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An improved infestation technique using eggs of the millet head miner (Heliocheilus albipunctella) (Lepidoptera: Noctuidae) in millet resistance screening

(*Keywords:* pearl millet, screening, *Heliocheilus albipunctella*, head miner)

OUSMANE YOUM*†, MALIKI YACOUBA‡ and K. ANAND KUMAR‡

†*ICRISAT BP 320, Bamako, Mali*
‡*ICRISAT, BP 12404, Niamey, Niger*

Abstract. The millet head miner moth, *Heliocheilus albipunctella* (De Joannis) (Lepidoptera: Noctuidae), is an important pest of pearl millet, *Pennisetum glaucum*, (L.) R. Br., in Sub-Saharan Africa and causes severe crop losses. Damage to the panicles is direct and caused by developing larvae. Management techniques are being developed among which is host plant resistance. Youm and Kumar (1995) reported little progress in identifying resistance due to lack of a repeatable and reliable screening method. Efforts for the past 5 years have been devoted to developing such a technique. Youm (1997) reported an improved technique based on the use of larvae where 35–45 larvae were efficient in causing 51–60% damage corresponding to a rating of 6 on a susceptible genotype. Though the use of larvae was promising, the present research shows that the use of eggs is more efficient than using neonate larvae. The use of 40 eggs per panicle resulted in 51–80% damage corresponding to a mean damage rating ranging from 5.5 to 8.4 across several genotypes. A higher and more consistent infestation was obtained with eggs than larvae. Finally, egg handling was easier and infestation cheaper than using larvae. This technique should significantly improve screening millet for reaction to the head miner. It is recommended for use for future resistance screening of genotypes against the millet head miner to avoid the release of highly susceptible varieties on-farm.

1. Introduction

Millet head miner (MHM), *Heliocheilus* (=*Raghuva*) *albipunctella* De Joannis (Lepidoptera: Noctuidae) is a major insect pest on pearl millet (*Pennisetum glaucum* (L.) R. Br.) in Western and Central Africa, where it causes annual losses estimated at US$116 million. The most affected areas are Sahelian West Africa, and the northern Sudanian zone (ICRISAT, 1995). MHM outbreaks were first recorded in the severe drought years 1972–74 (Vercambre, 1978), and since then the insect has consistently been a major yield-reducing pest. To date, studies have been carried out on its taxonomy (Matthews, 1987), its biology and pest status (Gahukar *et al.*, 1986; Bernardi *et al.*, 1988; Youm and Owusu, 1998), as well as its chemical control (Gahukar, 1990a; Jago *et al.*, 1993).

In Sahelian West Africa, because of the fragility of the environment and the subsistence nature of the farming system, the use of resistant millet varieties appears among the most desirable and adapted control strategies against this insect pest. Resistant varieties have the advantage of requiring minimum inputs from farmers and do not harm the environment (Nwanze, 1985; Nwanze and Harris, 1992). Screening for resistance against MHM has suffered however from the lack of an effective screening technique (Youm and Kumar, 1995). Youm (1997) reported an improved screening technique using larvae for infestation. The technique was, however, found to be tedious and expensive. Finding an effective and inexpensive technique would therefore contribute greatly to the identification of resistant varieties against MHM. This paper evaluates the implantation of eggs onto panicles as a screening technique for resistance to MHM.

2. Materials and methods

2.1. Field and experimental design

The experiment comprised of three trials conducted at an ICRISAT-Niamey research station. Trial 1 was conducted in 1997 and used the larval infestation technique of Youm (1977). Trials 2 and 3 were conducted in 1998 and involved the egg infestation technique (see below). In both Trials 1 and 2, five improved millet varieties from the ICRISAT Sahelian Center (ICMV IS 94206, 90309, 92222, 88212, 88305) were sown in 5×5 m plots. In Trial 3, 18 composite progenies from ICRISAT-India (EC91PVC-15, EC91-ORIGINAL, EC87PCV-15, EC87-ORIGINAL, HH-VBC-PCV-15, HHVBC-ORIGINAL) were sown in 4×3 m plots.

In all trials, a randomized block design was used with five replications for Trials 1 and 2, and four replications for Trial 3. Seed was sown on 0.75 m-spaced ridges. Spacing between millet hills on ridges was 0.5 m. Weeding, using a hand-held hoe, was carried out twice and all the hills were thinned to three plants at the first weeding.

After weeding, millet plants were observed until boot stage when four to five plants per plot were protected using a screen head-cage. When these plants reached about the one-third panicle exertion stage, they were infested with MHM eggs or larvae. After infestation, the panicles were kept covered with screen cloth-cages and monitored daily to keep off insects and ants as well as to maintain the cage structure.

*To whom correspondence should be addressed. Fax: +223-228683; e-mail: o.youm@icrisatml.org

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2.2. Cage structure

The screen cages used were identical to the one reported in ICRISAT (1993). Typically they were made of a cylindrical wire-mesh frame narrow at one end and covered with polyester cloth similar to a white mosquito net. It was about 100 cm long and 40 cm wide. In the field, the cage was slipped over the panicle, the narrow end attached to the last internode and the upper end tied to a supporting pole.

2.3. Eggs and larvae production

To obtain eggs and larvae, MHM adult females were caught daily from light traps and allowed to lay eggs overnight on sections of early planted millet panicle in oviposition cages in the laboratory. For the purpose of infestation by larvae, eggs were subsequently incubated for 2–3 days for hatching to occur.

2.4. Larval infestation technique

This infestation method consisted of depositing 35 MHM neonate larvae all round the exerted portion of the panicle with the help of a camel hairbrush. Depending on the availability of larvae, infestation was carried out when the air temperature was low early in the morning, late in the afternoon or in cloudy weather to avoid dessication.

2.5. Egg infestation technique

In the laboratory, 20 MHM eggs were placed onto the adhesive side of 4×3 mm paper stickers (Labcor Products, Inc., Frederick, MD, USA) using a soft-bristled, fine paint brush with manual manipulation under a binocular microscope. These stickers were then transferred to the field where they were pinned onto the covered panicle, sticky side outwards. Each panicle received 40 eggs (two stickers with 20 eggs each). Collected eggs were placed on stickers and put onto panicles within 24–48 h. The adhesive on paper stickers did not hinder the migration of the newly hatched larvae onto the panicles for subsequent feeding and development.

2.6. Data collection and analysis

Between 22 and 23 days after infestation (which allowed larval feeding and full growth), the screen cages were carefully removed and the panicles were cut and visually assessed to score the damage using the rating scale described in table 1, and manually checked to calculate a larval production index (LPI). LPI was calculated as:

$$\text{LPI } (\%) = \frac{\text{no. of larvae} + \text{no. of pupae}}{\text{original no. of eggs}} \times 100$$

Note that the number of larvae and pupae observed is always less than or equal to the numbers of initial eggs used. Both damage rating and LPI were analysed and compared for each method using analysis of variance (ANOVA) and general linear models (SAS Institute, 1987). Differences between means were assessed using Fisher's LSD tests at 5% probability. Correlation between damage rating and LPI was tested. Before analysis, LPI was square-root transformed (Gomez and Gomez, 1984).

3. Results

The results of Trials 1 and 2 are presented in table 2. The differences between varieties were not statistically significant ($p > 0.05$) in both types of infestation. The egg infestation method, however, gave significantly higher mean damage rating (6.3) (>60% severity in panicle damage) than the larval infestation (1.2) (>10% severity in panicle damage). Results were also more consistent using eggs than larvae, as shown in the lower coefficients of variation. Greater mean damage rating and low coefficient of variation indicate that the egg infestation method was more effective and consistent than the larval method.

The results of Trial 3 are presented in table 3. The entries were statistically different both in terms of damage rating as well as in term of LPI. With respect to damage rating, the lowest scores were recorded in varieties HH-VBC-PVC-3 (5.5) and HH-VBC-PVC-5 (5.6) while the highest scores were recorded in

Table 1. Rating scale used in screening the reaction to millet head miner (MHM)[a]

Rating scale[b]	Severity (%) of panicle damage	Classification
1	<10	highly resistant
2	10–20	resistant
3	21–30	
4	31–40	moderately resistant
5	41–50	
6	51–60	moderately susceptible
7	61–70	
8	71–80	susceptible
9	>80	

[a]After Youm and Kumar (1995).
[b]Rating scale (1–9) described and recorded based on visual assessment of panicle damage.

Table 2. Millet head miner damage rating (mean±SE), larval production index (LPI) and correlation coefficient of the varieties in Trials 1 (infestation by larvae) and 2 (infestation by egg)

	Mean damage rating[a]			
Varieties	Larvae infestation	Egg infestation	LPI (%)	Correlation coefficient, r[b]
ICMV94206	1.3±0.1b	6.5±0.3a	26.7±4.4	0.07[ns]
ICMV90309	1.1±0.0b	7.0±0.3a	31.3±5.1	0.21[ns]
ICMV92222	1.4±0.4b	5.8±0.4a	16.3±4.0	0.51[s]
ICMV88212	1.2±0.1b	6.6±0.0a	26.0±4.3	0.57[s]
ICMV88305	1.1±0.0b	5.7±0.3a	24.0±5.5	0.97[s]
Mean	1.2b	6.3a	24.8	
$LDS_{5\%}$	0.86	1.85	19.52	
CV (%)	39	24	79	

[a]Mean in the same row and followed by the same letter are not statistically different at 5% probability level (LSD).
[b]ns, Not significant at 5%, s, significant at 5%.
Number of panicles used ranged 18–22.

Table 3. Millet head miner damage rating (mean±SE), larval production index (LPI) and correlation coefficient (r) for varieties in Trial 3

Varieties	Damage rating	LPI (%)	Correlation coefficient, r^a
EC91PVC-1	8.1±0.4	32.0±7.0	0.24^{ns}
EC91PVC-2	8.3±0.1	46.9±5.0	0.58^{s}
EC91PVC-3	6.7±0.6	33.9±5.9	0.65^{s}
EC91PVC-4	7.9±0.2	50.7±5.1	0.29^{ns}
EC91PVC-5	6.6±0.5	30.7±5.9	0.81^{s}
EC91-Original	7.1±0.6	31.6±6.6	0.65^{s}
EC87PVC-1	7.0±0.5	29.6±6.6	0.42^{ns}
EC87PVC-2	7.6±0.4	41.1±6.2	0.31^{ns}
EC87PVC-3	6.7±0.7	34.0±5.8	0.60^{s}
EC87PVC-4	7.1±0.5	44.2±5.9	0.72^{s}
EC87PVC-5	8.2±0.2	41.5±4.7	0.25^{ns}
EC87-Original	7.6±0.3	35.5±6.2	0.33^{ns}
HH-VBC-PVC-1	7.0±0.7	30.6±5.4	0.86^{s}
HH-VBC-PVC-2	6.7±0.7	23.8±5.7	0.19^{ns}
HH-VBC-PVC-3	5.5±0.8	22.7±6.0	0.77^{s}
HH-VBC-PVC-4	5.8±0.8	21.3±6.3	0.75^{s}
HH-VBC-PVC-5	5.6±0.4	34.2±6.4	0.70^{s}
HH-VBC-PVC-Original	7.3±0.6	44.2±6.6	0.70^{s}
Mean	7.0	35.0	
$LSD_{5\%}$	1.6	10.42	
CV (%)	29	61	

[a]ns, Not significant at 5%, s, significant at 5%.
Number of panicles used range 11–14.
F=1.91, $p<0.03$ for damage rating; F=2.65, $p<0.002$ for LPI.

varieties EC91PVC-1 (8.1), EC91PVC-2 (8.3), EC91PVC-4 (7.9) and EC87PVC-5 (8.2). Despite statistically significant differences between varieties, however, they were all in the susceptible class.

The LPI (tables 2 and 3) indicate that in general 20 to >50% of implanted eggs developed into full-grown larvae or pupae. In addition, LPI was correlated significantly with damage rating for >60% of varieties. Varieties with higher damage rating generally scored higher LPI.

4. Discussion

LPI measures the proportion of eggs that become full-grown larvae and pupae out of the 40 eggs used for infestation. A 100% production index would indicate that all hatched infesting eggs became full-grown larvae. This is unlikely, however, because many factors, including the nature of the panicle, weather conditions, fertility of eggs, the level of tolerance or resistance of varieties, interact to affect larval survival. Actual LPIs for the egg implantation method are relatively high indicating that the egg infestation technique is more effective than larval infestation. Furthermore, the use of eggs reflects a situation closer to the natural infestation where hatched larvae settle readily in the panicle for feeding, whereas the use of larvae was more difficult due to their possible drifts and movements before settling in the panicle. In addition, the time spent to collect larvae and transfer to panicles was consuming due to larval movements, whereas eggs could be handled easily once collected and placed on stickers.

LPI can be used to measure larval success in feeding, survival and development on a wide range of varieties. It is expected that varieties that are resistant or tolerant would suffer less damage and contribute to few larvae completing development (antibiosis).

Damage ratings produced by the egg infestation method in the current study suggest that the varieties are all in the moderate to highly susceptible range. These results should, however, be interpreted in light of previous studies and reports such as those of Painter (1951) and Harris (1979), as reported by Youm and Kumar (1995) who stated that abnormally high infestation levels could overwhelm the expression of useful resistance. In the future, it is desirable to apply the technique taking into account the infestation levels and different sources of genetic material and varieties.

Varieties such as HH-VBC-PVC4, HH-VBC-PVC5 were significantly less damaged, probably due to their high head volume characteristic. The relationship between panicle characteristics and MHM damage has long been reported. For instance, Vercambre (1978) reported lower levels of damage on long and compact panicles. Gahukar (1984) and Guevremont (1983) found high a correlation between panicle compactness and pest damage. Youm and Kumar (1995) expressed, however, the need to reassess findings and varieties due to a lack of a uniform, reliable, repeatable screening technique at the time when these studies were done.

The high damage rating shown in tables 2 and 3 can be interpreted as indicative of the effectiveness of this method. In fact, as stated above, various numbers of larvae have previously been used as artificial infestation materials but very few full-grown larvae were reported and the highest damage rating ever recorded was 5–6 (ICRISAT, 1992, 1995; Youm, 1997).

It is interesting to observe that in Trial 2 the mean damage rating and LPI following infestation by eggs were 6.3 and 24.8% respectively (table 4). In Trial 3 the means of these variables were 7.0 and 35.0% respectively. When the material in Trial 3 is divided on the basis of genetic material used to constitute the two populations (EC=early composite; HHV=high head volume) it was found that the reaction of HHV composite was similar to the material in Trials 1 and 2 (table 4). The genetic material in Trials 1 and 2 is derived from locally adapted landraces from within Niger whereas in Trial 3 it is derived from an early maturing bold-seeded landrace *Iniadi* found in northern Togo, Benin, Ghana and Burkina Faso (Rai and Kumar, 1994; Clement, 1985). The observations here corroborate visual observations made in the field that varieties generated using landraces from within Niger maturing in 90–100 days are less prone to MHM infestation (interpreted as having a flowering–maturity cycle close to the popular western Niger landrace *Haini-Kirei*, 110 days) and early materials that mature in 70–80 days

Table 4. Millet head miner damage rating (mean±SE) and larval production index (LPI) of the genetic materials

Genetic material				
Designation	No. of genotype	Trial no.	Mean damage rating	LPI (%)
ICMV-IS-	5	1 and 2	6.3	24.8
EC	12	3	7.4	37.6
HHV	6	3	6.3	29.4

are more susceptible to MHM. The results of the screening support this observation as the technique eliminates flowering time as a variable influencing reaction to infestation.

5. Conclusion

The egg infestation technique was more effective than the larval infestation and gave a more consistent and higher damage rating and larvae production index and also eliminated the effect of flowering among test materials.

A further refinement of the technique in terms of number of eggs per panicle could be undertaken while critically assessing millet genotypes for resistance to the head miner. This is particularly important since abnormally high infestation or low infestation could affect detection of resistance.

This technique should significantly improve screening millet for reaction to the head miner. It is recommended for use for future resistance screening of genotypes against the millet head miner to avoid the release of highly susceptible varieties on-farm.

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