Estimating *Lygus lineolaris* (Heteroptera: Miridae) Population Densities in Sugarbeet

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ABSTRACT

Two relative sampling methods, the commonly used *in situ* visual inspection technique and an alternative Allen-vac (A-vac) method, were compared with absolute (total plant capture) sampling to estimate densities of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), in late-summer sugarbeet fields in the Red River Valley. A-vac counts regressed on total plant capture resulted in the best relationship with R^2 values of 0.81 and 0.67 for combined life stages and nymphs, respectively. Visual counting had correlation coefficients of 0.45 and 0.34 when regressed on the absolute method for combined life stages and nymphs, respectively. Visual counts regressed on total plant capture counts resulted in the best relationship (R^2 = 0.50) for adult *L. lineolaris* population density estimates. The A-vac technique is slightly more accurate than visual counting of nymphs and mixed infestations of nymphs and adults; however, visual inspections also will provide reliable estimates of *L. lineolaris* infestation levels when the use of specialized equipment is not preferred.

Additional key words: *Beta vulgaris* L., relative sampling, absolute sampling, Lygus bug, tarnished plant bug

The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), was first reported causing feeding injury to sugarbeet, *Beta vulgaris* L., grown for processing in the Red River Valley of Minnesota and

North Dakota in August of 1998 (Armstrong, 1999). Several thousand hectares of sugarbeet have since been treated for TPB control. Boetel et al. (2003) reported that growers in eastern North Dakota and western Minnesota applied insecticides to at least 2,800 ha of sugarbeet due to a TPB outbreak in 2001; however, debate exists regarding the potential for TPB to cause economic yield loss in sugarbeet.

The tarnished plant bug is widely distributed across North America (Schwartz and Foottit, 1992), and feeds on a diversity of host plants. It has a documented host range of at least 385 plant species, and 130 are economically important crops (Young, 1986). Insects in the *Lygus* genus prefer feeding on actively growing portions of host plants such as reproductive and meristematic tissues (Strong, 1970). Adult and nymphal stages of the tarnished plant bug inflict injury to sugarbeet by using piercing-sucking mouthparts to feed on petioles of new and emerging leaves, with damage symptoms including wilted and curled leaves, seepage of blackened exudate from feeding sites, raised necrotic scars, and occasional discoloration and necrosis of leaf tips (Boetel et al., 2005; Yun, 1986).

There are typically two generations of TPB per year in southern Alberta, Canada (Gerber and Wise, 1995), whereas the insect is capable of producing four or more overlapping generations per year in the southern United States (Anderson and Schuster, 1983; Day, 1987). Red River Valley TPB populations are thought to occasionally produce a third generation, which leads to late-season infestations in sugarbeet after other host habitats senesce or are harvested (Boetel et al., 2005; Knott, 2005). Infestations in sugarbeet are sporadic and can vary both spatially and temporally. The insects frequently can be found on flowering redroot pigweed, *Amaranthus retroflexus* L., and common lambsquarters, *Chenopodium album* L., in late-season sugarbeet fields (J.O.K., personal observation).

Sugarbeet producers and agronomists typically assess tarnished plant bug populations by visually inspecting individual plants. Visual inspection is the preferred method for sampling TPB in sugarbeet because specialized equipment is not required and no effective relative method is currently available. Garcia et al. (1982) concluded that sampler bias is the largest potential drawback to visual counts for sampling arthropods in cotton, and that varying levels of sampler experience can result in sampler biases. Adult TPB occasionally fly away when disturbed. Early instar nymphs are small and usually feed near the crowns of sugarbeet plants. These behavioral attributes can decrease sampling precision and, thus, result in underestimation of the actual population density in a particular field.

The Allen-vac (A-vac), developed for use in strawberry (Allen et al., 1988), is an alternative to visual counts. It consists of a modified leaf blower/vacuum that collects insects by suctioning them from plant canopies. The A-vac is preferred over sweep net sampling in strawberry because it allows for less mechanical injury to the fruit. The objective of this study was to compare the efficiency of two relative sampling methods, visual counts and A-vac, in relation to absolute sampling (total plant capture) for estimating tarnished plant bug densities in sugarbeet.

MATERIALS AND METHODS

Populations of TPB were sampled in six Red River Valley commercial sugarbeet fields between 15 August and 5 September during the 2002 growing season. The experiment was arranged in a randomized complete block design with twenty-four replications (four replications per field) of the following treatments: 1) whole-plant visual counts; 2) A-vac and 3) total plant capture. Replicates were parallel to the field margin at each location to remove potential variability associated with edge effects on TPB abundance. Twenty randomly selected plants were assessed per sampling method in each block. Insects positively identified as tarnished plant bugs emigrating from a randomly selected plant immediately before it was sampled were included in the counts.

Whole-plant visual counts. Each plant was examined for the presence of TPB adults and nymphs for a period of twenty seconds. Sampling began with inspection of the outer leaves, then followed with searching inward and downward from the leaf tips into the sugarbeet plant crown. After completing inspection of an individual plant, the soil surface beneath its canopy was examined for dislodged insects, which were included in the counts.

Modified A-vac. A two-cycle gasoline-powered blower/vacuum (Weed Eater BV 2000, Poulan, DeQueen, AR) with a nylon stocking placed over the air intake served as a modified A-vac. The device was moved in a circular fashion over the foliage of a single plant for three seconds, then placed directly above the crown for three seconds on each plant. Care was taken to prevent the air exhaust of the A-vac from inadvertently being directed toward and disturbing nearby plants that were subsequently sampled. A small foam rubber toy football was placed in the air intake of the A-vac immediately after sampling an individual plant but before the instrument was turned off, thus preventing escape by captured insects. The stocking and football were then removed and

the stocking was inverted to transfer the insects into a transparent plastic bag. Specimens from the A-vac samples were counted and recorded in the field.

Total plant capture. The total plant capture method required two samplers. One person gathered and held the sugarbeet plant foliage together while a second placed a trash bag over the plant and tightened drawstrings around the plant crown. Each plant was then cut off at the soil level and sealed into the bag. Both samplers maintained close watch for escaping insects. Additionally, the soil surface directly beneath the sampled plant was examined for dislodged insects. Those observed escaping or beneath the canopy of sampled plants were recorded and included in the total plant capture counts. All bags were taken to the laboratory and stored at 3°C pending subsequent processing of samples for captured *L. lineolaris* adults and nymphs.

Statistical analysis. A-vac and visual count sampling techniques were compared to the total plant capture method in a fashion similar to that of Browde et al. (1992). Simple linear regression was used to measure the correlation of each of the two relative sampling methods (used as dependent variables) with the absolute (i.e., total plant capture) method, which served as the independent variable. Fidelity of each relative sampling technique, measured by the R^2 value for its relationship with the absolute method, was compared for TPB nymphs, adults, and combined life stages. Previous work has shown that pooling observations by environment improves correlation (Browde et al., 1992; Luna et al., 1982; Rudd and Jensen, 1977; Zalom et al., 1993). Therefore, our regression analyses were performed by using means of the replicates.

Counts obtained by using relative sampling methods require conversion to absolute population levels that are standardized per unit surface area before density estimates can be used for population or pest management studies. The regression equations in this study were solved for probable tarnished plant bug counts to generate absolute population density estimates for twenty plants. Calculated absolute population densities were multiplied by a conversion factor of 0.43245 to standardize them on a per-unit-area (m^2) basis according to a typical sugarbeet plant population (86,500 plants/ha) for the region.

RESULTS

A-vac sampling. A-vac and total plant capture were significantly (F = 91.40; $P < 0.0001$) correlated ($R^2 = 0.81$) when TPB life stages were

Table 1. Linear regression statistics for *L. lineolaris* sampling method comparisons.

Regression	Life stage	*a* ± SE	*b* ± SE	*P*	R^2
A-vac to	Nymph	0.07 ± 0.08	0.92 ± 0.14	< 0.0001	0.67
total capture	Adult	0.10 ± 0.09	1.73 ± 0.64	0.0124	0.25
	All stages	-0.10 ± 0.12	1.57 ± 0.16	< 0.0001	0.81
Visual to	Nymph	0.19 ± 0.09	0.52 ± 0.15	0.0030	0.34
total capture	Adult	0.10 ± 0.05	1.74 ± 0.37	0.0001	0.50
	All stages	0.20 ± 0.15	0.89 ± 0.21	0.0003	0.45

combined (Table 1). A-vac sampling was more effective at capturing combined life stages of the insect than total plant capture, and the relationship between A-vac and absolute sampling was linear ($b = 1.57 \pm 0.16$). The A-vac method had greater overall fidelity ($R^2 = 0.81$) with the absolute (total plant capture) method than visual counts ($R^2 = 0.45$) for estimating densities of all life stages combined. The A-vac technique and total plant capture were also highly correlated for nymphs ($F = 45.55$; $P < 0.0001$). Nymphs were collected with the same relative efficiency by using the A-vac and total plant capture sampling methods. This was evidenced by the slope with its associated standard error not deviating significantly from 1 ($b = 0.92 \pm 0.14$; $P < 0.0001$; $R^2 = 0.67$). The A-vac method had a relatively poor relationship with total plant capture for estimating adult populations ($b = 1.73 \pm 0.64$; $P = 0.0124$; $R^2 = 0.25$).

Visual counts. Visual counts and total plant capture were significantly ($F = 17.87$; $P = 0.0003$) correlated for all TPB life stages combined; however, the regression generated a relatively low R^2 value (0.45). The slope with its associated standard error ($b = 0.89 \pm 0.21$) was not significantly different from 1. Therefore the visual count and total plant capture sampling techniques were considered not significantly different from each other for estimating densities of all life stages combined. Visual counting and total plant capture had a significant relationship for nymphs ($F = 11.10$; $P = 0.0030$) and adults ($F = 22.35$; $P < 0.0001$). Visual counts consistently underestimated nymphs ($b = 0.52 \pm 0.15$; $R^2 = 0.34$) and overestimated adult densities ($b = 1.74 \pm 0.37$; $P = 0.0001$; $R^2 = 0.50$) when compared with total plant capture sampling. Conversions of relative sample counts to absolute population estimates per m^2 are presented in Table 2.

DISCUSSION

Both A-vac and visual whole-plant counts were highly correlated with the total plant capture method when life stages were separated or combined. The A-vac resulted in the highest fidelity with total plant capture for all life stages combined, and appeared to also be effective at estimating infestations of TPB nymphs; however, it tended to overestimate adult densities. This could have resulted from adults being pulled into the A-vac air stream from the airspace of adjacent sugarbeet plants. This is consistent with the findings of Schotzko and O'Keeffe (1986) and Pruess et al. (1987) in sampling lentil and alfalfa habitats, respectively, who observed that relative vacuum methods overestimated

Table 2. A-vac and visual counts of *L. lineolaris* (nymphs and adults combined) converted to absolute population densities.

A-vac sample (*L. lineolaris* /20 plants)	**Population estimate (*L. lineolaris* /m^2)**	**Visual counts (*L. lineolaris* /20 plants)**	**Population estimate (*L. lineolaris* /m^2)**
1	0.3	1	0.5
2	0.6	2	1.0
3	0.8	3	1.5
4	1.1	4	1.9
5	1.4	5	2.4
6	1.7	6	2.9
7	1.9	7	3.4
8	2.2	8	3.9
9	2.5	9	4.4
10	2.8	10	4.9
11	3.0	11	5.3
12	3.3	12	5.8
13	3.6	13	6.3
14	3.9	14	6.8
15	4.1	15	7.3
16	4.4	16	7.8
17	4.7	17	8.3
18	5.0	18	8.7
19	5.2	19	9.2
20	5.5	20	9.7

A-vac absolute estimate = (A-vac + 0.1 / 1.57 = total plant capture) * 0.43245.
Visual count absolute estimate = (visual count - 0.2 / 0.89 = total plant capture) * 0.43245.

adult TPB populations in comparison to absolute sampling due to an inflated sampling area.

The visual inspection technique in our study estimated nymphs with relatively low fidelity to the absolute (total plant capture) technique. This could have been the result of small size and cryptic coloration of nymphs that allows them to blend in with the light green sugarbeet plant crowns and leaf petioles. In addition, many places are available for harborage by small nymphs in the sugarbeet canopy and understory. Visual counting tended to overestimate adult densities, although it achieved relatively strong fidelity with the total plant capture method. Adult tarnished plant bugs often were observed landing in the canopy and flying away when plants were disturbed by wind or as samplers approached. Thus, adults flying away from nearby plants could have been errantly counted as emigrating from randomly sampled plants. These effects disappeared when life stages were combined, and similar estimates of the overall population to that of total plant capture counts were achieved. Therefore, because damaging infestations in sugarbeet typically include all life stages (Knott et al., 2002), it is concluded that visual counts should provide accurate estimates of tarnished plant bug densities for making management decisions.

Both A-vac and whole-plant visual count sampling techniques have benefits and drawbacks for monitoring TPB infestations in sugarbeet. The A-vac method provides counts that most accurately reflect infestation levels of immature stages of the insect. A-vac sampling also has the advantage of quick assessment of sugarbeet foliage and penetration of the crown. Disadvantages of using a vacuum device include the expense of purchasing each device and slightly more labor associated with carrying it through the field during sampling. Both methods tend to slightly overestimate adult populations, although the visual method results in somewhat higher fidelity with the absolute (total plant capture) method. Visual counting and A-vac sampling techniques will likely be preferred over total plant capture for making management decisions because they can be carried out rapidly and provide immediate feedback; however, a tradeoff could be an occasional reduction in accuracy. Although the total plant capture method requires two samplers and subsequent laboratory processing, it is more accurate for deriving population estimates. Overall, the A-vac method gives the most precise population estimates when adult and nymph counts are combined. Producers and agronomists should be able to accurately and effectively estimate tarnished plant bug densities in sugarbeet by using either A-vac or visual sampling techniques.

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