FIELD EVALUATIONS OF A GENETIC SEXING STRAIN OF *Ceratitis capitata* IN HAWAII

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Abstract

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Large scale field studies of the Mediterranean fruit fly, *Ceratitis capitata*, were carried out in Hawaii using a genetic sexing strain based on pupal colour. In a ground release population suppression study, an all-male release was compared with a bisexual release; also included was a release of predominantly females. Following release, egg hatch measurements in the field indicated that the release of males was about four times more effective than the release of males and females. The release of females only, as expected, failed to show any effect on egg fertility. During the latter part of the releases it appeared that the wild medfly population was developing some form of behavioural resistance to the released sterile males.

1. INTRODUCTION

As reported in previous Research Co-ordination Meetings, organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, in Vienna (1985, 1987), Crete (1986, 1988), Guatemala (1990) and Italy (1992), the authors have been working with *Ceratitis capitata* (Wiedemann) (medfly) genetic sexing strains based upon sexual differences in pupal coloration [1]. One of these strains, Hawaiianized Robinson (RHW), was created through repeated backcrossing of males from *wp-23* [1] with standard Hawaii mass production females. This strain proved

itself superior to the original imported sexing strain (*wp-23*), received from A. Robinson, in a number of important fitness parameters, including: field cage mating competitiveness, small scale field dispersal and longevity, and rearing efficiency. The hybrid sexing strain also fared well against the standard bisexual laboratory strain (HI LAB) which has been in mass production for c. 38 years. On the basis of the encouraging preliminary test results with the RHW strain, in 1989 we began a programme of mass production and large scale field evaluation in order to evaluate the concept of releasing sterile males-only medflies in Hawaii.

2. PRELIMINARY LARGE SCALE FIELD STUDIES

Our initial field studies in coffee provided data comparing dispersal and longevity characteristics for a *C. capitata* pupal colour sexing strain and a standard bisexual strain. A third population (RHW-Mixed), differing from each of the other two populations by one parameter (either sex ratio or strain), served as a control. The inclusion of this third population proved to be important. Also of crucial value in evaluating the concept of releasing males-only strains is the need to maintain a highly pure stock and avoid genetic contamination and progressive breakdown. We endeavoured to provide as high a percentage of males as possible in the RHW-Males release population (98% or higher) and we established rearing and pupal sorting procedures to ensure the maintenance of this standard.

With respect to fly dispersal, the RHW-Males population dispersed significantly more than the HI LAB population, with c. 30% dispersing beyond 100 m compared with c. 10% for HI LAB. The ratio of the proportions of RHW-Males to HI LAB flies trapped increased with distance, exceeding a factor of 10 at the higher distances. The dispersal of the RHW-Mixed population could not be directly compared but, in general, was intermediate in magnitude between RHW-Males and HI LAB, on the basis of trap recaptures up to 400 m from the release point.

Regarding the longevity of the released populations, both the RHW-Males and RHW-Mixed flies had significantly lower mortality rates than HI LAB flies, and their estimated LT_{50} values were about twice that of HI LAB. This result indicates that the strain difference between RHW and HI LAB, rather than the males-only factor per se, was the significant factor in producing the observed difference.

3. GROUND RELEASE SUPPRESSION STUDIES

On the basis of the encouraging preliminary results of the ground releases discussed above, we proceeded with plans to attempt population suppression of medflies in coffee grown in Kauai [2]. For the first time in testing a medfly genetic

sexing strain, we collected sterility data to assess the competitiveness of released sterile flies. We compared two strains (standard HI LAB and RHW-sexing) and several sex ratios: (1) 50:50 (HI LAB and RHW), (2) 99:1 (RHW 'males-only') and (3) 5:95 (RHW 'high females'). The third treatment was a new one, made possible by the ability to save machine sorted white pupae (females). This treatment was included in order to better assess the effect of sterile females in the field.

The various strains involved were mass reared at the Tropical Fruit and Vegetable Research Laboratory in Honolulu, Hawaii. The RHW genetic sexing strain was reared at a level of c. 2 million adults per week, while the HI LAB standard strain was reared at a level of c. 5 million per week. Larval rearing followed the standard methods with an artificial wheat based diet [3]. Popping larvae were collected in water, then placed into fine vermiculite at the rate of 1 L of larvae to 4 L of vermiculite to allow pupation. At six days of age, c. 10 L of the RHW production was sifted, then rinsed briefly to remove any vermiculite, and allowed to dry overnight. At seven days of age, c. 8 L of clean RHW pupae were mechanically sorted by colour using a Sortex/Scancore Inc. Model 1121 bichromatic sorter at a speed of c. 300 000 pupae/h. Brown pupae were sorted twice to produce a c. 99.5% brown lot and white pupae were sorted three times to produce a 95–98% white lot.

After RHW pupae were machine sorted at seven days' pupal age, pupae of each treatment were dye marked with distinct Dayglo^{R} fluorescent dyes at a rate of 3 g per litre of pupae. Each week, 1 L (c. 60 000) of the bisexual treatments (HI LAB and RHW unsorted) and 0.5 L of the unisexual treatments (RHW males-only and RHW high females) were dyed and bagged for irradiation (at eight days). Pupae were gamma irradiated at 15 krad (150 Gy) under anoxia with a ^{60}Co pool type irradiator. While still bagged, irradiated pupae were shipped to Kauai and carried to field test sites.

A technique has been developed which can distinguish irradiated from wild sperm in mated female spermathecae [4]. The head lengths of irradiated sperm are significantly shorter than wild fly sperm head lengths (c. 26 μm compared with 30 μm average length), with less than 5% overlap in distributions. Measuring about 10 sperm per female with dark field microscopy at 1000× allowed one to make a correct determination as to the source of the sperm (sterile or wild). Presumptive cases of multiple mating can be recognized by the presence of bimodally distributed sperm head lengths. With the development of this procedure, for the first time the natural field mating behaviour of wild females can be monitored, and the resulting competitiveness of sterilized males deduced. Of course, the mating behaviour of sterile females in the field can also be determined, including the frequency of sterile–sterile matings. Two measures of sterility can be calculated, one based on hatchability of eggs and one based on the sperm identification technique described above.

Egg hatch measures were obtained from field collected coffee samples each week per test plot. Estimates were made each week as to the percentages of ripe and

infested berries at each trap site. Eggs dissected were scored first as to species type, *C. capitata* or *Bactrocera dorsalis,* the oriental fruit fly, then as either hatched or unhatched. During summer and fall months in Hawaii, *B. dorsalis* becomes the dominant tephritid species infesting coffee.

With only minor fluctuations, releases continued smoothly throughout the test. Pupal size, adult emergence and laboratory longevity remained high. Samples of pupae from the field buckets indicated, with a few exceptions, good adult emergence of 85–95%. Contamination of the RHW rearing stock remained low; nonetheless it did increase slightly as expected, resulting in a drop in purity of the brown pupae to c. 98% males and of the white pupae to c. 95% females.

Estimates of sterility induced by release of sterile flies were obtained by scoring egg hatch from coffee berry samples and correcting these by subtracting control field sterility. Egg sterility rose gradually over several weeks after the onset of pupal releases in all of the treatment fields except in Mahaulepu (wild population very low) and in Kapaa (RHW high females). Egg sterility remained low (<10%) in Kapaa throughout the test, indicating very little effect on sterility by sterile females. Indeed, the low sterility observed probably came from c. 5% sterile males emerging from ground released brown pupae in that field. In the two bisexual release fields, egg sterility rose to c. 45%, then dropped. This pattern differed sharply from that seen in the RHW males-only field. There, sterility rose to c. 40–50%, as happened with the two bisexual treatments, but instead of dropping off later, sterility rose quickly to 100% and remained there for several weeks until the end of the test.

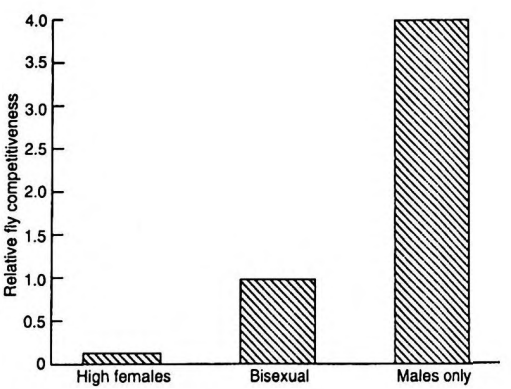

FIG. 1. Variation of sterile fly competitiveness with sex ratio.

In summary, the RHW males-only treatment caused significantly higher sterility in the wild population than bisexual populations. It was also trapped at significantly higher rates than bisexual populations in trimedlure (TML) traps. The high sterile female release did not reduce egg hatch or oviposition rates relative to control levels, and this resulted in a high trap-back of virgin flies coupled to a low trap-back of wild flies. In conclusion, males-only releases significantly increased the efficiency of the sterile insect technique (SIT) against wild medfly populations in coffee. The costs associated with each particular sexing system need to be accounted for in large scale SIT programmes.

Estimates of sterile fly competitiveness based on relative degrees of egg hatch suppression were calculated for each of the bisexual and unisexual treatments [2]. The competitiveness per fly for the high females treatment was estimated to be 0.04, where a value of 1.0 indicates that the sterile released flies are equally competitive with wild flies. The competitiveness of the HI LAB and RHW-Mixed treatments was estimated to be 0.14 and 0.21, respectively. The RHW-Males treatment, meanwhile, had competitiveness values of 0.62 and 1.33, depending on the formula used. These values indicate that the males-only treatment was 3–5 (average 4) times more efficient than the bisexual releases and c. 30 times more efficient than the c. 95% females treatment (Fig. 1).

In addition to relative field efficiency on the benefit side of the equation, one should consider the relative costs involved in rearing and releasing millions of sterile males. If the sexing operation is performed in the egg stage, one could save virtually 50% of the total costs by eliminating females that early, i.e. produce twice as many males for the same cost. The rearing efficiency in that case would be c. 2.0 (Table I). This maximum efficiency makes egg separation strains like the IAEA's current temperature sensitive lethal (*tsl*) strain very attractive, of course. Indeed, that strain is being field evaluated in Europe and Guatemala by several organizations. For the RHW pupal separation strain with which we worked, on the other hand, we have estimated a more modest rearing saving of c. 25%, leading to a relative efficiency of c. 1.25 (Table I). Combining both factors for rearing and field efficiencies, one can estimate the total increase in efficiency, considering only the benefits side of the cost–benefit equation. Clearly, our studies indicate that males-only releases, together

TABLE I. RELATIVE EFFICIENCY OF BISEXUAL AND MALES-ONLY STERILE FLY PROGRAMMES

Relative rearing saving/ million males sexed in:			Relative field efficiency (males-only/bisexual)		Total release efficiency
Pupal stage:	c. 1.25	×	c. 4	=	c. 5
Egg stage:	c. 2.0	×	c. 4	=	c. 8

with a rearing saving in the production of males, can result in very significant increases in efficiency — on the order of 8-fold for egg separation strains and 5-fold for pupal separation strains.

On the other side of the cost–benefit equation, one needs to consider the cost of creating a genetic sexing strain or system (if no new strain is involved), the cost of the sexing system in actually rearing all males and, finally, the cost of maintaining the sexing strain in a highly pure, stable condition. Depending on the strain (or system), the particular costs involved will be readily apparent. The ease of this exercise contrasts sharply with the difficulty in estimating the benefits side of the equation, as our lengthy field studies have shown. For the RHW pupal separation strain, for example, one has to consider the fixed, initial cost of one or more sorting machines, the labour costs to operate the machines, and the labour and materials costs to maintain the strain pure and the sorting machines operational. For egg separation sexing strains, the mass rearing and release costs will be much less, of course. However, the initial cost of creating such strains, e.g. the IAEA's *tsl*, can be high as rare genes are sought.

4. BEHAVIOURAL RESISTANCE TO THE SIT

Brimming with confidence after the exemplary performance of the RHW males-only releases in Kauai coffee, we initiated a programme to compare aerially dropped RHW males-only against the standard HI LAB strain already being air dropped in Kauai. Two plots each of 1 square mile (c. 2.6 km^2) were selected for the test. One of them, designated for the RHW males, had been kept as a control area free of flies until only several months prior to the onset of aerial releases in May 1992. The HI LAB test area was within the two-year-old aerial drop zone for standard HI LAB flies. Approximately 300 acres (c. 120 ha) of high yielding coffee were centred within each test area. Fly releases were made by a fixed wing aircraft twice weekly, yielding c. 1 million males dropped over each plot (i.e. c. 2 million HI LAB flies of both sexes). A series of standard male lure TML traps, and protein baited traps for both sexes, were emplaced in each area to monitor both sterile fly releases and wild fly populations. Fruit was routinely sampled weekly for dissection of eggs, as was done in the earlier ground release, small plot test. Sterility estimates were obtained from both egg dissections and sperm analysis, the latter from mated wild females caught in liquid protein traps.

Throughout the late spring and summer of 1992, the aerial releases proceeded smoothly, when good quality flies of both strains were dropped into their respective test areas. Sterility levels increased quickly in the males-only area, as expected, reaching 70% by the first week of September. Fly competitiveness was also good for the RHW strain since the sterile to wild ratio was relatively low — c. 20:1 during this

period of low wild fly population. However, in the HI LAB area, sterility remained at the relatively low level of 10–20% that it had been maintaining for about one year. Indeed, because the sterile to wild ratios were higher in this area (c. 50–100:1), the competitiveness per fly was even lower.

The appearance of a well controlled experiment, proceeding smoothly with clear-cut, consistent differences again showing a males-only advantage, was dramatically shattered by the advent of Hurricane Iniki on 11 September 1992. This storm, with winds in excess of 200 mph (320 km/h), devastated the island of Kauai, including the coffee growing area. It would be two months before any trap monitoring or coffee dissections could resume.

Unfortunately, the wild fly population recovered rapidly, no doubt owing to the protection offered pupae underground during the storm. Possibly as a result of some selective power of the storm, or simply through mere coincidence, the sterility levels in the males-only area never again reached 50%, and hovered around only 25% for most of the remainder of the aerial release period, which continued until May 1993.

During the whole period of aerial releases, we began investigating the mating behaviour of the RHW and HI LAB strains in outdoor mating cages. These tests were designed to measure the level of compatibility between laboratory reared flies and their wild counterparts from Kauai and other Hawaiian islands. Over the course of 1992 and into 1993, at the same time as problems developed in the field regarding low sterility, we began noticing problems with sterile males not mating with wild Kauai females in the outdoor cages. Interestingly, the reciprocal cross involving laboratory females and wild males remained at normal levels, indicating that the problem lay with a selective wild female. The corresponding declines in mating behaviour between sterile flies and wild flies from other non-SIT areas of Hawaii did not occur. These other sites included coffee growing areas on the islands of Maui and Hawaii, where, in the latter instance, even the same varieties of coffee, Red and Yellow Catuai, are grown.

In spite of these disappointing developments, yet another effort was made to compare RHW males-only with HI LAB releases in new 1 square mile plots within the previous HI LAB aerial release area. We hoped to increase greatly the number of flies actively participating in the field by ground releasing adults from roving vehicles thoroughly covering the entire test areas. This desired result was achieved as sterile to wild overflooding ratios reached 100:1 or higher for several weeks after releases began in June 1993. However, in both test areas the sterility again remained low (10–30%), though consistently somewhat higher for the males-only treatment. These results again strongly indicated a relatively poor mating performance by sterile released males with wild females. All lines of evidence — field sterility rates, wild population levels and cage mating studies — pointed to the dreaded monster, resistance to the SIT. It would appear as though a rapidly increasing level of resistance took hold about two years previously in Kauai in those areas which had received

continuous sterile fly pressure for several years. In other areas, such as the initial aerial release site for RHW, where no significant sterile fly releases had been made, sterility started out high, then dropped. Though such reported resistance is unprecedented in medflies, resistance to sterile insect releases was first reported for the melon fly, *Bactrocera cucurbitae*, during the eradication programme in Japan [5].

Faced with the reality of failure for even the vaunted males-only strain, we terminated fly releases in September 1993. Since that time we have focused our efforts on finding an effective way to counter the resistant population. We collected several wild populations from the resistant area and have been rearing and testing them in mating cages, in hopes of finding one which can successfully mate with resistant flies. Also, we have been trying to dissect the courtship behaviour of resistant and non-resistant flies through both visual observations in outdoor cages and analysis of video recordings of indoor and outdoor cage matings. This work is continuing at the present time. It is hoped that a solution will be found to the resistance problem in order to complete final testing on one or more males-only strains, and vindicate the initial highly encouraging results of males-only sterile medfly releases.

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