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RECENT FINDINGS ON MEDFLY SEXUAL BEHAVIOR: IMPLICATIONS FOR SIT

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Abstract

The preceding papers presented in this issue represent some of the activities of a group of researchers working on fruit fly mating behavior as it relates to the use of the Sterile Insect Technique (SIT). The group was co-ordinated and partially funded by the FAO/IAEA Joint Division of Nuclear Techniques in Food and Agriculture, Vienna Austria. A variety of approaches were used to examine lekking and courtship behavior of wild and mass-reared fruit flies including video analysis, morphometrics, physiological status, geographical variation and field cage evaluation. No major qualitative differences could be demonstrated between mass-reared males and wild males, although there were some specific changes related to mass rearing and irradiation. Many studies were carried out using field-caged host trees and the results of this work have led to the establishment of a standardized protocol that is now followed by all fruit fly programs using the SIT. Using these cage studies it was shown that there are no barriers to mating between medfly populations from many parts of the world. This information is of major relevance as it permits sterile flies to be shipped from one program to another. It also has some significance for the eventual commercialization of the SIT. The use of various compounds to improve the mating success of mass reared males could have a major impact on the efficiency of SIT programs. However, the initial experiments reported here will need to be expanded before this approach can be integrated into operational programs.

Key Words: medfly, *Ceratitis capitata*, sterile insect technique, genetic sexing strain, behavior, lek

Resumen

Los trabajos anteriores presentados en esta edición representan algunas de las actividades de un grupo de investigadores que trabajan en el comportamiento de apareamiento en mosca de la fruta y su relación con el uso de la técnica del insecto estéril (TIE). El grupo fue coordinado y parcialmente financiado por la Division Conjunta de Técnicas Nucleares en la Alimentación y la Agricultura de la FAO/IAEA, Viena Austria. Una variedad de métodos fueron utilizados para examinar el cortejo y el comportamiento de apareamiento de moscas de la fruta salvajes y criadas en forma masiva incluyendo análisis por video, morfometría, estatus fisiológico, variación geográfica y evaluación en jaulas de campo. No se pudieron demostrar diferencias cualitativas mayores entre machos criados masivamente y machos salvajes, aunque hubo algunos cambios específicos relacionados con la cria masiva y la irradiación. Muchos estudios fueron llevados a cabo utilizando árboles hospederos en jaulas de campo y los resultados de estos trabajos nos han dirigido al establecimiento de un protocolo estandarizado que actualmente es utilizado por todos los programas de moscas de la fruta que usan la TIE. Al utilizar estos estudios en jaula se demostró que no existen barreras a el apareamiento entre poblaciones de la mosca del Mediterráneo provenientes de muchas partes del mundo. Esta información es de gran relevancia ya que permite que moscas estériles puedan ser enviadas de un programa a otro. También tiene algún significado debido a la eventual comercialización de la TIE. El uso de varios compuestos para mejorar la eficiencia de apareamiento de los machos criados en masas podría tener un gran impacto en la eficiencia de los programas TIE. No obstante los experimentos iniciales reportados aquí necesitaran extenderse antes de que este método pueda ser integrado en programas operacionales.

A successful medfly Sterile Insect Technique (SIT) program requires that released sterile males locate a lekking site (Whittier et al. 1992), perform a courtship (Féron 1962), attract wild females (Whittier & Kaneshiro 1995), inseminate them and elicit the appropriate behavioral response from their mates (Jang et al. 1998). Consequently, mass reared sterilized medfly males must be able to deliver a behavioral repertoire that approximates as closely as possible that of their wild counterparts. Any departure from the "wild" behavior, or lack of field competitiveness could cause the failure of an SIT program. The female medfly is the final arbiter of male success or failure and this component cannot be influenced by any activities of the program. All strategies for maintaining a high level of mating between the sterile male and the wild female have to be directed towards the mass reared and sterilized male. An improved knowledge of what makes a successful, sexually competitive and attractive sterile male can contribute to increasing the efficiency of SIT field programs.

Sterile males are the key players in the SIT and competitiveness is usually discussed in terms of this sex and their ability to mate with field females (Hooper 1972, Tsubaki & Bunroogsook 1990, Shelly & Whittier 1996a). It is quantified as a function of the mating success of the sterile male compared to that of the field male (Orozco & Lopez 1993). Competitiveness of the sterile male in the field represents the cumulative effects of rearing, sterilization and release procedures of that particular cohort of insects as well as the "behavioral history" of the colony from which the cohort was reared. These two components of competitiveness can exert quite different effects on the insect. The history of the strain in terms of its behavioral adaptation to mass rearing conditions will tend to set the upper limit of field competitiveness (Cayol 2000) while the rearing, handling and sterilization of a particular cohort will determine the actual competitiveness of that cohort in the field. Both of these components are subject to a large degree of variability. Each strain has a different colonization history and is continually evolving and new rearing procedures, such as the Filter Rearing System (FRS) (Fisher & Caceres 2000), contribute to the behavioral makeup of a strain. Experimental approaches that would tease apart the historical and contemporary components and assign values to their overall contribution to field competitiveness would be very useful for SIT programs.

The preceding papers in this volume are an attempt to do this by 1) improving our understanding of the different components of fruit fly mating behavior; 2) evaluating how mass rearing and sterilization influence these components and 3) assessing how changes impact on the competitiveness of the flies in the field. Two questions can be posed in this last chapter, can the results of these different approaches be assimilated into a unified description of male mating success in medfly and can the results at one level (e.g. the laboratory) predict outcomes at a second level (e.g. the field)? An affirmative answer to the first question could enable mass rearing strategies to be developed which preserve male mating "success" and an affirmative answer to the second could enable QC protocols to be revised so as to improve their predictive value for the field effectiveness of sterile males.

A reading of the papers in this issue indicates that these questions cannot yet be answered fully although there is now a much better understanding of which components are crucial and perhaps, more importantly, which are not. In this paper the implications for the SIT of the methods and findings presented in the preceding papers are discussed.

What Makes a Medfly Male Successful?

Lekking Behavior

To be successful medfly males first need to join a lek (Prokopy & Hendrichs 1979). Field et al. (2002) discuss in detail lek formation in medfly and examine the different theoretical concepts that have been used to explain the origin of lek formation in different species. Although there is no doubt that medfly males do form a lek to which females are attracted, the functional basis of lek formation in this species has still to be fully elucidated. In medfly there are probably several interacting components which taken together can explain why medfly males form leks. Field cage and field observations of mass reared medflies have repeatedly shown that they do indeed participate in lek formation (Zapien et al. 1983, Cayol et al. 1999) and appear to do so in numbers which suggest that this behavioral component is not changed following mass rearing. Their preferences among foliage types on which to establish leks is also the same as in wild males (Katsoyannos et al. 1999). This is surprising as all the requirements for lekking behavior are removed during mass rearing and it suggests that there is little genetic variance for this particular character for selection to act on. Despite the extensive knowledge of medfly mating behavior in the field, key questions still remain to be answered. What proportion of males in the population take part in mating? Do males change their "position" in the lek as leks dissolve and re-form? If wild males change their position in different leks over time do mass reared flies do the same?

Participation in a lek is the first step that released males must successfully complete in order to have a chance to participate in mating. This can be partially studied in a field cage but confinement in a cage does not truly mimic lekking behavior in the open field. Some important spatial and temporal components of lek formation are absent in the field cage. Field cage tests are generally carried out during a single day using a single tree and they cannot monitor the behavior of the released males over time and space as they attempt to participate in new lek formations. Another restriction of field cage tests is that generally a constant number of flies are used whereas in the field, fly densities vary and leks of different size will be formed. Are mass-reared males capable of participating in leks of different size both in terms of the number of flies and the physical distribution of the lek within the leaf canopy? These observations are very difficult, if not impossible, to make in the open field and reliance will still have to be placed on results coming from field cages. In conclusion mass reared medfly males appear to participate fully in lek formation under field cage conditions, but it is not known if there are preferential positions within the lek and, if there are, whether mass reared medflies can identify them.

Lek behavior in other economically important *Ceratitis* spp was also analysed (Quilici et al. 2002). In general a similar picture emerged but there were species specific differences in the timing of lek formation as well as trend towards a more "simplified" courtship behavior.

Morphometric Traits

Does a female medfly see anything in a male that makes him attractive as a mate? This question was addressed by several authors (Hasson & Rossler, Hunt et al., Rodriguero et al. 2002) focusing on male morphometric traits. Hasson & Rossler (2002) addressed the question of fluctuating asymmetry (FA) of sexual characters that is known to play a role in sexual selection with symmetric males having a slight advantage in the mating arena. In medfly, there is a positive correlation between mating success and the symmetry of the superior frontal orbital setae (SFO) (Hunt et al. 1998). It is also known that stress during development can lead to an increase in asymmetry as measured by the size of bilaterally produced structures. The question can then be asked concerning the effects of mass rearing stress on the physical attractiveness of sterile medfly males to wild females. Hasson & Rossler (2002) have attempted to answer the fundamental question as to whether developmental homeostasis operates at the level of the individual or is regulated independently for each set of characters. By generating differently sized adults using different larval densities, measurements were made on both sexually dimorphic and shared bilateral characters. No effect of larval density could be demonstrated on the FA of any of these characters and even with thorax length, the best quality indicator, the association was weak. However, FA was highly correlated with the size of the character. The authors conclude that for medfly, character specific homeostasis plays a major role in FA but that whole body regulation is also implicated. The question of the relationship between the FA of sexually dimorphic characters and quality remains contentious. It is also likely that many other factors will over-ride any small effects that FA will have on the quality of flies that are released in an SIT program. Whatever its cause, asymmetry is present in the SFO bristles and as indicated above males symmetrical for this character are more successful in obtaining mates than are males showing asymmetry for this trait (but see Rodriguero et al. 2002). Mendez et al. (1999) showed that the removal of the two male bristles affects sexual competitiveness, even though it does not affect courtship behavior. Hunt et al (2002) examined in more detail the role of asymmetry in these bristles for male mating success. They artificially induced the ultimate asymmetry by surgically removing either one or two bristles and examining the effect this has on male mating success. The authors showed that the loss of one bristle did not have an effect on mating success as compared to a two bristle male. However a one-bristle male was more successful than a male with no bristles. These, in some way contradictory, results do not provide many more clues as to the real function of the SFO bristles in relation to male mating success.

The question of whether or not large males are sexually more competitive and attractive for females than small males has very often been raised (Partridge & Farguhar 1983), especially in the light of mass rearing (Burke & Webb 1983, Churchill-Stanland et al. 1986). Blay & Yuval (1999) showed that male size correlated positively with hatch rate in later stages of female reproductive cycle, however, in a recent study, Taylor et al. (2001) showed that there was no correlation between male size, copula duration and insemination success. Rodriguero et al. (2002) concluded that larger males are more sexually competitive than smaller ones. This was already demonstrated by several other authors (Burk & Webb 1983, Churchill-Stanland et al. 1986, Krainacker et al. 1989, Bloem et al. 1993, Orozco & Lopez 1993) who showed that larger male tephritids, in addition to being more sexually competitive, are stronger fliers and live longer than smaller flies. Pupal size is therefore an essential component of the fruit fly QC protocol (FAO/IAEA/USDA 2002).

Physiological Status

Liedo et al. (2002) examined the effect of the age of the fly on its propensity to mate in field cages, both laboratory and wild flies were evaluated. The authors showed that the optimal age for mating ranged between 7 to 13 days for wild flies against 3 to 5 days for the mass reared flies. This work was used to standardize the age of flies used in field cage tests to enable results between different laboratories and with different strains to be compared. They also showed that when sexes are held separately, insects are more prone to mate. This finding supports the use of all male strains (Hendrichs et al. 1995, Rendon et al. 2000) since sterile males will be virgin by the time of the release. Releasing 2 day-old sexually immature flies, as is still the case (Pereira et al. 1997, Cayol & Zarai 1999, Barbosa et al. 2000), wastes resources since most of the sterile insects will be eaten by predators before they can mate (Hendrichs et al. 1993). Male only releases can be improved by holding emerged flies longer (providing them food and water) and releasing 4 day old sexually mature virgin males.

Feeding status is another physiological character that can have direct implication for SIT. Shelly et al. (2002) showed that when fed with protein, medfly males attracted more females when calling in leks. Protein-fed males also achieved more matings than protein–deprived but sugar-fed males. This finding confirms the work by Blay & Yuval (1997), and would suggest that sterile males should be fed with a mixed diet of protein and sugar before being released into the field, rather than sugar-only as is the case in current SIT projects. However, even though Yuval et al. (2002) confirmed that protein-fed males are sexually more attractive and competitive that sugar-fed or starved males, Kaspi & Yuval (2000) found that, after 24 hours of starvation, 4 day-old protein-fed males suffered higher mortality than sugar-fed ones. The benefits, or not, of feeding mass reared sterile males with protein prior to their release will need further investigation.

Shelly (1994, 1995) reported that when fed with methyl-eugenol, a male attractant of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), irradiated males of this species were more sexually competitive. Shelly & Whittier (1996b) showed that medfly males exposed (not fed with) to trimedlure exhibited higher level of pheromone calling and were more successful in mating than not exposed males. This advantage, though of limited duration (24 h), could theoretically, be put into practice as a preliminary treatment before male release. Recently it was also determined that exposure to wounded orange peel substances conferred to males a mating advantage over unexposed males and this advantage lasted at least ten days following exposure (Papadopoulos et al. 2001). Other recent developments in the same field (Shelly, 2001) show that, medfly males fed on ginger oil are sexually more competitive than normal males. These findings, though under development, would represent a major step forward in increasing SIT effectiveness.

Can Wild Female Remating be Limited and/or Controlled?

The probability that a wild female will remate after mating with a sterile male is highly relevant to the effectiveness of SIT and a successful male should ensure that the female he mates with uses only his sperm to fertilize all her eggs. Males must be able to transfer sufficient sperm and accessory fluid during mating in order to change the behavior of the female from mate seeking to oviposition and so prevent remating. *Drosophila melanogaster* males have elegantly solved the problem of female remating by transferring a peptide in the accessory fluid that changes the behavior of the female in two ways. It makes her refractory to mating and initiates oviposition behavior (Fowler 1973, Clark et al. 1999). A similar behavioral switch does exist in medfly (Jang et al. 1998). Accessory gland fluid in medflies has been shown to contain biologically active compounds which cause a switch in wild female behavior from attraction to pheromone to attraction to fruit odors implying a shift from mating to oviposition (Jang 2002). Interestingly, laboratory females did not show this switch but irradiated laboratory males were equally good at causing this switch as wild males, when mated to wild females. It appears that laboratory colonization does not lead to any change in the production of these compounds even though its behavioral requirement in the female is probably completely suppressed under the laboratory conditions. This might explain why laboratory females fail to exhibit this behavioral switch. The fact that mass reared males elicit the correct response from wild females would suggest that this is not a major component of reduced effectiveness of sterile males in the field.

Multiple mating of wild medfly females seems to occur in field populations (Prokopy & Hendrichs 1979, Saul et al. 1988, Saul & McCombs 1993a, 1993b) and it is thought to enhance female fitness (Whittier & Shelly 1993). Two studies, one using linear measurements of sterile and fertile sperm (McInnis 1993) and the other using the numbers of sperm in the spermatheca (Yuval et al. 1996), provided the early evidence that wild medfly females engage in multiple mating. The lack of data on this point is not surprising, as it is a very difficult parameter to approach experimentally. However molecular analysis of progeny from field collected females has provided the first preliminary data on the level of remating in wild populations (Gasperi, pers. comm.). Measurement of rematings in field cages (Hendrichs et al. 1993) indicated that wild females, initially mated with laboratory males, tended to remate more frequently than the wild females initially mated with wild males. Other field cage studies (McInnis et al. 2002) used individually marked flies so that both male and female remating could be followed for a wild and a mass reared strain. The authors could show no statistically significant difference in the tendency of females to remate depending on which male they mated with first. The authors also concluded that wild males remated more frequently than wild females. In laboratory studies, Whittier & Kaneshiro (1995) demonstrated a similar non-randomness in the mating frequency of medfly males and females with ca. 27% of males not mating at all.

In the laboratory, female multiple mating in medfly is the rule as shown by the detailed studies on mating frequency (Vera et al. 2002). There is however the possibility that this is a laboratory artifact and of limited relevance to field populations. However, interesting differences were revealed in the study which, although compounded by the laboratory environment, will probably be of wider relevance. Females initially mated with laboratory males tended to remate more frequently than the females initially mated with wild males and there was a negative correlation between duration of mating and the chance of remating. Under laboratory conditions the wild flies performed very poorly and the experimental conditions of the test were in some way designed so that multiple mating was encouraged. Other factors were also identified which affect this parameter such as fly density, and male status. These studies confirmed that long term rearing in the laboratory significantly shortens male mating duration which indirectly increases the chances of female remating.

The whole subject of the relevance of female multiple mating to the effectiveness of the SIT through the part played by released sterile males will only be satisfactorily answered when more is known of this phenomenon in a wild medfly population using the molecular approaches indicated above.

IMPROVING THE SIT

Mass Rearing

Under "traditional" mass rearing conditions the requirements for appropriate mating "behavior" are removed as cost effective production processes demand that important compromises be made in the environmental arena presented to the fly for mating. The most important biotic change relating to mating is probably adult density in the production cages. Here, fly density, in terms of unit volume and area, is orders of magnitude higher than in the field. This single change initiates a cascade of responses in the fly which leads to a degeneration in most aspects of the normal mating behavior that is observed in the field (Cayol 2000). The critical question concerns the long term effects of this distorted mating "behavior" on the effectiveness of a male fly when it is released into the field. In other words, how hard-wired is the courtship behavior of medflies?

Major abiotic changes are also made, including constant light and temperature regimes and the provision of an artificial diet for adults and larvae, that is much richer in proteins than the available diets in nature. These changes also impact directly on mating behavior. All in all, it would not be surprising if continuous rearing under these totally artificial conditions for a species such as medfly, which has a complex and refined mating behavior, led to permanent and irreversible changes in mating behavior. Briceno & Eberhard (2002) confirmed their previous conclusion (Briceno & Eberhard 1998) that, due to the crowded conditions in mass rearing cages, a shorter courtship of males evolved in an "effort" to avoid interruption. Mass reared females co-evolve and accept, more than wild females, males that exhibit shortened courtship duration. Briceno et al. (2002) found no consistent differences between the courtship of wild and mass reared flies from various origins, but a tendency for interruptions in the buzzing phase of the courtship to occur more frequently in mass reared flies. In return, such an interruption is more likely to result in a rejection by wild females. These modifications in medfly courtship behavior are more likely with flies originating from a strain with a long history in mass rearing. Calcagno et al. (2002) reports an increased aggressiveness from mass reared males, thought to be due to the crowded conditions that prevail in mass rearing. These results suggest that changes in mass-rearing protocols may lead to improvements in the quality of males.

The Filter Rearing System (FRS) as described by Fisher & Caceres (2000) represents a major step forward in insect mass rearing philosophy and can perhaps be used to improve insect quality. At the heart of the FRS is a small colony that is maintained under relaxed rearing conditions and optimized environmental conditions and from which eggs are harvested on a regular basis. The eggs are the starting point for colony development and following 2 or 3 generations of mass rearing sufficient flies are produced for irradiation and release. In this way no behavioral changes induced by mass rearing are accumulated over time as all the insects that are mass reared are released and are not returned to the FRS. The size of the FRS will depend on the number of flies required for release and the reproductive potential of the strain. Care must be taken to maintain those adaptations, such as oviposition behavior or appropriate mating behavior, in order for large production lines to function efficiently and without changes in field fitness of the flies (Fisher & Caceres 2000). The FRS provides a unique opportunity to evaluate the use of "field-like" conditions in the small colony, introducing a host tree in a large room (no cage), natural light, selection for predator avoidance etc. The FRS is already in place in rearing facilities Guatemala (Caceres et al. 2000), Argentina, Chile, Madeira, South Africa and Australia.

Reducing the negative effects of irradiation

The negative effects of irradiation on sexual competitiveness of fruit flies are well documented

(Holbrook & Fujimoto 1970, Hooper 1972, Rossler 1975, Wong et al. 1983, Kanmiya et al. 1987, Moreno et al. 1991, 2002). Lux et al. (2002a) described in detail these negative effects on courtship behavior of mass reared medflies. The authors recommend re-evaluating the sterilization strategy and irradiation doses for males used in SIT programs. One way to approach this is to irradiate flies in nitrogen. The protective effects of nitrogen on the induction of somatic damage are well known and have been shown to increase the competitiveness of the sterile male (Fisher 1997). In addition, increasing the irradiation dose beyond the 99% egg sterilizing dose greatly reduces the mating competitiveness of males (Fisher 1997). However program managers still demand doses of radiation giving 100% egg sterility resulting in reduced quality of the males for a marginal gain in sterility.

One Strain for all Programs

The current geographical distribution of medfly probably represents its dispersal, from eastern Africa, over the last 150-200 years as a result of man's agricultural and commercial activities. Such a short evolutionary time would be unlikely to lead to significant pre or post-mating isolation barriers. Genetic analysis has also shown limited divergence of medfly populations at the protein and DNA levels (Gasparich et al. 1997). Against this background, field cage studies have confirmed that there are no significant pre-mating isolation barriers when populations from different parts of the world are confined in field cages. The only current exception to this is the population from Madeira, Portugal and of course not all populations over the whole distribution of the fly have been evaluated. Cayol et al. (2002) and Lux et al. (2002 b) summarize these studies and reach the same conclusions. The lack of any mating barrier between the tested populations has major implications for the SIT in that a particular mass reared strain can be used at any location, as well as to deal with outbreaks where the medfly population to be eradicated is of unknown origin. This has already enabled the large medfly rearing facility in El Pino, Guatemala to provide sterile male medflies, from a genetic sexing strain, to California, Florida, South Africa and Israel. Despite these developments, many program managers still request that a strain with the genetic background of their particular geographical area be developed for mass rearing and release. Their appears to be very little, if any, scientific justification for this type of request. The lack of pre-mating isolation barriers in medfly has encouraged the development and use of generic genetic sexing strains such that a particular strain can be used in any country. Any future commercialization of the SIT will depend on this principle being widely accepted.

Field Cage Test: a Regularly Requested QC Test

Assessing the behavioral quality of mass reared fruit flies under small cage conditions in the laboratory is of little, if any, relevance to SIT programs (Chambers et al. 1983). Field cages were used for the first time by Boller et al. (1977) to assess the mating activity of the European cherry fruit fly, *Rhagoletis cerasi*. Since then, the protocol for field cage tests has evolved considerably and, through the work done by the authors of this volume, has resulted in the inclusion of a field cage test in the FAO/IAEA/USDA (2002) QC manual. Field cage tests have also been proved to be useful to compare the mating behavior of wild and sterile flies of other fruit flies of economic importance such as *Anastrepha* spp. (Aluja et al. 1993, Moreno et al. 1981), *Bactrocera* spp. (Jackson & Long 1997, Samoeri et al. 1980) and other Ceratitis spp. (Quilici et al. 2002).

The research group agreed on 3 main conclusions concerning the use of field cage tests. 1) Field cage tests with host trees are the best compromise between laboratory conditions and costly and impractical field observations to assess fruit fly mating behavior under semi natural conditions. 2) Improved standardization is required e.g. nutritional status, sex ratio and density of flies in relation to available canopy surface in the field cage. 3) Wild flies remaining on the tree canopy reflect adequate environmental conditions within a field cage. Based on these observations the FAO/IAEA/USDA (2002) QC manual has already been modified.

This series of studies did not reveal all the intricacies of medfly courtship behavior as it relates to the SIT, nevertheless, the following conclusions were reached:

- There is a need to devise improved rearing systems (the benefits of the Filter Rearing System is now recognized worldwide) which can maintain critical aspects related to male courtship,
- It was demonstrated that medfly has not yet evolved pre-mating isolation barriers between different geographical populations enabling transboundary and intercontinental shipment of sterile flies to be carried out,
- The lack of pre-mating isolation barriers among medfly populations enables the development and use of generic genetic sexing strains in medfly SIT programs,
- Field cage tests are now included in the routine quality control test for SIT programs.

Conclusions

An SIT program can only be successful if wild females are mated with sterile males in a propor-

TABLE 1. THE MAJOR CONCLUSIONS OF THE CRP REGARDING THE DIFFERENT ASPECTS OF MEDFLY MATING BEHAVIOR.

Type of study	Conclusions of the CRP
Courtship behavior	• Wild and mass reared flies from different origins exhibit similar courtship behavior patterns. • A high variability is noticed in the quantitative aspect of the behavior of mass reared and wild flies. • Mechanisms determining female acceptance of a mate are complex and not yet fully understood. • Mass rearing conditions do affect the quantitative aspects of the courtship behavior. • Irradiation has a negative effect on the behavior of mass-reared males.
Lekking behavior	• Compared to other stages in the reproductive sequence of the medfly, leks are poorly understood. • Lek behavior is common to all medfly populations studied. • Mass reared, sterile males appear to have maintained the ability to find, join and participate in leks. • Leks appear at 2 different scales: the large scale, i.e. at the individual tree level and the small scale, i.e. at the microhabitat level within a tree canopy. • Predation and female preference appear to be of primary importance in driving and regulating lek behavior, • Laboratory cages are unsuitable for studying lek behavior.
Morphometric studies	• Multiple measures of body size are more reliable than single measures to determine levels of mating compatibility and competitiveness. • There are contradictory data on the importance of male body size on mating success. • The absence or abnormalities of SFO bristles can have a negative effect on male mating success. • Mass-reared males occasionally lack SFO bristles.
Nutritional aspects in relation to sexual behavior	• Studies on fruit flies indicate that ingestion of specific precursors affects pheromone production and male reproductive success. • Post teneral nutrition enhances reproductive success of male medflies by affecting: a) the ability to emit pheromone in leks, b) the copulatory success, c) the renewal of female receptivity.
Remating studies	• Remating in wild medfly females is common in nature. • Sterile males are less able than wild males to suppress remating in wild females. This effect is increased in males that have been colonized for a longer time. • Mass reared males have reduced copulation times when compared with wild males. These reduced copulation times are associated with increased female tendency to remate. • There is a strong evidence that male accessory gland fluids influence female receptivity and olfactory behavior. However, the mechanisms and variables involved are not well understood.
Compatibility and sexual isolation	• Wild strains from different geographic origins show a high degree of compatibility. • Mass reared strains originating from any wild population can potentially be used worldwide. • There is evidence that some mass reared strains of medfly exhibit a degree of incompatibility when tested with different wild populations. • Regular field cage monitoring of mass reared strains is required to assess the compatibility with the local target population. • In case of incompatibility, the role of visual, sexual pheromone and acoustic signalling is unknown.
Mating test on field caged host trees	• Field cage tests with host trees are the best compromise between laboratory conditions and costly and impractical field observations to assess medfly mating behavior under semi-natural conditions. • Results of field cage studies are still not fully comparable due to insufficient standardization of test protocols. • The nutritional status, sex ratio and density of flies in relation to available canopy surface in the field cage influences test results.

TABLE 1 (CONTINUED). THE MAJOR CONCLUSIONS OF THE CRP REGARDING THE DIFFERENT ASPECTS OF MEDFLY MATING BEHAVIOR.

Type of study	Conclusions of the CRP
Mating test on field caged host trees	• Adequate environmental conditions within a field cage are reflected by wild flies remaining on the tree canopy.
Field evaluation of sexual competitiveness	• Field cages are the closest approximation to open field evaluation. However, results obtained under these conditions may not always fully represent field performance. • Given the variability in control programme environment, field evaluation becomes a necessity for major field programmes. • Currently field evaluations are based primarily on trapping, fruit infestation, and dispersal and survival studies. • Field evaluations are used to determine effectiveness of mass-reared strains in inducing sterility in target populations.
Comparative approaches to sexual behavior in Tephritidae	• Courtship behavior and morphology studies in related Tephritid species have provided valuable information to understand medfly courtship behavior. • The phylogenetic relationships of members of the Ceratitini tribe is poorly understood at the present time.

tion that will cause an irreversible decline in the size of the wild population. It is therefore imperative that protocols are in place to monitor any change in the ability of the released male to successfully mate with wild females. The major outcome of this research network has been the provision of a standardized field cage test to be used by all medfly SIT programs. This test serves two purposes; 1) it enables a program to monitor male quality over time and also to assess the effects of any change in the production process and 2) it enables comparisons to be made between the quality of males being mass-reared in different programs. The inclusion of the test in the FAO/IAEA/USDA (2002) QC manual is a testament to its relevance for SIT programs.

The detailed video analysis of courtship behavior of mass reared and wild type flies revealed relatively few major qualitative differences. Most of the component behaviors of the wild males could be identified in the mass reared males. However, it played a major role in developing a deeper understanding of courtship and female choice decisions and certain new behavioral components were identified. In addition quantitative differences were identified between different populations.

The nutritional and physiological state of released sterile males is an area of growing interest in relation to their effectiveness in the field. Exciting work on increasing the mating success of fruit flies by feeding or exposure to compounds related to pheromones may have a major impact on the SIT efficiency. The use of these compounds could even compensate for the negative effects associated with mass-rearing and radiation. It is also possible that the nutritional status of flies could be improved by a better understanding of the role that gut microflora play in the nutrition of fruit flies.

Despite the significant expansion in our knowledge of fruit fly mating behavior as documented in this issue (Table 1), many questions remain unanswered. Continuing improvements in the application of the SIT will surely come from a better understanding of fruit fly mating behavior.

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