Techniques for Efficient Mass Rearing and Infestation in Screening for Host Plant Resistance to Corn Earworm, *Heliothis zea*

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The International Maize and Wheat Improvement Center (CIMMYT) is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center is engaged in a worldwide research program for maize, wheat, and triticale, with emphasis on food production in developing countries. It is one of 13 nonprofit international agricultural research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). The CGIAR consists of 40 donor countries, international and regional organizations, and private foundations.

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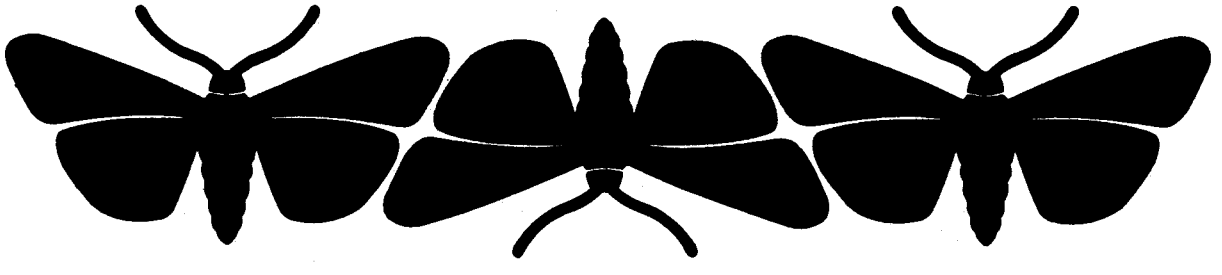

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Nearly mature *Heliothis zea* larva is pictured damaging the ear of a susceptible maize plant.

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 present cultivars utilized by farmer/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 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 used over the past six years for the efficient mass rearing and infestation in screening and improving host plant resistance to the corn earworm, *Heliothis zea* (Boddie), in maize. (The species of *Heliothis* occurring in Mexico can be seen in Figure 1.) The techniques described show promise of adaptation to other pest species, crop species, and screening/breeding initiatives in other parts of the world.

These techniques include the establishment of the insect colony and provision of the basic requirements for efficient mass rearing. The latter focuses on the rearing facility, diet, containers, and procedures for the various life stages (Figure 2). A method of efficient field infestation is presented along with a description of the rating scales used to evaluate resultant damage and aid in the identification of resistant genotypes.

Figure 1. Species of *Heliothis* occurring in Mexico.

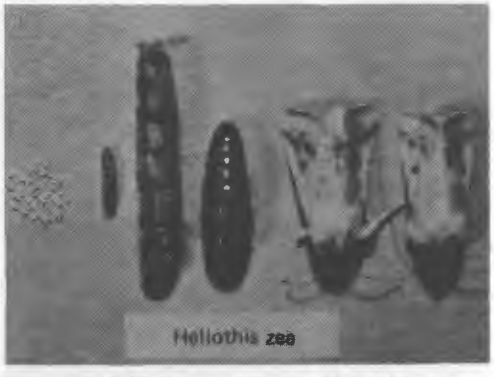

Figure 2. Life stages of *Heliothis zea* (Boddie).

Establishment of the Insect Colony

Guidelines established and recommended by some entomologists who have developed crop cultivars with resistance (Guthrie, 1980), and proven by experience under CIMMYT conditions, are followed to maintain a healthy, vigorous *H. zea* colony.

As there is only one crop and infestation cycle per year in the tropical highlands of Mexico, the earworm colony is replaced or rejuvenated by using (1) progeny of larvae collected from a late-planted trap crop of sweet corn (Figure 3), or (2) progeny of adults captured in a light trap in spring at the beginning of the rainy season (Figure 4). The colony is replaced or genetically mixed with wild stock at least every ten generations.

Figure 3. Collecting *H.zea* larvae in late-planted sweet corn trap crop for establishing the laboratory colony.

Figure 4. Light trap for collecting *H. zea* adults for laboratory colony establishment or renovation.

Efficient Mass Rearing

The basic requirements for successful insect colonization and rearing were listed by Needham *et al.* (1937), and include (1) food, (2) protection from enemies, (3) a suitable physical environment, and (4) fit conditions for reproduction.

The components necessary in an efficient mass-rearing operation include (1) the physical facility, (2) diet(s), (3) rearing containers, (4) rearing procedures or management of the various life stages of the insect (Figure 2), and (5) qualified trained personnel.

Rearing facilities. In many countries, physical facilities may consist simply of a room or two, a few boxes and cages, electrical power, and perhaps some means of temperature and humidity control. In some of the most developed countries, insect "factories" exist. Leppla and Ashley (1978) have compiled a valuable reference on the types of physical facilities which are presently being used for insect rearing, from small chambers to grand scale, semi-automated production. Anyone contemplating starting or expanding rearing programs should consult this reference for ideas which may apply to their conditions.

The physical facility should be simple, practical, and functional. Entomologists with experience in rearing the insect or species desired should be involved in the design or modification of the facility as applicable to their situation. If the entomologist has not had a great deal of experience in mass rearing those species, he should visit one or more facilities where the species are being successfully reared. In most cases, he will gain ideas on how he might design or modify his facility to make it more efficient. He should, however, be aware that not everything he observes will be appropriate for his conditions, and that he may need to modify or adapt existing techniques to his circumstances.

The rearing facility that serves the CIMMYT maize program is a simple, inexpensive structure which satisfies the most basic requirements for insect rearing. It has undergone many changes and modifications, as necessary, and this process is expected to continue. Most of the changes which have been made since its establishment fall into three categories: improvement in general sanitation, in storage facilities, and in making it more independent from other facilities.

Insect rearing is a seven-day-a-week job at CIMMYT. Four or five species are produced twice a year for field infestation at appropriate plant-growth stages over two-month-long periods. Therefore, the laboratory has to be independent of other units which operate only five or six days a week. This includes separate facilities for electrical power, refrigeration, water, storage, and general supplies.

One useful component in the CIMMYT facility that many rearing facilities do not have is a small workshop. It has the necessary tools and materials for basic maintenance of much of the physical facility and for the construction of rearing dishes, cages, or any spur-of-the-moment necessities. This small facility makes the rearing operation much more efficient.

Diet. Singh (1977) lists seven meridic diets which have been used successfully for rearing *H. armigera* Hubn., and 16 diets which have been used for either *H. virescens* (Fabricius) or *H. zea* (Boddie) or both. The adults of these *Heliothis* species are illustrated in Figure 1.

The diet used at CIMMYT for rearing CEW is presented in Table 1. Use of the checklist-register (see Appendix) is recommended in order to avoid errors in diet preparation and for use as a record to identify material lots that may coincide with

Figure 5. Simple, locally made container for rearing *H. zea*.

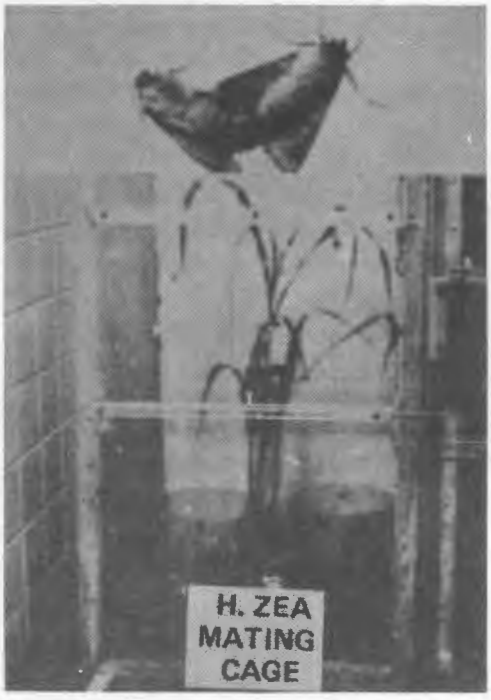

Figure 6. Pupal containers are placed inside a 1/2 x 1/2 x 1 m. mating cage, with potted maize plants and 10 percent sugar solution for adult food. Adults are transferred to oviposition cages every 24-48 hours.

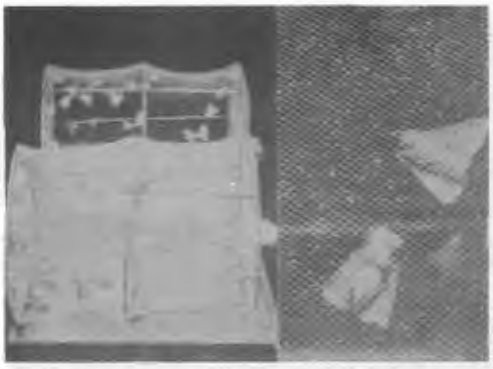

Figure 7. Oviposition cages consist of a simple wire frame support and a bag made of nylon mesh (bridal illusion). Females oviposit and attach single eggs to the mesh.

Figure 8. Cages are changed daily by placing a clean nylon bag over the mouth of the egg-laden one.

problems encountered in rearing. The only item unique to this diet is dried, sterilized maize tassel powder used at the rate of 20 g/kg of diet. (The tassels are collected and processed prior to pollen shed.) In tests under CIMMYT conditions, better larval establishment, shorter larval period, larger pupae, and better oviposition were obtained from insects reared on the diet with tassel powder than those on the standard diet.

Table 1. Meridic diet used successfully for mass rearing *H. zea* at CIMMYT

DIET FOR CORN EARWORM *Heliothis zea*	
(Amount to make 10kg Diet)	
Water	8 lts
Agar	100 g
Soybean Meal	500 g
Ground Opaque Maize	960 g
Brewer's or Torula Yeast	400 g
Wheat Germ	40 g
Sorbic Acid	20 g
Choline Chloride	20 g
Ascorbic Acid	40 g
Methyl p-Hydroxybenzoate	25 g
Salt Mixture W	70 g
Vitamin Mixture, Vanderzant	150 ml
Formaldehyde	25 ml
Aureomycin	50 g
Streptomycin	1 unit
Maize Tassel Powder (autoclaved)	200 g

Guthrie *et al.* (1969) found that *Ostrinia nubilalis* larvae could survive to pupal stage on only maize pollen, indicating that it is a nutritious food source. Trials at CIMMYT indicate that it acts as a feeding stimulant and/or makes the diet more palatable. It has been used with consistent results for the past four years in their diets for rearing five lepidopterous species. And since maize is continually undergoing improvement at

CIMMYT, tassel powder is a low-cost diet ingredient.

Prepared commercial diets for rearing *Heliothis* sp. are now available from several sources in the USA. CIMMYT experience with these, however, has shown that they need a few ingredients, mainly supplemental vitamins and microbial inhibitors, for successful use in the rearing facility. They have the advantage of saving time and effort in preparation, while providing the necessary quality assurance.

Walker *et al.* (1966) list criteria for diet suitability: (1) high larval survival, (2) vigorous adults with high reproductive capacity, (3) normal rate of larval development, (4) low-cost ingredients, (5) easy preparation from readily-available ingredients of uniform quality, and (6) good keeping quality. No one diet exists, however, which will measure up to all these criteria for mass rearing a given species under all conditions or at all locations. However, after testing several diets that have been used successfully by other scientists, and after experimentation with various concentrations of ingredients, it is possible to develop a suitable diet.

New information on diets, diet ingredients, suppliers, and rearing techniques is available in the "FRASS Newsletter," (Anon. 1981), published bi-annually by the Insect Rearing Group, which is composed of 575 scientists involved in rearing insects in 27 countries. It is provided free of charge to interested scientists and is a valuable reference.

Rearing containers. Containers suitable for rearing *Heliothis* sp. range from individual glass or plastic vials or cups (Sparks and Harrell, 1976), Hexcel units (Roberson and Noble, 1968; Raulston and Lingren, 1969), polystyrene light diffusion cell blocks (Raulston and Lingren, 1972), and cell webs processed and infested by a modified in line form-fill-seal machine (Sparks and Harrell, 1976).

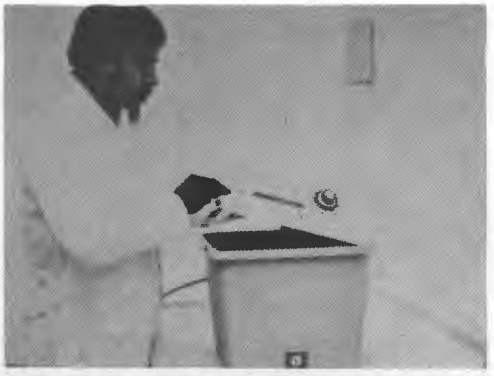

Figure 9. Egg-laden bags are agitated for 2 minutes in a small portable washing machine containing a 0.2 percent solution of sodium hypochlorite to loosen the eggs from the mesh.

Figure 10. The egg-bearing water is decanted into a fine mesh screen.

Figure 11. The eggs are then rinsed under tap water to wash off the sodium hypochlorite.

Figure 12 The rinsed eggs are then decanted into a graduated cylinder to estimate production. There are approximately 2,000 eggs per milliliter.

Any of the above may be utilized efficiently in a mass rearing program. Choice may be dictated by the size of the rearing operation, the cost and amount of labor available, and the supply, availability, and durability of a given container. The ultimate and most efficient system would appear to be the Inline form-fill-seal machine and system. However, at CIMMYT and in many other locations in the world, it is probably not the best choice, because of the cost of the initial unit and subsequent materials and the problems likely to be encountered in its operation and maintenance. In fact, because of high costs associated with such production, a study was done to see if *H. zea* could be produced more economically on maize plants in field cages (Sparks *et al.*, 1971).

CIMMYT has adapted the system used by Raulston and Lingren (1972) to meet its needs (Figure 5). The cell grids are made from polystyrene light-diffusion louvers available in Mexico, the boxes are made locally from 3 and 6 mm Plexiglas, and the cap for the unit is a layer of paper toweling, a 50-mesh bronze screen, and a sheet of the polystyrene grid, held in place by inexpensive large rubber bands. To minimize problems with microbial contamination, the units are sterilized by soaking in a 10-percent sodium hypochlorite solution, and 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 are forced into the diet manually. The unit is exposed to UV radiation prior to infesting to further minimize microbial contamination.

Rearing procedures and colony handling

Adult stages. When CIMMYT first began rearing *H. zea* in 1975, colonies were frequently lost because of sterility. Callahan (1962) reported that one of the major problems in rearing *H. zea* involved their unpredictable mating habits in the laboratory; consequently, he obtained a higher incidence of mating by using large cages containing host plants, with controlled temperature and humidity, and a 10-percent honey solution for adult nutrition.

Since 1977, a similar mating cage (Figure 6) has been used with continued success. It consists of a 0.5 x 0.5 x 1.0m Plexiglas cage with screen on two sides so that the moths can hang and rest easily. A pot containing several whorl stage maize plants is placed within the cage; a dish containing cotton moistened with a 10-percent sugar solution is also provided. Moths are left within the cage for 48 hours before they are transferred to oviposition cages.

Oviposition cages used at CIMMYT consist of a simple wire frame support and a bag of nylon mesh (Bridal Illusion) material (Figure 7). This system has been found to be superior to cotton cheese cloth either placed over paper ice cream cartons (Burton, 1969; Raulston and Lingren, 1972), or on the front or sides of other style cages (Callahan, 1962; Knott *et al.*, 1966). Its advantages include ease in changing oviposition substrate without adults escaping, ease in cleaning, maximum oviposition surface area, no need for cage liners, and no problem with hatching larvae since the entire cage walls are replaced daily.

Changing the cage (Figure 8) is accomplished by simply placing a new bag over the mouth of the egg-laden one. As the egg-laden bag is pulled off, the new one is pulled over the frame. A small

Figure 13. Eggs are decanted onto paper towelling and placed in box for incubation. They hatch in 2 days at 30°C and 95 percent RH.

Figure 14. Simple container for efficient infestation with *H. zea* larvae in rearing containers.

Figure 15. A mixture of sterilized corn cob grits and first instar *H. zea* larvae is applied to the rearing container until there are approximately 5 larvae per cell in the grid.

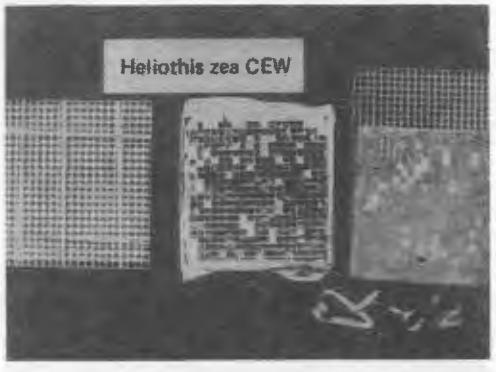

Figure 16. Infested boxes are capped with a layer of paper towelling and a fine mesh bronze screen. Rubber bands and a section of the plastic grid seal the cells during larval growth to pupation.

plastic box with cotton, moistened with a 10-percent sugar solution, is placed inside for food.

Egg stages. Egg-laden bags are placed in a small, inexpensive portable washing machine and agitated for 2-3 minutes in a 0.2-percent sodium hypochlorite solution. Egg-laden water is then decanted onto a fine mesh screen, and the eggs are immersed in a 10-percent sodium thiosulfate solution and then rinsed with water. (Figures 9-13). Eggs are washed onto a paper towel, the excess moisture is removed, and they are placed inside plastic dishes for incubation.

Once larvae have hatched (0-8 hours old), they can be stored in a refrigerator (at 10°C) for up to five days, or used immediately to reinfest diet or plants in the field.

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, and the dish is rotated gently to mix uniformly. The mixture is transferred to a simple shaker jar (Figure 14) and shaken over the boxes containing diet and the cell grid unit until there are 2-5 larvae per cell (Figure 15). After capping, the rearing boxes are moved to shelves in rearing rooms at 70-80 percent R. H. with temperatures ranging from 20-32°C, depending on how quickly the next generation is needed.

Depending on temperature, larvae mature and begin pupating in 18-30 days. The developmental stage can be easily checked through the clear Plexiglas box. Boxes are not opened until the pupal stage. Only one larva per cell survives to pupate (Figures 16, 17). Other rearing programs

(Raulston and Lingren, 1972; Burton, 1969; Sparks and Harrell, 1976) use eggs for infesting diet and rearing containers because they are more appropriate to their systems.

Pupal stage. Many rearing operations, particularly those where much or all of the procedure is mechanized, have developed various machines for pupal extraction (Harrell *et al.*, 1968; Raulston and Lingren, 1972; Harrell *et al.*, 1974; Sparks and Harrell, 1976).

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

Pupae are placed one layer deep in boxes or dishes of various sizes, depending on the quantity desired, and provided with a screen so that newly-emerged adults can hang and spread their wings (Figure 18). These containers are then put inside the mating cages when the first adults have emerged (Figure 6).

Efficient Field Infestations

Infestations with *Heliothis* sp. have been done with both eggs and larvae (newly hatched, second instar, and third instar). Manual infestation with newly-hatched larvae (using a camel's hair brush) was first done over forty years ago (Blanchard *et al.*, 1942). It was an effective method (Josephson *et al.*, 1966), but very inefficient because of the time and labor involved.

Figure 17. Rearing container with mature larvae. As *H. zea* are cannibalistic, only 1 larva survives per cell to pupation.

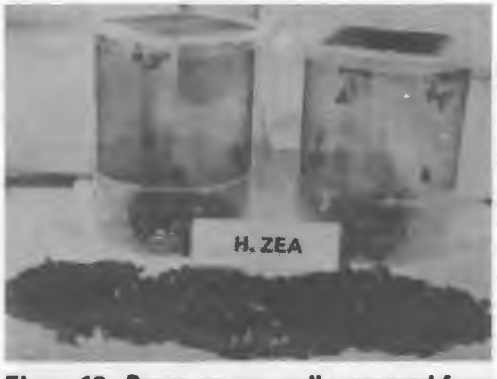

Figure 18. Pupae are manually removed from larval rearing containers and placed in simple screen cages for adult emergence.

Figure 19. Plants with fully-emerged, fresh silks to be infested are tagged with date of infestation for later identification. This is done prior to infestation to avoid dislodging the larval grits mixture.

Figure 20. About 10 larvae are applied per plant. Within minutes, they move into the silk mass and begin attacking the developing maize ear.

More efficient methods in use today include (1) infesting with eggs suspended in a 0.2 percent agar solution and applied to the plants in controlled amounts (hypodermic syringes or pressure applicators) or uncontrolled amounts (squeeze bottles) (Wiseman *et al.,* 1974), and (2) infesting with uniform numbers of newly-hatched larvae, using the Bazooka applicator. The second technique was developed by Mihm and colleagues at CIMMYT in 1976 (CIMMYT Review, 1977). The use of the technique and its advantages for use with several lepidopterous species are described in detail by Ortega *et al.,* 1980. Infestation of maize with *H. zea* larvae is illustrated in Figures 19, 20.

Larval infestation is more efficient than other means of infesting because it is more rapid, uses fewer insects per plant, and is more effective (fewer escape plants) than other techniques. The Bazooka, in original or modified versions (Wiseman *et al.,* 1980), has been used efficiently and effectively for field infestation with at least 11 species of lepidopterous insect larvae (*Diatraea saccharalis, D. grandiosella, D. lineolata, Ostrinia nubilalis, Chilo partellus, Sesamia cretica, S. calamistis, Busseola fusca, Heliothis zea, H. virescens,* and *Spodoptera frugiperda*) and one leafhopper (*Dalbulus maidis*) in three crop species—corn, sorghum, and cotton. To use the technique in cotton, the plants were simply sprayed first with water (Hall *et al.,* 1980). If done after rain or heavy dew, infestations in cotton would be even more efficient as it would then be unnecessary to spray the plants.

For infesting corn in the whorl stages, the larval-corn cob grits mixture is simply dispensed into the whorl. For infesting developing maize ears, the mixture is dispensed onto the fresh silks. Care must be taken not to disturb the plant for a few minutes after infestation, so the larvae have time to attach themselves.

If corn cob grits are not readily available, other materials may be used in preparing the larval mixture; corn meal (Hall *et al.,* 1980), millet seed (Pers. comm.), and sorghum meal have been used successfully. Other materials will likely be reported as they are tried.

Damage Evaluation

Rating scales are generally used to quantify the resistant (or susceptible) performance (Figure 21) of the plant(s) after infestation in the field or greenhouse.

For corn earworm damage in whorl stage corn, a scale similar to the one devised by Wiseman *et al.* (1976) is generally used. It is a 0-10 scale, where 0 is no damage and 10 is a completely destroyed plant. For damage to ears, the revised centimeter scale (Table 2 and Figure 22) developed by Widstrom (1967) is recommended as the most effective in indicating plants with heritable resistance.

Table 2. Use of the revised centimeter scale helps classify more exactly the plant reaction in screening and selecting in variable or segregating maize genotypes.

REVISED CENTIMETER SCALE FOR CLASSIFYING CORN EARWORM DAMAGE TO MAIZE. (Widstrom, 1967)

CATEGORY	VALUE	DESCRIPTION
RESISTANT	0	No damage
	1	Damage to silks only
	2	Feeding to 1 cm beyond the ear tip
INTERMEDIATE	3 +	Value increases by 1 for each additional centimeter of feeding beyond the tip of the ear
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	.	
	.	
SUSCEPTIBLE	...N	

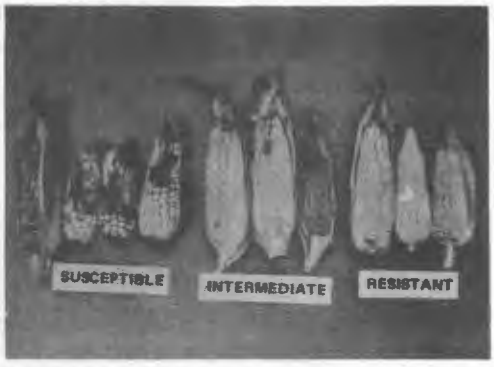

Figure 21. After deliberate infestation, plants may be categorized as susceptible, intermediate, or resistant.

Figure 22. This ear shows an intermediate to susceptible reaction.

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 the *Heliothis* sp. pest populations.

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APPENDIX

DIET CHECKLIST REGISTER
(Diet for Corn Earworm, *Heliothis zea*)

Supplier	ICN Pharm.	Bio-Serv.	Lot. No.	Ingredient	Amount to make 10 kg Diet	Day, Month Year	18 July 1981	22 July 1981											
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-Hydro-xybenzoate	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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