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Efficient Mass-Rearing and Infestation Techniques to Screen for Host Plant Resistance to Fall Armyworm, *Spodoptera frugiperda*

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A nearly mature fall armyworm larva causing atypical feeding damage to a maize seedling.

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summary

The practice of growing varieties, lines, or hybrids resistant to attack by insects, and their subsequent effectiveness in reducing pest populations and corresponding crop losses, is well documented for several agricultural crops and pest species.

The development of many of these resistant cultivars has resulted from or been facilitated by (1) many years of study of the insect pests, (2) the development of techniques to mass rear the insects, artificially infest the crop species, and screen the germplasm of the species (or their wild relatives) for resistance, and (3) the successful application of appropriate breeding procedures for improvement of the resistance characteristic over succeeding cycles or generations of population improvement (Guthrie, 1974, 1980).

The basic components necessary to identify or develop germplasm with resistance, or with higher levels of resistance than cultivars presently utilized by farmers/producers, include:

(1) A colony of the insect species, which exhibits the vigor and vitality of the damaging pest population within the geographical area that is affected.

(2) The capability to efficiently mass culture the species, including the rearing facility, trained personnel, natural, meridic, or defined diets, and rearing procedures and containers.

(3) Germplasm resources that are representative of the genetic variation

within the crop and/or its closely related species.

(4) Methods for uniform artificial infestation.

(5) Methods for assessing resultant damage, or lack of damage, to the plants subjected to deliberate infestation (rating scales to determine classes or categories of resistance or susceptibility).

(6) Screening to determine whether adequate levels of resistance exist within suitable agronomic types (equivalent to or better than currently grown cultivars), and an effective selection/breeding scheme established to improve either the resistance levels or agronomic characteristics of the "improved" materials.

introduction

This bulletin presents the techniques developed at CIMMYT and other places for efficient mass rearing and infestation in screening and developing maize with host plant resistance (HPR) to the Fall Armyworm (FAW), *Spodoptera frugiperda* J.E. Smith. The techniques described are likely adaptable to other Lepidopterous pest species, crop species, and screening/breeding initiatives in other parts of the world.

These techniques include colony establishment and maintenance and requirements for efficient mass rearing. The latter focuses on rearing facilities, diets, rearing containers, and rearing procedures for the various life stages (Figure 1). Finally, methods of efficient field infestation are presented along with a description of the rating scales used to evaluate resultant damage and aid in the identification of resistant genotypes.

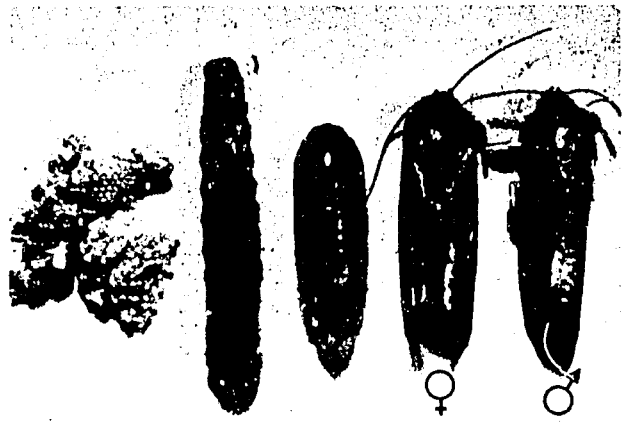

Figure 1. Life stages of *Spodoptera frugiperda* J.E. Smith.

establishment of the colonies

For some insect species to maintain a healthy, vigorous colony, it is necessary to replace or genetically mix it with wild stock at least every year (about 10 generations). With FAW, such frequent rejuvenation may not be essential, especially if a large colony is maintained. By maintaining at least 5000 moths in each generation, we have not found any notable reduction in the ability of larvae reared up to 33 generations in the laboratory to cause typical damage under field conditions.

Mayo (1972) found no difference in damage caused by FAW larvae which had been reared 17 generations on diet compared to those reared only 4 generations on diet.

Entomologists at Gainesville, Florida, have a FAW colony that has been maintained on diet in the laboratory since 1967 (Pers. Comm.).

When rejuvenating the FAW colony at CIMMYT, either eggs or larvae are collected from the field and reared in isolation for a generation to guard against the introduction of parasites or diseases into the laboratory colony.

efficient mass rearing

Rearing facility. Because the FAW is a very hardy insect, it can be successfully reared in any room with moderate temperature and relative humidity. Efficient mass rearing is possible with the addition of slightly more space and equipment. Basic requirements include a separate diet preparation area, a large chest type freezer, a larval rearing room, and an adult emergence/oviposi-

tion room. The rearing and oviposition rooms require controlled temperature (18-30°C), humidity (50-95°/o R.H.), and photoperiod. This is essentially what is used at CIMMYT to produce up to 10 million larvae per year.

Mass production on a grand scale (Knipling, 1980, proposed and deemed technically feasible the rearing of 100 million FAW moths/year) would likely require a more sophisticated rearing facility and a "factory" larger than the custom insect rearing facility described by Leppla *et al.* (1978).

Diet. Several diets have been successfully used to rear FAW. Singh (1977) lists five of them. Because of the FAW's polyphagous nature, it can be successfully reared on many diets that have been developed for other species. At CIMMYT, we successfully mass produce both FAW and corn earworm (CEW) on the same diet, a fairly simple meridic one. The major ingredients are ground high quality protein maize and soybeans (see Appendix). FAW production using this diet was equal to production on the imported commercial Vanderzant wheat-germ diet. Hence, we use the one based on locally available, cheaper ingredients. Burton and Perkins (1972) found that FAW could be reared more economically on a wheat-soy blend (WSB), but not on the other low cost CSM (Blended Food Product, Child Food Supplement, Formula no. 2) based diet.

Rearing containers. FAW have been reared in many types of containers: glass vials or cups, ice cube trays (Bailey and Chada, 1968), and "jelly cups" (Burton and Cox, 1966; Burton, 1967).

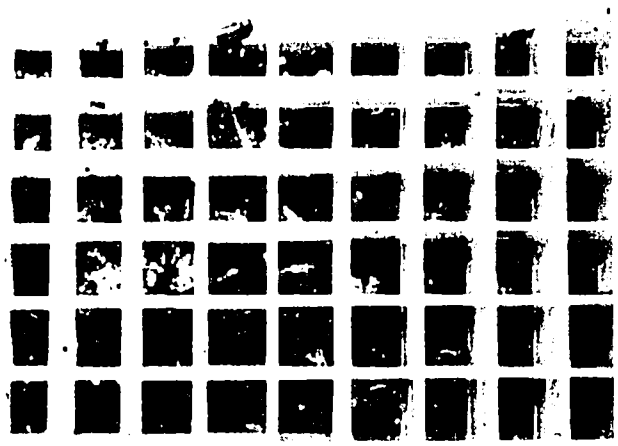

Figure 2. Rearing containers for FAW.

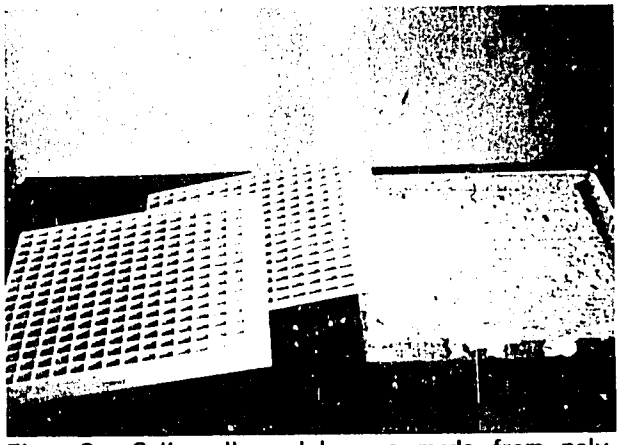

Figure 3. Split cell modules are made from polystyrene light-diffusion louvers.

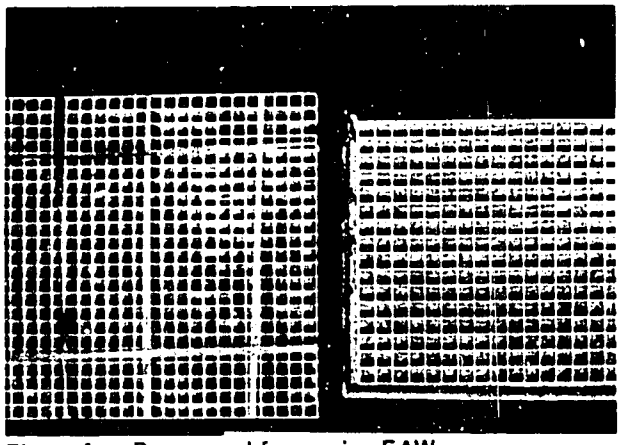

Figure 4. Boxes used for rearing FAW.

Sparks and Harrell (1976) hint that FAW can be reared in cell webs produced by an inline form-fill-seal machine, but they do not state that this has been done successfully. One obstacle to overcome is infesting the cells. As FAW lay egg masses, not single eggs as CEW do, the technique for separating the eggs from the masses would have to be used (McMillian and Wiseman, 1972).

Burton (1967) states that FAW are only semicannibalistic, and hence he was able to produce an average of 1.75 pupae per cup of diet. Our experience in Mexico shows that FAW are highly cannibalistic. Therefore, we use the same rearing containers as for corn earworm (Mihm, 1982) (illustrated in Figure 2). They are a modification of the containers used to rear *Heliothis virescens* by Raulston and Lingren (1972). The split cell modules are made from polystyrene light-diffusion louvers available in Mexico. The split modules (see Figure 3) aid in pupal extraction. The boxes (29 x 29 x 4 cm) are made locally from 3 and 6 mm Plexiglas. They are capped with a layer of paper towelling, a sheet of 50 mesh brass screen, and a section of the polystyrene grid, held in place by large rubber bands, (Figure 4).

To minimize microbial contamination, the units are sterilized by soaking them in 10 percent sodium hypochlorite solution for 24 hours. The boxes and grid blocks are surface treated by spraying with a 5 percent sorbic acid, 5 percent methyl paraben/alcohol solution. This treatment does not affect insect growth and aids in confining any chance contamination to a few cells within the box.

Hot diet is poured into the dishes and the grids forced into the diet manually. The unit is exposed to UV radiation to provide further decontamination.

Adult stages. In our initial attempts at collecting FAW eggs, we experimented with several types of oviposition cages. We continually had problems with the females ovipositing on whatever types of material were used as supports or frames. These masses were difficult to remove, and, as moths oviposit for up to 10 days and eggs need only 2 to 3 days to hatch, the result was that the cages were literally crawling with small larvae.

An intermediate solution was the use of paper bags, where the whole "cage" is substrate suitable for oviposition. The drawback to this was that the masses had to be cut from the bags, as punching machines were not available in Mexico (Figure 5).

The solution to this problem was the utilization of waxed paper bags. With a simple spatula/scraper, the masses can be removed from the cut open bag in a few seconds (Figure 6).

As FAW are very active flyers, newly emerged adults are inactivated by chilling them in a chest type freezer for a few minutes. Twenty pairs of moths are then placed in each bag (10 x 20 x 40 cm), which is closed by folding the open end and sealed with a piece of masking tape. A small plastic box with a piece of cotton moistened with five percent sugar

rearing procedures and colony handling

Figure 5. Egg masses can be cut from the paper bags.

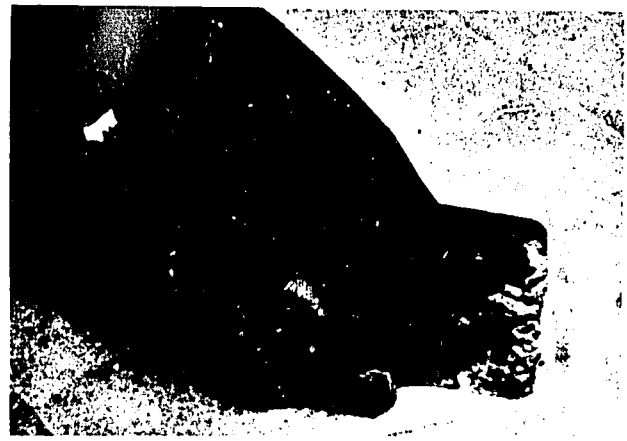

Figure 6. FAW egg masses are more easily collected with a simple spatula/scraper.

Figure 7. Adults are placed in a waxed paper oviposition bag.

Figure 8. Oviposition bags are changed daily; adults are shaken into a new bag.

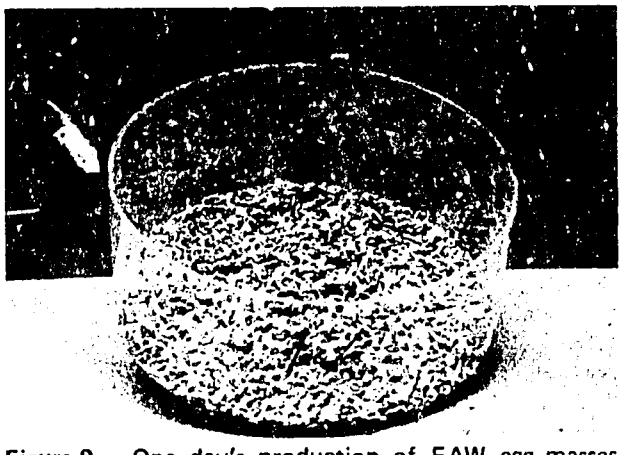

Figure 9. One day's production of FAW egg masses collected in round plastic dishes.

water is placed in each bag for adult food (Figure 7).

The bags are held in a room at 25°C and 80 percent R.H. until the first masses are oviposited (2 to 3 days), then changed daily for 5 to 7 days. Changing bags is simple: moths are shaken into a new bag (Figure 8), a new box with fresh sugar solution is inserted, and then the bag is closed.

Egg stages. Egg-laden bags are slit open with scissors and the egg masses are scraped off. Some eggs (less than 10 percent) are damaged in the process, but as normal production is more than 3,000 eggs per female, this loss is unimportant.

Egg masses are collected in round plastic dishes (Figure 9) and incubated until hatching (2 days at 30°C to 5 days at 20°C).

Once the larvae have hatched, they may be held for up to 5 days in a refrigerator at 10-12°C with no harmful effects. In this manner, up to a million or more larvae can be accumulated for large scale field infestations.

Larvae. At CIMMYT, newly hatched larvae (<12 hours old) are used for infesting diet to maintain the laboratory colony.

Infestation of the rearing boxes is accomplished easily and rapidly: 100-200 cc of sterilized corn cob grits are placed in the dish containing larvae; this is rotated gently to mix uniformly. The mixture is transferred to a simple shaker jar (Figure 10) and shaken over the boxes containing diet and cell grid until

there are 2 to 5 larvae per cell (Figure 11). After capping, the rearing boxes are moved to shelves in rearing rooms at 70-80 percent R.H., with temperatures ranging from 20 to 32°C, depending on how quickly the next generation is needed.

Depending on temperature, larvae mature and begin pupating in 18 to 30 days. The developmental stage can be easily checked through the clear Plexiglass box. Boxes are not opened until pupal stage. Only one larva per cell survives to pupate.

Other rearing programs (Burton, 1967; Raulston and Lingren, 1972; Sparks and Harrell, 1976) also use larval/grits mixture for infesting diet in rearing containers in their mechanized rearing systems.

Pupal stage. Many rearing operations, particularly those where much or all of the procedure is mechanized, have developed various machines for *Heliothis* sp. and FAW pupal extraction (Raulston and Lingren, 1972; Harrell *et al.*, 1974; Sparks and Harrell, 1976).

At CIMMYT, by modifying the polystyrene cell units into a split unit (three layers glued and one layer below), we eliminated the need for a special machine for pupal collection. Nearly all pupae are encountered below the surface of the diet in our boxes. The split cell unit, when removed, splits the diet layer and pupation cell so that the pupae can be gently dumped from the dish (Figure 12). The few remaining pupae which pupated above the diet plug can be removed by hand or simply discarded.

Figure 10. Simple container for efficient infestation of rearing boxes with FAW larvae.

Figure 11. The larval/grits mixture is shaken over boxes containing diet and cell grid.

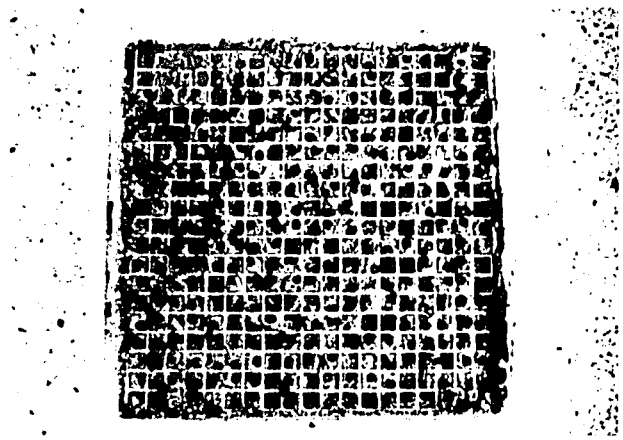

Figure 12. Pupae are easily extracted after the top of the split cell unit is removed.

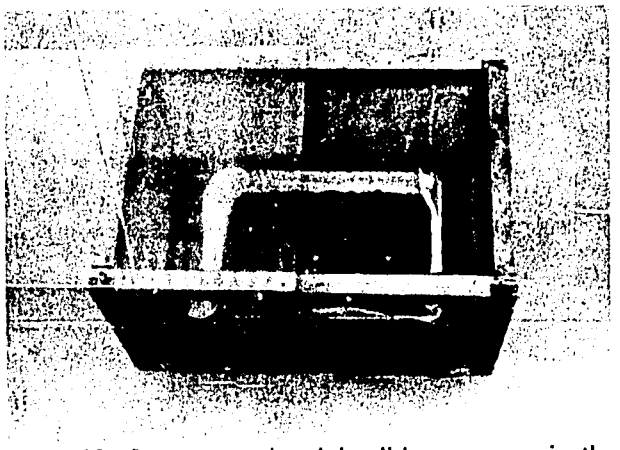

Figure 13. Pupae are placed in dishes or pans in the bottom of adult emergence cages.

Pupae are placed one layer deep in boxes or dishes of various sizes, depending on quantities, and provided with a screen from which newly emerged adults hang and spread their wings (Figure 13).

efficient field infestations

Limited artificial infestations with FAW have been done by manually applying larvae to plants with a camel's hair brush (Wiseman *et al.*, 1966; McMillian and Starks, 1967; Morrill and Greene, 1974; Widstrom *et al.*, 1972).

Wiseman *et al.* (1974) stated that the slowness and laboriousness of this technique virtually prohibit large scale screening. They further reported on a promising technique for separating FAW eggs from egg masses which, when subsequently suspended in agar solution and dispensed on diet or on maize seedlings in the green house, hatched adequately. Peairs (1977) subsequently found the procedure was not suitable for field infestations.

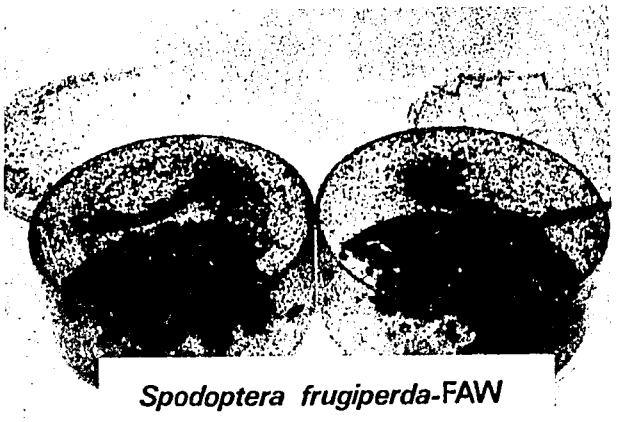

Figure 14. Boxes with newly hatched larvae ready for mixing with corn cob grits.

Since the development of the bazooka and larval infestation technique by Mihm and colleagues at CIMMYT in 1976 (CIMMYT Review, 1977), most programs doing host plant resistance or other field and greenhouse studies with

FAW now use it (Wiseman, *et al.*, 1980a; Davis, 1980). The use of this technique and its advantages for use with several lepidopterous pest species have been described in detail by Ortega *et al.* (1980) and Mihm (1982 and 1983).

Wiseman and Widstrom (1980) compared three methods of infestation, numbers of larvae, and number of plants per plot infested with FAW larvae, and concurred with the conclusion reached at CIMMYT: larval infestation of every plant to be screened with 20-40 FAW larvae gave the best results and was the most efficient.

The CIMMYT system of detaching FAW egg masses from the oviposition substrate and incubating to hatching in round plastic dishes (Figure 14) makes preparation of the larval-grits mixture easy. A measured amount of grits is simply poured into the box(es) and rotated gently to mix the newly hatched larvae into the grits. The mixture is then passed through a No. 14 US Standard brass sieve to remove any unhatched masses or debris. Then, by serial dilutions and counts, it is adjusted to the desired larval concentration of 15-20 larvae per shot.

The mixture is immediately taken to the field and the desired plots infested by making two successive passes of one shot per plant. Maize plants are usually infested in the seedling (3-4 expanded leaf stage, Figure 15) and midwhorl (7-9 expanded leaf stage, Figure 16). With experience, 1,500 plants per man hour can be infested; in one day at CIMMYT, approximately 1.5 million FAW larvae have been used to infest about 50,000 plants.

Figure 15. Maize plants are usually infested in the seedling stage.

Figure 16. Infestation of plants at the whorl stage.

damage evaluation

Figure 17. The yield differential technique is used to show tolerance type resistance—this is a susceptible family.

Rating scales are commonly used to quantify the resistant (or susceptible) performance of the plant(s) after infestation in the field or greenhouse (see Appendix).

For FAW damage in seedling or whorl stage maize or sorghum, a scale similar to the one devised by Wiseman *et al.* (1966) is generally used. It is a 1-9 scale, where 1 is a small amount of pinhole type injury or less, and 9 is a whorl almost completely eaten and a dying or dead plant. A scale devised by Wiseman and Davis (1979) is also used where 0 is no damage and 9 is a non-recoverable plant.

At CIMMYT, a 1 to 5 scale is frequently used, where 1 is slight damage and 5 is severe damage. Ratings are normally made at almost weekly intervals, starting a week after infestation and continuing until the larvae have ceased damaging the plants. Hershey (1978), Wiseman *et al.* (1980b), and Smith (1982) concluded that time(s) of rating can be critical to detecting differences in resistance or susceptibility.

In addition to categorizing the amount and type of damage (antibiosis type resistance reaction) that FAW cause to maize plants, CIMMYT has been using the yield differential technique of Hershey (1978) to try to capitalize further on tolerance type resistance. In this technique, yield comparisons between paired infested and protected plots or progeny rows are made and selection criteria include selecting progenies which are able to yield reasonably well in spite of the FAW damage sustained (Figures 17, 18). Results from using this tech-

nique to date (Hershey, 1978; Smith 1982) have not been as encouraging as had been hoped. Nonetheless, slow steady progress in resistance in materials undergoing recurrent selection is apparent. Trials to evaluate gains made over cycles of selection are now in progress, and results will soon be forthcoming.

Figure 18. Trial results show a tolerant family.

conclusion

The techniques and experience described in this bulletin for efficient mass rearing and infestation show promise of adaptability to other pest and crop species and to screening and breeding initiatives in other parts of the world. The final objective in the application of these techniques to any program of efficient mass rearing and infestation is to identify resistant genotypes for immediate use in farmers' fields or to identify the most resistant genotypes (plants) for use in a breeding program. Varieties with improved resistance can serve as one of the major components in the effort to manage *Spodoptera frugiperda* pest populations.

Commonly used damage rating scales for evaluation and development of resistance to Fall Armyworm, *Spodoptera frugiperda*

Categories of Resistance or Susceptibility Indicated by the Classes

Least Damaged ..... **Most Damaged**

Crop	Excellent / Highly Resistant	Very Good / Resistant	Good / Intermediately Resistant	Fair	Poor	Unacceptable / Susceptible	Reference
Maize	(0) No damage (1) Few pinholes (2) Several to many pinholes (3) Few shot holes and 1 or 2 elongated lesions (4) Several shot holes and a few elongated lesions		(5) Several shot holes and elongated lesions (6) Many shot holes, several elongated lesions and a few portions eaten away (7) Several lesions, portions eaten away and areas dying		(8) Elongated lesions, portions eaten away and areas dying (9) Whorl almost eaten away and several lesions and areas dying (10) Dying or dead plant		1
	(0) Slight pinhole damage (1) Pinholes on 2 + leaves (2) Shot holes and a few elongated lesions (3) Shot holes and several elongated lesions		(4) Many elongated lesions (5) Many elongated lesions and a few portions eaten away (6) Many elongated lesions and several portions eaten away		(7) Many elongated lesions, portions eaten away, and damage in whorl (8) Many elongated lesions, portions eaten away, and whorl destroyed (9) Plant dying or dead		2
	(0.6 per plant)		(2.0 per plant)		(4.0 per plant)		3
	Number of leaf nodes per plant having lesions longer than 1.3 cm at leaf node						
Sorghum	(0) 0-10% leaf area damage	(1) 11-20% leaf area damaged	(2) 21-40% leaf area damaged	(3) 41-60% leaf area damaged	(4) 61-80% leaf area damaged	(5) Beyond recovery	4
	(1) No leaf damage	(2) 1-10% plants with 1 + damaged leaves	(3) 11-25% plants with 1 + damaged leaves	(4) 26-40% plants with 1 + damaged leaves	(5) More than 41% plants with 1 + damaged leaves		5

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DIET CHECKLIST REGISTER
(Diet for Fall Armyworm, *Spodoptera frugiperda*)

Supplier	ICN Pharm.	Bio-Serv.	Lot No.	Ingredient	Amount to make 10 kg Diet	Day. Month Year											
1				Water	8 lts												
2				Agar	100 g												
3				Soybean Meal	500 g												
4				Ground Opaque Maize	960 g												
5				Brewer's or Torula Yeast	400 g												
6				Wheat Germ	40 g												
7				Sorbic Acid	20 g												
8				Choline Chloride	20 g												
9				Ascorbic Acid	40 g												
10				Methyl p-Hydroxybenzoate	25 g												
11				Salt Mixture W	70 g												
12				Vitamin Mixture	150 ml												
13				Formaldehyde	25 ml												
14				Aureomycin	50 g												
15				Streptomycin	1 unit												
16				Maize Tassel Powder (autoclaved)	200 g												
17				Other Ingredients if Used													
18																	

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