field data, the preserve-wash

Table 1. Significant paired means for instars by field and date for Berlese (B) and preserve-wash (PW) extraction systems representing only those fields and dates where there was a significant F value for the interaction of instars recovered and extraction method used in the factorial analysis.

		INSTARS							
		1		2		3		4	
Field	Data	B	PW	B	PW	B	PW	B	PW
IL-1	24/5		–		–	0.1	1.4*	0.1	1.6**
IL-3	11/4		–	71.7*	52.1	61.8**	28.9	35.3**	7.2
	28/4		–	113.0**	47.8	57.7**	13.3	17.9**	4.0
	5/5	10.2	20.2**	64.9*	60.3		–	45.1**	17.9
	20/5	4.7**	0.5	8.6*	1.8		–	18.6	40.2**
IL-6	24/4		–	49.0	108.2**		–	6.0	18.5**
	28/4	0.5	13.5**	0.4	14.3**	0.5	5.6**	0.2	1.7**
	5/5		–	25.2**	8.8		–	2.1**	0.0
IL-1, 3 & 6 (combined data)	24/4	56.2	77.2**	38.0	72.7**		–	5.0	9.4**

system was significantly higher than the funnel system for all instars except 3rd in the combined data. These samples were taken on successive dates following insecticide spray applications. Since the preserve-wash system did not differentiate between live and dead larvae (all larvae within the sampling quadrat were preserved), those larvae killed by the insecticide could be extracted. The funnels essentially extracted only live larvae.

In fields IL-6, 3 and 1, on 24 April, 20 and 24 May, respectively, there were significantly higher numbers of the 4th instars recovered by the preserve-wash system. Prepupae which had already spun cocoons, could have been dislodged in the preserve-wash procedures and counted as 4th instars. This would not be as likely to happen with the Berlese extractions. Another possibility is that some of the 4th instars may have pupated in the funnels during extraction, and consequently lowered those counts.

The preserve-wash again was higher for 2nd instars in field IL-6 on 24 April, and 1st instars in field IL-3 on 5 May. On several sampling dates, the alfalfa was wet because of rain or a heavy dew. Some 1st and 2nd instars may have stuck to the bottom funnels and could have been overlooked. Excess moisture from the sample was usually absorbed by the paper grocery bag while in transit. The preserve-wash system is not affected by wet sampling conditions.

The number of processing steps for the preserve-wash system probably subjects this system to more human error. Larvae could have been lost in transferring the samples to the fixative jars, or from the fixative jars to the washing sieves. Larvae may have splashed out of the top sieve during the washing process. First and possibly 2nd instars may have been overlooked in the bottom sieve. Even though the plants were dissected to extract the smaller instars, some larvae could have escaped detection. Any of these conditions could have been responsible for the instances where the Berlese counts were higher than the preserve-wash.

Although 1st and 2nd instars are lodged in the plant terminals and leaf axils, the Berlese system extracted all instars as well as the preserve-wash system for 80% of the individual field and 97% of the combined sample comparisons. Wet sampling conditions and pupation of 4th instars during extraction may cause an underestimated larval density when samples are processed by the Berlese system. Absolute density samples (processed by the preserve-wash system) most likely overestimated larval densities after insecticide applications until dead larvae were no longer present in the samples. Considering time for processing and no significant differences for at least 80% of the comparisons of the two systems, the Berlese funnel system is an acceptable method for approximating densities of alfalfa weevil larvae from the sample units. This Berlese extraction system can be adapted to a variety of insect-plant situations by changing bulb and hardware cloth size. The merits of this extraction system for population estimates of another insect species should be determined by similar data comparisons with absolute density samples of that insect.

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