Chapter 16

GENETIC CONTROL

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INTRODUCTION

The control of insects which annoy man or attack his food and fiber crops largely had been the exclusive domain of entomologists (and perhaps toxicologists) up to the early 1960s when geneticists became involved in certain new techniques called "genetic" or "autocidal" control procedures. A Russian geneticist suggested the use of chromosomal translocations to influence the reproduction of harmful species (Serebrovsky, 1940). However, this suggestion effectively was lost to entomological research until resurrected by Curtis (1968) [for a complete discussion of the history of genetic thought in insect control procedures see Whitten (1985)].

All methods of genetic control require the introduction of detrimental traits into the target population by the release of suitable carrier insects. Released insects are usually reared under laboratory conditions that emphasize mass-production. The quality of a released insect is a poorly understood concept that usually is secondary to production of high numbers. By its very nature, laboratory rearing often produces insects that are less fit than the native insects for life outside the laboratory. However, as LaChance (1979) indicated, the components of fitness for the released and native insects are not necessarily the same. Released insects need not be identical to natives to be effective. The released insects must mate with enough members of the target population to introduce their genes into that population, or, in the case of sterile insects, reduce egg hatch sufficiently to effect a negative rate of reproduction.

The most widely publicized and successful proposal for the use of genetic techniques in insect control is the Sterile Insect Release Method (SIRM) or Sterile Insect Technique (SIT) first conceived by E. F. Knipling in 1938 (Lindquist, 1955). Other well known genetic techniques are inherited sterility and backcross sterility. These three methods have been evaluated on cotton insects, particularly the boll weevil,

Anthonomus grandis grandis Boheman, the tobacco budworm, *Heliothis virescens* (F.), the bollworm, *Helicoverpa zea* (Boddie), and the pink bollworm, *Pectinophora gossypiella* (Saunders). With the exception of the pink bollworm, no method of genetic control has progressed much beyond the pilot test stage for any cotton insect. A USDA, APHIS directed program using sterile pink bollworms in California's San Joaquin Valley has been underway since 1969. It is funded primarily by cotton growers in California with some federal and state help.

At a *Helicoverpa/Heliothis* workshop, Stoneville, Mississippi, June 12-14, 1984, LaChance (unpublished) proposed adoption of the following terminology to avoid semantic difficulty in describing mechanisms for genetic control of species in these genera:

STERILE INSECT RELEASE METHOD

The Sterile Insect Release Method (SIRM) or Sterile Insect Technique (SIT) is a procedure wherein a fully sterilizing dose of radiation is administered to both males and females. Under these conditions the males are at least 99 percent sterile when outcrossed to normal females; the same is true when irradiated females are outcrossed to untreated males. Dominant lethal mutations induced in both the sperm and the ova (egg cells) of the treated species form the basis of the sterile insect release method.

Studies of insect reproduction have demonstrated that when insects are treated with X-ray, gamma radiation or certain mutagenic chemicals the treated insects become unable to produce the normal number of live progeny (Knipling, 1979). Treated males are usually at least 99 percent sterile when outcrossed to normal females, and the same is true when treated females are outcrossed to normal males. Treated insects are released in large numbers into a field environment and are expected to mate with the feral (wild, native) insects, thus interfering with reproduction. The number of insects released must be of such a magnitude that the proportion of normal X normal matings is essentially zero. If matings between treated insects and normal insects are successful, then reproduction of the field population will be disrupted, and the population will decline. The success of Sterile Insect Release Method depends on several factors:

1. Techniques for producing large numbers of the target insect;
2. Techniques for sterilizing large numbers of the target insect;
3. Reasonably competitive insects that can be released after treatment;
4. Tools that will assess field populations accurately before and after the release of the treated insects; and,
5. A treatment area large enough (or adequately isolated) to exclude the possibility of immigration of fertile females into the release area.

With the exception of item 2, these criteria also apply to other autocidal techniques.

Except for research or demonstration purposes, use of genetic methods for population suppression or eradication has been very limited. Eradication of the screwworm, *Cochliomyia hominivorax* (Coquerel), from the United States, conceived by E. F. Knipling (Lindquist, 1955) and completed in 1966 (Bushland, 1975), remains the clas-

sic example of insect control by Sterile Insect Release Method. Following the success of this program, this method was attempted on many other insect pests. The protection of California's fruit industry by the release of sterile Mexican fruit flies, *Anastrepha ludens* (Loew), and the short-term eradication of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), populations from Los Angeles County, California (LaChance, 1979) are other examples of successful implementation of the Sterile Insect Release Method. However, problems with reintroduction and possible establishment of these pests occur.

INHERITED STERILITY

Inherited sterility is the use of substerilizing doses of radiation administered to males and females. Depending on the dose given, the males and females can be partially fertile when outcrossed to untreated insects, or the males can be partially fertile and the females completely sterile. The dose can be adjusted so that the released males and females are completely sterile when they intermate. The F_1 progeny of these males and females can be completely to partially sterile, depending on the dose administered to the parents. Insects exposed to doses of radiation which do not produce full sterility produce F_1 progeny that can exhibit levels of sterility equal to or higher than those of their treated parents (North, 1975). This F_1 (or delayed) sterility has been suggested to be of use in control programs.

BACKCROSS STERILITY

Backcross sterility describes the release of sterile hybrid insects propagated by the use of backcross techniques. These insects have been derived from an original cross involving tobacco budworm, *Heliothis virescens* (Fabricius), males and *Heliothis subflexa* (Guenee) females (Laster, 1972). The fertile female progeny are backcrossed to tobacco budworm males each generation and continue to produce fertile females and sterile males. The backcross is a way to maintain the strain so that hybrid sterility can be expressed. Both the terms backcross and hybrid sterility are acceptable, but, because F_1 hybrid insects are not released and backcross insects are, backcross sterility has become the more used term.

OTHER GENETIC CONTROL CONCEPTS

Other genetic control concepts involve: (a) the release of insects homozygous for induced or natural chromosomal translocations; (b) selection and release of strains of insects bearing conditional lethal traits or recessive lethal genes; (c) releasing insects bearing compound chromosomes; (d) the overflooding of wild populations with cytoplasmically incompatible insects; (e) isolation and release of strains with distorted sex ratios; (f) forcing of deleterious genes through a population with segregation-distorting chromosomes (meiotic drive); or (g) release of sterile hybrids (progeny of crosses between closely related species). Each of these genetic techniques has a common requirement, the mating of a laboratory-reared insect with one from the field population. The Sterile Insect Release Method differs from other genetic approaches because all insects released are sterile. All of the other genetic

control techniques listed above require the release of at least one fertile sex so that the character can be transmitted to the field population. As will be discussed later in this chapter, this factor has been a stumbling block in the use of some of the more sophisticated autocidal control techniques.

The relative efficiency of various genetic methods for population suppression and a list of pertinent references has been compiled by Knipling and Klassen (1976). Additional information can be found in Hoy and McKelvey (1979) and Knipling (1979).

COTTON INSECTS

The subfamily Heliothinae of the family Noctuidae contains some of the most economically important insect pests of agricultural crops worldwide. Species of the corn earworm complex and the tobacco budworm complex are members of this subfamily. Traditionally, the bollworm or corn earworm and the tobacco budworm have been grouped together and referred to as the *Heliothis* complex. However, Hardwick (1965) revised the bollworm-corn earworm species. He divided them into seventeen species in five species groups and separated them from the genus *Heliothis*. He proposed the genus name *Helicoverpa* for this group. Acceptance of this genus name change has been met with mixed responses from entomologists. Poole (1989a,b) accepted the proposed revision by Hardwick (1965) as scientifically correct because *Helicoverpa* is morphologically distinct and phylogenetically separate from all other genera in the sub-family Heliothinae. Matthews (1987), in his classification of the Heliothinae, also agreed with Hardwick (1965). The corn earworm is identified as *Helicoverpa zea* (Boddie) in the Entomological Society of America (1989) list of approved common names. Because these changes have a sound scientific basis, it seems fitting that they should be adopted by the scientific community and used accordingly. The remainder of this discussion will follow the genus terminology of Hardwick (1965), and the species groups for each complex are listed in Table 1.

The bollworm and tobacco budworm are serious pests of a large number of agricultural crops. The bollworm is the only North American species within the genus *Helicoverpa*. Twelve species of *Heliothis* occur in North America (only three species are listed in Table 1), and the tobacco budworm, *Heliothis virescens*, is the primary pest in this genus. *Heliothis subflexa* is known to feed only on groundcherry, *Physalis* spp., and is not a pest in the Mid-South. It could be a serious pest of the husk tomato (tomatillo), *Physalis ixocarpa*, in Mexico or other areas where it is grown commercially. *Heliothis subflexa* is most important for hybridization with tobacco budworm, *Heliothis virescens* to produce genetic sterility. *Heliothis phloxiphaga* Grote and Robinson is not considered a pest, but it has been collected from safflower, *Carthamus tinctorius*.

STERILE INSECT RELEASE METHOD

Varying degrees of success have been demonstrated by this method, particularly with lepidopterous pests such as the codling moth, *Carpocapsa pomonella* (L.) (Proverbs and Newton, 1962a,b; Proverbs *et al.*, 1969), the tobacco hornworm,

Table 1. Grouping of *Helicoverpa* and selected *Heliothis* species, their distribution and economic importance (Hardwick, 1965; Poole, 1989b).

Species	Distribution	Economic importance[1]
Helicoverpa		
The *zea* group		
zea Boddie	New World	+++
confusa Hardwick	Hawaii	-
minuta Hardwick	Hawaii	-
pacifica Hardwick	Jarvis Island	-
assulta Guenee	Old World	++
toddi Hardwick	Africa	-
fletcheri Hardwick	Africa	-
tibetensis Hardwick	Tibet	-
The *punctigera* group		
punctigera (Wallengren)	Australia	++
The *armigera* group		
armigera (Hübner)	Old World	+++
helenae Hardwick	St. Helena Island	-
The *gelotopoeon* group		
gelotopoeon Dyar	South America	+
bracteae Hardwick	South America	-
titicacae Hardwick	South America	-
atacamae Hardwick	South America	-
The *hawaiiensis* group		
hawaiiensis (Quaintance and Brues)	Hawaii	-
pallida Hardwick	Hawaii	-
Unassigned group (Poole, 1989b)		
tertia Roepke	Indonesia	-
Heliothis		
virescens (Fabricius)	New World	+++
subflexa (Guenee)	New World	-
phloxiphaga Grote and Robinson	New World	-

[1]+++ = severe pest, ++ = moderate pest, + = occasional pest, - = not economically important or pest status unknown.

Manduca sexta (L.) (Cantelo *et al.*, 1973) and the pink bollworm. Irradiated pink bollworm moths have been used in Sterile Insect Release Method programs since 1968 (Miller *et al.*, 1984) to keep this species from becoming established in the San Joaquin Valley of California where approximately one million acres of cotton are grown.

Bollworm—Eradication of the bollworm from St. Croix was attempted in 1968-69 using irradiated insects (Snow *et al.*, 1971). This program was confronted with a number of complicating factors and was terminated without reaching its eradication goal (Snow *et al.*, 1971).

Tobacco Budworm—Following termination of the bollworm eradication program on St. Croix, a cooperative sterile insect release program for the tobacco budworm was initiated in 1971 between USDA, ARS, Brownsville, Texas, and St. Croix. Pilot test funds were made available to support this effort in 1972 (unpublished report of the tobacco budworm study on St. Croix from September 1971 to October 1973). This program did not accomplish its suppression objectives due largely to the non-competitive ability of the irradiated laboratory-reared insects that were released.

North and Holt (1968) reported that lepidopterous insects are extremely resistant to irradiation treatment when the criterion is induced male sterility. For example, 5 krads are required to sterilize adult male screwworms (Bushland and Hopkins, 1951), whereas 35-45 krads are required to sterilize adult tobacco budworms (Flint and Kressin, 1968). These high doses of radiation result in deleterious effects on the competitive ability of the treated insects. This lack of competitiveness can probably be attributed to radiation-induced somatic damage (North and Holt, 1968). Large amounts of radiation reduce the ability of the male to transfer sperm (Flint and Kressin, 1967, 1968; North *et al.*, 1975; Snow *et al.*, 1972). The successful mating of a bollworm or tobacco budworm is believed to require the incorporation of both eupyrene (normal sperm with nucleus) and apyrene (without nucleus) sperm in the spermatheca (sac connected to the female organ that receives and retains the sperm). The large nucleated eupyrene sperm are capable of fertilization, but they do not become motile until they are transported to the spermatheca of the female. The anucleated apyrene sperm possess motility when they enter the seminal vesicles and are involved in the transport of eupyrene sperm to the spermatheca (North and Holt, 1971). Transfer of eupyrene sperm by lepidopteran males is important in changing the female postcopulatory behavior. Females that have mated and received no sperm or only apyrene sperm continue to "call" for mates, tend to remate, and generally refrain from ovipositing (laying eggs) until they receive eupyrene sperm. Large amounts of radiation often reduce the ability of the male to transfer sperm; the irradiated sperm does not survive as long within the female; and, male vigor and longevity can be drastically lowered (LaChance, 1979). This combination of factors results in insects that are not competitive in Sterile Insect Release Method programs and dictates the need for ways to lower the doses of radiation for lepidopteran species. Consequently, inherited or partial sterility offers the probability of much greater success than total sterility for controlling the bollworm or tobacco budworm in Sterile Insect Release Method programs.

Pink Bollworm—Experiments on the use of Sterile Insect Release Method for the pink bollworm started in the early 1960s at Brownsville, Texas. The first tests used cobalt-60 gamma radiation on pupae (Ouye *et al.*, 1964) and the chemical sterilant, metepa, on adult males (Ouye *et al.*, 1965). By that time, Richmond and Ignoffo (1964) had adapted the individual rearing methods of Vanderzant and Reiser (1956) to the rearing of large numbers of pink bollworms.

Releases of high ratios (50 sterile: 1 native) of irradiated or chemically sterilized pink bollworm males into field cages containing native populations were very successful in controlling the increase in the population over the growing season (see Henneberry (1980) for a review of six field cage release experiments). However, until 1987, except for the Sterile Insect Release Method project in the San Joaquin Valley of California (Stewart, 1984), where numbers of native moths are very low, no large scale release programs had been able to duplicate the cage results. In most pink bollworm infested areas, the large numbers of moths present in the field population and/or the immigration of fertile females from untreated areas masked the effect of the released moths on population numbers.

In 1987, a pink bollworm management trial using a combination of Sterile Insect Release Method and pheromone disruption was conducted on 1000 acres of cotton planted in the Coachella Valley, California (Staten, 1987; Staten *et al.*, 1988). In this trial, sterile insects were released over all cotton growing areas in the Valley throughout the growing season. High-rate pheromone ropes were used only in fields which were not maintaining a 60 sterile : 1 native ratio at pinhead square stage (Staten *et al.*, 1987). Conventional insecticides were applied based on the recommendations of the growers' pest control advisors.

In this management trial, the criterion for success was a reduction in the number of insecticide treatments that these fields had experienced in past growing seasons. In 1985, before any management trials, insecticides were first employed on June 1 and 7.2 treatments per field were applied valleywide. Fifty-six of 57 fields were treated. In 1986, high pheromone rope treatments were used valleywide without sterile insect releases and only 1.8 treatments were made per field with 17 of 31 fields receiving treatment. During the 1987 Sterile Insect Release Method trial no insecticides were applied in June or July; only six of 27 fields were treated with insecticides through August; and only 7 of 27 fields were treated through September. An average of 1.03 applications of insecticide per field were applied valleywide. The trial in 1988 was even more encouraging, since no conventional insecticide applications occurred in the management area of Coachella Valley (R. T. Staten, personal communication). Secondary pest populations (such as whitefly) were also observed to be lower.

Thus, it appears that the integration of Sterile Insect Release Method and pheromone disruption as control procedures, along with careful monitoring of insect populations, reduced the number of conventional insecticide treatments required in the Coachella Valley of California. Further integration of other management practices—such as pest-resistant (nectariless, okra leaf) and short-season cotton varieties, crop termination with a plant growth regulator, early plowdown, and use of non-chemical

sprays (such as *Bacillus thuringiensis*)—for control of leaf-eating insects, such as the bollworm or saltmarsh caterpillar, should lead to further reductions in pest populations.

Boll Weevil—A review of the status of boll weevil sterility and the technology available for eradicating the boll weevil was presented in 1983 (Wright and Villavaso, 1983; Knipling, 1983). A brief history of boll weevil sterility, the effectiveness of sterile weevils in the field, and the potential use of sterile weevils as a genetic means of population suppression will be presented here.

In the case of the boll weevil, the sterile male technique has been the only method of genetic control attempted. A paper on the theoretical release of boll weevils carrying recessive lethal mutations is available (LaChance and Knipling, 1962), but as of yet, no colonies of boll weevils with recessive lethals are in existence.

Irradiation was the first method used to sterilize the boll weevil. Dosages of irradiation large enough to produce sterility also caused what was then considered to be unacceptably high mortality (Davich and Lindquist, 1962). Longevity in both the field and laboratory also was significantly reduced, and levels of sterility were not consistent. From results of a field cage test, Davich *et al.* (1965) estimated the mating competitiveness of irradiated males to be roughly 20 percent that of normal males.

Chemosterilization was tried as an alternative method to sterilize the weevil, but it also reduced vigor and sterility was not permanent (Borkovec *et al.*, 1978; Earle and Leopold, 1975; Gassner *et al.*, 1974; Haynes, 1963; Haynes *et al.*, 1975; Lindquist *et al.*, 1964; McHaffey and Borkovec, 1976). However, chemosterilization with busulfan and hempa appeared to be the best sterilizing treatment available in the early 1970s, and it was chosen as the method for sterilizing the weevils released in the Pilot Boll Weevil Eradication Experiment [PBWEE] conducted in South Mississippi, Louisiana, and Alabama from 1971-1973. Males released in the experiment were both mass-reared and mass-sterilized. Tests of the competitiveness of weevils treated by the chemosterilization technique were conducted on weevils reared and sterilized on a small scale (less than 1000 or so insects) and then released into 1/16 acre screened cotton plots (Villavaso and Earle, 1976). These males were 25-33 percent as competitive as untreated males.

The eradication area for the experiment averaged about 2600 acres during the three-year test with the total eradication and buffer areas averaging about 20,000 acres (Boyd, 1976). Events leading to this experiment, results of the experiment, and the reports of two committees convened to evaluate whether eradication was achieved or would be achievable with the technology then available are presented in the report of Boyd (1976).

A sterilization treatment in which small doses of irradiation were given to adult male confused flower beetles, *Tribolium confusum* Jacquelin duVal, over a period of time rather than in one large dose became known as fractionated irradiation (Ducoff *et al.*, 1969, 1971). The treatment appeared to produce both high sterility and longer post-irradiation survival. This type of treatment had been deemed to be unsatisfactory for the boll weevil (Flint *et al.*, 1966), but was revived in the mid-1970s as a series of 25 doses of irradiation administered to adult boll weevils at four-hour intervals (Earle *et*

al., 1978; D. Birkenmeyer, D. Childress, and R. Leopold, USDA, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota, unpublished data).

The use of fractionated irradiation on boll weevil pupae was begun in the mid-1970s. Males emerging from pupae subjected to a series of 25 irradiation treatments of approximately 250 rad per treatment (Haynes *et al.*, 1977) were 23 percent as competitive as normal males (Villavaso *et al.*, 1979). In comparison, adult males allowed to remain on the surface of the larval media for 3-4 days after emergence and then treated with a single dosage (acute irradiation) of seven krad followed by a five second dip in a 0.02 percent solution of diflubenzuron (Dimilin®) in acetone (R. A. Leopold and D. T. North, personal communication; Earle *et al.*, 1978) were 36 percent as competitive as normal males. Although it worked relatively well, pupal fractionation was dropped because of its unwieldiness and its failure to produce males any more competitive than those treated with acute irradiation.

Diflubenzuron (Dimilin®) had been found to be an effective means of preventing hatch of eggs laid by irradiated females mated to fertile males without causing increased mortality (Moore and Taft, 1975; Moore *et al.*, 1978), but administration of diflubenzuron to males not yet hardened after emergence severely reduced their ability to inseminate females (Earle *et al.*, 1979). The mating ability of males allowed to age four or more days before treatment with diflubenzuron was not affected. However, diflubenzuron was applied as an acetone dip, and acetone was found to severely impair the flight ability of treated weevils (Earle and Simmons, 1979; Haynes *et al.*, 1981).

Pheromone production for both pupal fractionation and acute irradiation was approximately equal. Even though the pupal fractionation group was newly emerged, pheromone production averaged 2.0 micrograms per male per day for days one to three after emergence; this rose to 4.5 per male per day for days four to six. The weevils that received the single dosage of seven krad had been allowed to feed on the surface of the larval media for three to four days before treatment; however, their level of pheromone production was not significantly higher than that of the pupal fractionation group indicating that diet might be as important as age in the onset of pheromone production by males (Villavaso *et al.*, 1979).

The laboratory work of Leopold, North and Earle had stimulated renewed interest in acute irradiation as a method to sterilize the boll weevil. Acceptable levels of field competitiveness in male weevils sterilized by acute irradiation reestablished the feasibility of using this treatment in mass-release programs (Villavaso *et al.*, 1979). However, the use of acute irradiation would not have come about without the advent of the following three factors: (a) mass-rearing of boll weevils relatively free of pathogenic bacteria (Sikorowski *et al.*, 1977; Sikorowski, 1984); (b) use of diflubenzuron (Dimilin®) to bring about complete sterility of treated females (Moore *et al.*, 1978); and perhaps most importantly, (c) the lowering of the formerly acceptable standard of 50 to 70 percent survival of treated males for three weeks after treatment to a more realistic one. A sterilizing treatment is now considered to be acceptable if males are able to attract and inseminate females for at least seven days after treatment. (Villavaso *et al.*, 1980).

The first field tests designed to estimate competitiveness of irradiated males were conducted in 1977 (Villavaso *et al.*, 1979). Sterile and fertile males were released into isolated boll weevil-free plots of cotton along with virgin females. One week later, squares with oviposition punctures were collected from the plots. Hatch of eggs collected from these squares along with hatch from crosses between sterile males X normal females, normal males X normal females, and the ratio of sterile to normal males in the field were used to estimate competitiveness according to a formula derived by Fried (1971):

$$\frac{Ha - Ee}{Ee - Hs} \times \frac{N}{S}$$

Where

Ha = percent egg hatch for normal males X normal females
Hs = percent egg hatch for sterile males X normal females
Ee = percent egg hatch observed in the experimental plots
N = the number of normal males
S = the number of sterile males

The formula gives an estimate of the overall competitiveness of the sterile males as measured by egg hatch. No assumptions are made as to the individual factor or factors that might be responsible for the degree of competitiveness achieved. The Fried formula gives competitiveness as a decimal equivalent. Multiplying by 100 converts this figure to percentage. Use of the isolated plot technique and the formula of Fried are the standard methods for determining competitiveness of sterile boll weevils.

Using basically the same procedures established in 1977, small plot tests were conducted simultaneously in Louisiana and North Carolina (Villavaso *et al.*, 1980) to determine the competitiveness of males sterilized by three methods. The three sterilization methods were: (a) fumigation with bisazir followed by dipping in penfluron (Borkovec *et al.*, 1978); (b) irradiation with 10 krad of gamma irradiation followed by dipping in diflubenzuron (Leopold and North, personal communication; Earle *et al.*, 1978); and (c) treatment of pupae with doses of 250 rad every four hours until a total dosage of 6250 rads had been administered (Haynes *et al.*, 1977). The males sterilized by the three methods were 23, 17 and 12 percent, respectively, as competitive as untreated males of the same laboratory reared strain. The fumigated males and those given the single dosage of irradiation were fed artificial diet for five days prior to irradiation (Wright *et al.*, 1980).

In 1979 sterile males were released as part of the Boll Weevil Eradication Trial (BWET) on approximately 19,000 acres of cotton in Virginia and North Carolina. A fall diapause program in which all cotton acreage was treated with organophosphate insecticides significantly reduced the number of weevils entering diapause. It was followed by spring applications of sterile insects, pheromone trapping and aerial applications of organophosphates and the insect growth regulator diflubenzuron (Dimilin®). Though the boll weevil was eradicated from the trial area by this combination of tech-

niques, the effect of each technique could not be measured separately. Only seven native weevils were captured in the trial area prior to the release of 11.2 million sterile weevils; thus, the role played by the sterile insects in eradication remains unclear. The treatment selected to sterilize the weevils released in the Boll Weevil Eradication Trial consisted of feeding weevils on slabs of diet containing 0.01 percent diflubenzuron for the first five days after they had emerged followed by 10 krad of gamma-irradiation (Wright *et al.*, 1980). This treatment was chosen because of its simplicity and predictability and because of the potential health hazard associated with the fumigation treatment (Villavaso *et al.*, 1980). Diflubenzuron (Dimilin®) was administered in the diet rather than as an acetone dip because acetone was found to have an adverse effect on the flight ability of dipped weevils (Earle and Simmons, 1979; Haynes *et al.*, 1981). Administration of diflubenzuron to newly emerged weevils was known to have a serious detrimental effect on their ability to mate (Earle *et al.*, 1979), but it was considered to be the only available means of assuring complete sterility while avoiding the flight problem associated with dipping in acetone.

In 1979, 1980 and 1981, weevils treated by the same method used in the Trial were tested for competitiveness in the field. Competitiveness of the sterile males versus untreated laboratory-reared males averaged 10.6 percent for the first seven days following release when they were released with laboratory-reared virgin females. Competitiveness of sterile males versus native males averaged six percent when they were released with native virgin females. In general, the treated weevils were competitive only during the first four days of the seven-day period. Between days five and seven after release, competitiveness was no more than two percent indicating that biweekly releases of sterile weevils would be more effective than weekly releases. In fact, if weevils that are only effective for four days are released at seven day intervals, their pheromone might tend to concentrate the native weevils during these four days. Between days five and seven, the concentrated natives would have virtually no competition from sterile weevils, and this could increase the probability of native males mating with native females (Villavaso, 1981, 1982; Villavaso and Thompson, 1984). Additionally, Mitchell *et al.* (1983) reported no reduction in egg hatch when weevils treated by the method used in the Boll Weevil Eradication Trial were released against a very small native population on 120 acres of commercially grown cotton; this indicated that some factor or factors had prevented their being effective under field conditions.

In the early 1980s, a method of sterilization was developed that resulted in the highest competitiveness value that had been obtained for sterile boll weevils in small plot field tests. Males fed an ecdysteroid rather than diflubenzuron (Dimilin®) for five days prior to irradiation were 43.7 percent as competitive for laboratory reared females as untreated laboratory reared males. In comparison, the diflubenzuron fed irradiated males were only 12.5 percent as competitive (Villavaso *et al.*, 1983; Villavaso and Thompson, 1984). Weevils treated by the ecdysteroid plus irradiation technique were 50.4 percent as competitive as native males that naturally infested three small field plots (Villavaso *et al.*, 1986a). All of these estimates of competi-

tiveness were obtained from males reared and sterilized on a small scale (several hundred to 5000 at a time). However, when weevils treated by this same technique were reared and treated on a large scale (several hundred thousand per week), they were estimated to be only 11.4 percent as competitive as the natives infesting 180 acres of cotton in the Mississippi Delta (Villavaso *et al.*, 1986b). Bacterial contamination of the mass-reared weevils and/or the crowded conditions during the 1.8 hour period of exposure to irradiation appeared to have a severe detrimental effect on the released weevils.

In 1983, mass-reared and sterilized weevils were released into the cotton fields by two new methods. The first consisted of hanging small paper bags each containing about 75 weevils on cotton plants at the rate of four bags per acre. The bags were torn open to allow the weevils to escape. For the second method, weevils were suspended in a 0.6 percent solution of furcelleran and dispensed onto the plants by means of a specially designed pumping device (D. K. Harsh, J. L. Roberson and E. J. Villavaso, USDA, ARS, unpublished). Both methods of release effectively placed weevils directly on the plants instead of randomly dropping them into the fields where they might land either on the plant or on the ground. Dropping weevils onto freshly cultivated or hot soils (greater than 115°F) in early 1983 resulted in very low numbers of weevils reaching the cotton plants (Roberson and Villavaso, unpublished). The loose soil prevented the weevils from leaving the ground where they had fallen, and if soil temperatures reached lethal levels as they often did during the release periods, the weevils died on the ground without ever reaching the plants. The importance of developing a method of release that resulted in a large portion of the weevils reaching the cotton plants was clearly seen, and a method by which released weevils would be containerized for mass-release was subsequently developed.

In 1984, mass-reared and sterilized weevils were released by the furcelleran method into six fields of commercially grown cotton totaling 69.5 acres in north central Mississippi (Villavaso *et al.*, 1989a). The weevil population in the fields was low (approximately four per acre) during the test due to the effects of the severe preceding winter. Diflubenzuron wettable powder (Dimilin® 25 percent WP) was used as an aqueous dip (Roberson and Villavaso, unpublished) or as an acetone dip (0.4 percent) prior to treatment with 10 krad of gamma irradiation. Use of the aqueous dip avoids the flight inhibition caused by acetone. Treating four day old weevils rather than newly emerged ones with diflubenzuron allows the cuticles of these weevils to harden and increases their ability to mate. Release of weevils directly on to the cotton plants in the furcelleran solution counteracted the flight inhibiting effect of acetone.

Egg hatch in the six fields was reduced to 15.2 percent while hatch in the three control fields (46.5 acres) was 94.4 percent. This was the most significant demonstration of the effectiveness of sterile weevils against relatively low populations of natives. A population of four weevils per acre is at least twice as high as that which sterile weevils might be used against in an eradication program, and the population was probably underestimated. Additionally, the sterile weevils were estimated to be only about 12 percent as competitive as the natives. Some of the then unidentified problems associ-

ated with the status of mass-rearing, handling and sterilizing of weevils apparently were responsible for the lowered competitiveness.

In 1985, mass-reared and sterilized weevils (irradiation plus aqueous dip in diflubenzuron) were containerized and mass-released in a large scale test in South Carolina. The LT_{50} (the day on which 50% or more of the males had died) of the samples of males held on cotton plants averaged 7.7 days, and competitiveness of the mass-reared, mass-sterilized weevils was increased to 19 percent. Antibiotics added to the pre-irradiation diet may have been beneficial in increasing longevity of these weevils (Reinecke *et al.*, 1986).

In 1986, there were reports that the vision of mass-reared weevils was impaired (Agee, 1986), and that the addition of carotenoids to the diet would remedy the impairment (Dickens and Agee, 1987). The competitiveness of the visually impaired weevils (71 percent) and that of weevils whose visual impairment was corrected by the use of carotenoids (77 percent) was not significantly different, and it was determined that the visual impairment was not an important factor in competitiveness (Villavaso *et al.*, 1988). Also in 1986, the competitiveness of visually impaired sterile weevils was tested in small field plots in Arizona against the Arizona natives. Competitiveness averaged 83 percent (Villavaso *et al.*, 1989b). The released weevils were mass-reared and then handled and sterilized in small groups of a few hundred. The high degree of competitiveness indicated that the quality of the mass-reared weevils had improved significantly over the previous year.

Prior to 1985, most of the research on the competitiveness of sterile weevils had been done in small isolated plots of 1/4 to 1 acre or in commercial cotton plantings of less than 200 acres (Villavaso *et al.*, 1979, 1980, 1986a,b, 1988). In 1987 and 1988, a test of the effectiveness of mass-reared, sterilized (irradiation plus aqueous dip in diflubenzuron), containerized and aerially-dropped weevils was conducted on about 5000 and 3000 acres, respectively, in Fayette County, Alabama. In 1987 the test area had native populations that were too high for the sterile weevil to be very effective. However, even with the high populations, the fertility of the native females was reduced by about 39 percent. The LT_{50} of samples of sterile males held in individual screened containers on cotton plants averaged 9.1 days. This exceeded the previous high for a test of this type by 15 percent. The 1988 weevil populations appeared to be smaller than those of 1987, and fields selected for intensive sampling showed the effectiveness of the sterile weevils (Smith *et al.*, 1989).

The degree of competitiveness that sterile weevils must exhibit in order to eradicate indigenous populations of boll weevils has not been determined. Eradication was achieved in the 1979 Boll Weevil Eradication Trial, but the extent to which the released weevils contributed to eradication could not be partitioned from that of the other methods of suppression used. This remains one of the major problems in assessing the value of sterile weevils in eradication efforts. Before eradication by means of sterile weevil releases can be demonstrated in large acreages of commercially grown cotton, the target population must be very low. A highly competitive sterile weevil might be effective in eradicating populations as high as five natives per acre. However, the chances

of achieving eradication with sterile weevils alone probably decrease greatly as native populations increase to more than two per acre. When native populations are small enough to expect eradication, it becomes almost impossible to evaluate the effect of sterile weevils because of the difficulty in collecting data and the possibility of migration into the test area. The expense of testing over very large acreages (more than 3000 acres) where migration might be plotted by use of trap lines is too large for most research budgets to absorb. Different management practices from farm to farm, especially application of insecticides for other insects, confound the evaluation process.

When using small (less than 1 acre), isolated plots to evaluate the effectiveness of sterile weevils, a sufficient number of normal weevils must be released into the test plots to insure an adequate number of eggs for measuring egg hatch. This means that many more normal males and females must be put into the small plots than one would anticipate in any program where eradication was the goal. From the small plot data, the competitiveness of sterile weevils can be estimated. These estimates can then be used in models to predict the probability of eradicating very low weevil populations. However, many variables affect the performance of weevils released in the field, and they must be researched or assumed before models can be constructed. These include, but are not limited to, the number of native weevils entering the cotton fields, the time period over which they enter the fields, the expected rate of increase of the native population, their spatial distribution in the fields, and the relationship between the growth stage of the cotton plants and the temporal (of or relating to time) and spatial distribution of the native and the released weevils.

The temporal (time related) distribution of native populations emerging in the spring can alter the effectiveness of sterile weevils. Two populations of similar size might have dissimilar emergence patterns. In one year, most of the overwintered weevils might emerge before the cotton has begun squaring. In this case treatment with insecticide before the squares are large enough for larval development (pinhead square treatment) will have a devastating effect on the native population. In another year or the same year in a different location, most overwintered weevils might emerge after the appearance of squares large enough to support reproduction. In this case the effectiveness of the pinhead square treatment will be reduced. Thus, even though the spring populations were similar in size, sterile weevils will be competing with a much larger number of native weevils in the second case than in the first.

Sterile weevils will probably be released at a fixed number per week, but the ratio of sterile to native weevils will vary depending on how the natives emerge. If, for example, 200 native weevils fly into a 100 acre cotton field during the week after pinhead square treatment and sterile weevils that are effective for one week are being released at a rate of 100 per acre per week, the ratio of sterile to natives will be 50:1 for that week. If native weevils live for two weeks and the 200 natives emerge at a rate of 25 per week for eight weeks, then the sterile to native ratio will be much greater (400:1 for the 1st week and 200:1 thereafter). If the sterile weevils are 25 percent as competitive as natives, the 50:1 ratio becomes 12.5:1 and reproduction will probably occur. The 200:1 ratio becomes 50:1, and there is a much greater chance that the sterile weevils

will prevent reproduction in the field. Thus the odds of sterile weevils preventing reproduction in two native populations of exactly the same size can be quite different.

Another problem associated with suppression by sterile weevils is the spatial distribution of the native weevils in the field. An average population of one native per acre uniformly distributed over a field would be amenable to eradication. However, if 80 native weevils settled in a 10 acre portion of a 100 acre field over a short period of time, and the remaining 20 natives dispersed over the other 90 acres, then a higher than acceptable rate of reproduction is almost certain to occur in that portion of the field where the native population is actually eight times the average for the whole field. The use of sterile weevils would be effective only against very low populations of natives where aggregations of emerging overwintered insects would be small enough to be controlled by the released weevils. Therefore, their use wouldn't be effective in fields with spatial distribution problems.

If sterile males are unable to attract and mate with native females before the native females mate with a native males, the effectiveness of the sterile males will be diminished. The eggs laid by native females tend to be highly aggregated (Pieters and Sterling, 1974). The F_1 weevils emerge from these aggregations or clumps (Mitchell *et al.*, 1976) in close proximity to one another, and the ratio of sterile to native weevils in such aggregations will be much lower than that over the field as a whole. These aggregations will be difficult for sterile weevils to control.

Effects of both clumping (spatial distribution) and emergence pattern (temporal distribution) on the effectiveness of sterile weevils can only be speculated, but it is reasonable to assume that both can have significant impact on the success of a sterile insect release program. These effects might best be estimated with the aid of computer simulation models.

Use of insecticides to decimate boll weevil populations followed by the use of pheromone traps to identify surviving pockets of reproductive activity, followed by more insecticide applications and more trapping has been successful in eradicating boll weevils from North Carolina and most of South Carolina. This method of eradication is continuing in most of the cotton growing areas of Georgia and Florida and significant portions of Alabama, and as long as the method is acceptable, sterile weevils will probably not be used for eradication. The odds for sterile boll weevils ever being used for other than research or demonstration appear to be low at present.

INHERITED STERILITY

Bollworm—LaChance (1985) stated that all models comparing inherited sterility (see discussion of inherited sterility in earlier section of their chapter) with total sterility demonstrated that inherited sterility is more effective in suppressing native populations of lepidopterous species than an equal number of fully sterile insects. Proverbs and Newton (1962a) first reported the incidence of inherited sterility in the codling moth. Since that report, many researchers have studied inherited sterility and its potential for population suppression for a number of lepidopterous pests (North, 1975; Laster *et al.*, 1988a).

Knipling (1970), using population models, demonstrated the advantage of inherited sterility over the sterile insect release method. The bollworm has been suggested as a potential candidate for control by inherited sterility (North and Holt, 1971; Knipling, 1979; LaChance, 1985; Carpenter *et al.*, 1987a,b,c). North and Holt (1970) first reported inherited sterility in the bollworm. They observed reduced egg hatch from F_1 (first generation offspring) moths compared to P_1 (parental generation) moths, found that irradiated males transferred a normal amount and ratio of eupyrene: apyrene sperm (e.g. ratio of normal sperm: sperm without a nucleus), and suggested the possibility of population suppression by releasing partially sterile moths. Snow *et al.* (1972) studied the effects of irradiation on the ability of adult male bollworms to transfer sperm and the field attractiveness of females mated to irradiated males. They found that irradiated males transferred significantly less normal sperm than nonirradiated males, but the decrease was greater with sterile males than partially sterile males. Also, females containing irradiated sperm were as attractive as virgin females; females mated with untreated males were less attractive. They concluded there would be significant advantages, in terms of sperm transfer, from the use of partially sterile males in release programs.

The early work by North and Holt (1970) has been expanded with efforts directed toward refining the inherited sterility technique to control the bollworm. Carpenter *et al.* (1987a) found that females mated to normal males and males irradiated with 10 krads have the same mating propensity and experience the same intermating interval. Sperm competitiveness demonstrated by these irradiated males was reduced in F_1 males. Females mated to male progeny from the irradiated males outcrossed to normal females exhibited the same attractiveness and mating propensity as virgin females. These females apparently were able to detect the quality of a sperm complement and reduce their intermating interval if the quality was not satisfactory. Therefore, the sperm from the F_1 males would be less competitive than normal sperm because they would be displaced more quickly by sperm from a subsequent mating due to the shorter intermating interval.

Carpenter *et al.* (1987c) studied the effects of substerilizing doses of radiation and inherited sterility on reproduction of the bollworm. They noted a higher degree of sterility in the F_1 progeny than in the P_1 adults when irradiated males were mated with normal females, and radiation-induced deleterious effects were inherited through the F_2 generation. Carpenter *et al.* (1987a), using a population model to predict the effects of inherited sterility on a native population, projected that a single release of males irradiated with 10 krads at a 9:1 ratio (irradiated:normal) would reduce the native population by more than 99 percent after three generations. Therefore, inherited sterility appears to be the more promising means of suppressing the bollworm than any other release technology presently known.

Tobacco Budworm—Flint and Kressin (1967) noted that male tobacco budworm moths were 99 percent sterile after an irradiation dose of 35 krads. Female moths produced few eggs at this dose, but there was some egg hatch. These studies were not

expanded to determine the extent of inherited sterility. Proshold and Bartell (1970) reported the effects of inherited sterility on reproduction, developmental time and sex ratio of this species. They found that irradiation reduced mating and fecundity (the ability to lay eggs), increased developmental time, increased larval and pupal mortality, and distorted the sex ratio in favor of the males. Proshold and Bartell (1972) further indicated the potential for reducing tobacco budworm populations by inherited sterility and reported that sterility factors were nearly eliminated by the third generation.

Laster (1972) discovered hybrid sterility by crossing *Heliothis subflexa* females with tobacco budworm males. Knipling (1979) stated that the calculated effects due to the release of both hybrid sterile males, if fully competitive, and hybrid fertile females for one generation are among the most impressive of the various genetic mechanisms considered. Since hybrid sterility for the tobacco budworm was discovered and its population suppression potential recognized, little effort on irradiation sterility for this species has been pursued.

Pink Bollworm—In the pink bollworm, F_1 males from parents treated with radiation failed to transfer sperm to their untreated mates and the females continued to seek mates (LaChance *et al.*, 1973). Because of the apparent reproductive problems with F_1 males, as well as a lack of good isolated field populations, no experimental field release programs have been attempted using partially sterilized pink bollworms. The effects of low doses of radiation (1 - 10 krad) on the reproduction of P_1 and F_1 pink bollworms have not been examined fully, and the impact of such insects in field populations is unknown.

Boll Weevil—In the boll weevil, some reduction in the reproductive potential of F_1 through F_3 insects has been seen, but the results are highly variable (Haynes and Smith, 1989; Haynes, 1990; Villavaso, unpublished). The technique does not seem to offer much promise at present.

BACKCROSS STERILITY

Bollworm—Backcross sterility such as that which has been demonstrated for the tobacco budworm is not known for any other agricultural insect pest species. Because the sterility mechanism was found for the tobacco budworm, it is reasonable to assume that it may also occur in other phylogenetically related lepidopterous species. Efforts are in progress to search for a similar type of sterility for the bollworm.

Research was initiated in 1984 to search for backcross sterility for bollworm. This effort involves importing *Helicoverpa* species from various parts of the world into the Stoneville Research Quarantine Facility, Stoneville, Mississippi, crossing them with bollworm, and evaluating the progeny for male sterility. In a cytoplasmic incompatibility system, sterility may not be expressed in the F_1, but may develop in later backcross generations as the chromosomes of one species are transferred to the cytoplasm of the related species. For this reason, long term experiments involving several laboratory generations are necessary (Laster *et al.*, 1985).

The search for bollworm backcross sterility is dependent upon foreign exploration, importation and colonization of the "exotic" species in quarantine in order to carry out crossing trials over several generations. Primary emphasis is placed on obtaining and evaluating the *Helicoverpa* species described by Hardwick (1965) (Table 1). Species that have been evaluated thus far, their origin, and reproductive status are listed in Table 2. From the standpoint of hybridization with *Helicoverpa zea, Helicoverpa armigera* appears to be homogeneous across its geographic range. Although the incidence of mating between the two species is low, progeny are produced from successful matings with no evidence of sterility (Laster, unpublished). Matings between *Helicoverpa fletcheri* from Mali, West Africa, and *Helicoverpa zea* gave results similar to those between *Helicoverpa zea* and *Helicoverpa armigera.* All attempted matings between *Helicoverpa punctigera* from Australia and *Helicoverpa zea* or *Helicoverpa gelotopoeon* from Argentina and *Helicoverpa zea* resulted in the pairs permanently locked in copula and no reproduction. Progeny were obtained from one

Table 2. Reproduction from exotic *Helicoverpa* species imported into the Stoneville Research Quarantine Facility and crossed with bollworm.

Species	Origin	Reproduction
armigera	Australia	yes
armigera	Egypt	yes
armigera	Indonesia	yes
armigera	Pakistan	yes
armigera	Thailand	yes
armigera	Zimbabwe	yes
armigera conferta	New Zealand	yes
assulta	Pakistan	yes[1]
assulta	Thailand	no
assulta	Zimbabwe	no
fletcheri	Mali	yes
gelotopoeon	Argentina	no
punctigera	Australia	no

[1]F_1 Progeny were obtained from one mating of bollworm x *H. assulta.* Haldane's (1922) effect was expressed and the colony was lost.

mating of *Helicoverpa zea* x *Helicoverpa assulta* from Pakistan. Progeny from this mating were all male following Haldane's Rule (1922) which states: "When in the F_1 offspring of two different animal races, one sex is absent, rare or sterile, that sex is the heterozygous sex." The hybrid from *Heliothis subflexa* females mated to tobacco budworm males is an exception to this rule because, in Lepidoptera the female is the heterozygous sex (Robinson, 1971). Although no backcross sterility has been found for bollworm, there still remains a large number of candidate species in various geo-

graphical locations for crossing with bollworm. The potential for population suppression of bollworm with backcross sterility justifies the effort in continuing the program.

Tobacco Budworm—Laster (1972) crossed *Heliothis subflexa* females with tobacco budworm males and discovered that the hybrid males were sterile. Hybrid females, when mated to tobacco budworm males, produced progeny with sterile males and reproductive females. This sterile male trait continued through successive backcross (BC) generations when backcross females were mated to tobacco budworm males. After a few backcross generations, these insects are genetically almost identical to tobacco budworm except that the males are completely sterile (Laster *et al.*, 1988a). The tobacco budworm genome operating in the *Heliothis subflexa* cytoplasm results in male sterility. However, the mechanism causing male sterility has not been determined.

The potential for suppressing wild tobacco budworm populations through use of backcross sterility in mass rearing and release programs was recognized. The sterile male producing females provide the driving force for population suppression. A number of models have been developed that project the decline of the natural tobacco budworm population following release of backcross insects (Laster *et al.*, 1976; Makela and Huettel, 1979; Levins and Parker, 1983; Roush and Schneider, 1985).

Biological investigations showed that backcross insects utilized the same host plants as the tobacco budworm (Laster *et al.*, 1978, 1982; Martin *et al.*, 1984), and that the final mating of females took precedence over previous matings (Pair *et al.*, 1977). Egg hatch was reduced through sterile male matings and the sterile male trait was infused into the tobacco budworm population (Laster *et al.*, 1978). A pilot backcross release program conducted on St. Croix during 1977-1980 demonstrated that the tobacco budworm population was suppressed during this period when compared with the population on Vieques, a neighboring island, for the same period (Proshold *et al.*, 1982).

Evaluation of backcross sterility in a typical agricultural area in the contiguous United States is needed to determine its effectiveness for tobacco budworm population suppression. All biological data indicate that backcross insects are competitive with normal insects in the feral (wild, native) population. Also, characteristics such as insecticide resistance, to give the backcross insects a competitive advantage, might be incorporated into the backcross (Firko and King, 1990).

Evaluation of tobacco budworm collected over a wide geographical range (Arizona, California, Mississippi, North Carolina, Texas, Mexico, South America, Puerto Rico and St. Croix) indicated no differences in their response to hybridization (Laster *et al.*, 1988b; Laster, unpublished). This indicates that a backcross release program should be widely adaptable and would have the following advantages over the other sterility inducing systems: (a) no treatment is necessary other than the original cross; (b) any life stage of the insect can be released; (c) backcross populations are perpetuated by the backcross females; and (d) the desired backcross frequency can be obtained either by release of large numbers for one generation or fewer numbers for several generations (Proshold *et al.*, 1982).

Pink Bollworm—No measurable hybrid sterility has been found in crosses between pink bollworm collections from areas within the United States, Mexico, Puerto Rico or St. Croix, Virgin Islands, (A. C. Bartlett, unpublished results). Raina *et al.* (1981) reported no incompatibility between a strain of insects from southern India and two strains of pink bollworm (one was a long-term laboratory colonized strain, the other a newly colonized strain) from Arizona. We have not been able to import live pink bollworms from other areas (e.g., Egypt, China, Macedonia, Turkey, USSR) to pursue this research as thoroughly as should be done.

LaChance and Ruud (1979) made crosses between strains of the pink bollworm from Australia and Arizona and found full fertility. They also made reciprocal crosses between both strains of the pink bollworm and a strain of *Pectinophora scutigera* (Holdaway) from Australia. These crosses were characterized by reduced interspecific mating, low fecundity and low fertility. Some F_1 fertile progeny were produced, especially when *Pectinophora scutigera* females were crossed with pink bollworm males. Those F_1 individuals were fertile in backcrosses to *Pectinophora scutigera* but infertile in crosses to the pink bollworm. The authors suggest that interspecific hybrids between these two species will not be obtained easily and that these results may not be useful in control procedures. It seems possible, by artificial selection procedures, to improve the rate of interspecific mating, fecundity and fertility, so that increased numbers of F_1 progeny could be produced. Because the interspecific hybrids are sterile when crossed with pink bollworms, there is a possibility that they could be used in sterile releases without the debilitating effects of radiation. Such usage may entail more research effort than is justified, if radiation sterilized insect releases continue to be as efficacious as shown in the Coachella Valley trial in California.

CONDITIONAL LETHAL MUTATIONS

Pink Bollworm—Strains of insects can be manipulated by artificial selection procedures to carry traits that are detrimental to a field population but that do not affect the ability of the strain to exist in the laboratory For example, in areas of the world where diapause is mandatory for carrying populations through host-free or environmentally unsuitable periods, the inability of the pink bollworm to go into diapause would be a conditional lethal trait. A non-diapausing (ND) strain could be reared readily in the laboratory, but progeny produced by this strain in the field would not diapause, and could not reproduce during the host-free period.

Bartlett and Lewis (1987) selected strains of pink bollworms for the inability to respond to conditions in the laboratory (short photoperiod and low temperature) which normally induce diapause. The non-diapause character is controlled by dominant or partially dominant alleles and is polygenic.

The nature of the inheritance of the non-diapause character suggests that single releases of the non-diapause strain should be made in extremely large numbers near the end of the reproductive season. However, if females were released in the numbers needed to insert the character into field populations, they would lay fertile eggs and

almost certainly increase the numbers of larvae present in the bolls. Increased larval numbers would lead to increased crop loss. The increased loss coupled with the cost of the release program, is not likely to be accepted by most farmers. In common with other genetic control procedures, it would be most beneficial if only males carrying the trait could be released. In this way, crop loss due to the addition of fertile females to the population would be avoided since the released males would mate only with native females.

SEX-LINKED RECESSIVE LETHAL MUTATIONS

Pink Bollworm—Lepidopterous males carry two X chromosomes (homogametic), while the females have only one (heterogametic). Strunnikov (1979) proposed the use of strains of Lepidoptera with balanced recessive lethal mutations on the sex chromosomes of the male as a method for control of lepidopterous pests.

Males carrying balanced recessive lethal mutations have two different recessive lethal genes, one on each X chromosome at different loci. When such males are crossed with any female, no female progeny are produced, unless crossing-over occurs between the two loci. Strunnikov (1979) postulated that the use of such genetically altered insects would be 1.3 times as effective in the F_2 as the single release of fully sterile males and that the effect would increase over generations.

In addition to the usefulness of balanced sex-linked recessive lethal mutations in control procedures, such stocks would be useful in genetic sexing of strains where only males should be released to drive a detrimental character (such as non-diapause) into a field population. In fact, the two systems (conditional lethal and balanced lethal) would act in concert to reduce pest populations during the growing season and the host-free season.

Bartlett (1988) demonstrated that sex-linked recessive lethal mutations can be induced readily in the pink bollworm and, by means of a sex-linked recessive eye color mutation, can be maintained over many generations in the laboratory. The production of a balanced sex-linked lethal strain has not yet been accomplished in the pink bollworm, nor in the codling moth (Anisimov, 1988). However, the possibility of developing such strains is being investigated actively at this time.

TRANSLOCATIONS

Pink Bollworm—Pink bollworms have been exposed to irradiation for the production of chromosomal translocations in a number of experiments (A. C. Bartlett, unpublished). Visible eye-color genetic markers have been used to recover reciprocal translocations. However, radiation induces a number of detrimental mutations (recessive lethals, deletions, duplications, etc.) along with the reciprocal translocations. A single heterozygous translocation produces about 50 percent sterility in the insect carrying the translocation. Thus, the reduced fertility due to the translocation, plus the problems with fertility caused by induced detrimental mutations, lead to rapid loss of translocation-bearing strains. Implementation of control using translocations thus

awaits further experimentation with agents that will induce translocations without causing other reproductive problems.

CYTOPLASMIC INCOMPATIBILITY

Pink Bollworm—No information is available nor have any experiments been attempted to isolate cytoplasmic incompatibilities in the pink bollworm, or cases of meiotic drive. Part of the reason for this lack of information is the fact that only nine simply inherited visible mutations are presently available as chromosome markers. None of these markers are linked, so only 9 of 30 chromosomes are marked.

FUTURE POSSIBILITIES

BOLLWORM AND TOBACCO BUDWORM

Although backcross sterility for the bollworm has not been discovered, there remains a number of *Helicoverpa* species in various geographical locations (Hardwick, 1965) that have not been evaluated in crossing trials with the bollworm. Each of these species is a possible candidate for producing hybrid sterility when crossed with the bollworm. Efforts to obtain and evaluate these species should be continued. Even if backcross sterility is not developed for the bollworm, the potential for controlling this species with inherited sterility is very encouraging. This technology should be refined and thoroughly tested for practical application.

Much of the backcross sterility technology for the tobacco budworm has been developed and evaluated to a limited extent. This technology needs to be evaluated in an areawide program in a typical agricultural production area. Techniques for using this technology for areawide tobacco budworm suppression need to be refined for practical application.

Genetic control methods offer considerable promise for suppressing both bollworm and tobacco budworm populations. Total sterility and inherited sterility are effective for both species whereas backcross sterility is only available for the tobacco budworm. Problems associated with insecticide resistance in these species and their destruction of food and fiber crops dictate the need for alternate control methods. The potential benefits in controlling these species by genetic means make continued development of these programs worthwhile.

PINK BOLLWORM

Some of the technical limitations of genetic control procedures for the pink bollworm have already been overcome. Rearing techniques are well-developed and in place. Insects produced by these techniques are vigorous and competitive. The tools for accurate assessment of field populations and the evaluation of the effects of the procedures have been used and improved on in actual field trials. In fact, recent con-

trol programs have been successful using the sterile insect release method and pheromone disruption. These techniques can be integrated easily with other existing technologies to further ensure success. However, in case certain populations of the pink bollworm are reluctant to succumb to the encroaching of man into their territory, new methodologies are beginning to be developed. At present, the methods of molecular biology are being employed to refine early genetic techniques. For example, yolk protein genes have been cloned in three insect species. The expression of these genes is stage-, sex- and tissue-specific. Sufficient information is available on the effects of hormonal regulation of protein production of yolk protein genes to indicate that these genes could be used to produce single sexed progeny in genetically engineered strains. The practical use of this information awaits the development of germline transformation vectors for insect pest species.

The identification and testing of candidate genes to introduce into the genome of the pink bollworm will be an expensive and long-term proposition since so little has presently been accomplished in this species or in Lepidoptera in general. However, once such candidates are identified and transformation vectors isolated, specific phenotypes can be altered rapidly and placed into service utilizing the considerable rearing and control expertise presently available for this important pest of cotton.

BOLL WEEVIL

Though no definitive work has been done, one might reasonably assume that the effectiveness of sterile weevils would increase as postirradiation survival time and mating capability increased. Thus, experiments to improve the effectiveness of sterile boll weevils have focused on these two traits. A strain of boll weevil was genetically selected with postirradiation survival in the laboratory 1.65 times that of the control and with significantly increased mating capability (Enfield *et al.*, 1981). Differences in postirradiation survival reached a plateau at about the 12th generation, and relaxing selection pressure for five generations did not result in a decline of longevity (Enfield *et al.*, 1983). In greenhouse and field tests, males of the selected strain lived 1.25 times longer than those of the strain currently in mass production (19.5 vs 15.2 days). Attractiveness and mating propensity during the second week after irradiation was somewhat greater than that of the mass reared strain. These differences did not result in increased competitiveness in the field, apparently because the mass-reared strain lived much longer than had been previously observed (Villavaso *et al.*, in press). Preliminary experiments that minimized cross-contamination, crowding, and handling produced a much longer lived mass-reared sterile weevil. (J. L. Roberson and E. J. Villavaso, unpublished). Research is underway to develop, on the scale that would be required for areawide programs, a workable system for minimizing crowding and handling while maintaining sterility.

SUMMARY

For genetic control of an insect population to be successful, detrimental traits must be introduced into that population from a released carrier population. Most methods of genetic control use prevention of egg hatch in the target population as the final mode of action, e.g., sterile insect method, inherited sterility, and backcross sterility. Other methods include the use of chromosomal translocations, conditional lethals, and cytoplasmic incompatibility. With the exception of irradiated pink bollworm moths in the San Joaquin Valley of California, no method of genetic control has been used on any cotton insect for other than research or demonstration purposes. because genetic control is species-specific and environmentally benign, research to perfect commercially usable technology will probably be supported for the foreseeable future.