Sequential Sampling and Biorational Chemistries for Management of Lepidopteran Pests of Vegetable Amaranth in the Caribbean

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ABSTRACT Although vegetable amaranth, *Amaranthus viridis* L. and *A. dubius* Mart. ex Thell., production and economic importance is increasing in diversified peri-urban farms in Jamaica, lepidopteran herbivory is common even during weekly pyrethroid applications. We developed and validated a sampling plan, and investigated insecticides with new modes of action, for a complex of five species (Pyralidae: *Spoladea recurvalis* (F.), *Herpetogramma bipunctalis* (F.), Noctuidae: *Spodoptera exigua* (Hubner), *S. frugiperda* (J. E. Smith), and *S. eridania* Stoll). Significant within-plant variation occurred with *H. bipunctalis*, and a six-leaf sample unit including leaves from the inner and outer whorl was selected to sample all species. Larval counts best fit a negative binomial distribution. We developed a sequential sampling plan using a threshold of one larva per sample unit and the fitted distribution with a k_c of 0.645. When compared with a fixed plan of 25 plants, sequential sampling recommended the same management decision on 87.5%, additional samples on 9.4%, and gave inaccurate recommendations on 3.1% of 32 farms, while reducing sample size by 46%. Insecticide frequency was reduced 33–60% when management decisions were based on sampled data compared with grower-standards, with no effect on crop damage. Damage remained high or variable (10–46%) with pyrethroid applications. Lepidopteran control was dramatically improved with ecdysone agonists (tebufenozide) or microbial metabolites (spinosyns and emamectin benzoate). This work facilitates resistance management efforts concurrent with the introduction of newer modes of action for lepidopteran control in leafy vegetable production in the Caribbean.

KEY WORDS *Amaranthus* spp., callaloo, *Spodoptera* spp., *Spoladea recurvalis*, *Herpetogramma bipunctalis*

VEGETABLE AMARANTH, *Amaranthus viridis* L. and *A. dubius* Mart. ex Thell., are grown in Jamaica, other Caribbean islands, and parts of Africa, predominantly on small holdings of under two hectares. The local market is highly lucrative because vegetable amaranth is traditionally part of the Jamaican diet, where it is known as callaloo, and it is a potential nontraditional export crop with the United States and Canada as main markets. From 1991–1995, the crop's value to Jamaica's economy increased from U.S. $2.3 to $8.7 million, the price paid to growers increased ≈2.5-fold, and acreage increased 1.6-fold.

Vegetable amaranth is grown from transplants. Vegetative shoots (≈30–45 cm long) with wide basal leaves are harvested before the development of reproductive structures, and new vegetative shoots emerge from axillary buds. Hand-harvesting of vegetative shoots continues approximately weekly for 6–12 mo from a given planting. Plants selected for horticultural and culinary qualities ("land races," sensu Allard 1999) are allowed to produce seeds, which are then used to grow transplants in nursery settings. Nurseries are in the same geographical region as production fields, sometimes on the same farm. Thus, the crop is present continuously, with nearby fields and transplant nurseries in various stages of vegetative and reproductive growth.

This crop is attacked by numerous herbivores (Clarke-Harris et al. 1998). The major yield losses are from five lepidopterans in either the Noctuidae [*Spodoptera frugiperda* (J. E. Smith), fall armyworm; *S. exigua* (Hb.), beet armyworm; and *S. eridania* (Cramer) southern armyworm] or Pyralidae [*Herpetogramma bipunctalis* (Fabr.), southern beet armyworm; and *Spoladea recurvalis* (F.), Hawaiian beet armyworm]. Although only one or a few of these species typically predominate in a given field, any combination of these species can be present at any one time or field.

Growers currently rely on insecticides applied on a calendar basis (usually every 7–8 d) to control lepidopteran larvae. Field failures of insecticide applications have been observed (unpublished data). One potential cause of field failures is insecticide resistance, and preliminary data suggest the presence of pyrethroid resistance in at least *S. exigua* in Jamaica, which is consistent with reports of resistant *S. exigua*

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in the United States (e.g., Brewer and Trumble 1994). Insecticides with novel modes of action that are more selective for lepidopterans, and thus often termed biorational materials, are available in the United States. These include ecdysone agonists (e.g., tebufenozide, Dhadialla et al. 1998), the semisynthetic microbial metabolite from *Streptomyces avermitilis* [e.g., emamectin benzoate, (Lasota and Dybas 1991)], microbial metabolites from *Saccharopolyspora spinosa* (e.g., spinosyns, DowElanco 1997), and extracts from the neem tree, *Azadirachta indica* (National Research Council 1992). With the exception of neem, these materials are not generally available on the Jamaican island. However, *S. exigua* has the capability of developing resistance to spinosyns (Moulton et al. 1999) and tebufenozide (Moulton et al. 2002), and scientists have called for proactive resistance management with these valuable selective chemistries.

A proactive resistance management program would include a scouting program to limit applications to times when they are necessary. Preliminary work identified an action threshold of two larva per plant based on visual inspection of 12 leaves from each of 25 plants (D.C.-H., unpublished data), but this is a labor-intensive scouting system. Sequential sampling plans have the potential of reducing sampling labor and encouraging sampling, and thus the implementation of integrated pest management and resistance management. Sequential sampling programs have been developed for a co-occurring complex of species in sweet corn (Hoffmann et at. 1996). The goal of this work was to develop and validate a sequential sampling plan for the lepidopteran complex in vegetable amaranth. We modeled the frequency distribution of sampled counts, developed a sequential sampling plan, and examined the influence of this plan on sampling labor and insecticide inputs on farms in Jamaica. We also report on the relative efficacy of new biorational materials for lepidopteran control in vegetable amaranth.

Materials and Methods

We monitored lepidopteran populations twice per week in 137 m^2 (1,000 plants) field plots at Bodles, St. Catherine Parish, Jamaica, for three cropping seasons: April–July 1997, August–November 1997, and December 1997–March 1998. Each field plot was divided into four quadrats, each containing 250 plants. We selected eight plants per quadrat (32 plants per sampling date), choosing one plant every 10 paces while walking a zigzag path through each quadrat, using arbitrary starting points. We counted the number of larvae on six leaves of each plant, including three leaves from both the inner and outer whorl (n = 192 leaves per sampling date), and compared densities between these two within-plant positions with analysis of variance (ANOVA) (SAS Institute 1995) for *S. recurvalis*, *H. bipunctalis*, and for the combination of all *Spodoperta* spp. These ANOVAs combined data for all dates and plants, and compared densities on leaves from the inner whorl with densities on leaves from the outer whorl. We monitored relative densities of adults using a 61-cm diameter sweep net and a two-sweep sampling unit. Ten two-sweep samples were taken per quadrat (n = 40 per date) among the selected plants. All sweeps were done before conducting other sampling activities. Total sweep net capture of adults was tested for correlation against total larval density using Pearson's product-moment correlation coefficient (SAS Institute 1995). Identifications were confirmed with the Caribbean Network for Biosystematics (CARINET) and voucher specimens of adults were placed in Commonwealth Agricultural Bureau International in Trinidad.

Larval counts per six-leaf sampling unit were placed into categories of 0 to >20 (21 categories), and the resulting frequency distributions from 40 sampling dates were compared with expected frequencies from both a Poisson and negative binomial frequency distribution with a χ^2 test at $\alpha = 0.05$. A k parameter for a negative binomial distribution was estimated from the variance and mean (Elliot 1977, Davis 1994) for each sampling date and whole field. Independence of k with the mean was examined with regression (Elliot 1977), and a common k (k_c) was estimated by regressing y' on x' (Elliot 1977, Fleischer et al. 1991). Equations in Waters (1955) and Binns (1994) were used to calculate sequential sampling plans assuming a negative binomial distribution with a k_c based on the modeled probability density function. We used varying inputs of upper (m_o) and lower (m_1) class limits (which bracketed an action threshold of one larva per plant with a six-leaf sampling unit), and allowable type I and type II error rates. As in Fleischer et al. (1991), expert opinion (of D. Clarke-Harris) was used to choose parameters that resulted in a practical sampling plan. The final parameters chosen were m_o = 0.75, m_1 = 1.25, and estimated type I = type II error rates of 0.2. We conducted validation tests (see below) using a minimum of 10 and a maximum of 25 sample units. A minimum sample size of 10 sample units was set to reduce decision errors and the maximum number of sample units was fixed at 25 based on the estimated time required (45 min to 1 h) to take 25 sample units. The subjective error rates were chosen after reviewing the minimum sample size under varying error rates. Use of arbitrary minimum and maximum sample units nullifies the theoretical error rates, therefore the error rates achieved should not be equated with the error rates used to parameterize the sample plan, and achieved error rates are unknown.

As in Luna et al. (1983), we compared the pest management recommendation and sample size needed to reach this recommendation when using the sequential sampling plan or a fixed sample size of 25 plants. We conducted these validation tests using an action threshold of one larva per plant on 32 farms within a 28-km^2 radius in St. Catherine Parish during March 1998.

We also compared frequency of insecticide inputs and damage from lepidopteran larvae when using two decision-making criteria: (1) the sampling plan, versus (2) weekly application representing a grower standard. We conducted these tests on four farms in Bushy

Park, St. Catherine Parish, from 7 July 1998 to 3 May 1999. The trials occurred over 12, 22, 15, and 15 wk on farms 1 through 4, respectively. On each farm, two square plots of 400 plants each were established on the edge of a larger planting of vegetable amaranth. In one plot, insecticide applications were made when total larval densities exceeded thresholds as determined with the sequential sampling plan. On the grower standard plot, insecticide applications were made once per week. The same insecticide (550 ml/ha of Karate SG; 18.3 g [AI]/ha of lambda cyhalothrin) was used in both plots. In each plot, 25 plants were selected randomly and the number of lepidopteran larvae recorded from six leaves as previously described. Stalks of harvestable length (≥30 cm) were cut from all plots every 8 d, and the percentage of leaves with damage from lepidopteran feeding recorded. The influence of the decision-making criteria (sampling plan versus grower-standard) on the frequency of insecticide applications, and percentage loss from lepidopteran feeding, was compared using likelihood G^2 (SAS Institute 1995).

We compared efficacy of nonpyrethroid insecticides in a randomized complete block experiment (five treatments and four replicates) at the Caribbean Agricultural Research and Development Institute, on the campus of the University of the West Indies, Mona, Kingston, Jamaica, during 16 March–28 June 2000. Treatments were 263 ml/ha of Proclaim 5SG (13 g [AI]/ha of emamectin benzoate), 512 ml/ha of Confirm 2 F (123 g [AI]/ha of tebufenozide), 512 ml/ha of Spintor 2 SC (123 g [AI]/ha of spinosyns), 585 ml/ha of Ecozin (18 g [AI]/ha of azadirachtin), and the farmer standard (control) of 550 ml/ha of Karate SG (18.3 g [AI]/ha of lambda cyhalothrin). Each plot contained 56 plants (8 × 7 arrangement) in a 6.5-m^2 area; data were collected from the 20 central plants. Plants were fertilized approximately every 21 d and irrigated as needed. Treatments were applied once per week with a calibrated hand-held backpack sprayer beginning 2 wk after transplanting. The number of lepidopteran larvae was recorded from five plants using six leaves per plant as before. The influence of treatment on total lepidopteran larval density was tested with ANOVA (SAS Institute 1999) on each date, using a single mean per plot, and the replicate × treatment interaction as the error term.

Results

The range of densities under which the sequential sampling plan was parameterized is shown in Fig. 1. The total number of lepidoptera larvae per 32 plants, using the six-leaf sample unit, ranged from 19 to 238 during April to July, 0 to 322 during August to November, and 2 to 193 during December to March. Total adult density per 40 sweeps and total larval lepidoptera density was correlated for each species and for all species combined ($r = 0.71$, df = 47, $P < 0.0001$ for *S. recurvalis;* $r = 0.72$, df = 47, $P < 0.0001$ for *H. bipunctalis;* $r = 0.61$, df = 47, $P < 0.0001$ for *Spodoptera* spp.; and $r = 0.72$, df = 47, $P < 0.0001$ for all lepidoptera combined). Larval densities of *H. bipunctalis* were significantly higher on inner whorl leaves than outer whorl leaves ($F = 23.17$; df = 1, 2238; $P < 0.001$), but there was no difference for within-plant position for *S. recurvalis* ($F = 3.27$; df = 1, 2239; $P = 0.07$) or *Spodoptera* spp. ($F = 1.51$; df = 1, 2239; $P = 0.22$).

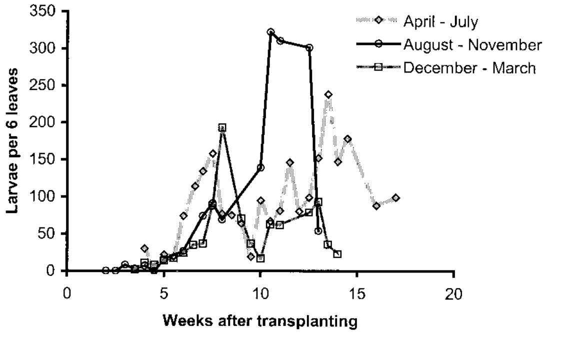

Fig. 1. Seasonal dynamics of total lepidopteran larvae per six leaf sampling unit on vegetable amaranth over three crop seasons in St. Catherine Parish, Jamaica.

The negative binomial model consistently gave a much better fit of the expected frequency distribution of total lepidopteran larvae per six leaf sampling unit than did a Poisson model. Of 40 sampling dates tested, frequency estimates differed significantly from expected in 78% of sampling dates using the Poisson model, and 10% using the negative binomial model (Fig. 2, $\chi^2 > 30.14$, $P < 0.05$, df = 19 for the Poisson model; $\chi^2 > 28.87$, $P < 0.05$, df = 18 for the negative binomial model). The regression of $1/k$ against the mean for each sampling date showed no statistical trend ($F = 2.2$; df = 1, 33; $P = 0.15$ when all data were considered, and $F = 0.6$; df = 1, 32; $P = 0.45$ when two outliers at the upper left of the scatterplot were deleted) (Fig. 3). Since the influence of the mean on k was weak, we assumed that a common k (k_c) which would be applicable to the range of growing condi-

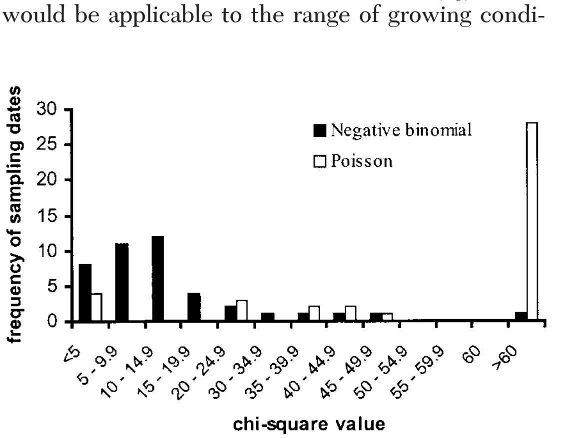

Fig. 2. Frequency distribution of χ^2 values generated from fitting 192 six-leaf samples against a negative binomial and a Poisson frequency distribution. Observed distributions were not significantly different ($P < 0.05$) than the expected when the χ^2 was <28.87 for the negative binomial and <30.14 for the Poisson.

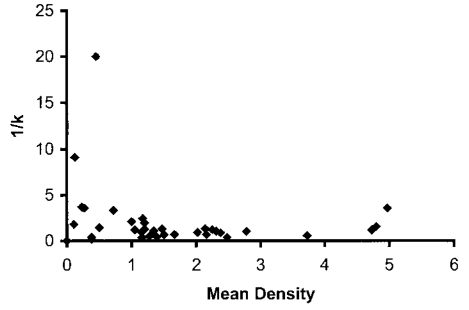

Fig. 3. Scatter plots of $1/k$ against mean larval density per six leaf sampling unit. Each point represents the results from sampling from one field on one date.

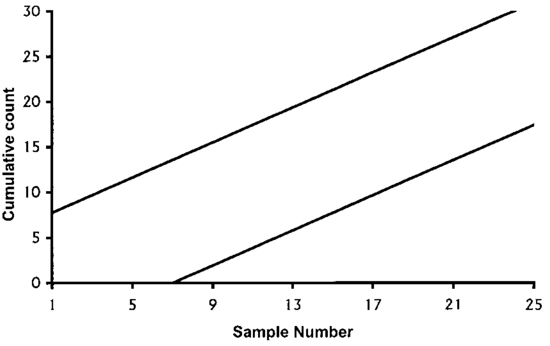

Fig. 5. Sequential sampling plan for a complex of lepidopteran larvae in vegetable amaranth assuming a negative binomial distribution with $k = 0.645$, alpha = beta = 0.2, $m_0 = 0.75$, and $m_1 = 1.25$. Stop lines are y = 0.966 x ± 6.779 for the upper and lower lines, respectively.

tions encountered in the callaloo growing areas in Jamaica could be used in developing the model. The inverse of the slope from the regression of y' on x' using all the data provided an estimate of k_c of 0.645 (Fig. 4).

Sequential sampling plans were generated using a negative binomial frequency distribution model with $k_c = 0.64$, an action threshold of one larva per plant (6-leaf sampling unit), and varying type I and II error rates. Plans were assessed using our subjective knowledge of farmer tolerances and potential sampling labor inputs. A sampling plan with both type I and II error rates set at 0.2 (Fig. 5) was selected as the most feasible. Stop lines were y = 0.966 x ± 6.779 for the upper and lower lines, respectively.

Validation trials of the pest management decision reached by the sampling plan occurred on 32 fields. As discussed in the methods, these trials imposed a minimum sample size of 10 sample units to reduce decision errors and a maximum of 25 sample units based on the estimated time required (45 min to 1 h) to take 25 sample units in a field. A fixed sampling plan using 25 sample units per field resulted in eight of these fields classified as above threshold, and 24 below threshold. The sequential sampling plan recommended the same pest management decision in all of fields that were above threshold, and in 79.2% of the fields below threshold (Table 1). Inaccurate recommendations were reached in 8.3% of the fields below threshold, and 12.5% of these fields resulted in no recommendation (after 25 samples) with the sequential sampling plan. In 27 of the 32 fields (84.4%), the same management recommendation was reached using the sequential sampling plan as a fixed sampling plan. With the sequential sampling plan, recommendations were reached with a mean of 13.5 sample units per field. This represents a saving of 46% compared with a fixed sampling plan of 25 sample units.

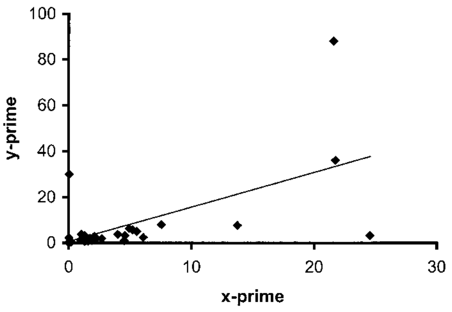

Fig. 4. Regression of y' on x' to estimate a common k (k_c) from the inverse of the slope as described in Elliot (1977). Estimate of k_c was 0.645, from the line $y' = 0.044\ (\pm 2.60) + 1.549\ (\pm 0.344)\ x'$, $R^2 = 0.38$.

Validation trials of the effect of using the sampling plan on foliar lambda cyhalothrin application frequency were conducted on four farms, ranging from 12 to 22 wk per farm (Table 2). Lepidopteran larval densities per 25 leaves ranged from 19.5 to 38.8; the number of pyrethroid applications ranged from 6 to 22, and the percentage loss from these larvae ranged from 10 to 46% among the farms. Basing spray frequencies on the sampling plan and its associated threshold, as compared with a grower standard of weekly sprays, significantly reduced the frequency of lambda cyhalothrin sprays (likelihood ratio $G^2 = 7.3$, df = 1, $P = 0.007$), while having no significant influence on the percentage loss from lepidopteran larva (likelihood ratio $G^2 = 0.02$, df = 1, $P = 0.89$).

In the comparison of biorational insecticides with grower-standards, larval densities were low (mean of <1 larva per six leaves) in all treatments for the first six sampling dates, but began to rise by the seventh sampling date, reaching peaks of 45 and 16 per six leaves in the neem and lambda cyhalothrin treatments, respectively, on 2 June (Fig. 6). A second peak

Table 1. Percentage of pest management decisions reached using the sequential sampling plan and action threshold compared with decisions reached with a fixed sampling plan

Decision using 25-sample plan	n	Decision from sequential sampling plan (%)		
		Correct	No decision	Incorrect
Above threshold	8	100	0	0
Below threshold	24	79.2	12.5	8.3

Table 2. On-farm validation trials comparing application frequency and percentage of crop lost due to lepidopteran larval feeding when applying lambda cyhalothrin either weekly (grower standard, GS) or based on results from a sequential sampling plan (SP)

Farm	Larvae (mean per six leaves ± SD)		Frequency of sprays[a]		Percent loss from lepidopteran damage[b]	
	GS	SP	GS	SP	GS	SP
1	36.7 ± 38.5	38.8 ± 27.2	12	8	46	44
2	19.5 ± 14.8	24.2 ± 14.1	22	13	30	33
3	30.4 ± 66.0	30.6 ± 38.9	15	6	11	10
4	24.4 ± 23.5	26.5 ± 24.1	15	10	20	22

[a] Likelihood ratio G^2 comparing frequency of sprays = 7.3, df = 1, P = 0.007.
[b] Likelihood ratio G^2 comparing loss from lepidopteran larvae = 0.018, df = 1, P = 0.89.

occurred as the experiment ended, on 28 June, when densities were 24 and 16 per six leaves on the neem and lambda cyhalothrin treatments, respectively. Significant effects as a result of treatment ($F = 4.32$; df = 7, 12; $P = 0.01$) first appeared on the seventh sampling date (27 April), and were present for six of the last nine sampling dates (Fig. 6) ($F = 2.96$; df = 7, 12; $P = 0.05$ on 11 May; $F = 3.15$; df = 7, 12; $P = 0.04$ on 18 May; $F = 6.15$; df = 7, 12; $P < 0.01$ on 25 May; $F = 12.33$; df = 7, 12; $P < 0.01$ on 1 June; $F = 8.90$; df = 7, 12; $P < 0.01$ on 8 June; and $F = 7.42$; df = 7, 12; $P < 0.01$ on 28 June). Mean lepidopteran larval densities remained low throughout this time in the tebufenozide treatment (<1.25 larva/sample unit), and in the emamectin and spinosyn treatments (<3.75 larva/sample unit).

Discussion

In the Caribbean, scouting to determine whether lepidopteran larvae density exceed one larva per six leaves should occur during all crop cycles (Fig. 1). The significant correlations between adult and larval density for all species suggest that the more visible sign of increased moth activity may be a useful signal to increase sampling activity for lepidoptera larvae. Pheromone or light trap capture of adults may provide a useful indicator of when to intensify in-field scouting.

There was a significant difference in within-plant distribution (leaves from the inner whorl versus those from the outer whorl) of at least one species (*H. bipunctalis*). To ensure samples would capture all species, we used sample units that contained leaves from both the inner and outer whorl. Expected frequency distributions using these sampling units were modeled for the range of conditions and pest densities observed in local agroecosystems. These probability density functions were combined with expert opinion regarding farmers' willingness to invest time for scouting to develop a sequential sampling plan that could optimize allocation of sampling labor resources. The estimate of k_c came from a regression that contained a lot of variation (Fig. 3), and thus may not be a stable estimate for each field and date. Although methods exist for more exact parameterization of frequency distribution models, Nyrop et al. (1999) have shown that the performance of sample plans are relatively robust to variation in the distribution of sample counts. They argue for investing resources in defining threshold densities and decision tools that will be adopted. Although further work for defining thresholds is warranted, we developed tools that would be adopted by evaluating the plan's ability for informing pest management decisions on 32 sampling dates, and on the effect of conducting spray decisions based on the

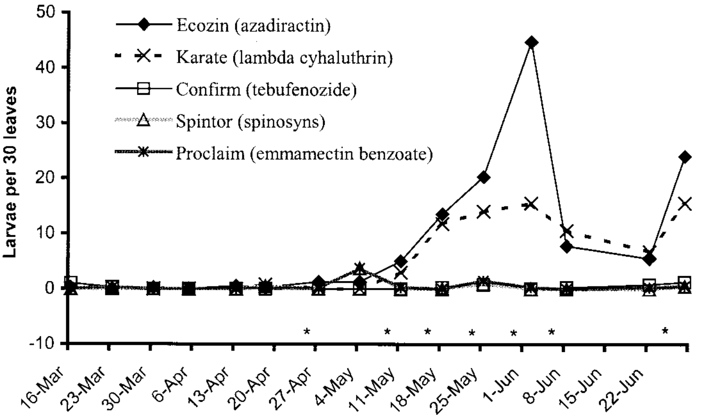

Fig. 6. Density of lepidopteran larvae totaled for five species in plots subjected to weekly sprays of a grower standard (lambda cyhalothrin), a botanical product (neem), an ecdysone agonist (tebufenozide), and two microbial metabolites (emamectin benzoate and spinosyns). Density from five 6-leaf sampling units (total of 30 leaves) per plot. (* indicates a significant difference, $P < 0.05$, as a result of spray treatment).

plan's pest management decision on four grower's fields.

Validation trials suggest that the plan is reasonably effective at informing management decisions. The plan produced the same pest management recommendation as a fixed sample plan in ≈80–100% of the fields, but with a sampling effort of <40%. Thus, under the levels of infestation experienced in the field validation trials, the plans operated approximately within the theoretical type I and II error rates used to parameterize them. Further work, using simulation, would be necessary to determine true error rates when arbitrary minimum and maximum numbers of sample units are imposed upon sequential probability ratio tests, as they were in this work. Where error occurred, it was more likely to be a case of spraying when not necessary, as opposed to not spraying when thresholds were exceeded. Validation trials also suggest that implementation could dramatically reduce foliar insecticide inputs with no effect on yield. The large variation in lepidopteran larval density in these spray validation trials allowed for testing at low and high pest densities. Basing decisions on the sequential plan and an action threshold reduced the number of sprays in all tests, with a range of 33–60% reductions in foliar insecticide input. It is important to note, however, that there were damaging lepidopteran densities regardless of the lambda cyhalothrin applications, which is consistent with a hypothesis of pyrethroid resistance by one or more of the lepidopteran pests.

Insecticides with novel modes of action showed dramatically improved lepidopteran control when compared with lambda cyhalothrin in this growing area (Fig. 6). Although a neem product was not effective in this trial, populations were significantly reduced with an ecdysone agonist (tebufenozide), and with two types of microbial metabolites (emamectin benzoate and spinosyns). These field results are consistent with two earlier studies conducted in 1998 and 1999 (unpublished data). The dramatically increased efficacy with the ecdysone agonist and the microbial metabolites are also consistent with a hypothesis of pyrethroid resistant lepidopteran populations in Jamaica, and makes it very attractive for growers to adopt these newer materials. Furthermore, these more selective, biorational options can be expected to improve farm-worker safety and reduce harmful residues. As of this writing, these materials are not available to growers, and efforts are being initiated to bring them into the country, in part, as a result of the work reported here. However, resistance by *S. exigua* to spinosyns and tebufenozide in other geographic areas has been documented (Moulton et al. 1999, 2002). Our work suggests that these materials would currently be very effective relative to current options in Jamaica, but we strongly agree with the call for proactive resistance management expressed by Moulton et al. (2002). Introduction of new biorational chemistries into this growing system should occur concurrently with a resistance management effort. This study provides the basis for implementing a scouting program in vegetable amaranth in the Caribbean, which would reduce insecticide and labor input in which the insecticides are applied with human labor, increase farm-worker safety, and contribute toward resistance management with the introduction of new biorational chemistries.

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