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INTERNATIONAL ORGANIZATION FOR BIOLOGICAL CONTROL OF NOXIOUS ANIMALS AND PLANTS
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QUALITY CONTROL

AN IDEA BOOK FOR FRUIT FLY WORKERS

Edited by
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INTRODUCTION

D.L. Chambers and E.F. Boller

It has been a major concern of all entomologists involved in rearing and utilizing fruit flies or other insects that the final products of rearing operations under laboratory conditions need to be adequate for their intended purposes. We felt strongly that the time has come to compile the many bits and pieces of information about quality control concepts and techniques that exist in fruit fly laboratories all over the world and to make them available to the community of fruit fly workers in the form of an "Idea Book".

Indeed, our invitation to fruit fly specialists working on basic and applied aspects of economically important fruit fly species to provide short contributions reflecting their expertise and ideas to the mutual benefit fell on fertile grounds. The outstanding cooperation of the many contributors as well as the full support of the IOBC Council exceeded all our expectations and allowed the preparation and publication of this document within 12 months. The editors gratefully acknowledge this world-wide support and encouragement.

The general concern about the quality of laboratory reared fruit flies becomes a vital issue in those cases where insects are produced at great expense in special facilities to achieve an objective of considerable socio-economic urgency and importance. The production of fruit flies in massive numbers for release in pest control strategies using induced or natural sterility is now considered to be a part of the armory of available suppression and eradication techniques. The sterile insect technique (SIT) in its application against fruit flies is, in fact, considered by many to be a model autocidal technique.

Nevertheless, the SIT for fruit flies is still principally available only to public agencies, is still conducted largely either as research or under emergency conditions, and still cannot be applied in "doses" known to be precisely graded to the need. That is, the SIT is primarily used under an overriding philosophy of "hit them with all we have and hope it's enough".

This "Idea Book" is hoped to have application for all who rear fruit flies, but we hope primarily for its use in developing better understanding of the function of fruit flies produced in "factories" for these large public programs, and for the commercial enterprises the public campaigns may engender. Reliable use of the SIT by public agencies and its adoption by commerce will both be dependent on the passage of the technique to its next level of application. That passage will require, in part, much more highly developed knowledge and technology concerning the production of large numbers of insects and of the capability of those insects to perform their function in a reliable, predictable, and quantifiable manner.

Lest the sensitive reader become defensive, we hasten to label this a self-critical exercise. The editors and most of the contributors have been part of the development of the SIT for fruit flies. We believe we all work toward its continued development. Also, we are well aware that the SIT is frequently employed under socio-political-economic conditions that preclude deliberate step-wise consideration of every ramification of this complex procedure. We do believe, though, that development of technologies and concepts, and particularly awareness of the need for quality control should be that aspect of the SIT that is next intensively addressed. Furthermore, we, the loosely but intimately grouped community of fruit fly workers, are in the best position to address this challenge.

Perhaps more thoroughly than those studying any other group of insects, we have developed the means to produce startling numbers of flies economically and reliably. We have examined the techniques for inducing sterility and perfected the most appropriate. We have devised cost-effective ways to handle, package, and deliver flies to any point, on schedule, with little error. And, we have conducted large successful campaigns of control and eradication. However, we cannot say with reliability that any fly we produce will have an impact of known quantity upon the target population against which it must act. Until we can, the SIT retains a large hit-or-miss, release-and-hope element. Gaining this ability is the objective of quality assessment; applying this ability routinely is the objective of quality control.

Quality control in fruit fly production finds two primary obligations: (1) The routine production of adequate numbers of insects, on schedule, with economy and efficiency; (2) the ensurance that the product has the behavioral and physiological requisites for carrying out its mission. The remarkable abilities of our rearing experts to discharge the first obligation explain our emphasis in this book on techniques for addressing the second.

Concern for procedures and plans for ensuring product quality has been a part of fruit fly SIT research since its inception. The breadth of the effort is apparent from the variety of techniques reported here and their many sources. Their incorporation into a unified doctrine for application in SIT programs seems to be the next step.

The evolution of such theories has progressed much farther in the industrial manufacturing sector for reasons of history, economy, and complexity. The proposals herein for incorporation of industry's techniques into our biological systems are just that, proposals. They, along with others, need to be dissected, tested, and recombined in schemes suitable for our programs. We hope that this book will help stimulate such efforts. It was developed by identifying areas of importance and petitioning potential contributors to address proposed topics. Therefore, each paper represents the author's individual perception of our objectives. We have emphasized currency rather than editorial perfection. Perhaps the reader will excuse redundancies and errors on the grounds that less than 12 months elapsed between the call for papers and publication.

The book is organized into sections covering those units of performance we consider most representative of and critical to the success of mass produced flies in a SIT program. The following chapter, CONCEPTS AND APPROACHES, identifies the major components of quality and the use of their classification in approaching quality assessment. Thus, the section MEASURING OVERALL PERFORMANCE includes procedures that attempt to assess overall quality as defined therein (Fig. 1). Historically, these techniques are the oldest; the ratio test has been the most frequently used assay of fly quality. It has been proposed to measure overall quality because it assesses effects that must be the sum of all of the individual traits that contribute, eventually, to imposed infertility. The weakness of the test as usually applied lies in, (1) the probability that not all individual traits are actually expressed at least in laboratory cage tests, and (2) the inability of the test to identify inadequently performed (or assessed) individual traits.

This weakness in overall quality tests has led to the development of specific tests for individual traits. These are discussed under headings that group them according to the major quality components to which they most contribute. Some of these assignments are more or less arbitrary, since most specific behavioral and physiological traits may be expressed in several ways. Following overall performance are the individual performance traits related to Motility, Orientation to Habitat, Sexual Activity, and Physiology.

Ensuring that production line continuity is maintained is an essential role of the production manager; we have included a section MONITORING PRODUCTION that identifies how continuity is maintained and how adaptation to colony conditions is assessed.

An attempt to standardize key terms was made as follows:

Propensity	The tendency for an individual insect to carry out an act, or for an individual event to occur. Used in favor of "drive", "urge", "willingness", etc., which terms carry implications of motivation.
Frequency	The number of events performed by an individual insect in a given period of time.

Acknowledgments

The editors are in particular debt to their secretaries, Mrs. Betty Brooke and Mrs. M. Flury, for their dedication and effort toward manuscript preparation, and to Mr. U. Remund for assistance in preparing the figures.

(propensity = disposition, incline, etc.)

CONCEPTS AND APPROACHES

E.F. Boller and D.L. Chambers

1. What is Quality Control?

Quality control is a relatively new aspect of mass production of insects despite the fact that concepts and techniques developed and applied by industrial manufacturers have been available for a considerable time. Indeed, the basic philosophy of quality control does not need to be invented and developed from point zero in our fruit fly rearing programs. It has already been defined and described in many comprehensive books written for industry. The basic principles do exist and require only adequate adaptation to biological processes. Persons responsible for the design and implementation of quality control in insectaries will find a wealth of useful information and stimulation in several textbooks, such as those of D.A. Simmons (1970) and A.G. Robertson (1971). Most of the concepts and approaches presented in this chapter have been taken from these two books.

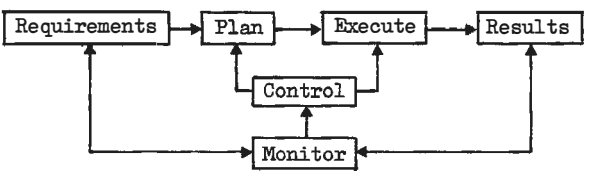
What is quality in the context of quality control? The European Organization for Quality Control (EOQC) has defined quality as "..... the degree to which a product meets the requirements of the customer". This market-oriented definition can be applied to mass reared insects when we substitute "objective or expected function" for the term "customer". The role of quality control is to provide and coordinate a production system that ensures that the operation will produce adequate numbers of an optimum quality at minimum product costs.

Two axioms reflect the direction the quality program should take: (1) Quality must be designed and built into the product, and (2) quality cannot be achieved by inspection (mere removal) of defective products).

Significant advances in industrial quality control were brought about during World War II when statistical quality control was established with its Shewhart control charts and other statistical tools. The present concept of total quality control was first introduced by Feigenbaum in 1961. The main difference between the total quality control concept and that of the early statistical era is that statistics is now a tool of the system and not the system itself (Simons 1970). Total quality control utilizes a systems approach and emphasizes planning and measuring methods to ensure product quality rather than increased inspection (sorting good from bad). Quality control deals with the whole system of production and all methods that are used to establish and achieve standards. Its aim is to identify the causes of deficiencies and to eliminate them by appropriate corrective action.

It is with the aid of cybernetics, the science of control, that a better understanding of the nature of the processes involved can be achieved. The following simple diagram (King 1975) shows the nature of the feedback mechanism:

Fig. 1



2. The Basic Steps in Quality Planning and Implementation

There are several chronological steps that can be followed in any situation where quality control programs have to be established.

a. Define the objectives. Define for what purpose the insects are reared and identify the requirements.
b. Establish standards. The required attributes of the insects produced have to be specified (specification).
c. Design and test the production methods that satisfy the specifications.
d. Implement quality control to ensure, within the confidence limits required, that the end product conforms to the specifications (via monitoring and corrective action).

2.1 Objectives

Fruit flies are mass produced for purposes that range from the culture of parasites upon a biomass of fly tissue to the induction of infertility in a target population by genetically altered strains. The characteristics required of the colonized fly are defined by the objectives that it must achieve. The strictest quality specifications can generally be assumed to be required in the latter case, where intraspecific action resulting in infertility is the important criterion for the definition of standards. For further discussion of this aspect we refer to the literature (Boller 1973, Boller and Chambers 1977, Chambers 1975, 1977, Huettel 1976, Mackauer 1972, 1976).

2.2 Standards

Quality can be quantified only by its measurement against standards, which are the basis for developing and applying quality control procedures. Too often standards, if present, are vague and poorly defined, whereas they should be precise and descriptive of both the average and the range of acceptable performance levels. Precise definition requires precise information however, and often our knowledge of fruit fly behavior, ecology, and genetics is inadequate.

The standard of reference generally felt to be most suitable in programs of sterile release is the target population against which the sterile fly must act. However, an internal standard (Chambers 1975, 1977, Huettel 1976) is an acceptable reference for the detection of variations in performance of colonies already deemed competent by more stringent standards. Thus, untreated samples from the colony may serve as internal standards for judging the effect of sterilization, diet, marking, etc.

Quantification of performance standards becomes more complex as the objectives of the program become more demanding. Furthermore, it becomes increasingly evident that overall quality must be dissected into numerous components that are amenable to numerical assessment. The development of a hierarchy of quality components may aid in developing measurable units and a model of such a hierarchy is shown in Fig. 2 (adapted from Boller and Chambers 1977).

The overall quality (Huettel 1976) of a laboratory population is measured in terms of how well it functions in its intended role, e.g., how effectively it interacts with and impacts upon the target population. There is temptation to proceed with a release program and let success or failure assess the quality of the production fly, but the hazard is great and unnecessary. Additionally, the costs inherent in such programs dictate that they be conducted efficiently, and efficiency can be assessed only through quality assessment. Thus, procedures that evaluate overall performance (the top of the hierarchy shown in Fig. 2) need to be developed and applied,

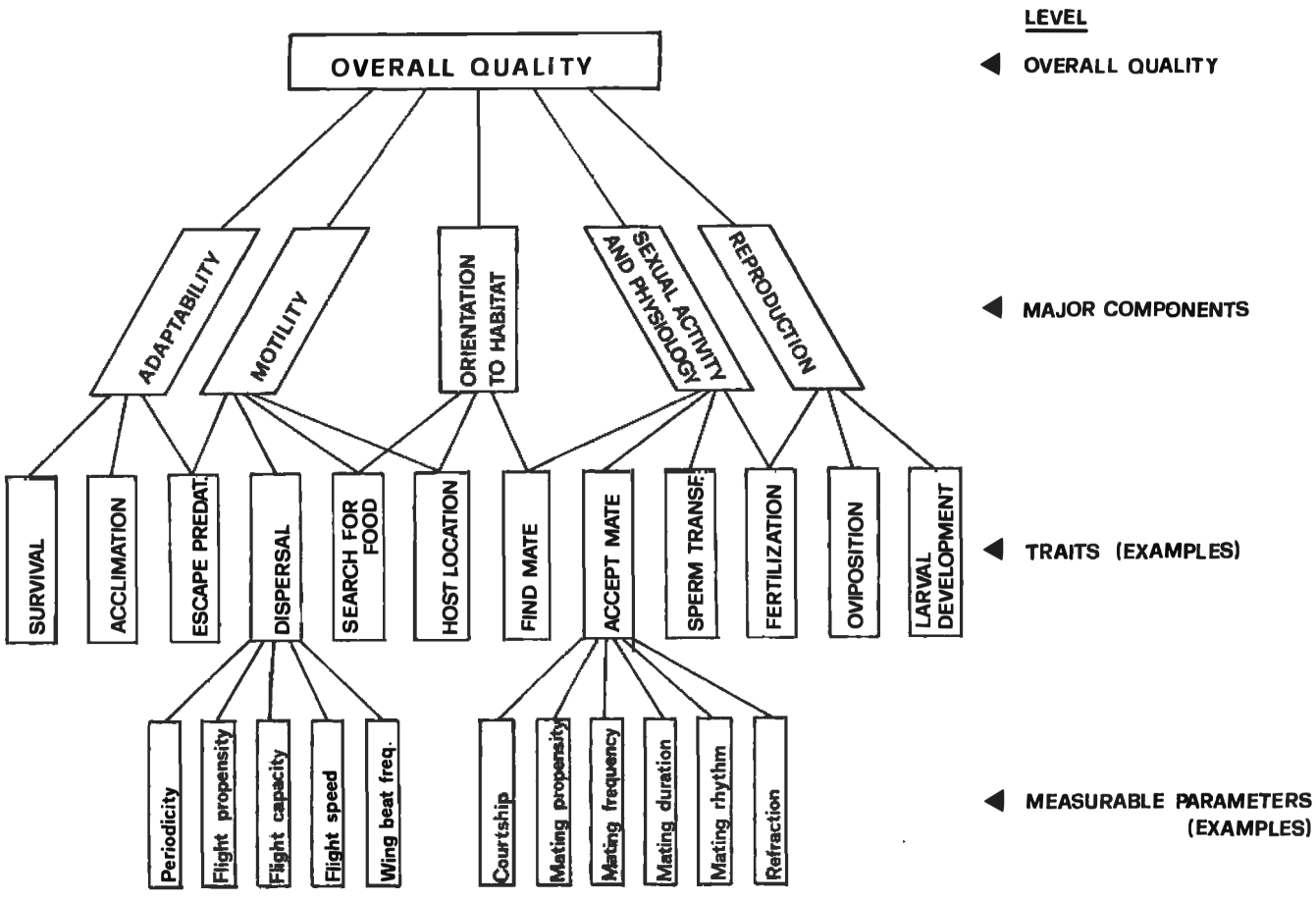


Fig. 2 Schematic presentation of a possible hierarchy of quality components with examples of second and third order interrelationships.

but they can be interpreted only through insights provided by analysis of more discrete quality components.

In Fig. 2 overall quality is divided into five major components covering adaptability, motility, orientation to habitat, sexual activities (courtship and mating), and reproduction. Such subdivision allows sorting of those activities that are most critical to success or subject to alteration. A challenge at this step is determination of the relative importance of each selected component. An attempt to weight the components in relation to the objective of production is shown in Fig. 3. Such a

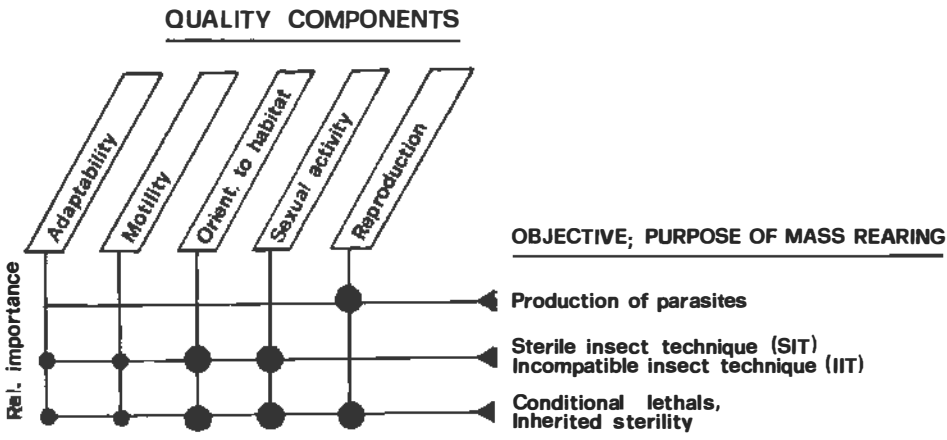


Fig. 3 Relative importance of major quality components according to the objectives of a rearing program.

subjective generalization has limited value but does aid in identifying aspects deserving of more intensive effort. Thus, sexual activities deserve special attention in programs of genetic control.

Determination of the level of effort needed in assessing components determined to be of less than top priority may be more difficult than for those at the top. Thus, while normal dispersive behavior can be readily seen to be of value (Coluzzi 1971, Boller 1972, Bush et al. 1976), it may be that a realistic assessment of adequacy and/or logistical considerations will allow a standard lower than anticipated (Chambers 1977). Finally, certain quality components are irrelevant in establishing standards (e.g., colonizing properties in the field in releases of sterile insects).

Determination of the major components allows one to proceed with somewhat more confidence in the selection of individual quality traits containing measurable parameters (Fig. 2). This level of the hierarchy shows, necessarily, a great variety of entries. Again, the listing should initially be as complete as possible and then reduced to those shown to have critical impact on overall performance.

2.3 Specifications: The Problem of Measuring and Evaluation

".... when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to the stage

of science, whatever the matter may be" (Lord Kelvin 1883). And, ". . . measurement is the comparison of an unknown with a standard" (Robertson 1971). And, ". . . quality in statistical quality control refers to some measurable property of the product that can somehow be translated into numbers" (Simmons 1970).

Indeed, the establishment of specifications will call for an even further division of the traits into individual parameters amenable to direct measurement. One example is the subdivision of the trait embracing the activities involved in the location of the mating site, the sexual partner, or the host. Because both physical and chemical stimuli are involved in directing the insect to the proper site (such as color, odor, shape, or sound) different techniques are required to measure and interpret visual, olfactory and acoustical processes. This is the final level where a variety of techniques is to be developed for measuring and monitoring quality, where a wealth of data will be produced, and where it must be determined what techniques will produce information relevant for the events in the field.

The relatively complex structure of quality we have presented brings us into conflict with a need for quality control procedures that should be relatively simple in order to be applied widely and routinely. This breakdown of quality into innumerable parameters might indeed lead to the wrong conclusion that quality control is so complex and sophisticated that it becomes the privilege (or pleasure) of a few specialists. This is not the case. Thanks to the rapidly accumulating experience, improved and simplified methods and devices, and not the least to the services provided by specialized facilities, entomologists should soon be able to analyze their problems and select or develop insects of the quality needed. The many techniques described in the larger part of this book reflect the increasing volume of ideas and options.

2.4 Adopting Techniques From Industry to Insect Production

Several interesting concepts and techniques have been developed for industrial quality control that merit the attention of entomologists responsible for the design and implementation of quality control in insect production facilities. Again, the key elements are described in detail by Simmons and Robertson, whose books might be studied with great profit. The following outline summarizes some of the salient features that may be adopted for our purposes.

In every process random variation is present. This variation is inherent but there is also induced variation that is directly attributable to specific causes. We often refer to these two types of variation as genetic and environmental variation; both act together and produce what we call the phenotype, or phenotypic expression of a certain quality trait. In essence, statistical quality control investigates processes and locates and separates these two types of variation in order that meaningful steps can be taken to control "quality". When only inherent variation is at work in a process, we consider the process to be under control. The ranges of inherent (genetic) variation of a trait that follows a normal distribution are called the capability limits of the system, which cover six standard deviations (Robertson 1971). The 3σ (sigma; standard deviation) is a key element for the evaluation of the dynamics of a production process and also the basis for establishing the specification limits (Fig. 4). Capability limits and specification limits are also called, according to their functions, the control (warning) and action limits, respectively.

When we study wild insect strains using the techniques described in this book we are measuring phenotypic expressions of a given quality trait under a defined set of circumstances. The frequency distribution of the measured values is the central part of our investigation as it tells us essential characteristics of the trait. In industry this basic study is called the process capability study. Process capability is defined (as shown in Fig. 4) as the 6σ range of a process under specified conditions. The calculation should be based on data from at least 50 samples. The data

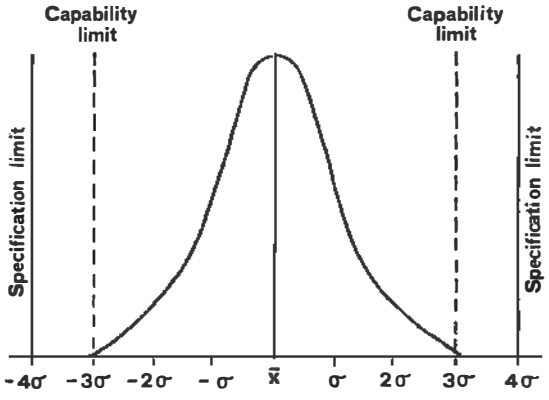


Fig. 4 Specification and capability limits of a quality trait.

should be plotted to ensure the presence of a normal distribution and, where doubts exist, the data should be tested with statistical methods. For most applications the visual (or "eyeball") test of the plotted frequency distribution should tell the story. The results obtained in these capability studies are the basis for the design of quality control tests and evaluation techniques.

Once the capability limits have been obtained we can proceed to the next important step, which leads to the establishment of the terms of reference in our quality control program, the specifications or tolerances of the trait with its upper and lower boundaries clearly defined. Specifications should always be realistic and, thus, as wide as possible consistent with satisfactory function of the insects produced. The larger the quantities being produced, the greater the need for wider tolerances and the bigger their impact on costs. When establishing specifications the first step is to separate the few vital ones from the many that rank lower on the priority list. It is a common practice in manufacture to set the specification limits for a range of 8 σ . Whether this practice can be applied to insects remains to be tested. Setting the specification limits tighter than necessary is an expensive exercise. Specifications must be feasible from a production and measurement point of view.

The Shewhart control charts were among the first statistical tools to be introduced in the era of statistical quality control. Their purpose is to plot a parameter with predetermined limits on a time scale and to present this information in an easy to interpret graphical form. By plotting sample results as averages ($\bar{X}$-chart) or ranges (R-chart) on a time scale, we can ascertain whether the variation from sample to sample is due to chance (random) variation or to assignable causes. The criteria used to make these decisions are the control limit lines (Fig. 5).

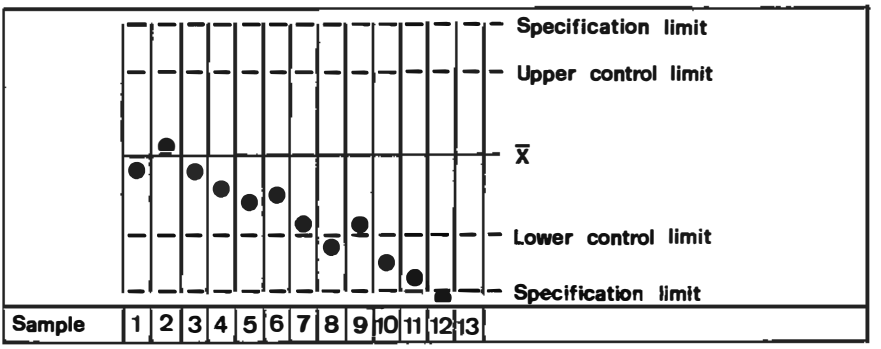


Fig. 5 Example of a quality control chart for averages ($\bar{X}$-chart)

Control charts are a type of hypothesis testing. The hypothesis being tested is: Is the process "in control"? In these tests two errors can be made:

1. If we reject the hypothesis that we are in control when in fact we are in control, we make an error (type I). The consequence is that we will look for nonexistent troubles, which will cause unnecessary efforts and costs.
2. If we conclude that the process is in control when in fact it is not, we make another error (type II). The consequence is that we do not look for trouble when it is present and thus continue to produce products that do not meet standards.

$\bar{X}$- and R-charts therefore provide information on three matters, all of which need to be known as a basis for appropriate corrective actions:

-Basic variability of the quality characteristics
-Consistency of performance
-Average level of performance of the quality traits

For further details on these techniques (sampling, rejection criteria, statistical background, etc.) we refer to the pertinent literature (e.g., Grant and Leavenworth 1972, King 1975, Ott 1975, Robertson 1971, Simmons 1970).

3. Organizational Aspects of Quality Control

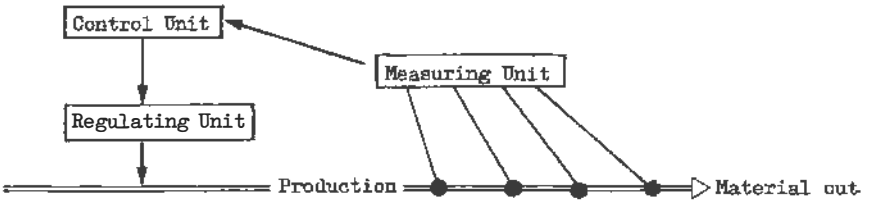
Most industrial enterprises have quality control groups or units. These do not exist in comparable form in most insect rearing facilities. However, certain aspects of quality control organization are of general validity and could be adopted accordingly.

All units or individuals responsible for quality control should have well defined responsibilities and authority and should have the organizational freedom to identify and evaluate quality problems and to initiate, recommend, and provide solutions. These points will deserve greater attention wherever quality control and production are not in the hands of the same persons (e.g., in large rearing facilities).

Major problems are likely to arise when the operation succumbs to the pressures of shipping schedules. If an adequate and acceptable procedure is not established and agreed upon by quality control personnel, project management, and personnel of the production line, the quality control program can degenerate into periodic end-of-the-month activities involving constant reworking or continual changing of tolerances and lowering of standards. When quality has been adequately defined and built into the product by careful preparation then the production flow should be smooth with substandard insects at a minimum and the need for concessions and modifications at a low level.

The postulated form of the relationship between quality control and feedback processes used in total quality control (cf., Robertson 1971) is shown in the following flow diagram and should be the aim of any organization that wants to achieve the most cost-effective method of producing items that meet a specified quality.

Fig. 6



4. The Economics of Quality

The economic aspect of quality control has never been analyzed in conjunction with insect production and there are no figures available that could support that analysis. The economics of quality might, however, become a major point of interest with increasing capacities of the rearing plants and need for their efficient operation. Although figures given by industry must be interpreted with great care when extrapolated to governmentally operated insect production plants it might be of interest to examine the order of magnitude of estimations made in the United Kingdom (Robertson 1971). Quality and reliability costs of mass-produced products fall into three categories: prevention costs, appraisal costs (when production gets out of control) and actual failure costs. The distribution of these three components under systems emphasizing product checks has been estimated as follows:

failure costs	65%	present average costs for quality = 4-20% of gross turnover
appraisal costs	30%	
prevention costs	5%	

Potential costs under systems implementing total quality control with emphasis on prevention:

prevention costs	10%	
appraisal costs	20%	Savings might range from 1.5 to 6.5% of gross turnover
failure costs	35%	
savings	35%	

5. Concluding Remarks

Quality control in mass reared insects: In what direction is it heading? Which of the present problems call for intensified investigation?

One thing has become evident. We force our fruit flies through a series of genetic bottlenecks as soon as we bring the wild material to the laboratory for mass production under artificial laboratory conditions. Two major bottlenecks often occur when all life stages are reared exclusively on artificial substrates: one that occurs in the adult stage reduces drastically the reproducing portion of the founder population. The second one occurs during the larval stage, and its effect is often more conspicious because the pupal yield in the early phase of mass rearing is usually very low. Mortality occurring during the larval stage will eliminate those individuals that cannot survive on the new diet and selection will favor those exhibiting the appropriate physiological disposition. Its effect on important behavioral traits will not appear to be dramatic, as it will influence mostly characteristics that rank low in the quality hierarchy outlined in Fig. 2. However, its impact on the numbers of insects produced (also a quality criterion) may be severe. The impact of bottlenecks that affect the adult stage are more severely felt by performance criteria, as such selection acts directly on those characteristics of adult behavior that have an immediate relationship with performance quality. Most of the techniques applied in fruit fly laboratories and described in this book deal with adult behavior. There is increasing evidence that this is justified.

Many opportunities remain to advance the state-of-the-art. These advances will probably occur in techniques, methods, and programs rather than in quality control philosophy. The lack of good measuring tools and the lack of suitable techniques to evaluate quality data has hampered our ability to demonstrate the effectiveness and value of quality control programs. The many bits and pieces of information and ideas contributed by as many fruit fly workers all over the world have found their first precipitation in this book. The idea book by itself will certainly not completely solve the problem of designing and implementing the quality control programs urgently needed in our fruit fly facilities. We hope, however, that it will stimulate discussion and initiate a fruitful exchange of ideas between entomologists working in theoretical and applied fields, resulting in further research, development and trial implementations. The book may also help to clarify the open questions and indicate areas of future actions.

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MEASURING OVERALL PERFORMANCE

INTROOUCTION

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The insects maintained in a mass rearing facility constitute a population which is isolated from other populations of the same species. This population is also adapted to an environment which is very different from that experienced by its counterparts in nature. Gene flow (operationally, the introduction of field collected material into the production strain) will in most cases be insufficient to overcome the effects of this adaptation to the rearing environment; it may however restore some of the genetic variability lost through random processes and inbreeding in the early stages of colonization. Strain replacement will quickly lead to a population similar to the one replaced if the rearing environment remains unchanged. Many types of selection will be operating simultaneously. Directional selection may, for example, shift the mean of a character far away from the natural population mean. Relaxed selection may increase the population variability in a character.

It is an established concept of evolutionary genetics that the individual is the unit of selection. Selection acts upon individuals with a strength proportional to their fitness, where fitness is defined as differential reproductive success. In other words, individuals with high fitness are selected because they contribute the greatest number of progeny to the succeeding generation. The individual expresses a multitude of traits which are variable within the population from which it comes. The total of the variations in the set of traits constituting an individual determines the fitness of that individual. The cumulative effects on fitness of many small variations may be as great or greater than a single severe defect in one important trait. Because the fitness of a trait is often dependent upon the environment in which the trait is expressed, it may be high in the mass rearing facility and low in the field. The more the rearing environment differs from that of the field the more likely this is to be the case.

In the rearing environment, perhaps more than in nature, nongenetic disabilities may also occur. Diet, disease, photoperiodic entrainment, and other factors such as the effects of sterilizing radiation may contribute largely to the failure of mass reared insects to perform in the field. In attempting to cure defects it is important to partition the trait into its genetic and nongenetic components, for the approach taken may be very different depending on the relative strengths of the two.

The quality of a mass reared population of insects is a function of the proportion of highly fit individuals within it; thus, we must have accurate methods for measuring the overall performance of our released insects. This is particularly true in the extreme case outlined above wherein low fitness may be the result of small deviations in many of the components of fitness. Some of these deviations may well be too small to measure with the individual tests described in later sections.

So far I have discussed the quality of the mass reared population relative to that of its conspecific natural populations. In our attempts to measure the overall quality of our released product we should not forget the capacity of the natural populations to adapt to the released insect. Several points may be made in support of such an idea. In the first place the population size of the mass reared insect is larger than the size of the natural population into which the release is made. In essence the SIT is an exercise in post-mating reproductive isolation; i.e., matings do not result in offspring. In the process of speciation, post-mating reproductive isolation produces intense selection for pre-mating reproductive isolating mechanisms. The released insect, being sterile, cannot itself evolve but because of the high numbers released it must act as a selective force on the natural population. Therefore in the SIT we are carrying out a huge selection experiment on a natural population, the magnitude of which is beyond the means of even the most affluent evolutionary biologist. We may now look at the three ways in which sterile insects are used and examine the probability of the occurrence of evolution of reproductive isolation between a "high quality" mass reared strain and a natural population.

The classical cases of successful employment of SIT have occurred on islands or other geographically restricted areas. In these cases the population may be driven to extinction (until the area is reinvaded) because both the area and population size are limited and manageable. In the second case we operate at the edge of a species range either to push the population back or to prevent its expansion. In this case we are applying the SIT in a buffer zone against that portion of the species that is operating at the edge of its range. While the possibility exists that pre-mating reproductive isolation could evolve in some species with limited dispersal capabilities, in most cases gene flow from the center of the population will tend to overcome evolutionary advances made at the edge. In the third case, which is the most advanced and as yet untried technique, we attempt to eradicate or control a species over its entire range within a broad continental area. These populations are adapted to a wide range of niche parameters and may be further substructured in their behavior patterns. The release of sterile insects into these geographically variable populations has the very real potential for producing selection for pre-mating reproductive isolation. It is paradoxical that the strength of the selection pressure exerted on the native population will be directly related to the quality of the mass reared strain. Furthermore, if the wild population is used as the standard for comparison with the cultured population we will find that the standard changes in response to this selection.

It is therefore incumbent upon the managers of SIT programs to be able to assess the quality of the mass reared insect relative to the wild population and to detect changes in either population. For this reason several methods have been developed that attempt to measure the fitness of the mass reared insects. The earliest of these is the ratio test carried out in small laboratory cages. In it irradiated insects are caged with untreated insects in various combinations of sexes and in various ratios. The per cent egg hatch is used as the criterion of the effectiveness of the irradiated insects in competing for mates. As Hooper points out in this description of this technique below, the small cages typically employed for these tests will exclude the operation of certain factors which may affect competitiveness. Large field cages may be more appropriate as more of these factors will be brought to bear on the populations being tested. Harris describes such tests briefly below. It is likely that both laboratory and field cage tests will continue to play a role in quality control monitoring because of the ease with which they are established and the control that the investigator has in manipulating them.

The results of cage tests become more meaningful after sterile insects are actually released into the field and their performance is evaluated. These tests are described in the papers by Harris and by Iwahashi. Ratio tests carried out in the field have three major advantages. (1) The target population in the target habitat is the standard for quality. (2) All aspects of the program which ultimately affect insect quality are tested at one time. (3) Mark-release-recapture data and egg-hatch data give two ratios for comparison. The first is the absolute ratio; the second is the effective ratio. If the absolute ratio of sterile-normal insects is larger than the effective ratio then the competitiveness of the irradiated insects may be questioned.

The ratio tests described briefly above and in greater detail in the papers to follow should be the methods of choice for the initial evaluation of overall competitiveness. However, if the insects are not competitive a large number of potential factors involving rearing and/or treatment and genetic and/or environmental problems may be in operation. The method using genetic markers described by Huettel et al. provides a first step toward partitioning the effects of irradiation from those in operation earlier. It may also provide insight into the dynamics of the intermating of mass reared and wild populations not obtainable by other means.

In conclusion, a number of techniques are available to test the effectiveness of our mass reared insects in mating with native populations. Considerable subtlety will be required in the interpretation of these data in order to determine whether the mass reared population is diverging from the natural one or vice versa. In addition, none of these techniques is likely to pinpoint the specific defects leading to the loss of quality. This must be attempted by application of the specific tests outlined in the sections which follow.

EVALUATING PERFORMANCE USING RATIO TESTS

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Description and Objectives

The term "ratio tests" is used to describe experiments in which males (on the assumption that they are the determining sex) sterilized by ionizing radiation or chemicals are combined in defined ratios with untreated (U) males and females. The observed egg hatch can be compared with the egg hatch expected on the basis of equal performance i.e., competitiveness, of irradiated (I) and U males. Conclusions may then be drawn about the competitiveness of the I males under the experimental conditions. These ratio tests are of considerable importance since in the final analysis a field suppression/eradication program is simply a large scale ratio test. Proposed field overflooding ratios may need to be modified in the light of the competitiveness of the I flies as determined in ratio tests. Ratio tests can be used to compare the competitiveness of males sterilized by different means e.g., gamma radiation, neutrons, or chemicals, and to obtain information on the optimal sterilizing dose. They provide an overall assessment of the competitiveness of I males which results from the interaction of a number of factors (see Hooper and Katiyar 1971, Hooper 1975).

Materials and Methods

The most common procedure involves combining I ♂ with U ♂ and ♀ , soon after eclosion and before mating can occur, in the desired ratio(s) e.g., 3 I ♂ : 1 U ♂ : 1 U ♀. However, since in field programs both sexes are released, the test can be modified e.g., 3 I ♂ : 3 I ♀ : 1 U ♂ : 1 U ♀, so that when considered in conjunction with the first procedure the role of the female can be evaluated. The impact of the ♀ alone can also be determined e.g., 3 I ♀ : 1 U ♂ : 1 U ♀. The cages are provided with water and appropriate food and maintained in a controlled environment. When the flies are sexually mature eggs are collected on a number of occasions e.g., twice per week for three consecutive weeks. The eggs, preferably 150-200 per collection, are incubated on moist filter paper in petri dishes for determination of hatch. The experiment must be replicated and each replicate should, in addition to the ratio treatments, include the crosses U ♂ x U ♀ and I ♂ x U ♀ to account for day to day variation. Ideally a further control of 3 U ♂ : I U ♀ should be run to determine if an excess of males effects fecundity and egg hatch.

Variables, Design, and Analysis of the Experiments

If the survival of I o is known to be significantly reduced, and if the time for sexual maturation is appreciable, all I and U insects should be sexed immediately after eclosion, held separately, and combined in the desired ratio(s) close to the time of sexual maturation. If this is not done the desired ratio(s) may be drastically changed by the time mating can occur. Variability is high in this procedure and adequate replication is essential (Hooper 1972). Each replicate should be established with a different batch of insects and ideally on different days to account for biological and experimental variation. After arcsin transformation of the percentage hatch data an analysis of variance is appropriate with differences between means being tested by Duncan's (1955) multiple range test. If required, treatment hatch data can

be corrected for the control (U ♂ X U ♀) hatch to enable comparison of ratio hatch data between replicates:

$$\text{Corrected \% hatch} = \frac{\text{observed \% hatch}}{\text{control \% hatch}} .100$$

The observed egg hatch (Ho) can be compared with the egg hatch expected on assumption of full competitiveness of the I o :

$$\text{Expected \% egg hatch (He)} = \frac{N.Hn + S.Hs}{N + S}$$

where Hn is the % hatch from cross U ♂ x U ♀, Hs is the % hatch from cross I ♂ x U ♀, N is the number of U ♂ and S is the number of I ♂. If Ho is greater than He, reduced competitiveness is indicated. Fried (1971) proposed a procedure to quantify this competitiveness. If we assume that the competitiveness of I ♂ can be affected by a factor "c" which can vary from 0 to 1 (1 indicating full competitiveness), then we can modify the above equation to give:

$$\text{Observed \% egg hatch (Ho)} = \frac{N.Hn + c.\ S.Hs}{N + c.\ S}$$

and solving for "c" we obtain:

$$\text{Competitiveness Value (c)} = \frac{N}{S} \cdot \frac{Hn - Ho}{Ho - Hs}$$

The terms Hn, Hs and Ho are experimentally determined and have associated error terms. Consequently, for optimum precision the test ratio should be such that the differences between Ho and both Hn and Hs are appreciable. In practice ratios between 2:1:1 and 5:1:1 should be adequate. While these ratios are not representative of probable field overflooding ratios, large ratios can be run if care is taken to minimize density effects and maintain adequate numbers of U ♀ in each cage (see Hooper 1972).

Possible Modifications and Expansion of the Technique

Most ratio tests are carried out under laboratory conditions and in small cages. Data more relevant to a field program are obtained when these tests are carried out in large cages in outdoor situations. A trend in this direction is occurring and should accelerate.

Limitations

This technique does not define which factors may be responsible for reduced competitiveness, and there are problems in investigating large ratios e.g., 100:1. When carried out in small cages it eliminates some factors which can be presumed to be important in the field e.g. location of food and mates and flight capability. If different sterilizing techniques are to be investigated, the levels of sterility must not be significantly different since sterility and competitiveness are inversely correlated (Hooper 1971 a,b).

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PROGRAM EVALUATION USING RATIO TESTS AND RECOVERY DATA

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Description and Objectives

In Hawaii, releases of sexually sterile Mediterranean fruit flies, Ceratitis capitata, oriental fruit flies, Dacus dorsalis, and melon flies, D. cucurbitae, have been made to evaluate their performance in the field in competition for mates and natural resources with that of native flies of the same species. Depending upon program objectives, such releases were made to evaluate the effect of laboratory conditions, handling, irradiation, and subsequent field distribution on the competitiveness of sexually sterile laboratory reared flies. The methods with minimum adverse effects were adopted for release of sterile tephritids to suppress native fruit fly populations or to achieve eradication with integrated control methods combined with sterile fly releases.

Materials and Methods

Laboratory ratio tests. Within 24 h after eclosion male and female fruit flies are segregated and held separately in screen cages under standard laboratory conditions. Equal lots of sexually mature, treated (marked with fluorescent dyes, irradiated, and prepared for release) males and females (50-100) were mated in large outdoor screen cages and compared with native flies using the method of Holbrook et al. (1970). After the irradiation and handling methods were proven to be satisfactory (see Ashraf et al. 1976 and Ohinata et al. 1977 for details) they were used routinely to release sexually sterile medflies. These methods may be used to release sterile oriental fruit flies and melon flies.

Field tests. Steiner traps (1957) and tub traps (Nakagawa et al. 1975) were placed in the test areas (2 per square mile) to obtain pre-treatment population estimates of fruit flies and fruit infestation rates and monitor these factors routinely throughout periods sterile flies were released. Weekly releases of from 200,000 to 20 million medflies were made depending on program objectives. Trap recoveries of native and sterile flies were made once every 1 or 2 weeks. Overflooding ratios were determined by counting the fly catches and segregating the marked sterile flies under black light to obtain total number of each category. For suppression or eradication we try to obtain a minimum 100:1 overflooding ratio in the most favorable host plant areas. We collect fruits from the entire test area and record the fruit infestation rates weekly. A sustained decline in fruit infestation rates is considered to be the ultimate criterion by which the effectiveness of the sterile releases can be determined.

Variables, Design, and Analysis of the Experiments

In outdoor cage mating tests adjustment must be made in the age classes to allow for differences in age at sexual maturity between the laboratory reared flies and their native counterparts. In case of the medfly, the laboratory fly reaches sexual maturity within 5 days whereas the wild medfly requires 10 days. The results obtained in field experiments are affected by field conditions particularly rainfall and the distribution and abundance of host plants. These factors must be considered in evaluating the results of sexually sterile fruit fly releases.

Possible Modification and Expansion of the Technique

These techniques can be used to conduct a large-scale SIT program to eradicate new outbreaks of fruit flies as well as long-established populations.

Limitations

An understanding of the biology of these insects is required to carry out these tests. However, careful thought has to be given to the design of the field tests to minimize the effect of uncontrolled variables. Adequate personnel and material resources must be available to carry out large-scale tests.

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EVALUATION OF THE QUALITY OF RELEASED DACUS CUCURBITAE IN JAPAN

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Description and Objectives

Aspects of the functional quality of sterile flies used in an eradication program can be evaluated by monitoring the ratio of sterile:wild insects in the field, the hatchability of eggs, the infestation of the crop and the trends in population densities. We found that the assessment of sexual competitiveness as described by Fried (1971) is a useful approach in our quality control program. Also, in most cases the ratio of marked to unmarked individuals is underestimated due to loss of the fluorescent powder marker. A simple method of examination is described that alleviates that particular problem.

Materials and Methods

The method for estimating the ratio of marked and unmarked flies in the field has been described by Itô and Iwahashi (1976). Females are collected with sweep nets in the experimental areas. These flies are reared individually and oviposit into artificial oviposition devices (Iwahashi et al. 1976). The hatch-rate of the eggs is determined for the release and control areas.

Applying Fried's equation based on these percentages of egg-hatch we can estimate the ratio of sterile to normal males in the field as follows:

$$\frac{S}{N} = \frac{H_n - H_c}{H_c - H_s} \cdot \frac{1}{C}$$

where H_n = Per cent egg-hatch in the control area (no sterile flies released)

H_s = Per cent egg-hatch in matings between sterile males x normal females in the laboratory

H_c = Per cent egg-hatch in release area

C = Sexual competitiveness of sterile males obtained by ratio tests in the laboratory.

Sterilized females of D. cucurbitae (6 or 7 krad) lay no eggs. Therefore we can also estimate the ratio of sterile-to-normal females in the field:

$$\frac{S_f}{N_f} = \frac{T_f - R.T_o}{R.T_o}$$

and $$R = C_f/C_o$$

where C_f = Number of females collected in the control areas

C_o = Number of females collected in control area and which produced eggs

T_f = Number of females collected in the release areas

T_o = Number of females collected in treated area and which produced eggs

Variables, Design, and Analysis of the Experiments

The following variables require standardization in order to reduce overall variation of the data: the number of females examined, the temperature in the test room, and the period between oviposition and examination of egg-hatch.

The following approach is suggested for the experimental design: 1. Define the optimal time for the collection of females. Females should be collected simultaneously in the control and release areas; 2. Verify the developmental time of the eggs at defined temperatures and check the hatch-rate when hatching has been completed; 3. Determine the minimal period for the examination of the fertility rates. In D. cucurbitae some 40 days are required because some wild females exhibit preoviposition periods up to 30 days in the laboratory.

The methods for the analysis and comparison of estimated ratios of sterile and wild flies have been described by Iwahashi et al. (1976) and Iwahashi (1977).

Possible Modification and Expansion of the Technique

The method can be applied for the estimation of the sexual competitiveness of sterile males under field conditions when the accuracy of marker identification is known. Also we can estimate the rate at which the marker is lost under field conditions provided the number of females collected in the release area reaches several hundreds.

Limitation

These methods require no special skill and equipment.

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MEASURING INTERBREEDING OF NATIVE AND MASS REARED INSECT POPULATIONS IN THE FIELD

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Description and Objectives

The objective of the following technique is to evaluate the ability of mass reared fruit flies to mate successfully and competitively with their native conspecifics in the field.

The procedure outlined is essentially a genetic mark-recapture technique. The parents are genetically marked, and the progeny resulting from their mating with the native population are "recaptured". The technique assesses a summed value of all relevant quality measures except the effects of sterilizing irradiation (Huettel 1976).

Materials and Methods

A mass reared strain is developed that carries a genetic allele in high frequency that is known to occur in low frequency in wild populations. This marked strain, consisting of approximately equal numbers of males and females, is released into the native population by the methods used in a normal sterile release program. Adults are then sampled sequentially and by suitable methods throughout the expected life span of the released insects.

At intervals after the release, infested fruit are collected, and larvae are reared to the stage appropriate for analysis. The timing and number of these collections are dictated by the life span of the insect being studied, but they should be frequent enough so that trends in the data may be distinguished from sample variation.

The marker allele frequency is then determined in these adult and larval samples. If morphological mutants are used, the marker allele frequency is determined by crossing individual insects in the samples with appropriate tester stocks and scoring the resultant progeny (e.g., Whitten et al. 1973). If biochemical markers (allozymes) are used, they are assayed in the sample insects by gel electrophoresis and histochemical staining (e.g., Huettel et al. 1976).

Variables, Design, and Analysis of the Experiment

The proportion ($\underline{M}$) of the released individuals in the population after the release is obtained by

$$\underline{M} = \frac{p_F - p_R}{p_R - p_N} \qquad (1)$$

where p_N and p_R are the frequencies of the marker allele in the native and released populations prior to the release, and p_F is the frequency of the allele in the samples taken after the release (Wallace 1968).

In the absence of selection, allele frequencies are conserved when 2 populations are mixed. The values of $\underline{M}$ for both the adult and larval samples should therefore be the same if the released insects are competitive with the natives. In addition, the marker

alleles in the larval samples should be in Hardy-Weinberg equilibrium if the two populations freely interbreed.

An estimate of the native population size (n_N) may be obtained from

$$n_N = \frac{n_R}{M} - n_R \qquad (2)$$

where (n_R) is the number released and M is from (1). The similarity of the above to the Lincoln Index is obvious, and the latter could easily be incorporated into a study of this kind by the use of marker dyes on the released adults.

The type of genetic marker chosen will depend upon the markers available for each species and the resources of the investigator. We have chosen genetically variable enzyme proteins (allozymes that are separated by starch gel electrophoresis and stained by histochemical techniques). Morphological mutants may also be used (e.g., Whitten et al. 1973). The advantages of allozymes over morphological mutants have been summarized by Huettel 1976. The simultaneous use of several loci greatly reduces the variance of the estimates of (1) and (2).

Possible Modification and Expansion of the Technique

The algebraic model developed above has been extended to the case where only males are released, where a proportion of females are monogamous, and where a refractory period occurs between matings. These will be outlined by us in more extensive publications elsewhere. Positive assortative mating between released and native populations may be measured as a function of the excess of homozygotes over that expected from Hardy-Weinberg equilibrium. Makela and Richardson (1977) have developed the mathematical techniques necessary for this analysis.

Limitations and Requirements

This technique requires at least a minimal knowledge of genetics and techniques of genetic manipulation. The electrophoresis techniques for allozyme analysis require trained technicians and some equipment and supplies that may be difficult to obtain in certain locations. The authors will provide assistance or additional information to interested parties.

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MEASURING INDIVIDUAL PERFORMANCE TRAITS

Motility

INTRODUCTION

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The cultured fly must upon release display a characteristic well-maintained by selection in its wild counterparts -- that is, flight. The efforts put into its production may be wasted almost immediately if the released fly cannot or will not escape predation and environmental stress and seek shelter, food, and water. Thereafter, it must move over greater or lesser distances in seeking its native target within appropriate habitats. If the targets or habitats are distributed non-uniformly then the released flies must distribute themselves in the same manner. If the native mate is coy or fleet then the suitor must seek and pursue its objective.

Factory production imposes a degree of crowding on the colony, and the stress of crowding must select against irritability and perhaps against capability for flight. Nutrition and treatment may also induce alterations in locomotory characteristics. These alterations, if undetected, could readily render futile the release of flies that otherwise appear totally competent.

Assessment of motility can be divided into two categories: (1) Studies of the propensity for flight; i.e., the tendency for flies to commence flying. (2) Studies of the capability of flight; i.e., the duration, velocity, and distance of flight, once it is initiated. It is important to recognize the difference between these factors and that they may be affected separately and by different mechanisms.

A third aspect, rhythmicity of locomotion, should also be studied, either separately from or in concert with the two aspects listed previously. Thus, a strong tendency or capacity for locomotion may be of little value in a release program if it occurs at the wrong time. Such deviations will be most significant in their relationship with mating behavior.

Examination of behavior related to movement can be carried out in the laboratory and in the field. Laboratory studies are most useful in developing indices for comparing flies treated in various ways and in discerning the components of complex effects, endogenous or induced. Field tests provide data most applicable to the situation in which the released fly must compete. However, their interpretation is often made difficult or limited by variables such as climate, habitat and unobservable behavior. Thus, the value of either type of experiment is limited to specific objectives and by conditions.

An essential difference exists in the principal kind of data gathered in laboratory and field experiments. Tests of locomotion in the laboratory produce records of behavioral events or patterns of events, e.g., numbers of flights, landings, revolutions, etc. Field data have primarily been obtained through variations of trapping, a procedure that stimulates little intuition as to the behavior that resulted in

capture. More subtle variations or alternatives to trapping need development and application in order to provide the interpretive capability needed to dissect behavioral components in the field.

Movement of fruit flies may be characterized as either local or dispersive. Minor changes in location within the local habitat, as among trees or tree quadrants or cover types may be called local movements. These may occur due to changes in the habitat, such as fruiting stage, food or shelter availability, or microclimate or to small changes within the fly as might result from circadian rhythms. Dispersive movements are more or less permanent relocations among areas that may result from general changes in the environment or in the physiological condition of the fly. The importance of this difference in each species, and whether it is an essential difference (i.e., is dispersive movement the sum of many local movements), has not been adequately considered in sterile releases. It is an aspect of quality control that deserves more attention. Dispersal and distribution maps may be prepared from recoveries in a grid of traps of wild or released flies (Boller et al. 1971). The rate of movement through the grid, its randomness, and the persistence of contagious distributions can provide an estimate of the nature of the movement (Dr. Boller also described these procedures in a mimeographed booklet on fruit fly movement prepared for the International Biological Program Working Group on Fruit Flies). Traps and attractants are, thus, necessary and potent tools for studying movement. However, an attractant trap while providing data on dispersive movement may seriously affect local movement. Thus, the advantage that large recoveries give in terms of statistical significance must be contrasted with the accuracy of observations made with passive techniques; the use of both may be advisable.

In a discussion of adult fruit flies (Bateman 1976), Dr. Fletcher and I have reviewed recent literature on the topic. Bateman (1972) prepared a review article on fruit fly ecology; adult movement as an ecological phenomenon is treated therein. Because movement is a process that is promiment in the study of genetics, physiology, ecology, and behavior we can predict its importance in all of these categories to quality control. Although the following papers appropriately treat it primarily in terms of its behavioral significance, we should keep in mind the broader implications of locomotion in colonization and release.

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FLIGHT MILL STUDIES WITH *ANASTREPHA SUSPENSA*, *DACUS DORSALIS* AND *DACUS CUCURBITAE*

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Description and Objectives

Flight mills consist of a rotor attached to a low friction hub that rotates freely on a vertical axle in response to insect flight. They vary from simple designs that provide data observationally to complex systems interfaced with data analysis devices. They provide data on the effects of age, sex, etc., on the propensity to initiate flight and on in-flight parameters (duration, velocity, distance, and frequency of flights). However, once a fly loses tarsal contact it cannot recover it *ad libitum*, so flight thresholds remain at lower levels than if the insect is able to rest with tarsal contact. The tests are conducted in the laboratory under defined, controlled conditions to provide indicative data that can be extrapolated to indicate the need for field studies.

Materials and Methods

Flight mills of simple designs have been made of aluminum sheets suspended from pin pivots mounted in cork bases (Yates 1969). Mills of intermediate complexity consist of a hub of teflon that supports opposing like pole magnets (Chambers et al. 1976). Complex ones have hubs made of jeweled bearings (Schoenleber et al. 1970). Sophisticated mill systems are coupled to digital counters (Kishaba et al. 1967) or to event recorders that transduce results directly to computer-compatible analytical procedures (Ashley et al. 1976). For tests, individual flies are fastened (in our laboratory by plastic rubber) on the dorsal thorax to an L-shaped harness that permits normal wing movements. The free end of the harness is attached by a rubber sleeve to a horizontal rotor arm that is supported by a hub. The positioning of a fly on the rotor must be standardized. For our work each insect is positioned with the body tipped, head down, at an angle of ca. 13 degrees from horizontal to enable it to produce the greatest thrust of power by wing movements. For other details refer to Sharp 1976, and Sharp and Chambers 1976.

Variables, Design, and Analysis of the Experiments

The identified variables requiring standardization to reduce variation are: size, weight, and design of the flight mill (hub, axle, rotor arm); tethering procedures; and time required for cold recovery. The following should also be standardized: rearing procedures; temperature and humidity in rearing and test rooms; photoperiod and light intensity; size, age, and sex, and sexual and nutritional condition of the flies; and time of the day of the tests. Suggested approach for experimental design: 1. Establish time limitations that define a flight period (minutes) and a rest (seconds). For example, in the field unrestrained flies rest and fly for indefinite periods. Under controlled conditions in the laboratory, a fly tethered to a rotor arm demonstrates similar behavior. Perhaps it flies a short distance of 1 m in 2 sec, rests for 10 sec, then resumes flying for several minutes (sustained flight). 2. At

temperature and humidity conditions that are appropriate for rearing, evaluate age, sex, and sexual conditions to find the optimal group for other tests. 3. Define the proper number of replications to reduce standard error of the mean since the investigator should expect much individual biological variation among test flies.

Possible Modifications and Expansion of the Technique

The flight mill technique can be used in long-term studies to determine whether flight ability is genetically controlled, and to test and compare strains and ecotypes. Incorporating sound monitoring and analysis equipment with flight mills enables simultaneous measurements of the wingbeat frequency. It also offers another advantage: Since the hub rotates on an essentially friction-free axle, the rotor and hub do not stop abruptly when an insect ceases flying. Indeed, inertia enables the tethered fly and rotor and hub to continue to rotate (coasting). This incorrectly increases the distance flown. However, if the wingbeat sound triggers an event recorder (an alternative to interruptions by the rotor arm of a light-saturated phototransistor) the effect of coasting is negated. As a result no erroneous data are collected though the rotor arm is turning. Also, wing dimensions can be taken and insect weight determined to broaden information.

Limitations

Some flight mills require a power source, and they can be designed to operate on dc current. Automated units are expensive to design and construct and may occasionally require repairs; however, they reduce labor costs.

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MEASURING FLIGHT PARAMETERS IN R.CERASI AND D.OLEAE

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Description and Objectives

Laboratory flight studies utilizing flight mills and stroboscopes provide early detection of significant deviations of the flight parameters of test strains measured under defined conditions and compared with wild standards. The following parameters can be measured in the laboratory test: Wing beat frequency (with stroboscope); flight propensity (expressed as per cent of flies showing immediate flight activity at the start of the test); total distance flown during a defined period (in our case 24 h); number and distance of individual flights flown by every fly within 24 hours; and flight speed (calculated from the event recorder chart). Comparative flight tests have provided evidence for the detrimental effect of marking techniques in R. cerasi and D. oleae (Remund and Boller 1975a, 1975b) and for a substantially decreased flight potential of D. oleae reared for many generations on artificial diets (Remund et al. 1977). Laboratory flight tests are considered to be precursors of field dispersal studies.

Material and Methods

The flight mill used in our investigations has been described in detail by Remund and Boller (1975a). The difference from other types of flight mill systems (cf. Sharp) lies in the design of the low-friction rotors, which are suspended in series on a steel wire. The test procedures have been described by Remund and Boller (1975a) and Remund et al. (1977). They are summarized as follows: The test flies are starved for 24 h before the test. Then they are chilled at 2°C for 5-10 min, and the head of a fine insect pin is glued to the thorax. Before testing the flies they are fed a defined amount of sucrose in order to obtain comparable energy levels (a 10% sucrose solution is sprayed with an atomizer on 5-mm diam styrofoam spheres. The sugar-coated balls are grasped by the flies and rotated, whereby they may consume approx. 0.2 mg of sucrose).

Before the insect is attached to the rotor of the flight mill we measure the wing beat frequency with a stroboscope (Turbo-Strob 551 XW, 220 V, 50 Hz, range 3.3-300 Hz). For this purpose the styrofoam ball is removed and about 5 measurements carried out until the wing beat frequency has become stabilized.

Thereafter the flies are attached to the rotor arm in the optimal position. The angle between pin and rotor should approach 95°, and best flight performances are achieved when the insect's head is lowered by 5° below the horizontal axis. One revolution of the rotor equals a flight distance of 1.0 m. The rotors are monitored by photocells, and the electrical signals are processed by digital counters and a 10-channel event recorder.

Variables, Design and Analysis of the Experiments

The following experimental variables have been identified and should be standardized in order to reduce overall variation: body size of the insect (cf. Remund and Boller 1976); sex; age; energy level (starved or fed); temperature, humidity and light intensity; chilling temperature and period; friction coefficient of rotors; proper adjustment of stroboscope; and number of replicates.

These variables should be defined and kept constant during the test period. One experimental series consists of 50 males and 50 females of each test and standard strain.

The evaluation of the flight data is relatively simple and does not require computer processing. The distance flown during the 24-h period can be read directly on the digital counters. The flight pattern (number of individual flights and resting periods, duration of consecutive flights, etc.) can be taken directly from the recorder chart. The average flight velocity can be calculated easily from flight distance and flight duration. However, we found that flight speed is not an appropriate parameter for analytical purposes.

The statistical analysis is carried out either with parameter-free tests (Wilcoxon) or with the classical analysis of variance following an adequate transformation of the data (Remund and Boller 1975a, Remund et al. 1977).

Limitations and Expansion of the Technique

Laboratory flight studies do not provide behavioral data that can be extrapolated to describe the anticipated behavior in the field. However, the flight indices obtained in comparative tests carried out under defined experimental conditions can serve as guidelines for the investigations to be conducted under field conditions (e.g., Fletcher and Economopoulos 1976, Prokopy et al. 1975). The technology involved in flight studies can be most simple or highly sophisticated, especially with respect to data processing. The proper servicing of the devices and the preparation of the test insects requires a certain amount of skill that can, however, be acquired by adequate training. Comparative tests should always be carried out by the same persons.

The application of flight mill studies is unlimited. Flight data can be analyzed together with allozyme data of the test animals (see Bush); shifts in flight performance levels in the course of adaptation of strains to laboratory conditions can be compared with similar changes observed with respect to other activity patterns such as locomotor activity (see Baily) or sexual activity (see respective contributions).

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TAKE-OFF FREQUENCY AS A CRITERION OF FLIGHT PROPENSITY

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Description and Objectives

Flight is essential for maintaining the population as well as individuals of a fly species. Therefore, flight behavior is an important criterion for assessing the physiological and genetic status of a species or strain. Internal and external stimuli eliciting the starting and ending of flight establish the flight phase. The technique described here provides an assessment of the propensity to enter the flight phase, i.e., take-off. When environmental factors are carefully controlled it is possible to establish the influence of internal factors on flight propensity; if the latter are known to be constant the effects of environment on take-off propensity can be studied.

Materials and Methods

The equipment consists of a flight cage (40x40x40 cm) suspended from an electronic balance that registers each weight reduction occurring due to the take-off of a fly that was resting on the cage wall. The resulting electric signal is transformed into a pulse of a form appropriate for transduction by an event recorder. External vibrations can largely be filtered out. For technical details see Haisch et al. (1976).

Variables, Design, and Analysis of the Experiments

The flight propensity of Rhagoletis cerasi has been shown to be determined by internal factors such as age, sex, hunger, thirst and exhaustion acting together with the specific individual constitution of the fly. In other species endogenous rhythms may play an important role. The experimental design must include standardization of these internal factors to avoid misinterpretations.

We use five pairs of flies 1-4 weeks of age for each experiment, taking into account mutual excitation and sexual stimulation. These individuals are taken from a population of at least 200 flies to ensure randomness. Each test lasts 2.5 h and three tests per day are conducted (0830-1100, 1100-1330, 1330-1600 h). Food and water are provided for ad libitum consumption during the test. Each test is repeated with completely independent experimental units. Using flies standardized in this manner it has been possible to study the effect of radiation, as well as light intensity and temperature and the interplay of these two factors. Currently the technique is being applied to the study of host races of R. cerasi and it could be adopted for comparing wild and laboratory strains.

Possible Modifications and Expansion of the Technique

Take-off frequency can be most meaningfully interpreted as an aspect of studies that include flight capacity and landing behavior. In tephritids the latter may be largely a study of orientation.

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MEASURING WINGBEAT FREQUENCIES

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Description and Objectives

Wingbeat frequency, a measure of flight ability, is the speed at which the wings oscillate through the wing stroke angle. It depends upon the ratio between the power of the thoracic muscles and the resistance they must overcome. Wingbeat frequency tests are conducted in the laboratory under controlled conditions and provide insight on the possible detrimental effects to the flight musculature due to various treatments to immatures or adults. Among tephritid fruit flies, measurements of frequency (Hz) with electronic stroboscopes have shown significant differences due to temperature, humidity, sex, age, and radiation and provided insight on subtle changes to flight behavior in test insects not detectable with flight mill measurements.

Materials and Methods

Individual flies of the proper sex and age are immobilized at a temperature of 7°C and then fastened by glue on their dorsal thorax region to an L-shaped wire in a manner that does not interfere with the normal movement of the wings. The free end of the wire is inserted into a stationary mount. The flies are allowed to recover from the cold exposure, and after a designated time of sustained stationary flight, the wingbeat frequency is obtained under defined temperature and humidity regimes by using an electronic stroboscope. For other details refer to Sharp 1974; Sharp et al. 1975, Webb et al. 1976, Sharp and Webb 1977, Van Veen 1966.

Variables, Design, and Analysis of the Experiments

The following variables have been identified that require standardization in order to reduce overall variation of the data: time and temperature required for immobilization and time allowed for recovery, tethering procedures, time allowed in stationary flight before measurements are taken, temperature and humidity in the test room, size, age, and the life history of the fly (strain, generation).

Suggested approach for experimental design: 1. Calibrate the stroboscope or test instrument; 2. Carry out tests with increasing age of both sexes at the temperature and humidity established for rearing and determine the optimal age groups; 3. Establish the number of replicates needed to provide a low standard error of the mean.

Possible Modifications and Expansion of the Technique

The measurement of wingbeat frequency in flying insects should be a routine, integral part of any quality control program. Together with data that are collected using other techniques such as flight mill systems, actographs, and propensity tests, comprehensive studies can be conducted that expand the production of data and provide several avenues of attack to observe effects or predict them.

Limitations

Reliable stroboscopes (that are easy to operate) currently cost $ 200-700. Instruction manuals are usually provided by the manufacturer. General Radio Strobotac® is available from representatives in West Concord, Mass., USA; Zurich, Switzerland; and London, England. Type 1538 Strobotac can be operated from a 115- or 230-volt, 50-60 Hz or 400 Hz line as well as from a 24-volt dc supply.

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MEASURING THE STARTLE ACTIVITY IN MEDITERRANEAN FRUIT FLY, CERATITIS CAPITATA, POPULATIONS

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Description and Objectives

It has been observed that the propensity for flight can affect mating and the ability of flies to successfully seek food and shelter in the field. The threshold of response resulting in flight can be measured for different populations of flies in "startle test" chambers under reproducible laboratory conditions. After establishing the startle activity for a population, individual effects of various treatments on this activity can be determined. There are also individual flies within each population that have lower startle activity than the mean. By exposing the population to predators in field cages, one can eliminate flies with the lowest activity. Survivors can then be used as parent stock to maintain and increase startle activity.

Materials and Methods

The chamber for measuring the startle response (Fig. 1) consists of 2 round cardboard containers, a 4-liter upper "catching container", and a 1-liter bottom "holding container" (for details see Schroeder et al. 1973). Flies are chilled and segregated by sex, then 25 are placed in each holding container. A tray holding 10 containers (5 with males and 5 with females) is then returned to ambient temperature. Three paper clips are placed on the closed butterfly valves of each holding container. After the catching container is positioned over the holding container, the butterfly valves are opened for 3 min. The paper clips fall into the holding container, and this sudden movement produces the startle effect. Flies stimulated to flight enter the catching container where they are captured on the sticky surfaces and are counted. The test populations are paired and results are analyzed as for a paired t-test.

To select flies with a greater potential for survival in a field cage, we make releases of ca. 50,000 flies. When the population is almost depleted, survivors are captured and used as parent

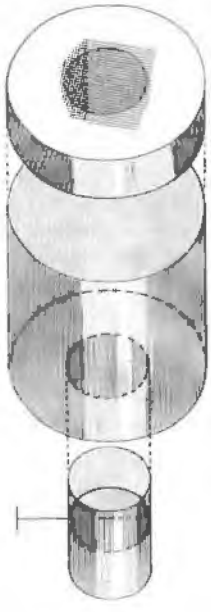

Fig. 1. The startle-test apparatus consisting of 2 cardboard containers.

stock. A tray protected from ants and suspended within the cage provides sugar, water, and hydrolyzed protein.

Variables, Design, and Analysis of the Experiments

In the test for startle response, the variables are minimized by pairing the test populations. The entire test is repeated 5 times, i.e., with 50 units; however, data for the 10 units/tray constitute a paired treatment and are analyzed as for a paired t-test. Important biological variables include age, sex, and nutritional history. Time of day of the test and temperature and humidity conditions prior to and during the test should be controlled.

In the outdoor large-cage test, the sex ratio of the residual population is determined periodically. This is done to avoid loss of either sex.

Possible Modifications and Expansion of the Technique

The techniques are easy and inexpensive and can be utilized to select insect populations with a lower threshold for startle activity. This selection will increase startle activity and probably increase the ability of flies to successfully seek food and shelter in the field. The startle response for each rearing generation can be determined and used as an indication of insect quality and of the impact of treatment, diet, age, etc.

Limitations

This technology requires the building of at least 50 chambers but no special skill.

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MEASURING LOCOMOTOR ACTIVITY IN DACUS CUCUMIS

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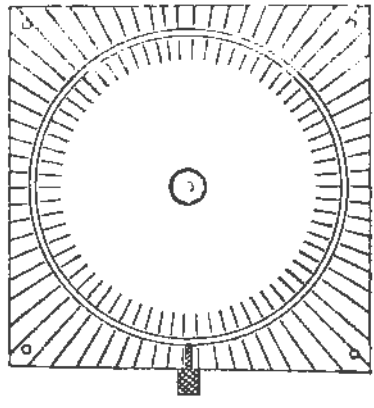
Description and Objectives

Wild populations of fruit flies include individuals with capabilities over a range of locomotor performance. Laboratory selected strains may differ from wild strains in the average locomotor performance of their individuals. Further, various treatments and the environment of fruit flies (e.g., diet, temperature, insecticides and ionizing radiation) may affect locomotor activity.

Locomotor activity is easily and directly measured using a simple apparatus that costs less than $2.00. The tests take little time, and the technique is suitable for routine monitoring.

Materials and Methods

The apparatus used to measure locomotor activity in Dacus cucumis is similar to that used by Connolly (1966) to select active and inactive strains of Drosophila (Fig. 1); this was slightly modified for use with the larger D. cucumis (Bailey 1975).



Briefly, the apparatus consists of a perspex base, 11 cm square, 1 cm thick, into which is cut a circular groove, 8-cm outside diam, 7.2-cm inside diam and 0.5 cm deep. The base is fitted with a clear perspex top with radii engraved from the centre at 5° intervals so that they intersect the runway beneath. A single fly is introduced to the runway by means of a hole, 0.5 cm square in section, cut into one side of a square base. The hole is then plugged with a close-fitting piece of perspex.

A single fly is induced to enter the runway by placing a light opposite the entrance hole; this method of introduction could also be used for other species of fruit flies that respond positively to light. This obviates the need to anaesthetize insects before introduction to the runway. Anaesthesia may affect the subsequent performance of the test insect.

Once inside, the fly is allowed 3 minutes to habituate. Then, for the following one minute the number of lines that the fly crosses is counted. This total, the activity score, can be easily converted to distance travelled for comparative purposes. After testing, the fly is removed and a new fly is introduced.

The number of replicate tests necessary to detect a difference between two groups of flies depends upon the magnitude of that difference, and the degree of confidence required for detecting that difference. For example, a difference (at the 95% level of confidence) between male and female locomotor activity in D. cucumis was detected by using 50 replicates of each sex. Routine monitoring to detect changes in successive generations of laboratory reared and wild flies of the same sex may require fewer replicates.

Variables, Design, and Analysis of the Experiments

Variables that require standardization are: 1. Uniform light conditions, preferably in an enclosed room with diffuse, uniform illumination on the test apparatus. 2. Test at a specific time of day to eliminate any effects of diurnal rhythm of locomotor activity. 3. Age of test flies, although tests with D. cucumis did not reveal any difference in locomotor activity with age (4-15 days). 4. Because of sex differences in locomotor activity, male and female scores should be kept separate. 5. Temperature and humidity.

Procedures for analysis of the data will depend upon the information sought. In quality control this will commonly consist of establishing a "standard" performance of wild flies, then making routine comparisons with successive generations of laboratory strains. These data may be simply displayed in a histogram control chart. However, where large numbers of flies are to be tested over a long period of time, the time taken to develop a statistical control system and a variable sample size procedure (as outlined in Moroney 1962, Chapters 11 and 12) may save considerable time in testing.

Possible Modifications and Expansion of the Technique

This technique may be used to select active strains of flies to improve laboratory cultures.

Limitations

The apparatus is simple and cheap, and no special skills are required for its use. No limitations for use of this method are foreseen beyond those of interpretation arising from the controlled and restricted test conditions.

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ACTOGRAPHIC MONITORING OF ANASTREPHA SUSPENSA COLONIES

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Description and Objectives

The frequency and amplitude of locomotor activity of Caribbean fruit flies, Anastrepha suspensa, are determined routinely to indicate overall vigor of the colony. Populations of flies are confined in individual cages that are monitored simultaneously by actographs to determine the variation among samples or to evaluate different sexes, strains, rearing procedures, etc. Relative adaptedness is determined by maintaining cages under different environmental conditions during the tests.

Materials and Methods

Currently, the vibration-sensitive actograph system includes 2 controlled environment rooms, 2 banks of 6 insect cages each, 12 sensors, 12 amplifiers, and a strip-chart recorder (Leppla and Spangler 1971, Chambers 1975). The sensors are ordinary contact microphone cartridges mounted in metal cans and attached to the cages. For each test, pupae and teneral flies are held at 26±1°C and 80±15% RH with a 14-h photophase (310-750 µm, 183-312 lux), provided with water, and given a food strip made of a brown sugar and yeast hydrolysate paste. Adults are sexed at 1 to 2 days postemergence and transferred to similarly provisioned 14.4x14.4-cm diam cylindrical wire screen test cages held under appropriate environmental conditions. For three consecutive days, hourly levels of locomotor activity of sexually mature (8-to 12-days old) populations of 40 males, 40 females, or 20 pairs per cage are recorded (Leppla and Turner 1976).

Variables, Design, and Analysis of the Experiments

For each trial, the rooms and equipment are cleaned, the sensors, electrical circuits, and recorder are activated, the photoperiod clock is inspected, the environmental monitoring instruments are serviced, and the insects are checked. Records are kept of the source, species, stage, sex, number, age, and mass of these test populations. All aspects of the system are rechecked, and mortality is noted daily.

Electrical signals generated in the sensors by insects landing or falling against the cages are stored in capacitors for an hour, amplified, and printed as a single line per cage. Relative activity is determined by manually plotting the amplitudes of these hourly values. Digital displays, printers, and other data transformation equipment are used for more immediate determinations (Hamilton 1977). Regardless of the electronics, data are analyzed from at least the second day of the 3-day trials. The mean 24-h and hourly diurnal activity levels, thresholds relative to light intensities during phase transitions, and overall periodicity of activity of test populations are compared.

Possible Modifications and Expansion of the Technique

Phonograph and microphone cartridges, speakers, and other devices that produce output pulses may be used as sensors. Microphones and speakers detect both sound and substrate vibration, and the ceramic-element types are durable, relatively impervious

to water vapor, and stable at laboratory temperatures. Applications and options for the current techniques are essentially unlimited; however, automated data acquisition and processing would facilitate the analysis of results from a large number of samples. A variety of actographs are available for monitoring specific kinds of behavior (Cloudsley-Thompson 1955, Andrieu 1968, Miller 1977).

Limitations

Actographic analysis is sensitive, inexpensive, and versatile, but it does not identify specific locomotory behavior patterns. Also, since sensors and circuits are not uniform, records are averaged for each cage and compared as percentages of activity per time interval. Then these results are related to other measurable parameters. The audio amplifiers currently in use are obsolete and should be replaced with less sophisticated voltage amplifiers. The expense of establishing banks of cages is negligible, and a 6-channel event recorder may be purchased for ca. $250.00.

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ACTOGRAPHIC MEASUREMENT OF ACTIVITY IN DACUS OLEAE

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Description and Objectives

There are indications that certain activities of the artificially reared olive fruit fly, such as mating, flight, walking and feeding have been changed when compared to wild flies. Two of these activities, mating and total activity, can be studied with an actograph using a device to detect and record the sounds produced. The method employed involves a continuous recording of the sound that is produced by the fly. This sound consists of 4 elements: 1. Flight sound; 2. Stridulatory sound, produced only by males during the mating time; 3. Sound that is produced when aggressive males attack other males or females; 4. Walking sound of the fly.

Methods and Materials

The fly container: One or more flies are kept in a bottomless jar (8 cm diam, 12 cm high), standing on a piece of plexiglass (0.8 cm thick), supporting a microphone in a central hole. A round aluminum dish with solid diet, a water bottle, and a ceresin dome or green olives for oviposition are placed in the jar. Twelve fly containers are placed in a semi-soundproof box (external measurements 157x54x50 cm). The 12-cm thick box walls are made of successive layers of wood, cork, rubber, and perforated cardboard. The lid is made of two sheets of 0.8 cm plexiglass separated by a 5 cm-wide air space. Each recording channel consists of a crystal microphone, a preamplifier, an amplifier, and a channel of an Esterline-Angus event recorder. In essence, the microphone transforms the sound into an electrical signal which is amplified in two steps and then rectified. When the signal reaches a certain voltage and has passed an RC circuit it will deflect the ink pen of the event recorder, which records the signal on a paper roll moving at a speed of 2 cm/h. The following combinations of wild and artificially reared (sterilized or normal) flies have been used: 1. One or more males; 2. One or more females; and 3. Mixed sexes. The tests were run in a light-tight room at 25°C, 60±5% RH, and under different light regimes and light intensities. Simultaneous direct observations complete the studies.

Variables, Design, and Analysis of the Experiments

The following variables have been identified and require standardization: 1. Biological variables: Sex, age, sexual maturation, mating history, body size, and the number of insects in the fly container. 2. Environmental conditions: Temperature, humidity, light intensity, light quality (white or monochromatic), and length of the scotophase (Zervas, unpublished data). 3. Technical variables: Sensitivity of the microphones, acoustical isolation, and the influence of the electrical current on the electronic device.

In the case of virgin flies, sexual activity could be distinguished as increased activity during the mating period. In females the total activity also contained elements of oviposition activity.

Careful examination of actograms revealed that: 1. In both sexes activity is exhibited only during the photophase. 2. Female activity is equally distributed throughout the photophase and is more intensive than male activity. 3. Males, less active in

general than females, are mainly active during the mating period. 4. The activity pattern in wild and artificially reared flies is similar, but wild flies are more active than the latter.

Possible Modifications and Expansion of the Technique

The potential uses of the device can be considerably increased by using special equipment such as an oscillator, type recorder, digital printer, special noise filter, and by changing the fly container and the sensitivity of the microphones. Thus it would be possible to record separately stridulatory, flight, or walking activity of the fly for more complete interpretation of the total activity recorded.

Limitations

The method requires special equipment, which is not available on the international market, and knowledge of basic electronics.

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FIELD DISPERSAL STUDIES WITH DACUS OLEAE

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Description and Objectives

The normal dispersal of artificially reared or sterilized flies is of major importance in SIT programs. In laboratory studies it has been found that the flight capacity of artificially reared flies is drastically reduced as compared to wild flies (Remund et al. 1977). The objective is to study such differences in the natural environment of the fly. Artificially reared and wild flies or untreated and sterilized flies can be compared within a 2-3 week period using a network of McPhail traps (or other traps). Marked flies are released in the center, and their dispersal is studied.

Materials and Methods

A sufficiently large number of wild pupae (if possible 5000) are collected from infested fruit. Fruit are collected and placed in a screen-bottomed box so that emerging larvae can pupate in sawdust or sand underneath the screen. If the fruit are still green, a moist cloth is placed over them to prevent desiccation and induce pupation outside the fruit. A similar number of artificially reared pupae (Tsitsipis 1975) are collected the same day. If it is not possible to collect an adequate number of pupae in a single day, adult emergence from pupae collected over a period of several days is synchronized by low temperatures (Tsiropoulos 1972). For ptilinal marking, pupae are placed in trays and covered by successive layers of fine sand (1 mm), 30% fluorescent dye in talc (1 mm), and sand (8-10 mm) on the day the first adults emerge. In studies of sterilized flies, irradiation is done just before the dye is applied. Only water and sucrose are given to the flies prior to release. On the day of release, the flies are immobilized at 3-6°C, sexed, counted, and placed in paper bags which are immediately taken to the field. Flies are released in an olive tree in the center of a grid of traps.

Standard McPhail glass traps charged with protein hydrolysate are placed in the olive grove, 42 m apart. They are hung in the north-west side of the trees, 3-4 m above ground. The total area covered is 0.5-1 km^2. If labor availability permits a larger area should be covered by traps. Six to eight groups of 5-10 traps each, are also set out in the surrounding olive groves at ca. 0.5-1 km from the boundary of the experimental grove, to check flies that will possibly disperse beyond its boundaries.

Traps are activated at the time of release and are checked and serviced every second day. Trapped flies are crushed between two rectangular pieces of glass and the presence of fluorescent dye is determined by observation under UV light (Fletcher and Economopoulos 1976).

Variables, Design, and Analysis of the Experiments

Age, sexual maturation, body size, and area of origin should be considered carefully, especially when artificially reared flies are compared to wild ones. The optimal age for flight performance and sexual maturation should be determined before the dispersal of lab and wild flies is studied (lab flies are known to mature earlier). This can be studied with a flight mill apparatus. Wild flies should originate, if possible, from the area where dispersal studies are planned. Since the wild flies

exhibit a greater dispersal capacity, an adequately large area should be used in the experimental design.

The dispersal patterns of flies at various distances around the release point have been described by Fletcher and Economopoulos (1976). The method was similar to that used by Fletcher (1974) with D. tryoni.

Possible Modifications and Expansion of the Technique

The technique has been used to study the dispersal of artificially reared and wild flies through narrow valleys to olive groves at higher altitudes (Economopoulos et al. in preparation).

Limitations

The technique requires a rather large number of man-hours to check and service the traps, and to examine the flies for markers (especially in the fall when the wild flies are abundant). No special skills or equipment are required.

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FIELD EVALUATION OF THE MOVEMENT OF CERATITIS CAPITATA

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Description and Objectives

Mass rearing procedures for the Mediterranean fruit fly can seriously affect the field performance of released flies. Strains of different geographic origin used in a sterile insect technique program may exhibit different physiological and ecological traits resulting in additional deviations in important quality traits due to the specific environment. The study of the movement of different strains under field conditions is a valuable supplement to preliminary studies in the laboratory and in field cages. Comparative tests with different strains can cover the following aspects: 1. Local movement (measurement of the dispersal rate and pattern); 2. Host finding (measurement of arrival on host plants in time and space); 3. Localization of fruit (measurement of arrival on fruit in time and space).

Materials and Methods

The basic method used in these experiments is the mark-release-recapture technique. Pupae of wild and/or laboratory populations are differentially marked with fluorescent powder and emerging adults are transferred daily in equal numbers to release-cages containing food and water. The release is carried out according to the experimental design described later. The recapture of marked flies is achieved either by plastic olfactory traps (e.g., Nadel trap baited with trimedlure and DDVP), or by knocking the insects down with insecticides and collecting them on plastic sheets spread under the trees, by sticky coated nylon nets (Chambers et al. 1972), or with sticky-coated plastic fruits. Captured flies are examined under UV light for the marker. Traps are serviced daily until no more marked flies are captured for 3 days.

General information about these topics has been provided by Chambers (1976), Chambers et al. (1972) and Cirio et al. (1974). Methods for the study of local fruit fly movement have been described by De Murtas et al. (1972), Fletcher et al. (1976) and Sonleitner et al. (1963), for the study of host finding by Moericke et al. (1975) and Prokopy et al. (1975), and for the localization of fruit by Prokopy (1968) and Prokopy et al. (1973).

Variables, Design, and Analysis of the Experiments

The following variables should be standardized in order to reduce the overall variation: age of the flies, type of larval substrate, number of laboratory generations, larval density per kg of fruit or artificial diet, and conditions during rearing and storage of the release cages.

The following experimental design is suggested: Local movement: 1. Select one experimental crop of at least 2-ha surface and homogeneously distributed. 2. Release the flies at a central point of the experimental area in a ratio of about 20 flies per tree. 2. Start the recapture 2 days after the release by arranging the sampling locations in a grid, block, or cross system, each point 10 trees apart. 4. Increase the number of released flies or density of trapping devices according to the recapture data. Host finding: 1. Select a tree in the central part of the experimental area according to the stimulus to be investigated (e.g., shape or olfactory stimuli). 2. Surround the tree with a fence made of sticky-coated nylon nets (2 m higher than the tree). 3. Release marked flies under all surrounding trees and start checking the

horizontal and vertical strata of the net for captured flies in 2-h intervals. Keep the net clean of trash insects. Fruit localization: 1. Select a tree in the central part of the experimental area and remove all natural fruits. 2. Suspend sticky-coated plastic spheres in the different vertical and horizontal quadrants of the crown. The perforated spheres should be of the same color and size, and contain a natural fruit in prime stage for oviposition. 3. Release marked flies under the tree and check devices for captured flies every 2 h.

The analysis of data can be carried out by standard procedures described by Lewis et al. (1967) and Southwood (1965).

Possible Modifications and Expansion of the Techniques

The study of local movement can easily be expanded to an investigation of the flight range of the target species under various environmental conditions, provided that an equal trap density can be maintained over the entire range from the release point. Recapture data can be used for the assessment of the flies' longevity under field conditions.

Limitations

One serious limitation with any field experiment is the multitude of environmental factors that makes replication in time impossible. Another factor that might hamper comparative movement studies is the possibility that differences could occur with respect to the response of various strains to olfactory traps (e.g., to trimedlure). This aspect should be investigated in preliminary tests.

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INTERPRETATION OF MARK-RELEASE-RECAPTURE DATA

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Description and Objectives

Successful eradication or suppression using the sterile insect technique requires releases of adequate numbers at appropriate times. Also, release of a super abundance of sterile flies is inefficient. Assessment of the number of flies in the target population (along with an estimate of the competitiveness of the released flies) allows timed release of suitable numbers of sterile insects. Therefore, to determine the number of sterile insects required for the eradication of the melon fly, Dacus cucurbitae, from Kume Island, Okinawa, attempts were made to estimate population parameters (density and mortality rate) of wild melon flies based on the mark-release-recapture technique. Analysis was made using a set of new equations developed by Itô (1973) and Hamada (1976).

Materials and Methods

Male flies of known age, obtained from our mass-rearing facilities, were anaesthetized with carbon dioxide and individually marked with a dot of lacquer on the dorsal part of the thorax. These flies were then released in a 4-ha square plot including vegetable fields, bush and small woods. Recapture was made with 25 plastic traps, baited with a male attractant (cue-lure) and naled and distributed over the plot. The number of insects caught in 25 traps was checked every 5th day.

Analysis of Data

The following equations (Hamada 1976) were used to analyze our recapture data:

$$z_i = \frac{10^4 m_i}{u_j M'_{0(i)}} \qquad (1)$$

$$M'_{0(i)} = M_0 - \sum_{j=1}^{i-1} m_j \qquad (2)$$

where M_0, u_i and m_i represent the number of marked male flies released at time 0, and the number of unmarked and marked flies recaptured at time i, respectively. To avoid large negative bias due to trapping mortality, modified values of M_0 and $M'_{0(i)}$ were used to calculate z_i, which is an index of the number of recaptures when we postulate that 100 flies were released at time 0 and 100 flies were caught at time i. If adult mortality was constant, plots of z_i on a semi-logarithmic paper against time should be arranged on a straight line. This is the case in our experiments.

Here

$$\log z_i = \log z_0 + i \log S. \qquad (3)$$

where S is the survival rate (for 5-day intervals) and z_0 is the theoretical value of recaptures at time 0, when neither mortality nor recruitment takes place. Then we can calculate the number of wild males using an equation

$$\hat{U} = 10^4/z_0 \qquad (4)$$

where $\hat{U}$ is the estimated number of unmarked flies at time 0.

An example of the results is shown below.

Density of male flies per 4 ha ($\hat{U}$) and survival rate (S) per 5 days

Station	A (Nov.)	C (June)		D (June)	
Mark	Red	Red	Blue	Blue	White
$\hat{U}_h$	2611	1359	1222	1220	1048
$\hat{U}_j$		983		960	
$\hat{S}_h$	0.651	0.226	0.407	0.318	0.403
$\hat{S}_j$		0.277		0.428	

Note: $\hat{U}_h$ = Hamada's method; $\hat{U}_j$ = Jolly's method.

Good agreement of three estimated values for $\hat{U}$ and S at station C and D indicates high accuracy of our method. Based on the estimations made at Kume Island with different density levels and on different vegetation we estimated that the number of wild males reached about 2.5 million at the peak of the season. The survival rates per 5-day intervals were 0.3-0.6.

Modifications, Expansion and Limitations of the Technique

This method is convenient for the estimation of fruit fly densities applying a single release-multi-recapture census. It can be used in those cases where population density fluctuates gradually without large amplitude.

The assumption that the population is constant should be satisfied but this limitation can be solved by shortening the period between release and recapture. The negative bias in the estimation of density remains in the method described. However, Hamada (1976) presented a table that can be used for the evaluation of the intensity of that negative bias. Computer simulations for increasing and decreasing population densities should be made in the future to study this inherent bias in greater detail.

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Orientation to Habitat

INTRODUCTION

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The natural habitat of tephritid flies consists of those host and non-host plants on which members of the wild population may be found. The plants may provide the flies with the resources of food, rendezvous sites for mating, oviposition sites, and shelter (Southwood 1973, Hocking 1975, Prokopy 1977). If released laboratory cultured flies are to survive and compete successfully with wild flies, they must be comparable to wild flies in ability to locate these resources. This implies comparable levels of response to specific stimuli emanating from the resources. What are some of these stimuli?

The principal adult food of tephritids is insect honeydew, with additional food being pollen, nectar, plant sap exuding from wounds, bird dung, and insect frass. Because the bulk of the food is situated on vegetation, the process of food detection is closely linked to vegetation detection. Tephritids locate vegetation evidently primarily by vision, with the color of foliage and/or the darkness of plant silhouette being especially important cues (see Prokopy 1977 for references). Within the insect-visible spectrum of ca. 330-650 nm, foliage reflects maximally between 500-600 nm, and it is this band of energy which seems most important in guiding tephritids to vegetation. Olfactory attraction to specific compounds emanating from vegetative tissues may also play a role in some species. Besides vegetation, odors emanating directly from fly food or from the hydrolytic, oxidative, or microbial breakdown products of food are also attractive.

In some tephritids, mating is initiated at the oviposition site (Prokopy et al. 1971). In others, it is initiated on the host plant but not necessarily at the oviposition site (Zwölfer 1974, Prokopy 1976). In still others, it is apparently never initiated on host plants but rather on nearby non-host plants rich in fly food or sheltering sites (Nishida and Bess 1957, Leroi 1975). In some tephritids, confinement of mating to a particular rendezvous site may be extremely important to recognition of conspecifics and avoidance of interspecific matings (Prokopy and Bush 1973, Zwölfer 1974). If released laboratory flies were unable to readily locate the mating rendezvous site characteristic for its species and instead disperse to other sites, they could expend much wasted energy courting individuals of other species instead of conspecifics. The plant stimuli guiding males and females to mating rendezvous sites are, as far as we know, the same sorts as those guiding females to oviposition sites or both sexes to food or shelter.

The odor of host fruit is a specific stimulus attracting several tephritids to hosts in suitable stage for oviposition (see Prokopy 1977 for references). Little is known, however, about the chemical nature of the particular fruit volatiles eliciting such attraction. At least one species, Dacus oleae, is repelled by the odor of non-host fruit (Orphanidis and Kalmoukos 1970), suggesting that tephritid host finding may involve a combination of attractant, repellent, or neutral olfactory processes. The visual cues of foliage color, plant size, and plant shape do not seem specific to hosts.

After arrival on a plant, leaf stimuli acting as contact arrestants (if a host) or deterrents (if a non-host) can be important to the host selection process. Whether these stimuli are chemical or physical (or both) is unknown, but one can readily conceive that flies continually in contact with the inanimate surface of cage walls in the laboratory might have less sensitivity than wild flies to arresting or deterring leaf surfaces in nature.

Once the flies have arrived on a host plant, detection of individual fruits is principally, if not exclusively, on the basis of the physical characteristics of fruit shape, color-contrast against the background, and size (see Prokopy 1977 for references). The stimuli eliciting oviposition after arrival at a potential oviposition site include shape, color, size, surface structure and condition, and chemistry of the site (see Prokopy 1977 for references). Learning and induction also may be factors in fly recognition of potential oviposition sites.

Plants may shelter tephritid flies against intense sunlight, wind, water loss, and natural enemies. Indeed, individuals of several species have been observed "resting" on the leaves of densely foliated non-host plants in the vicinity of hosts (see Prokopy 1977 for references). How the flies locate favorable shelter is unknown, but reaction to light could play a role in some instances.

The habitat of tephritids also includes conspecific and interspecific competitors for feeding, mating, oviposition, and sheltering sites. Intense intraspecific competition for food has been shown to cause dispersal of released D. tryoni flies (Monro 1966). Pritchard (1969) suggests existence of intraspecific competition among D. tryoni for resting sites, while males of several species aggressively defend mating rendezvous sites against competitors (see Prokopy 1977 for references). Competition for available oviposition sites may involve post-ovipositional deposition of fruit juices or marking pheromones on the fruit surface to deter repeated boring attempts, or aggressive interactions between females. Contact with the deterrent can arrest males and stimulate dispersal of females from the immediate area. Laboratory cultured flies should have adequate ability to compete for these resources if they are not to be displaced by wild flies.

While the foregoing suggests that we have some generalized understanding of the nature of tephritid fly orientation to habitat, it is clear that we do not yet know enough about the specific characteristics of stimuli eliciting most responses. Also, we know little about the true nature of the response pattern, whether it be directed oriented movement (taxis), or movement resulting in fly arrival or dispersal through ortho- or klino-kinetic processes (Kennedy 1965). We have nearly no knowledge of the frequency and direction of movements of individual wild flies in nature. Nor do we understand much about how the type and distribution of non-host vegetation in the vicinity of hosts may influence the movements and density of tephritids.

The test procedures outlined in this chapter are based upon the better understood habitat stimuli and wild fly responses. As our knowledge of wild fly orientation to feeding, mating, oviposition, and sheltering sites increases, then additional and more precise field and laboratory procedures for comparing wild and laboratory fly responses can be devised. Until that time, however, the appropriate direction of selection for optimal levels of laboratory fly response to habitat stimuli will remain less defined than we might wish.

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MEASURING ATTRACTION TO FOLIAGE AND FRUIT MODELS

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Objectives

Many frugivorous tephritids utilize visual cues to detect foliage and individual fruits. Several studies have shown that foliage color and fruit shape, color, and size can be effectively mimicked by appropriate inanimate models. For example, Rhagoletis pomonella flies are attracted by the green color of foliage. However, they are even more attracted by yellow, which reflects maximally in the same part of the spectrum as green foliage but at greater intensity. Yellow, especially daylight fluorescent yellow paint, is therefore considered a super-intense or super-normal foliage type stimulus (Prokopy 1972). The shape of Rhagoletis, Dacus oleae, and Ceratitis capitata host fruits is roughly spherical, and wooden or plastic spheres mimicking fruit shape have proven highly attractive to these species. Spheres which are dark in color (black or red) and somewhat larger than the host fruit (e.g., 7.5 cm) have proven the most attractive because the dark color stands out in strongest contrast against this background of foliage and other reflected light and the larger-than-normal fruit size renders the spheres more readily detectable.

The ability of released lab-cultured tephritids to detect foliage and host fruits is important to their location of potential feeding, mating, and oviposition sites. This ability is also important if visual traps incorporating foliage and fruit type stimuli are to be employed in monitoring populations of released lab flies. There is already evidence to show that some lab-cultured D. oleae and Anastrepha suspensa flies are less visually discriminating than their wild counterparts (Agee and Park 1975, Prokopy et al. 1975, Prokopy and Haniotakis 1976).

Materials and Methods

Before any comparison of lab and wild flies is made, the wild population should be studied to ascertain the precise visual characteristics of foliage and fruit models most stimulating to that species. This can be accomplished by constructing wooden or plastic models of different colors, shapes, and sizes, coating them with Bird Tanglefoot to capture arriving flies, and hanging them in host plants harboring a wild population. An array of models should be employed, such that some (the standards) approximate as closely as possible the actual spectral reflectance pattern, shape, and size of real foliage or fruit, while others comprise a range from slight to large departures from the standard. For example, a study of response to fruit shape might involve comparison among wooden spheres, ovals, cylinders, cubes, cones, discs, and squares, each having the same color and surface area. Once the nature of the visual responses of the wild population has been ascertained, wild and lab-cultured flies can be released in the plants and their responses compared (see Prokopy et al. 1975 for further details). It is important that the models placed within a plant be frequently rotated in position to avoid possible artifactual location effects.

Variables, Design, and Analysis of the Experiments

Interactions between color, shape, and size of model can be important. Thus, careful attention must be paid to the design and probable biological significance of each model tested to avoid possible mistakes in interpretation of fly response. Standardizing fly rearing procedures to insure as much as possible that all released flies are in equivalent maturational and motivational state will aid in reducing experimental variation.

Possible Modifications and Expansion of the Techniques

Combining synthetic olfactory feeding, mating, and oviposition stimuli with appropriate visual models eliciting feeding, mating, and oviposition activities can enlarge the experimental scope. Also, synthetic attractants whose precise behavioral effects are unknown (e.g., trimedlure, methyl eugenol) may be better understood after being assayed in combination with a variety of visual models eliciting known types of behavioral response (see Nakagawa et al. 1978). To increase the percentage of released flies captured on the test models, releases could be made into screened field cages containing potted host plants as a substitute for releases into host plants in nature. The validity of this procedure depends on the degree to which the responses of wild flies released in the cages duplicate the responses of wild population flies.

Limitations

A spectrophotometer for analyzing reflectance patterns from foliage, host fruit, and prospective paints mimicking foliage and fruit would be a valuable aid. Otherwise, no special equipment is needed other than a variety of paints and models and a UV lamp to check on dye-marked flies.

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TESTING THE RESPONSE OF FRUIT FLIES TO TREE MODELS

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Description and Objectives

Experiments with large two-dimensional tree models have shown that Rhagoletis spp. are attracted by visual stimuli that are associated with trees (color, shape, size and position) (Moericke et al. 1975). Yellow rectangles capture increasing numbers of flies as model size increases from 15x20 cm to 122x244 cm. However, the reactions of R. fausta and pomonella to dark red rectangles differ: R. fausta is not attracted by dark red panels regardless of their size, whereas pomonella is captured in increasing numbers with increasing size of the model. This response was considered to be a reaction to a silhouette. The purpose of such experiments is not only to find orientation patterns characteristic of the different species but also, as part of a quality control program, to detect possible deterioration of these traits in laboratory reared strains.

Materials and Methods

The suggested comparative experiment involves the assessment of the number of flies attracted by sticky-coated rectangles of two sizes and two colors. Plywood panels painted with a dark red and a pure yellow color are prepared in sizes of 15x30 cm and 60x120 cm, respectively. They are attached to and supported by vertical wooden poles spaced 6 m between the models and approximately 5-20 m from the closest host tree. The height above ground at the lower edges of the small boards should be 105 cm; that of large models should be 60 cm above ground. A test series of these panels with adequate replicates is installed in an open field, preferably just outside an orchard. The frequency of observation of the boards and of collecting the flies (stored in small test tubes with gasolene for later examination) depends largely on the flight intensity of the test species. The models should be rotated three times in order to avoid positional effects. The captured flies are examined in the laboratory with respect to sex and ovarian development.

Variables, Design, and Analysis of the Experiments

The distance between the rectangles and the closest trees should be large enough to provide a flight range of normal inter-tree flight. However, too large a distance between models and orchard considerably decreases the number of arriving flies. Thus, the optimum distance should be determined by experimentation. The experiments should start at the beginning of the flight period and be repeated at periodic intervals in order to determine the influence of the changing physiological state of the fly population.

Possible Modification and Expansion of the Technique

Instead of investigating the orientation behavior of wild populations in orchards the experiment can be carried out in an open field without host trees. Two or more adequately marked strains of flies are released at a given distance from the models and their responses tested simultaneously. In this situation we recommend transporting the flies in adequate containers to the release site and letting the flies emerge from

confinement ad lib. in order to avoid escape reactions. Small branches of a host tree that cover the release containers and provide a suitable starting platform should be included in the experimental design.

Additional data can be obtained in the same experiments. The height of flight can be determined by counting the flies captured on the lower and upper halves of the rectangles or, by an even finer stratification, in the center or at the margin of the model. To find the most favourable height of the panels, 4 small rectangles can be installed at 4 different heights above ground (e.g., 60, 90, 120 and 150 cm, respectively). The position of the small rectangle inducing the greatest fly catches could be used in comparative tests with large models.

Models with different shapes, edge conformations, or colors can be tested. A combination of shape with host-specific odor was tested with success in our experiments. Species infesting low plants such as vegetables should be tested by comparison of vertically and horizontally oriented models.

Limitations

These experiments do not require special skill and no expensive equipment.

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TESTING RESPONSE TO HOST LEAVES IN DACUS OLEAE

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Description and Objectives

The response to the host tree is of major importance in quality tests comparing artificially reared and wild flies. Possible differences may affect the dispersal and distribution of released populations. Since olive flies utilize both olfactory and visual cues to locate host trees from a distance, their ability to respond normally is highly important, especially in ecological studies and SIT projects. The comparative response to host leaf and non-host leaf color, and empty space (simulated by clear plexiglass) can be studied by glueing fresh-picked leaves onto clear plexiglass rectangles and trapping alighting flies on Tangle-Trap ^{R}.

Materials and Methods

Freshly picked leaves can either be cut into rectangles or squares and glued contiguously to leave no empty spaces in between, or glued as they are onto the plexiglass. Glueing is accomplished by pressing the leaves onto a dry but tacky coating of contact cement covering the plexiglass. Leaf color should not be affected by this process, provided the cement is dry prior to glueing. The best method for capturing arriving flies is to stretch white nylon fish net (8x8-mm mesh, 0.3 mm thick) a few cm in front of each clear and leaf-covered panel and brush the strands of netting with molten Tangle-Trap. Direct coating of the leaves with the sticky material can lead to leaf discoloration on exposure to strong sunlight. Tangle-Trap was found to have little effect on spectral reflectance properties of various surfaces. Olfactometer tests can be utilized to test possible olfactory effects of leaf odor, contact cement, and sticky material on fly responses.

For fly release, artificially reared and wild pupae are placed under successive layers of sand (1 mm), 30% fluorescent powder in talc (1 mm) and sand (8-10 mm) just before emergence, to mark the ptilina of the flies with dye. Emerging flies are held in lab cages until mature. Then, they are chilled, sexed, counted and taken to the field for release, either in olive tree canopies in which small panels are hung or in an open field at various distances from large (e.g., 130x200 cm) leaf-covered panels. Releases in open fields are best made under large cut olive branches pushed into the ground. This helps to ensure that flies leaving the release site are not in an "escape mood".

Variables, Design, and Analysis of Experiments

By using leaves glued on panels one eliminates variation due to tree size, shape, and leaf orientation and movement. Sun shining on the panels can be avoided by proper panel orientation.

The details of the design and analysis of relevant experiments are described by Prokopy and Haniotakis (1975) and Prokopy et al. (1975).

Possible Modifications and Expansion of the Technique

The technique can be used to study fly color responses to various leaf and color surfaces, at various times in the day, at various light intensities.

Limitations

This rather simple technique requires substantial manual labor for leaf cutting and glueing. Except for a UV lamp to check marking of flies by fluorescent dyes, no special equipment is required. If reflectance curves are needed, a spectrophotometer should be available.

Selected References

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THE ELECTRORETINOGRAM -- AN INDICATOR OF POTENTIAL RESPONSIVENESS TO COLORED OBJECTS

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Description and Objectives

Electroretinographic (ERG) methods have already proved useful in measuring the visual threshold of laboratory-reared (LR) flies relative to a standard such as their wild counterparts (see report by Agee). In this manner the ERG is a useful monitor of general adaptedness. However, the utility of the ERG technique may be extended to use as an indicator of potential responsiveness of LR fruit flies to specific stimuli such as foliage- and fruit-colored objects. Electroretinographic comparisons between LR and wild flies are typically made only at the wavelength of light to which the wild insects are most sensitive, usually in the green or yellow-green region of the spectrum (ca. 530 nm). It is in this region that most leaves reflect best also.

Because fruit are used as well as foliage as a rendezvous site for mating by many tephritids, fruit perception ability is significant in sterile-insect programs, and it is therefore important to determine the visual acuity of LR flies in the region of the spectrum to which they respond when seeking fruit. For Rhagoletis species, ERG responses in the yellow region would be most important when considering potential responsiveness to foliage; responses in the red region would be important when considering potential responsiveness to fruit. However, the Caribbean fruit fly, Anastrepha suspensa, appears to respond behaviorally to orange (590 nm) rather than red objects when seeking fruit (Greany et al. 1977).

Materials and Methods

Electrophysiological methods are described in detail in the accompanying report by Agee. By these methods, the responses of wild and LR insects can be assessed throughout the visible spectrum, and a spectral response curve can be developed. Behavior tests are conducted in the field by employing colored sticky traps of various sizes and shapes (see reports by Moericke plus Bateman 1976, and Greany et al. 1977 for examples).

The influence of specific regions of the visible spectrum upon capture rates is assessed by comparing the capture rate for a given color with the proportion of reflected light emitted in discrete bandwidths across the spectrum. This requires that reflectance measurements be made for each color tested, and the proportion of light in each 10 nm bandwidth may be determined by finding the area occupied in this region as compared with the area occupied by the total reflectance curve. The bandwidths that yield the highest correlation coefficient may be considered the most important in attracting the flies. By plotting the regression of the per cent total reflected visible energy emitted in the most influential region vs. the mean per cent flies captured for each color tested, it is possible to visualize the relationship between hue and intensity of reflected light and their influence upon capture rates (Greany et al. 1977). Application of this analysis to responses of A. suspensa indicated that in predicting the responsiveness of LR flies to fruit-colored objects, light at 590 nm should be used for ERG tests even though the ERG responses are greatest at 530 nm (Agee, unpublished).

Variables, Design, and Analysis of the Experiments

Consideration should be given to the reproductive status and sex of the flies; this applies to both behavioral and ERG tests. The age of the flies may also be important in ERG tests. Trapping results may be influenced by compass position about the periphery of the test tree, distance from the tree trunk, height above ground, seasonal effects, and the species of host tree.

Possible Modifications and Expansion of the Technique

It would be desirable to develop laboratory bioassays to determine behaviorally the responsiveness of LR flies to visual stimuli (i.e., a color perception and orientation test) and to correlate these responses to visual acuity measurements.

Limitations

Electrophysiological apparatus is required for the ERG analyses. A spectral reflectance radiometer (or reflectance data from the paint manufacturer or from a collaborator) is required for reflectance measurements. No special techniques or skills are required for the field trapping studies.

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TESTING RESPONSES TO HOST ODOR IN RHAGOLETIS SPP.

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Description and Objectives

In numerous Rhagoletis species, mating as well as oviposition occurs on the host plant. For reproductive success, it is therefore essential that both sexes be able to locate the host. While Rhagoletis flies do indeed utilize vision to locate vegetation, the visual cues of foliage color, plant size, and plant shape are apparently not specific to hosts (Moericke et al. 1975). The odor of host foliage is also apparently not a cue, but the odor of host fruit is a specific stimulus known to attract at least one Rhagoletis species (pomonella) to hosts in suitable stage for oviposition (Prokopy et al. 1973). After arrival on a host plant, Rhagoletis flies detect individual fruits principally, if not exclusively, on the basis of fruit physical characteristics (Prokopy 1977). After arrival on a fruit, ovipositional behavior of the females and movements of the males may again be influenced by fruit odors, but these particular behaviors will not be considered here. Rather, attention will be focused on the comparative ability of wild and lab-cultured flies released in the field to locate real or artificial trees baited with host fruit odor.

Materials and Methods

If real trees are utilized, they can be either of a host or non-host type. They should be located in a relatively open area free of neighboring fruiting host trees to avoid competing fruit odors, divided into sets of pairs of similar type ca. 20 m apart, and stripped of all fruits. Three or four sticky-coated non-odoriferous visual traps (e.g., yellow rectangles or dark-colored fruit-size spheres) should be hung in each test tree to capture arriving flies. If artificial trees are utilized, they may be constructed of plywood ca. 1x2 m in size, painted yellow to simulate the color aspect of a tree, coated with a sticky substance to capture alighting flies, and erected vertically ca. 20 m apart in an open field. One or two ca. 30-liter containers filled with fresh-picked host fruit in prime stage for fly oviposition should be hung from the upper part of one member of each pair of real or artificial trees. Similar but empty containers should be hung from the other member of the pair. The containers should permit fruit odor to diffuse into the surroundings but exclude any possible visual or tactile contact of flies with the fruit.

Wild and laboratory cultured flies should be caged in the laboratory, appropriately marked with dye for identification, and released ca. 1 week after reaching maturity. Care should be taken that the flies' wings are not damaged during caging. Equal numbers of each sex of wild and laboratory flies should be released downwind from each baited and unbaited tree. The distance from tree to release site may vary from 5-25 m, depending on fly species, tree size, and wind speed. Releases are best made under large cut fruitless host tree branches pushed into the ground. This helps insure that flies leaving the release site are not in an "escape mood". Releases should be made in mid-morning on warm sunny days with a slight wind blowing. A second release should be made several days later (using new fresh-picked fruits), with the positioning of the baited and empty cages reversed.

Variables, Design, and Analysis of Experiments

Utilizing a sufficient amount of fresh-picked fruit, standardizing fly rearing procedures to insure as much as possible that all released flies are in equivalent motivational state, and releasing flies under conditions appropriate for downwind odor detection and upwind flight will all aid in reducing experimental variation.

Further details of design and analysis of relevant experiments are given in Prokopy et al. (1973).

Possible Modification and Expansion of the Technique

Possible attraction to odor of potential new host fruits can be evaluated by this method. Also, possible attraction to host foliage or bark odor can be assessed by comparing fly response to foliage-or bark-baited vs. unbaited containers placed in non-host or artificial trees.

Limitations

This technique requires availability of a substantial amount of host fruits, and a UV lamp to check dye-marking on flies. Otherwise, no special equipment is needed.

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TESTING THE RESPONSE OF CERATITIS CAPITATA TO HOST ODOR

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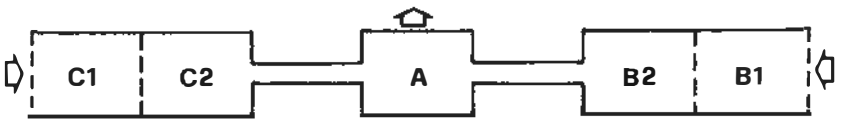
Description and Objectives

The Mediterranean fruit fly tends to congregate on ripening fruit in nature. Host fruits are known to be the oviposition and mating sites for many tephritids (Prokopy 1968, Prokopy et al. 1973). Bush (1974) considers the attraction to and congregation on a specific host fruit to be an important factor in the formation of races and the sympatric speciation of fruit flies. Lack of fruit stimuli in the mass rearing of Mediterranean fruit flies may eventually produce flies that will no longer respond to such cues in nature. Thus, they may be of little value for SIT programs where meeting and mating of released flies with wild individuals is the objective. Therefore, it is important to monitor the ability of the laboratory reared flies to respond to various host odors (sub-test 1) and to compare their responses to those of field-collected populations (sub-test 2).

Materials and Methods

Sub-test 1: The olfactometer used (fig. 1) is a modification of the apparatus described by Haniotakis (1974). It consists of five cylindrical plastic containers (60x45 mm).

Fig. 1

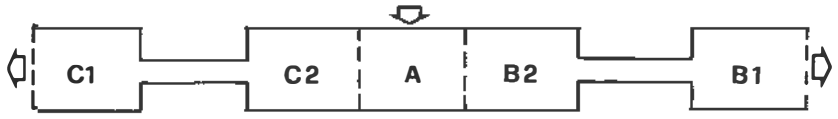


Cell A is connected to the assemblies of cells B and C by a 100-mm-long glass tube (10 mm diam). A screen cloth separates the units B1 and B2 as well as C1 and C2 and also covers the external surface of units B1 and C1. Air is pulled from cell A by a vacuum pump generating an air flow from cells B and C towards the central cell A. A number of such olfactometers are placed under laboratory conditions and illuminated uniformly from above. The number of olfactometers used depends on the number of treatments and replicates.

Twenty-five adult males or females (5 days old) are placed in cell A. A 10-mm disc of the peel of the host fruit to be tested is placed in cell B1; cell C1 remains empty. Flies reaching cells B2 and C2 are recorded every 30 min for a period of 6 h and their numbers plotted against time. The number of flies reaching cells B2 and C2 at the termination of the experiment is compared by Chi^2-test to determine whether the movement towards the host fruit was random or directed. The slope of the curves obtained is a measure for the speed of the response.

Sub-test 2: The five plastic units are arranged as shown in fig. 2. Air is pushed into cell A generating an air flow towards cells B and C. The host fruit peel is placed in cell A and 25 adults (males or females, 5 days old) of the two test populations are placed in cells B1 and C1. Flies reaching cells B2 and C2 are recorded every 30 min

Fig. 2



for a period of 6 h and their numbers plotted against time. The slope of the curves obtained measures the speed of response; the number of flies reaching the target after 6 h is compared by Chi^2-test to detect significant differences in the response of the two populations.

Variables, Design, and Analysis of the Experiments

There is a marked intrinsic variation in the behavior of the flies within a given population. This is especially true for response to chemical stimuli, and minimizing extrinsic variability in behavioral tests is of utmost importance. The following variables should be standardized: Uniformity of illumination of the test arena, temperature, humidity and air-flow. Sub-test 1 with flies of increasing age should be carried out first in order to identify the optimal environmental factors as well as the age of the test flies for achieving maximum response.

The size of the peel and the zone on the fruit from which the peel was taken should be standardized. A single fruit should serve as odor source in comparative experiments in order to assure homogeneous material.

The two tests described can be used alone or in combination to measure the following behavioral components: 1. Response of males and females of a given population to a variety of host fruits. 2. Response of two different strains to a given host fruit.

Limitations

The technique tests a highly variable and complicated behavioral pattern. Therefore, tests should be carried out under defined conditions in order to achieve replicability.

No sophisticated equipment is needed.

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TESTING RHAGOLETIS CERASI FOR DISCRIMINATION OF FRUIT SIZE

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Description and Objectives

Physical stimuli such as shape, size, color and texture are important factors for the selection of oviposition sites in nature as well as for eliciting oviposition into inanimate objects under laboratory conditions (Prokopy and Boller 1971, Prokopy and Bush 1973). Fruit size preference in Rhagoletis spp. is related to their host range and is therefore an important trait for a comprehensive quality control program. Fruit sizes accepted by R. cerasi females for oviposition range from 4 to 24 mm (Wiesmann 1937), and intermediate sizes within this range are preferred. The technique described here defines the fruit size preference of wild strains and provides early detection of significant deviations from that standard caused by laboratory adaptation. The fruit size test stands as an example for similar tests involving other criteria of physical and/or chemical nature.

Materials and Methods

The technique used is similar to that described for testing the oviposition-deterring pheromone (see Katsoyannos). Six artificial oviposition domes are equally spaced in the standard test cage. Two different sizes (10 and 18 mm) at randomly chosen positions are offered simultaneously in this choice experiment. One mated female is tested in each of 20 replicates.

At periodical intervals the number and position of the eggs are counted providing an observation series with an increasing number of eggs laid. The position of the first egg laid provides information on the initial preference for one of the two fruit sizes. This initial choice of a given size by the test female is verified after 3-6 eggs have been laid. At that level the choice of the female is not yet influenced by the marking pheromone deposited after each oviposition. At the level of 20 and more eggs laid per female we can investigate the persistence of the preference for a given fruit size; i.e., we can determine whether the initial choice of a fruit size persists despite the increasing concentration of oviposition-deterring pheromone (conflict situation between fruit size preference and pheromone avoidance). This division of the evaluation into initial and persisting preference patterns proved to be of great help in our analysis of wild host races and laboratory reared strains (unpublished).

Variables, Design, and Analysis of the Experiments

The following variables have been identified and should be standardized in comparative tests: age, oviposition drive and conditioning of the females; fly density; and number of domes per size offered to test females.

It is desirable to standardize oviposition drive and to avoid conditioning to a given fruit size before the test. Mated but immature females are kept during the pre-oviposition period in holding cages without any oviposition devices. At the beginning of the oviposition period (usually at the 5th day after eclosion) the test females are transferred to the test cages. By using single females instead of groups the influences of negative interactions such as territorial and aggressive behavior among females can be avoided; also the performance of individuals can be measured. The influence of oviposition-deterring pheromones necessitates the use of more than one dome per size, and equal numbers of domes of each size tested should be provided.

Suggested approach for the experimental design: 1. Determine the range of host sizes accepted by the wild standard, the size preference, and the plasticity of this preference at higher oviposition drives and oviposition levels. 2. For comparative tests with standard and test strains select two appropriate intermediate sizes within the two extremes of the range that provide real alternatives to the females. The combination of an intermediate size with an extreme will probably mask minor shifts in the flies' response and produce significant differences in favour of the intermediate size. 3. Standardize the variables that influence the overall variation.

Expansion and Limitations of the Technique

The method can be applied for testing the preference for other factors influencing host selection such as color, shape, contrast to background, etc. It can be combined with chemical factors such as oviposition-deterring pheromones in order to test the plasticity of the trait under more difficult conflict situations.

The method requires no special equipment or skill of the investigator.

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TESTING A. SUSPENSA FOR DISCRIMINATION OF OVIPOSITION SITE

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Description and Objectives

To maintain laboratory colonies of fruit flies most similar to their wild counterparts, it is important to define the ovipositional requirements of the wild flies and then provide the conditions conducive to oviposition, insofar as is possible, in the laboratory (Prokopy and Boller 1971). This may help prevent selection through unnecessarily restrictive genetic bottlenecks. As fruit serve not only as an oviposition site but also as a mating site for many fruit fly species (Bateman 1976), changes in the oviposition behavior of a laboratory strain could also adversely affect the mating competitiveness of laboratory reared insects, with concomitant deleterious effects on sterile-insect releases. Techniques were therefore developed for evaluating the influence of physical and chemical characteristics of the oviposition site on the behavior of Anastrepha suspensa. These techniques may be applicable to other fruit fly species as well.

Materials and Methods

The oviposition substrate employed for testing A. suspensa consists of thin (ca. 0.4 mm) 5-cm diam hemispherical wax domes. The wax is prepared by using a mixture of 2 parts household paraffin (41°C m.p.) to 1 part petroleum jelly, and is either left uncolored or is dyed with fat soluble dyes. Domes are formed by covering a round bottomed flask with a single layer of cheese-cloth and dipping the unit first into melted wax (104-107°C) and then into cold water. Release of the dome is facilitated by first spraying the flask with an aerosol lecithin solution. The domes are mounted in holes in petri dish lids and sealed with melted wax. Tests of the influence of size and shape can be performed by altering the conformation of the oviposition substrate.

Bioassays of the influence of internal chemical stimuli (such as sugars, salts, etc.) are performed by incorporating the test material into a 2% agar solution and forming a composite dome by inserting a wedge of treated agar plus a wedge of plain agar (separated by a Parafilm^{R} septum) underneath the wax surface. For tests of the influence of volatile external chemical stimuli, an aliquot of the solution to be tested is applied to the surface of the treatment dome, and an equivalent amount of solvent is applied to a control dome. Bioassays may be conducted by using 25 reproductively mature flies (ca. 14 day old), exposing test domes in a cubical screen cage (25 cm on each edge) for 3 h during the peak oviposition period. Because of the phototactic responses of the flies, the domes are supported in an upright position near the top of the cage.

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Variables, Design, and Analysis of Experiments

Care should be taken to standardize the following factors to avoid undue variation in the data: number of flies, age of flies, time of day and duration of bioassays, position of domes in cage, light intensity, ambient temperature and humidity, and thickness of domes. It is better to use composite domes whenever possible (e.g., when testing non-volatile compounds) to increase the likelihood that the flies will sample both treated and control sites. If two separate units are provided, the flies will sometimes concentrate their attack on only one unit without investigating the other unit at all. We found it of value to analyze treatment vs. control responses using Wilcoxon's matched pairs signed rank test. The criterion for discrimination employed was the number of eggs present at treated and control sites.

Possible Modifications and Expansion of Technique

The techniques described here could be used to test a wide range of colors, shapes, sizes and chemicals and to evaluate possible synergistic effects of combinations of physical and/or chemical factors. These tests could also include provision for monitoring the number and activities of flies present at treated and control sites throughout the bioassay period, such as by time-lapse photography. However, a multichannel monitoring and recording technique would be highly desirable for sake of expediency.

Limitations

No special skills or equipment were required for these tests, but considerable time is involved in setting up each bioassay unit in the case of tests of internal chemical stimuli.

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TESTING RESPONSES TO OVIPOSITION-DETERRING PHEROMONES IN TRYPETINAE

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Description and Objectives

Following oviposition, females in the trypetine genera Rhagoletis, Anastrepha, and Ceratitis drag their ovipositors around the fruit surface and, in so doing, deposit marking pheromone deterring repeated boring attempts in that fruit. Small fruits capable of supporting only one larva to maturity usually receive no more than one egg before deterrence becomes operative, whereas larger fruits receive proportionately more eggs and associated dragging before deterrence becomes operative. It has been observed that if wild females repeatedly contact pheromone-marked fruit, they become hyperactive and prone to flight, presumably resulting in movement away from the area. Hence, possible differences between wild and laboratory reared flies in ability to produce or respond to these pheromones may differentially influence female distribution and dispersal patterns in nature. In this regard, at least one colony of R. pomonella females is known to produce marking pheromone in less quantity or quality than wild flies (Prokopy et al. 1976), while at least one laboratory colony of C. capitata females is known to be much less responsive to the pheromone than their wild counterparts (Prokopy et al. 1977b). Comparative production of and responses to marking pheromone can be readily assayed in laboratory cages by alternately presenting marked and unmarked fruits to each type of fly and observing boring attempt frequencies.

Materials and Methods

Experimental flies should be caged immediately following eclosion and tested ca. 1 week after reaching maturity. The fruit used for bioassay should be uninfested and water-washed, equally acceptable as oviposition sites to both fly types, of small size (ca. 10-14 mm diam) varying no more than 1 mm, and attached to a dissecting needle or stiff wire for ease of handling. Real fruit, but not necessarily host fruit, should be employed (see Prokopy et al. 1976, 1977a, 1977b).

The number of dragging circles around the surface of a clean fruit should be observed in at least 25 individuals of each fly type. Since the dragging pattern is random in direction, the total number of dragging circles usually consists of some complete circles plus some partial circles, which should be added together and recorded in terms of complete circle equivalents. The highest number of dragging circles recorded for an individual wild fly should be adopted as the standard, with the average number for all wild and laboratory flies noted. All pheromone-marked test fruits should receive the standard number of circles. Because fruit odors emanating from boring punctures may act as ovipositional stimulants, there should be no more than one boring hole/fruit. Therefore, to accumulate the standard number of dragging circles, it will usually be necessary to transfer dragging females onto the marked fruit. This is easily accomplished by attaching a small (ca. 4x8 mm) piece of paper to a dissecting needle and placing it under the fore tarsi of a female ovipositing in another fruit. Just as the female withdraws her ovipositor from that fruit, she should be gently lifted onto

the marked fruit where she will usually commence and complete dragging.

To conduct the experiment, first present an unmarked control fruit to a wild (or laboratory) female, and transfer the female to an empty cage undisturbed by other flies. If she rejects this fruit (i.e., leaves without attempting to bore), she should be eliminated from further consideration. If she accepts this fruit (i.e., attempts to bore), she should be allowed to complete ovipositor dragging. Then she should be offered a fruit marked by wild (or laboratory) flies, with rejection or acceptance noted. Next she should be offered a fruit marked by laboratory (or wild) flies. The female should be allowed to rest ca. 5 min between fruit presentations. If she accepts the last marked fruit offered, the replicate is complete, and a different female employed for the next replicate. If she rejects it, she should be offered a control fruit. If she rejects this control, then none of the data for that female should be included in the results, inasmuch as rejection of marked fruit may have been due to low oviposition "drive" rather than influence of marking pheromone. At least 25 replicates of each fruit type should be carried out for each type of female assayed.

For comparison between wild and laboratory females to be meaningful, wild assay females should accept 75% or more of the control fruit but no more than 25% of the wild-marked fruit. If this is not the case, then possibly the ovipositional drive of the assay females is too high (see next section), or the test fruit is too large relative to the amount of pheromone deposited.

Experimental Variables

The natural variability of ovipositional threshold levels among wild and cultured assay flies is the major variable influencing experimental results, provided the above conditions have been met. Too low an ovipositional threshold (i.e., high drive) can result in boring attempts despite the presence of a substantial amount of pheromone. Standardization of assay females with respect to ovipositional drive is therefore essential. This can be accomplished by providing the flies with continuously available oviposition sites, and then, ca. 1 h or less before testing, allowing prospective assay females to oviposit in fruits until only one egg (or egg clutch) per fruit visit is laid. Initiation of a boring attempt without departure from the fruit after ovipositor dragging is not the normal behavior of the flies in nature and therefore represents an abnormally high oviposition drive.

Possible Modifications and Expansion of the Technique

Artificial oviposition substrates could be substituted for real fruits. Multiple numbers of control and each type of marked fruit could be simultaneously presented to several caged assay females and the number of boring punctures or ovipositions into each recorded, but this method is not as sensitive as the method described here and is confounded by unwanted additional deposition of pheromone after an oviposition.

Limitations

Except for appropriate test fruits, no special supplies or equipment are necessary.

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TESTING THE RESPONSE OF R.CERASI TO FRUIT-MARKING PHEROMONE

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Description and Objectives

Rhagoletis cerasi females deposit a marking pheromone immediately after oviposition by dragging their ovipositor over the surface of the oviposition substrate. This pheromone prevents repeated ovipositions in fruit already infested and also plays a rôle in the sexual behavior of the flies as a male arrestant and aphrodisiac.

Production, detection and discrimination of the pheromone by both sexes are therefore important quality traits of high behavioral, physiological and evolutionary importance because they are directly related to host and mate selection. The technique described here measures the qualitative, and by further expansion the quantitative aspects of the phenomenon.

Materials and Methods

The experimental unit consists of a cylindrical 1-litre standard multipurpose cage we use in this laboratory (Katsoyannos 1975). In addition to water and food supply it contains 10 ceresin wax domes as artificial oviposition substrates (Prokopy and Boller 1970). Five randomly chosen domes are coated with a defined amount of the pheromone solution (either aqueous raw extract or ethanol solution; the latter provides a more homogeneous application of the pheromone; Katsoyannos 1975), whereas the other 5 domes remain untreated or receive a control treatment with the pure solvent. One mature, mated female is introduced and the positions and number of eggs laid are recorded at frequent intervals. In our routine tests the experiment is terminated when a total of about 10 eggs has been laid. Each experimental series consists of 20 replicates.

The same initial procedures are adopted to test the response of males. After introduction of the test male the number of visits to treated and untreated domes and the time spent on the two classes of domes are assessed by continuous direct observation during a defined period of time (30-60 min). Additional details concerning the collection, partial purification, and application of the pheromone are given in recent publications (Hurter et al. 1976, Katsoyannos 1975).

Variables, Design, and Analysis of the Experiments

The most important variable is the oviposition drive of the females. The mating status, age, and holding conditions before the test influence the oviposition drive. Holding young females in crowded condition with no or very few oviposition devices over a longer period of time increases the oviposition drive. This tends to decrease discrimination ability until, after an initial intensive egg laying activity, the drive has reached a normal level. Test females are therefore held in small groups in standard cages providing sufficient numbers of oviposition domes and are used in the test shortly after maturation (5-8 days after eclosion).

In the male tests the following variables are standardized: age, time of the day, observation period (duration), and temperature and light intensity. The mating status seems to play a minor rôle.

Several methods can be applied to analyze the data using either absolute egg counts or parameter-free sign tests. It is also possible to use the calculation of discrimination coefficients (DC) such as that published by David and Herrewege (1970).

Possible Modifications and Expansion of the Technique

Female tests: The qualitative yes/no reaction tested can be refined by testing the response to a series of logarithmically diluted pheromone solutions. The lowest concentration eliciting a response is called the discrimination threshold. These thresholds can be used for interstrain comparisons.

In special cases females can be used directly for the application of pheromone to the treated domes. An example is the cross-recognition experiment described by Prokopy et al. (1976). The use of females as direct pheromone source is also indicated in those cases where only a limited number of flies is available. Five domes are exposed to mature, mated females until all domes have received at least 1 egg. Then the females and eggs are removed. The test cage is then supplied with 5 additional new control domes.

The experimental design described can also be used for direct continuous observation of the test female for a defined period of time. Flow diagrams, such as those presented by Katsoyannos (1975), can provide additional information about the behavior of the females as a function of time. A further expansion of the technique is the incorporation of host fruits in choice experiments or the continuous observation of 1 female and 1 male per experimental unit (mating activity, site of mating etc.).

Male tests: Instead of direct and continuous observation of a single male, groups of 5 or more males can be used per cage and their position and actions recorded at periodical intervals. This test can be carried out either as choice or no-choice experiments (all domes treated or some untreated). In this case the frequency of homosexual activities (mating attempts) could be used as a measure of the aphrodisiacal effect of the marking pheromone.

Limitations

This technique requires no special facilities and skill. However, knowledge of the flies' behavior increases the information output.

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TESTING THE RESPONSE OF DACUS OLEAE TO OVIPOSITION DETERRING SUBSTANCES

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Description and Objectives

Studies of the oviposition behavior of Dacus oleae have revealed that the acceptability of the host fruit is largely related to the presence of a natural chemical oviposition deterrent. The active compound has been identified as beta-3,4-dihydroxyphenyl-ethyl-alcohol, a component of the olive juice that is spread by the female over the olive after oviposition. The objective of the method described is the detection of deteriorating sensitivity of laboratory reared flies to this compound or to other oviposition deterrents of natural origin.

Materials and Methods

The experiments are carried out under standard laboratory conditions. Gravid females are transferred 10 days after eclosion to a 20x20x20 cm plexiglass cage and checked for their propensity to oviposit into paraffin domes (Hagen et al. 1963). In addition to a supply of food and water, the experimental cage contains two domes, one receiving a treatment of a defined concentration of the deterrent, the second one serving as an untreated control. The following deterrents can be tested in this two-choice test: beta-3,4-dihydroxyphenyl-ethyl-alcohol; the acetone soluble fraction of soy lecithine; 2-phenyl-ethyl-alcohol and the various natural glucosides containing this compound.

Three mature females are placed in the test cage and the number of eggs are counted every 24 h. The positions of the two domes are rotated after each egg count. At least 10 replications per test have to be carried out.

Variables, Design, and Analysis of the Experiments

The following experimental variables should be standardized in order to reduce overall variation: age of females; oviposition drive and rhythm; density of the females in the test cage; chemical and physical characteristics of the oviposition domes; temperature, humidity and photoperiod in the test room; mode of application and concentrations of oviposition deterrents. We suggested the following experimental design: 1. Perform preliminary tests with females of increasing age to determine optimal response; 2. Calculate the discrimination index for increasing concentrations of the compounds; 3. Define the concentration of the deterrent that can be used for the detection of differences in the sensitivity thresholds of different strains; 4. Determine the persistence of the deterrents under laboratory conditions.

The analytical methods have been described by Dethier (1948), Cirio (1971), Cirio et al. (1976), Vita et al. (1974) and Katsoyannos (1975). The analysis of the two-choice test and calculation of the discrimination index has been described by David and Herrewege (1970).

Possible Modifications and Expansion of the Technique

A multispeed video tape recorder can be used to monitor and count the number of visits of the flies to the two domes, to measure the exploratory period, and to assess the number of oviposition attempts. Other olfactory or chemotactile deterrents of natural or synthetic nature can be tested. This method might also be used for a selection of Dacus oleae populations exhibiting very low or high sensitivity thresholds for a given compound.

Limitations

No special equipment is required to carry out the basic test. If a video tape recorder is used it should have a time-lapse recorder (e.g., Scibaden model SV-612E), and, if possible, a closed circuit TV camera (e.g., Chuomusen QIT-IOIA) and a monitor (e.g., Philips LDH 20).

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Sexual Activity

INTRODUCTION

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The sexual compatibility of laboratory reared and wild insect strains has been recognized as a predominant prerequisite for the successful introduction of a genetic load into a wild target population. It is therefore not surprising that the first quality tests performed in insect colonies (such as the ratio test and the sexual aggressiveness test) aimed at the evaluation of the insects' sexual activity and potential. Indeed, sexual behavior plays an important rôle whenever insects are released to exert a maximum impact on the target in intraspecific action.

However, the exclusive concentration of efforts in a quality control program on monitoring and manipulating sexual activities of mass reared insect colonies might be a mistake because successful copulation between the released and wild partners is only one of many aspects that can be affected by the potential premating isolating mechanisms acting in sequence. A normal or even increased level of sexual performance obtained in laboratory reared strains cannot be exploited to the full extent when the released individuals fail to disperse properly in the field and fail to locate food sources, host plants and sexual partners. These aspects are discussed in other chapters of this book and should find the attention they deserve in the planning stage of a quality control program.

From the methodological point of view we can divide the causes of sexual deficiencies observed in comparative tests with laboratory and wild strains into two broad categories. One group of potential factors influencing the sexual characteristics of a strain is of physiological and physical nature. These factors are important olfactory, acoustic and visual stimuli produced by the flies and needed for successful location of the sexual partner and during courtship preceding copulation. The second group of factors deals with the mating intensity and capacity of individuals or populations, described in this chapter as mating propensity (drive, speed, motivation), mating frequency, mating duration, and assortative mating. Some of these mating characteristics are intimately related to the premating stimuli (e.g., mating rhythm), whereas others (e.g., mating frequency and duration) seem to be independent traits.

A review of the techniques and ideas presented in this chapter allows certain conclusions with respect to the present status of our knowledge and to possible lines of research that might be needed. First of all, the reader will look in vain in this section for the ratio- or competitiveness-test widely used for measuring traits that have been described vaguely as "vigor" or "sexual competitiveness". The multiple causation of significant differences between expected and observed egg-hatch data obtained in ratio-tests has been widely recognized; therefore, the conclusion that the observed deviations have been caused exclusively by sexual traits is not justified. The complex of factors influencing the data (e.g., mating frequency, female fecundity, sperm motility, etc.) seems to justify listing this technique with other methods measuring overall quality or performance. The problem of measuring sexual traits belonging to the second group is evident: most of the techniques described are applied under laboratory conditions, although it cannot be ignored that several traits (such as mating propensity, assortative mating, or mating rhythmicity) are often studied in the laboratory as well as in field cages. Certain important characteristics (e.g., mating frequency and duration) are very difficult or even impossible to investigate by direct

observation under true field conditions. An exception is the interesting approach reported by Zouros and Krimbas (1970) who investigated the mating frequency of field-collected Dacus oleae females by means of the electrophoretic analysis of the allozyme polymorphism in the adults and their progeny. However, observation of the mating frequency of fruit fly males under field conditions is still an unsolved problem.

On the other hand there is increasing evidence that conclusions drawn from laboratory tests measuring sexual traits can, in general, be confirmed by data derived from field cage tests that are conducted on the basis of previous laboratory findings. The main value of laboratory tests carried out under defined experimental conditions is the provision of early indications about shifting threshold values that might not be detectable if field tests were carried out alone. The correlation between mating propensity (speed) and mating frequency in Rhagoletis cerasi, for example, would not have been detected if field cage tests had been the only technique used.

The aspect in greatest need of intensified investigation is the evaluation of the data obtained by various techniques measuring sexual activity. How serious is the effect on the field performance of released flies of deviations of 10, 20 or more per cent in a given trait measured in laboratory and wild strains? Where is the rejection line for elimination of mass reared flies from a release program? What test produces relevant data, and how can we define the standards and specifications? These problems can only be solved by the thorough comparison of data obtained in both sensitive laboratory tests and appropriate field experiments. An encouraging step in this direction is the increasing availability of techniques that can be applied both under laboratory and field-cage conditions that do not just measure indices or static values of a given sexual trait but provide information about the dynamics of the respective trait in a time profile. Measuring mating activities in regular intervals over a defined period of time has increased considerably the information output of mating propensity studies and provided new insight into the mechanics of the complex aspects of sexual behavior. Furthermore, the adaptation of this and other techniques used in Drosophila research to the requirements of the applied problem of quality control in fruit flies might prove to be useful for another purpose that has hardly been investigated so far. This is the study of the heritability of important traits and the selection of strains that match or even surpass these features in the wild target population.

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TESTING SEX PHEROMONES IN DACUS TRYONI

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Description and Objectives

Studies on Dacus tryoni indicated that the males release a multicomponent sex pheromone which is produced in glands associated with the rectum (Fletcher 1969). The pheromone is used at dusk by sexually active males to attract and sexually excite responsive females. Many other male tephritid fruit flies also appear to use sex pheromones to facilitate pair formation and mating. Any experimental treatment that causes a deterioration in the quantity or quality of the males' sex pheromone might be expected to reduce sexual competitiveness. Quantitative bioassays have been developed for D. tryoni which can be used to compare the activity of the pheromone of experimental males with that from wild males, or a standard control group, by measuring the response of females.

Material and Methods

The responsiveness of females to the male pheromone can be evaluated in both laboratory and field bioassays, and as the pheromone(s) facilitates both orientation towards the source and increased sexual excitement at close quarters it is desirable to utilize both kinds of bioassays when making comparisons, as different components may be involved. The two laboratory bioassays are similar in that they measure the percentage of females responding to known quantities of pheromone (expressed in male equivalents) impregnated on filter paper discs. The test females are either enclosed in nylon gauze-covered cages as described by Fletcher and Giannakakis (1973 a,b) or in glass units with a controlled air flow (Giannakakis and Fletcher 1977). For both bioassays the females used were sexually mature virgins 2-3 weeks old that had been kept from shortly after emergence in a photoperiodic regime of 14 h light (10,000 lux), 1 h dusk (ca. 10 lux) and 9 h darkness. For the field cage bioassays large nylon mesh cages (3 m3) were erected over suitable sized fruit trees. Approx. 1000 mature virgin females were introduced into each cage on the day prior to the experiments and supplied with sugar, protein hydrolysate and water sprayed onto the leaves. At dusk (the normal time of sexual activity) the pheromone preparations to be tested were impregnated on 1-cm circles of filter paper placed into holes in the centers of 10-cm diameter perspex discs covered with stickem. These were then suspended in pairs 1 m apart (one experimental and control per cage) 2 m from the ground and 0.3 m from the cage wall on the upwind side of the tree. The females that respond stick to the discs and can be counted after darkness, or if necessary next morning.

Variables, Design, and Analysis of the Experiments

For the laboratory bioassays it is possible to use whole pheromone glands as described by Fletcher and Giannakakis (1973 a,b). It is also possible to use serial dilutions of pheromone (in the range of 10 - 10^{-4} male equivalents) extracted from a known number of glands in a suitable solvent (e.g., ether). For the field bioassays, concentrations of between 10 and 50 male equivalents were found suitable.

It is essential to ensure that the females are sexually mature and to determine the optimum time of day, light intensity, temperature etc., for the bioassays, as

studies with D. tryoni indicated that the females only become sexually receptive and therefore responsive to the male pheromone under low light intensities at the normal time of dusk in their daily cycle. For a comparison of the pheromone activity of experimental and control males in the laboratory bioassays it is best to obtain the per cent response of females to a logarithmic series of pheromone concentrations. Equal numbers of replicates (at least 10) should be carried out for both the experimental and control groups for each concentration in a randomized block type experiment and the results analyzed by probits (Finney 1971) to check for significant differences. For the field bioassays in which the tests of the pheromone from the experimental and control groups are carried out simultaneously the results can be analyzed as paired comparisons.

Modifications and Expansions of the Technique

If possible all comparisons of experimental flies should be carried out against pheromone from wild flies as laboratory populations may undergo changes in sex pheromone quantity and quality even when not being mass-reared or sterilized. If this is not possible then the pheromone of the population that is used as a standard should be checked against that of wild males from time to time. If facilities are available it is possible to compare directly the number of volatile components and their relative concentrations in the secretion from the pheromone glands using gas-liquid chromatography.

Limitations

The equipment for the cage bioassays is cheap and easily manufactured, while the glass units, although more convenient, require a greater initial outlay unless glass blowing facilities and personnel are available. To ensure controlled conditions, a constant temperature room equipped with photoperiod cabinets and a fume hood with an exhaust fan are highly desirable but not absolutely essential.

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TESTING SEX PHEROMONES IN ANASTREPHA SUSPENSA

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Description and Objectives

Bioassays are necessary to determine if either sex produces a sex pheromone, to detect aggregation pheromones, to test artificial lures, and to determine an age vs. response profile with both attractants and pheromones. A bioassay can be used to detect differences between laboratory and field populations in responsiveness to sex pheromones or attractants, and to select strains of flies with a broad or variable response to production of pheromone.

Materials and Methods

Laboratory Bioassay: Flies are sexed after eclosion and held separately in incubators at 26°C, and given a 14:10 LD cycle. Flies can be tested at any age for response to attractants, but they respond to the sex pheromone only as they sexually mature (about 9 days in the lab strain, 14 days in the wild strain). To initiate a test 200 virgin females are placed in a cubical (45 cm each dimension) plexiglass assay box. Live males or extracts of the pheromone are placed in traps fitted into the back wall of the assay box. A removable back in the assay box facilitates different arrangements of trap ports (in a circle, in a straight line) for different purposes. A slow air flow (1-3 km/h) over the source of the pheromone, and directed into the assay box of females and out through an exit port is required. Natural daylight and/or artificial light must be available during a test. Tests are continued for 15-18 h, although usually an unambiguous indication of the presence of pheromone can be observed after 6 h. The sex pheromone response is best when tests are started about 1400-1600 h. Generally all females are replaced after each test, but they can be retested if the supply is short. Extracts work best when made with 100 flies homogenized in a final volume of 5 ml hexane, or equivalent flies/volume ratio. One to 2 ml of extract are pipetted onto filter paper, laboratory absorbent wipes, or other absorbent paper and the solvent is allowed to evaporate at room temperature. The paper is then rolled tightly, folded, and wrapped inside another paper of the same type. Results are expressed as the number of females attracted through the trap port and into the body of the trap.

Field Bioassay: Assays may be conducted with wild populations or with marked, lab-reared flies. Metal wing traps, or single sheets of wood, metal, or plastic (15x20 cm) are coated with tanglefoot and baited with live males in screen cylinders containing food and water, or with dental roll wicks treated with extracts or the pheromone. Traps of certain colors may be more attractive than others. Control traps should be baited with food and water when live flies and food are used as bait in experimental traps. A Latin square trap arrangement is used when possible. In another arrangement a control trap and experimental trap are placed about 6-8 m apart at the same height in a tree and their positions are rotated at intervals. All tests are replicated and data are analyzed statistically.

Variables, Design, and Analysis of the Experiments

Adult age and nutrition influence female response and male production of pheromone. Mated status influences female response.

Typical tests follow these steps:

1. Determination of the sex and age of flies producing and responding to the pheromone: One-half of the traps are baited with males and one-half with females, starting at one day of age. On successive days older ages are tested. In a field assay catches are recorded after 48 h, and in the lab assay after 18 h. In the lab assay all flies in the assay box must be the same sex in any one test.

2. Determination of synergism between 2 or more compounds or determination of the best ratio of components with a multi-component pheromone: Use the field assay and a Latin square design.

3. Location of pheromone in eluted fractions from column chromatography: The lab assay is rapid and useful in this procedure. Combine 1 ml from each of 5 fractions in 1 trap. Arrange up to 6 traps in a circle at the back of the assay box. The upper-most trap should serve as a control since there may be a slight bias toward greater catches in the traps near the top of the box. Repeat assays with individual fractions when activity has been located in 1 or more groups of 5 fractions. Confirm the presence of the attractant in the identified fractions by field assay when possible.

Possible Modifications and Expansion of the Technique

The lab and field assays can probably be extended to most of the tephritid fruit flies. The lab assay box can be constructed from a variety of materials, including sheet metal, wood, plastic, plexiglass, or glass. Since light is needed for the flies to be active, 1 or 2 sides or the top should be constructed of a light-transmitting material. By designing a trap that captures flies alive, one can use the field assay to select breeding stock for culturing flies that give a rapid or very strong response to the pheromone or to certain ratios of pheromone mixtures. The assays can be used to determine effects of rearing procedures, diet, light regimes, and other factors on response to or production of the pheromone.

Limitations

The lab assay requires construction of 1 or more boxes, a small electric fan for each box, a source of electricity, and possibly a rheostat to slow the speed of the fan. The field assay requires only traps and tanglefoot. No special skills of the investigator are required for either assay.

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TESTING SEX PHEROMONES IN DACUS OLEAE

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Description and Objectives

Mating in Dacus oleae is influenced considerably by chemical signals. Sexually mature females release a sex attractant that attracts mature males (Haniotakis 1974). The attracted male releases a chemical signal received by chemoreceptors at the female antennae that elicits female receptivity. Disruption of this male signal significantly affects mating propensity and percentage of successful matings (Haniotakis 1977b). Qualitative or quantitative differences between D. oleae strains in pheromone production or in time of release or response are expected to affect mating. Differences in sexual attraction between various strains have been tested by means of bioassays in lab olfactometers, field cage and greenhouse tests. Live females or natural material collected from females by various methods (Haniotakis et al. 1977) can be used as sources of pheromone.

Materials and Methods

Olfactometer tests: Insects are anesthetized by chilling and sexed before any mating occurs. Males and females are held separately in screen cages under standard laboratory conditions. Starting from 4-5 up to 15 days of age, lab strains are compared to wild strains that are 5 days older because sexual maturation of lab strains occurs sooner (Economopoulos et al. 1975, Zervas 1977). For laboratory bioassays various types of olfactometers can be used (Haniotakis 1974, 1977b; Haniotakis et al. 1977). All olfactometers are based on the same principle, i.e., one cage, preferably of glass or plexiglass for easy cleaning and large enough to allow insect flight, contains the males. Another cage, smaller in its dimensions (flight being unnecessary) contains the females or the attractant. Between the two insect cages a third cage similar to the second one can be introduced to receive the responding males. Mating is thus avoided and insects can be used again the next day for the same or other tests. A funnel-shaped trap placed between the 1st and 2nd cage prevents responding males from returning to the first cage. Responding males are counted at regular intervals in the middle cage during the last 2-4 h of the photophase.

Field cage or greenhouse tests: Greenhouses should be used only when weather conditions do not allow field cage tests. Details of techniques used in field cage tests have been described by Haniotakis et al. (1977). A more convenient trap which has been used recently is a cylinder (7 cm diam, 20 cm long) made of wire screen (0.5 cm mesh). The trap is coated with sticky material and the pheromone source is placed inside the trap.

Variables, Design, and Analysis of the Experiments

There are indications that sexual maturation of wild flies occurs at different ages during the various seasons of the year. Thus, before the tests the sexual maturity of the wild insects must be verified and then compared with the appropriate physiological state of lab insects. In these tests the insects are considered mature when 50% of the females of a sample of 10 have been mated. Temperature, humidity, photoperiod and feeding of the flies must also be kept constant for laboratory tests. For field cage or

greenhouse tests the male density must be kept constant, and the traps must be rotated because the efficiency of the traps may be affected by location and by changes in the direction and velocity of the wind. Due to the high variability in these experiments a large number of replicates is usually required, especially in identifying small differences in attraction between various strains. Analysis of sexual attraction between strains can be made by standard statistical tests.

Possible Modifications and Expansion of the Technique

This technique can be also used to study the effect of various environmental and physiological parameters on the sexual attraction of the olive fly, as well as the diurnal rhythm of pheromone production, release, or response. The technique is also used in studies of collection, purification and identification of sex attractants.

Limitations

This technique requires no special skills or equipment.

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TESTING SEX PHEROMONES IN CERATITIS CAPITATA

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Description and Objectives

A simple, quantitative method for bioassaying the sex pheromones of the Mediterranean fruit fly, Ceratitis capitata, or for assessing differences among strains or treatments of medflies through response to standards is described. Initial tests are done in small cages in the laboratory, additional evaluations are done in an outdoor cage or against a natural population of flies in the field.

Materials and Methods

Laboratory bioassays. Males and females are segregated within 24 h after eclosion and held separately in screen cages at standard laboratory conditions. Populations of 50-100 virgin, 4-10 day old females are transferred into screen cages ca. 1 day before the test. When attractants are to be evaluated, they are applied to short rolls of filter paper placed in traps made of inexpensive and disposable Dixie ^{R} bathroom cups coated with Stikem ^{R} (for details see Ohinata et al. 1973). An untreated trap is placed adjacent to a treated trap in each cage to provide the flies a choice. The reference standard is filter paper previously exposed to a population of sexually mature males. The standard treatment usually attracts ca. 50% of the responder population of laboratory reared virgin females. Possible differences in the ability of males of different strains or treatments to produce pheromones can also be evaluated in small cages by comparing the response of females to filter paper soiled by males to be tested, or to live males (5-10) in small fiberglass screen cylinders placed in the Dixie cup traps. The tests are done in the morning when the mating activity and sex pheromone response of this species would be strongest. Standard laboratory conditions of temperature, humidity and lighting suffice for these studies. The tests are usually run for 30 min; the traps are then removed from the cages and counts made of captured flies. When only limited amounts of pheromones are available for testing, additional information may often be obtained by transferring the previously used filter paper wicks into fresh sticky-cup traps and exposing them to fresh cages of flies for a separate exposure period.

Additional tests of pheromones or assessments of fly quality are made in an 8-m^3 outdoor cage. Ca. 500-1000 sexually mature virgin females are released in the cage about an hour before the test is started. Treatments (including a standard, as well as an untreated blank) in Dixie-cup traps or delta-shaped "Jackson" cardboard sticky traps (Harris et al. 1971) are placed on the rim of a 1.2-m diam wheel which is rotated slowly to reduce positional influences within the cage. Tests are done during the morning for 30 min to 2 h, depending on the speed of response which may be affected by environmental conditions. Counts of captured flies are made at suitable intervals and the behavior of responding flies is observed. The behavior characteristics of normal virgin medfly females responding to the male pheromone include rapid orientation to the odor source, brief sessions of wingbeating, upward curving of the abdomen and ovipositor, and confrontation with, and "bumping" of, adjacent females. In quality control tests, effects due to treatments or strain differences on male pheromone production may be assessed by observing the response of females to groups of 25-50 males in small screen containers placed in Jackson sticky traps. When wild males are evaluated in these tests, they are usually reared out of fruits naturally infested in the field, in order to utilize flies of uniform ages, however, males caught alive in traps in the field could also be used after a suitable holding period.

Field bioassays. Attractants showing high levels of activity in the outdoor cage are further evaluated in the field. Treatments are applied to cotton dental roll wicks and placed in Jackson sticky traps which are hung on medfly host trees. The residual activity of substances to be tested in the field may be prolonged by applying treatments to cotton wicks inserted in polyethylene vial-caps. The reference standard in field bioassays is a treatment of live males. One hundred sexually mature, laboratory reared males are confined in a cylindrical fiberglass screen cage which is placed in the cardboard trap. Food provided by means of a wick in a small vial containing a solution of sucrose will allow the flies to survive for ca. 10-15 days in the field if the traps are placed in shade. Fresh groups of flies in screen containers are replaced in the traps as needed. Tests are usually run for 4-6 weeks and trap catches are recorded once or twice weekly. Effects of treatments or selection on pheromone production of males may be tested under field conditions by comparing wild female responses to groups of laboratory reared, wild and treated males.

Variables, Design, and Analysis of the Experiments

Some of the variables which may affect the bioassays include: sexual maturity and age of flies of both sexes, mated or unmated status of females, temperature and humidity. Flies of different ages of both sexes should be treated to ascertain whether the effects of treatment or selection on response to, or production of, pheromone may become evident with age. Testing in outdoor cages, utilizing appropriate reference standards to monitor response, is recommended as the most practical method for conducting these studies.

Possible Modifications and Expansion of Technique

This method could be used to monitor the quality of medfly males, not only by their degree of attractiveness to females, but also by their response to synthetic attractants. Also, the quality of females of different strains or treatments could be assessed by comparing their response to pheromone standards or to live males in outdoor cage and field tests. When flies of several different treatments are to be compared simultaneously, they could be marked with appropriate dyes.

Limitations

This method requires no special skills of the investigator nor any special equipment.

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FIELD TESTING OF SEX PHEROMONE IN RHAGOLETIS CERASI

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Description and Objectives

The discovery that males of Rhagoletis cerasi attract the females by the release of a volatile sex pheromone (Katsoyannos 1976) has stimulated research on this behavior under laboratory and field conditions. It was observed that the distance of attraction in the field is larger than previously assumed (20 m or more). Also it was observed that many factors influence this important behavioral trait, such as the time of the day, the age of the flies, and the mating history of the females. Obviously, significant deviations of laboratory reared or geographic strains from the patterns of the wild target populations with regard to the release and detection of the sex pheromone can hamper orientation toward the sexual partner in a genetic control program. Therefore, the incorporation of a field test monitoring this important aspect in a quality control program might be desirable.

Laboratory tests with olfactometers have been described in adequate detail by other authors (e.g., Haniotakis 1974, Nation 1972, 1975); therefore, we report here some techniques used in field experiments.

Materials and Methods

The method consists basically of the release of assay flies in a cherry orchard and of monitoring their orientation toward cages containing test males as pheromone source. The details of the test cages have been described by Katsoyannos (1976). The cages containing the males (and empty control cages) consist of plastic 1-liter cylinders that prevent visual contact between test and assay flies, or other attractive stimuli (such as color and odors other than the sex pheromone) and have a mechanism to capture the attracted females (sticky-coated paper above the ventilation hole, fig. 1).

Assay females can be released in large field cages (2x2x2 m) placed around small fruitless cherry trees in which the test cages with males are suspended. Alternatively, females can be released in orchards free of wild flies where the test cages have been suspended on host or non-host trees. In this case we recommend release of the assay females in small batches at different points located around the test cages in order to take into account the influence of changing wind direction. The distance between the pheromone sources and the release points depends on the purpose of the experiment (e.g., testing

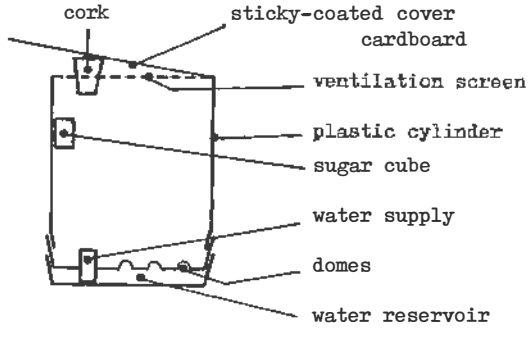

Fig. 1

the distance of attraction or the quantitative response of females in comparative experiments). Similar methods have been described by Prokopy (1975), Perdomo (1974) and Haniotakis et al. (1977) for Rhagoletis pomonella, Anastrepha suspensa and Dacus oleae, respectively.

Variables, Design, and Analysis of the Experiments

The following variables have been identified that should be standardized in order to reduce overall variation: age of the flies, mating history of females, number of males in the test cages,and time of the day. The following environmental factors should be measured during the experiments: wind direction and velocity, temperature, and light intensity. We suggest the following approach for the experimental design: 1. Define in preliminary laboratory tests the optimal age of the flies, the influence of mating (refractory period), and diurnal rhythms of wild standard strains or determine these factors under field conditions with appropriately marked flies. Experimental designs have also been described by Roelofs and Cardé (1977, p. 384); 2. Compare the performance of wild and test strains under optimal as well as extreme conditions; 3. Whenever qualitative results indicate significant differences between strains, try to obtain more quantitative data by increasing the number of replicates carried out on the same day (elimination of variation due to changing environmental conditions).

Possible Modifications and Expansion of the Technique

This method can be used with minor modifications to test the response of flies to other olfactory stimuli such as odors generated by host fruit or leaves (see contributions of Cirio and Prokopy).

Limitations

This technique requires no special equipment or skill of the investigator.

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MONITORING ACOUSTICAL SIGNALS RELATED TO MATING IN ANASTREPHA SUSPENSA

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Description and Objectives

The male Caribbean fruit fly, Anastrepha suspensa, makes a signaling sound prior to pair formation that is believed to coincide with the release of one or more sex pheromones. It is generated by very rapid and precise wing movements and consists of a 2-part pulse train. The waveforms in the pulse train are analyzed with a Fourier analyzer where they are broken down into discrete frequency bands. The amplitude of the fundamental wingbeat frequency and all harmonics included in the waveforms constitute a unique signature of each series of waveforms. This signature is analogous to a voice print. When changes occur in waveforms due to wing movement changes, there are corresponding changes in the frequency bands within the signature. These bands may change in amplitude or frequency, or disappear completely. Comparisons of signatures can identify changes in waveform generated by wing movement. Aberrations in the frequency signature, such as those caused by irradiation or dietary influences, can be readily detected (Sharp and Webb 1977, Greany et al. 1977). The frequency signature data are obtained in ca. 1 min and the test individuals are unaffected and can be used for other purposes.

Materials and Methods

Males and females are sexed after eclosion and held separately under standardized laboratory conditions. At 6 to 10 days posteclosion, 10 of each sex are placed together in screened test cages (Webb et al. 1976b). The test cages are then transferred to an anechoic chamber, and a microphone is positioned 12.5 mm above a signaling male fly. The chamber is maintained at 24±2°C and 50% RH with ca. 3.1-lux light levels to stimulate mating. After the mating signal is recorded for 1 min, the fly is removed from the cage. The analyses can be made in real time and either displayed on an oscilloscope or plotted on an X-Y recorder directly if no tape recorder is available or if gross changes are to be detected quickly.

Variables, Design, and Analysis of the Experiments

The analog data are stored on magnetic tape; then a frequency spectrum (signature) is developed for each fly and for an average of several flies exposed to each treatment by using a Fourier analyzer (Webb et al. 1976a).

Signature analysis is a relatively new technique for describing changes in physical phenomena. The technique has proved to be very valuable and is used routinely in industry where rotating or oscillating machinery is involved. For example, standard signatures are developed for waveforms generated by a given bearing, shaft, etc., when the machinery is in good working condition. Signatures are then developed periodically and compared with the standard to determine changes related to wear in the machinery. Then the worn parts can be replaced at the convenience of the company or before damage

occurs. The same techniques are being used here to describe changes in physical activities of insects. Standard signatures are established for "normal" insects under known conditions. The signatures of "normal" insects are compared with those of insects exposed to a given treatment. The following variables were identified in establishing standard signatures: the age of the males, temperature in the test room, population density in the test cage, and dietary history of the fly.

Overall variation is reduced by defining the daily peak mating period, by using low population density (approximately 10 to 20 pairs), standardizing the distance between the microphone and the fly, and calibrating all equipment prior to testing.

Possible Modification and Expansion of the Technique

Presently, the method requires expensive and highly technical equipment. However, less expensive and less sophisticated equipment may be adapted by conducting preliminary research and identifying those elements in the frequency signature that are important for quality control.

Limitations

The technique, at present, requires specialized equipment and a basic knowledge of signal processing. Use of the technique as a quality monitor will indicate subtle changes in the motor behavior of the insect. Direct interpretation of the impact of these changes on gross behavior is not possible by this technique alone.

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MONITORING ACOUSTICAL SIGNALS RELATED TO MATING IN DACUS OLEAE, CERATITIS CAPITATA, AND OTHER TEPHRITIDS

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Description and Objectives

Our investigations and observations reported by several authors (e.g., Feron 1960, 1962, Monro 1953, Rolli 1976) show that a number of tephritid species produce typical acoustical signals associated with mating. These sounds are not only produced before and during copulation but also during aggregation. They can be recorded with special techniques and can be analyzed with regard to their rhythm, frequency and intensity. The comparative investigation of the sounds generated by different strains of fruit flies (e.g., wild and laboratory reared strains) could help to detect significant differences between strains and be incorporated in a quality control program as an additional and sensitive performance test.

Materials and Methods

Facilities that we have found adequate consist of a sound-proof observation chamber equiped with the necessary recording devices and analyzers: Two tape recorders (frequency range 0-17 kHz, 0-300 kHz, respectively); TV camera and video-recorder and TV monitor (suitable for close-ups); oscillograph and frequency analyzer; impulse generator; Luminoscript for graphical display of sound frequencies; and various microphones. A stroboscope is available for measuring wing beat frequencies. The sound-proof chamber is temperature and humidity controlled, and light can be provided in the range of 0-20000 lux by incandescent or mercury vapor lamps.

Limitations

These investigations require an adequately equipped laboratory and knowledge of basic electronics.

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MEASURING MATING PARAMETERS IN DACUS TRYONI

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Description and Objectives

The mating process can be divided into a number of parameters, each of which can be measured and used in assessing the quality and sexual competitiveness of experimental flies. In the case of males, the three major components of the process are 1. the ability to attract females, 2. copulation and sperm transfer, 3. switching off of receptivity in the female. For females, the ability to locate males and stimulation of males to attempt copulation are the two most important factors, as from then on the females role in mating is largely a passive one. However, it should be borne in mind that it may be possible to select for a characteristic that, although abnormal, might be advantageous for a particular method of control, e.g., continuation of receptivity after mating in females used for booby-trapping (Smart and Gilmour 1976).

Materials and Methods

Methods for determining the quantity and quality of pheromone produced by males and the responsiveness of females to the male pheromone are described in other sections of this handbook, as are methods of recording and analyzing acoustic signals produced by male tephritids during periods of sexual activity. The effects of temperature, light intensity, age, time of day, etc., on mating in experimental flies as compared with control flies can be tested in experiments similar to those described by Tychsen and Fletcher (1971) in which the percentage of matings in a 30-min period was recorded. For this, batches of 50 experimental or control males can be tested with 100 control females, and vice versa in the case of females. Mating propensity (speed) can also be tested by a similar technique except that pairs are removed from the cage as they form and a cumulative mating speed curve obtained (Boller et al. 1977). To test the effectiveness of switch-off of receptivity, batches of females that are known to have mated on a particular date with either control or experimental males can be tested for propensity to remate in experiments similar to those described by Tychsen and Fletcher (1971). Alternatively, after the initial mating their response to the male pheromone can be used to test the subsequent effectiveness and duration of the switch-off (Fletcher and Giannakakis 1973).

Variables, Design, and Analysis of the Experiments

In all the experiments it is essential to standardize the conditions so that only the parameter being studied is varied during the experiment. The age of the flies, their previous sexual history, time of day, temperature, lighting conditions and the period over which matings are recorded have a marked effect on the results and, therefore, need to be taken into account in the design and conduct of the experiments.

Possible Modifications and Expansions of the Technique

It should be borne in mind that wild flies frequently perform badly in experiments carried out under laboratory conditions and, therefore, parallel experiments in large field cages should be carried out whenever possible.

Limitations

Apart from the desirability of doing the laboratory experiments in a room in which light intensity, temperature and humidity can be controlled, the experiments are straight forward and no special equipment is necessary.

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MEASURING MATING RHYTHMICITY IN DACUS TRYONI

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Description and Objectives

Two parameters of the daily rhythm of sexual behavior of Dacus tryoni (Tychsen and Fletcher 1971, Tychsen 1975) can be assessed in quality control procedures. They are the average maximal sexual responsiveness of flies in a culture and the daily timing of sexual behavior of individual males.

1. Maximal Sexual Responsiveness

Materials and Methods

Males and females are sexed soon after eclosion and held separately under an artificial light dark cycle of 14 h at 10,000 lux, 1 h dusk at 10 lux, and 9 h darkness. When the flies have reached sexual maturity groups of 100 males and 50 females are placed in standard cages during the light period. Then at the time dusk would normally begin the light intensity is dropped to 10 lux and the percentage mating of females in 30 min recorded by removing copulating pairs as they form (for details see Tychsen and Fletcher 1971).

Variables, Design, and Analysis of the Experiments

A number of variables must be standardized: age of flies, temperature, light intensity of test and of the light dark cycle, photoperiod, exact time in the daily rhythm to apply the test. To control these variables an artificial light-dark cycle in a constant temperature room is desirable.

Comparison of adequately replicated values is useful for assessing variations in the fitness of established laboratory cultures. However, the assessment of divergence from wild strains with this method is difficult since wild flies either brought into the laboratory as adults or raised from fruit collected in the field mate less readily than laboratory adapted flies under these conditions.

2. Daily Timing of Sexual Behavior

Materials and Methods

The daily timing of sexual behavior can be monitored using the males' habit of stridulating when sexually motivated. In D. tryoni males stridulate readily when isolated in tubes 2.5 cm diam x 12 cm. A recording device is constructed to display the time of each burst of stridulation on a 24-h chart. Each unit of the device consists of a horizontal tube containing a single male fly plus sugar and water. A microphone in the end of the tube leads via a circuit to a recording pen which marks on slowly moving paper. The circuit has as its main function the discrimination of the sound of stridulation from background noise and from other noises the fly makes. It achieves this in two ways: 1. It is tuned to a fairly high frequency which,

nevertheless, contains a considerable amount of the energy of the stridulation sound (in D. tryoni a 400-cycle bandwidth centered at 1750 Hz is appropriate); 2. It is only triggered by sounds of more than a couple of seconds duration. (This is particularly effective at discrimination against sounds produced by the short flights possible in the container). As many units as are available can be used in parallel, feeding into a multipen event recorder (e.g., an Esterline Angus) or a single pen recorder where each channel causes a different amount of deflection of the pen.

Variables, Design, and Analysis of the Experiments

Standardization of the age of the males used and of temperature is required. The light cycle under which the timing is determined is critical. The time of mating of flies under a natural light-dark cycle depends on the interaction of a light intensity preference of the flies with an endogenous circadian rhythm of responsiveness. Therefore testing the time of mating under an artificial light cycle with large sudden changes in light intensity will obscure significant changes in the light intensity preference or the rhythm that may be occurring. An artificial cycle that simulates the light intensity change on a cloudy day is most appropriate, though exposure to a natural cycle through a large window is a reasonable alternative.

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MEASURING MATING PROPENSITY (SPEED) OF IRRADIATED AND UNIRRADIATED DACUS CUCUMIS

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Description and Objectives

A primary requirement of the sterile insect technique for population suppression or eradication is that released sterilized flies must successfully mate with the wild population. To be successful, the released flies and the wild flies must be compatible, mating propensity of the released flies must be high, and the times of mating of released and wild flies must be synchronized. For example, Ceratitis capitata males treated with 10 krad gamma radiation mated less than did untreated males, and the time of peak mating response was delayed (Holbrook and Fujimoto 1970). Mating speed and propensity have been determined for untreated and irradiated Dacus cucumis under "natural dusk" and "artificial dusk" conditions and the techniques should be appropriate for other crepuscular-mating species.

Materials and Methods

To prevent uncontrolled mating, irradiated and untreated flies are sexed within 8 h of eclosion, and the sexes held separately with food and water in a controlled environment lit by natural light until the flies are sexually mature (approximately 15 days in D. cucumis). In experiments under "natural dusk" conditions two large, clear plexiglass cages are set up at noon on the test day. Each contains 100 pairs of males and females. One cage contains irradiated males and the other untreated males of the same age. The two cages are placed side by side in front of a window facing west. Once mating commences copulating pairs are caught in glass tubes and removed, and the number of males mating in successive 10 min periods is recorded. These are plotted against time to give mating speed curves (Hooper 1975).

It has been shown with D. tryoni that the optimal light intensity for mating is 8.6 lux (Tychsen and Fletcher 1971) and that mating decreases above and below that value. Similar work with D. cucumis showed that the optimal light intensity for mating was 16.2 lux (Hooper 1976) and this approximates the light intensity when maximum mating occurs under "natural dusk" conditions. At about noon on the test day 50 pairs of virgin males and females are placed in each of two clear, plexiglass cages; one cage contains untreated males and the other irradiated males of the same age. These cages are placed side by side, in an artificially lit controlled environment room, under two flood lamps which give a light intensity of 33,000 lux in the cages, and this inhibits mating. One hour before the time of meteorologically defined sunset the light intensity is dropped in one step to 16.2 lux, obtained from a lamp in a white opaque shield connected to a dimming device. Mating is initiated immediately and mating pairs are captured and removed. Mating pairs in successive time intervals can be recorded to give mating speed curves, or simply the number of males mating in a specified period, e.g., 1 h.

Variables, Design, and Analysis of the Experiments

In order to employ this technique for crepuscular species the following parameters need to be determined: 1. The time of mating in relation to environmental light intensity; 2. The time required for sexual maturation (and here it should be appreciated

that the rate of increase of mating activity of irradiated flies can differ significantly from that of untreated flies (Schroeder et al. 1973); 3. The light intensity that will inhibit mating; 4. The optimal light intensity for mating (see Fletcher and Giannakakis 1973, Hooper 1976); 5. Whether a single step down to optimal light intensity initiates mating or whether a gradually decreasing intensity is required; 6. The duration of copulation, which will determine the length of the successive capture periods (in D. cucumis this is no problem since mating pairs remain in copula for 12-14 h). In addition, environmental conditions, fly density in the cages and age of flies should be standardized.

Ideally tests in both "natural dusk" and "artificial dusk" conditions should be carried out initially to confirm that mating performance in the "artificial dusk" situation approximates that in the "natural dusk" situation. It is important that the time of the artificially determined dusk period corresponds with the time of natural, or artificial dusk in the photoperiodic regime in which the flies are held prior to the test. In the "natural dusk" procedure replicate variation is high, due in part to varying conditions at sunset. The "artificial dusk" procedure is much less variable and fewer replicates are required. The latter test can also be completed in a shorter time (1 h vs. 2-2.5 h). Results are analyzed by the paired comparison procedure.

Possible Modifications and Expansion by the Technique

Measurements of mating speed and propensity can be used to monitor performance of mass reared flies, and conceivably selection for increased mating performance could be initiated. These tests can be used to assess difference between irradiated and untreated flies, and to compare males sterilized by different agents. The natural dusk procedure can be used in field cages (Holbrook and Fujimoto 1970) where it may be presumed that replicate variation will be high. The artificial dusk procedure can be used to assay the response of females to male sex pheromone (Fletcher and Giannakakis 1973), and to determine the temperature threshold for mating. If both sexes of untreated and irradiated flies (flies of one treatment having been suitably marked) are combined in one cage, then mating compatibility can be determined (Schroeder et al. 1973).

Limitations

No specialized equipment or skills are required.

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MEASURING MATING PROPENSITY (SPEED) IN RHAGOLETIS CERASI

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Description and Objectives

It has been observed that wild populations consist of fast, intermediate, and slow mating individuals or genotypes occurring in apparently well balanced proportions. The mating speed as expressed by the time-interval between the pooling of the two sexes in the test cage and mating not only indicates the mating propensity but it is also related to the mating frequency of at least the males (frequency = number of matings per individual male or female within a given period of time). Therefore, the cumulative mating speed curve for the test population describes the population pattern with respect to important mating characteristics and can be used for the detection of significant deviations of test strains from wild standards. Mating speed data can be obtained in a relatively short time (within 1 h) and the test individuals can be reused immediately for other purposes.

Materials and Methods

Males and females are sexed after eclosion and held separately until used for the test at standard laboratory conditions. When the flies have reached the required age (in our routine tests 5 days) 10 to 50 individuals of both sexes are put together into plexiglass cages (for details see Boller et al. 1977) at low light intensity to avoid mating. The test cages are then transferred to the test room where the light has been turned off, and kept in darkness for a short period of time to allow the flies to recover from disturbing influences of previous handling. At time zero or start of the test the light is turned on at optimal intensity and pairs in copula removed from the cage at regular intervals (in our tests every 10 min). The test is terminated at a defined point (e.g., after 60 min, or after 70% of the wild standard population have copulated). The numbers of pairs formed during each consecutive interval are added and expressed in percentages of the total number of pairs in the test. These cumulative per cent figures plotted against time give the cumulative mating speed curve for the test population. If fast and slow mating flies are selected, the criterion for the fast and slow mating category can either be the time required for pair formation or e.g., the first 20% of flies copulating in the case of fast mating individuals. In our routine tests with large numbers of replicates run at the same time, with 10 pairs as an experimental unit, we no longer remove the flies in copula but reduce our observations to one single reading of pairs formed after 30 min.

Variables, Design, and Analysis of the Experiments

The following variables have been identified that require standardization in order to reduce overall variation of the data: age of females, temperature in test room, density in test cage, and diurnal rhythm of mating activity.

Suggested approach for experimental design: 1. Define peak mating period in relation to the prevailing light regime; 2. Carry out tests with increasing age of both sexes to find optimal age groups; 3. Standardize observation intervals and duration of test taking into account minimal duration in copula (gives indications with respect to observation intervals) and time required for 60-80% pair formation (gives indications

with respect to test period); 4. Determine crucial period where largest differences between strains occur. In Rhagoletis cerasi the steepest slope of the mating speed curve occurs between 10 and 40 min, and largest differences occur during the 30 and 40 min period. These differences tend to become much smaller towards the end of the test.

The analysis and comparison of mating speed curves have been described by Boller et al. (1977). Mating indices and their analysis have been described by Manning (1961), Spiess et al. (1966), Kessler (1968), and Prakash (1968).

Possible Modifications and Expansion of the Technique

The method can also be applied in field cages but variable environmental influences call for an increased number of simultaneously run replicates. The technique is also applicable for testing assortative mating or competitiveness when differently marked strains are included in the same experiments. Fast mating flies can be selected for improving mating activities of laboratory strains.

Limitations

This low technology method requires no special skills of the investigator and no special equipment.

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MEASURING MATING PROPENSITY IN DACUS OLEAE

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Description and Objectives

Mating propensity, especially in the female, has always been of special interest in studies of the sexual behavior of Dacus oleae. The differences found between the two sexes (the male mates more often) and the mating activity of wild and laboratory populations have also been considered in relation to the effectiveness of sterile insect technique programs involving releases of artificially reared insects.

Materials and Methods

Flies to be studied are placed either individually in small cages or in groups in larger cages. Glass-tube cages (10x8 cm) are convenient for such studies (Zervas, personal communication). They are maintained at 25±2°C and 60±5% RH, and 12 h photoperiod. (The latter could also be 13 or 14 h in order to relate to the system established in the rearing facility or the natural conditions during the insects' breeding season.) The light intensity at the cage level is ca. 2500 lux, while in most experiments one-half or one-fourth of the lights are turned off during the last 3-4 h of the photophase to obtain better mating conditions.

For studies with individual flies, 1 pair of virgin flies is placed in the cage during the last 4 h of the photoperiod (mating time for D. oleae) each day. If it is impossible to watch the flies for 4 h, or to make several observations during that time, then one observation just before dark will cover the great majority of matings. Sexes are separated just before the end of the light period, unless they are in copula (these are separated early the next day).

If flies are studied in large numbers, equal numbers of each sex are combined in the same cage. The separation of sexes before dark proved to be necessary after we observed that the males were often successfully copulating after the end of the photophase (Zervas unpublished). The combining of sexes is usually done after sexual maturation has been reached. In studies with only one type of fly the sexes can be together from the beginning of the test. In cases where different types of flies are put together (e.g., wild, artificially reared, normal, irradiated, from different climates etc.), the age at which they reach sexual maturation should be determined before the time for combining the sexes is chosen. In mixed populations identification of the strains is facilitated by marking them with a small dot of oil paint on the notum. For marking, the flies are first immobilized in glass tubes that are put in water with ice cubes, and then placed on waxed paper on ice.

Variables, Design, and Analysis of the Experiments

Age, sexual maturation, body size, nutritional status, previous sexual activity, duration of matings, and environmental conditions (temperature, humidity, light intensity) are factors that may influence the results. These variables should be standardized, especially when mating is studied in mixed populations.

For the design and analysis of experiments see Tzanakakis et al. (1968), Tsiropoulos and Tzanakakis (1970), Economopoulos (1972), and contact Dr. G. Zervas of the "Demokritos" laboratory for unpublished information.

Possible Modifications and Expansion of the Technique

The method can also be applied in field cages in various periods of the year. Such studies are important, although environmental conditions cannot be controlled.

Limitations

No special equipment or skill are required for these studies. On the other hand a long series of tests is needed to study and standardize the variables mentionned. Several hours of intense watching is also needed, often in late afternoon hours (field cages). This technique provides information that may well differ from the true picture in wild populations. Flies are kept in captivity which may alter their mating patterns (high density of population and altered significance of chemical communication).

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MEASURING MATING RHYTHMICITY, DURATION, AND REFRACTION IN CERATITIS CAPITATA

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Description and Objectives

Mass rearing and irradiation can influence, among other important quality traits, the sexual behavior of the flies. Direct comparison of mating rhythmicity, duration, and refraction between wild and laboratory adapted strains can provide early warning of significant deviations. We describe herein three simple methods that can quickly provide such data.

Materials and Methods

Males and females are separated after eclosion and held separately under standard laboratory conditions (25°C, 70% RH, and 18-h photoperiod) in 20x20x25 cm screen cages. Smaller cages are used (8x8x10 cm) when single pairs are studied. The standard food consists of a 10:2:1 mixture of sucrose, brewers' yeast and enzymatic yeast hydrolysate. Sexually mature males and females are placed together in the cages; a perforated plastic orange containing a moist filter paper provides the oviposition substrate.

For the study of the mating rhythmicity of the males, a series of small cages is prepared, each receiving one male and five virgin females of appropriate ages. These females are replaced by other virgin females as soon as they have mated or died. When the rhythmicity of the females is investigated, groups of virgin females are held with three times their number of males. Copulating pairs are removed and transferred to a second cage where males are constantly removed as soon as mating is terminated. Mated females are again exposed to males the following day and then held in separate cages identified as to the number of matings they have performed. The tests are carried out under either natural or artificial light conditions. Under natural light conditions, sexual activity starts at the beginning of the photophase, reaches its peak in the late morning, and decreases considerably in the early afternoon. Mating starts on the third day after eclosion. It is important to verify mating by examination of dissected samples of spermathecae.

For the assessment of mating duration males and females of known age are kept under direct observation and the beginning and end of copulation is recorded. Where these observations are not combined with other studies requiring groups of flies, they are usually carried out with single pair units.

In our studies of mating refraction we observe the behavior of mated females that become unreceptive (they avoid mating attempts by movements of the abdomen or by escape). Females are kept in groups of those that performed identical numbers of matings, or if the refractory period is to be investigated, groups of those with equal periods since the last mating. The females are tested with males of various ages at a ratio of 1:3.

Variables, Design, and Analysis of the Experiments

The following variables should be standardized in order to reduce experimental variation: 1. Rhythmicity tests: age, size and physiological status of the test insects, and temperature, photoperiod and diet. 2. Duration tests: temperature.

3. Refraction tests: age of both sexes, the number of previous matings, and the time interval between two consecutive matings. We suggest the following approach for the experimental design: 1. Define the influence of age on the parameters measured; 2. Investigate the influence of various population densities; 3. Define the observation intervals, if continuous observation is not feasible, by taking into account the rhythmicity of sexual activity and the minimum duration of the various traits.

Possible Modifications and Expansion of the Technique

The disturbing effects of handling immediately before the tests can be reduced by keeping the two sexes in a double cage with a removable separating wall. Experiments carried out in small cages could be repeated and verified in larger field cages. A further expansion could include the assessment of sperm depletion and its effect on refraction, and the influence of the physiological condition of the males on the transfer of the "male factor" to the females (e.g., by using aspermic males).

Limitations

These simple methods do not require special equipment or special skills of the investigator other than the ability to dissect specimens and to examine the spermathecae.

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MEASURING MATING FREQUENCY IN RHAGOLETIS CERASI

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Description and Objectives

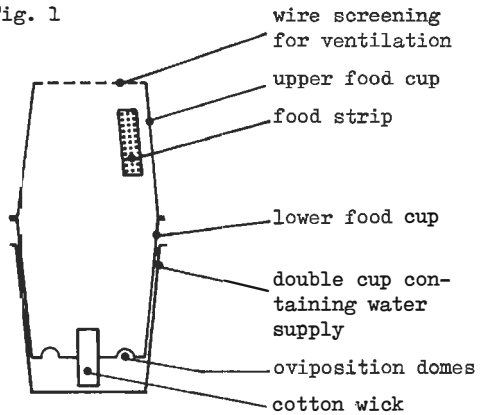
The mating frequency test measures the number of matings performed by individual flies (usually the males) within a given period of time. By comparison of the average mating frequency of a given test strain with that of a wild standard strain we intend to detect early signs of deviations from the standard under defined laboratory conditions. If significant differences are observed we hope to identify the causes and find ways to solve the problem. Since we are primarily interested in the mating performance of released sterile males, and since we have indications that mated females become unreceptive for a certain period of time (refractory period), we have geared the mating frequency test entirely to the study of male activity.

Materials and Methods

Previous experiments have shown that the mating frequency of individual males tends to increase whenever the male has a choice among several virgin females (Katsoyannos, unpubl.). Therefore, the sexes are kept separated from eclosion until testing.

At the age of 5 days 1 male and 5 females are transferred to the minicage (figure) 1 h before the start of the test (6th h of the photoperiod). The cages are made of 2 plastic food cups (180-ml capacity) containing 3 oviposition domes (Prokopy and Boller 1970), water supply, and a small piece of food-impregnated filter paper. Light intensity of 1500 lux at the dome level is provided from above by light bars. At least 20 replicates per strain (test strains and wild standard) are operated at the same time.

Fig. 1



Beginning at the 7th h of the photoperiod, the cages are checked regularly at 15-min intervals for 8 h and the number of mating pairs recorded. The total number of matings performed by the male is the mating frequency. Also, the time spent in copula (expressed in 15-min units) and the average mating duration can be calculated easily from the data. As the observed matings are recorded on a time scale we can also detect possible deviations in the mating periodicities.

Variables, Design, and Analysis of the Experiments

The following variables have been identified that require standardization in order to reduce overall variation of the data: age of the flies, number of females per male, temperature in the test room, and period of the light cycle.

Suggested approach for experimental design: 1. Define peak mating period in relation to the prevailing light regime; 2. Carry out tests with increasing age of both sexes in order to find optimal age groups; 3. Standardize observation intervals and duration of the test. If the assessment of the mating duration is an important part of the test, then the observation intervals should be relatively short.

Possible Modifications and Expansion of the Technique

This technique can be used for long-term series of observation covering, for example, the entire adult stage in order to study age dependent activities. Other behavioral aspects such as interaction of the sexes, place of mating, mating-feeding-oviposition periods etc., can also be studied.

Mating frequency tests could be used in conjunction with the allozyme technique used successfully in Dacus oleae by Zouros and Krimbas (1970).

Tests of mating frequency in the laboratory are useful for comparing the mating activity of different strains under extreme temperature and light conditions.

Limitations

This low technology method requires no special skill of the investigator and no expensive equipment.

Selected References

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Prokopy, R.J., and E.F. Boller. 1970. Artificial egging system for the European cherry fruit fly. J. Econ. Entomol. 63: 1413-17.

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EVALUATING EFFECTS OF ACCLIMATION ON MATING PROPENSITY AND MATING COMPETITIVENESS IN DACUS SPP.

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Description and Objectives

Meats and Fay (1976) have shown that mating frequency and mating competitiveness are affected by thermal history. It is therefore important to determine the extent to which laboratory flies are competitive with those that are acclimated to relevant field conditions - which may be hotter or cooler than the laboratory.

Materials and Methods

Acclimation. Studies on temperature and survival thresholds (Meats 1976a, 1976b, 1976c) have shown that full acclimation to any regime can be achieved in the pharate adult stage; insects exposed to a given regime during the last sixth of puparial life emerge as adults fully acclimated to that regime. Cold-acclimation can be produced by using a constantly cool regime (e.g., 15°C) or by using one that alternates between warm and cool (e.g., between 25°C and 12°C) on a daily basis. The extent of acclimation in D. tryoni produced by any given constant or varying regime can be calculated from the model of Meats (1976c). Acclimation to new conditions is a lengthy process in adult flies, taking several days or weeks depending on the degree of change. The aforementioned model can also be used to calculate the amount of change expected in adults in a given time.

Flies are therefore most readily acclimated by exposing them to the desired regime from the late puparial stage onwards. In the case of cold-acclimation, developmental thresholds must be considered. Adult D. tryoni mature very slowly at constant 15°C but as mentioned earlier, good cold acclimation can also be achieved in regimes that fluctuate between warm and cold. A regime in which 12°C and 25°C alternate at 12-h intervals will allow flies to mature at half the rate expected at constant 25°C.

Performance tests. To measure the mating propensity of a test population, sexes must be separated before maturity and only mixed at the start of the experiment. It is possible for one person to observe, record and remove mating pairs from four replicate cages, each containing 40 males and 40 females. Mating competitiveness experiments differ only slightly from mating propensity experiments. For instance, 40 laboratory-acclimated males (suitably marked) can be caged with 40 field-acclimated males to compete for 40 field-acclimated females.

D. tryoni mates at dusk. Mating behaviour is controlled by a circadian rhythm but low light intensity (ca.10 lux) is a key requirement. Thus, in the case of this and similar species, tests should be run in cabinets in which photoperiod can be controlled and where 1 h of dusk illumination (ca.10 lux) comes between the light (10,000 lux) and dark part of the photocycle. Ideally the test chamber should have controls so that temperatures fluctuate on a daily cycle approximating the mean cycle expected in the field at the time of release. If this is not possible the most pertinent constant temperatures to use are those expected to prevail in the field at dusk at the time of release. In the case of species that mate at other times of the day the temperatures expected at those times in the field should be used and the dusk part of the photocycle can probably be omitted without affecting the results.

Variables, Design, Analysis, Modifications and Expansion of Technique

Reference should be made to the equivalent sections in the article in this publication on "Measuring mating propensity of Rhagoletis cerasi populations".

Limitations

Cabinets capable of simulating natural fluctuations of temperature are essential if the full potential of the technique is to be realized.

Selected References

Meats, A. 1976a. Developmental and long term acclimation to cold in the Queensland fruit fly (Dacus tryoni) at constant and fluctuating temperatures. J. Insect Physiol. 22: 1013-19.

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Meats, A. 1976c. Seasonal trends in acclimatization to cold in the Queensland fruit fly (Dacus tryoni), and their prediction by means of a physiological model fed with climatological data. Oecologia 26: 73-87.

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TESTING FOR ASSORTATIVE MATING IN CERATITIS CAPITATA

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Description and Objectives

A measure of sexual isolation between allopatric populations of the Mediterranean fruit fly may be obtained by use of the multiple choice test with direct observations (Ehrman 1965). The test can also be used for comparing the mating propensity of males and females of two populations (Merrel 1950). We used the test to compare laboratory reared and field collected flies, and populations differing at one or several genetic loci (Rössler and Koltin, in press). The technique has been applied with various modifications by geneticists to measure assortative matings in Drosophila strains (Elens et al. 1973, Elens and Wattiaux 1964, Faugeres et al. 1971).

Materials and Methods

The mating arena is a 1-liter, ventilated transparent plastic box provided with water and food. Adults of the test populations are sexed after eclosion and held as virgins for 4 days (26°C). Individuals of the two test populations are distinguished either by the use of a morphological genetic marker or by the topical marking of one population with an appropriate marking system. Twenty adults of each sex and strain are then introduced into the mating arena (a total of 80 flies per box) in the morning and observed every 2 h during the light phase of a 24 h period. Immobilization of the flies for handling should, if absolutely necessary, be done by chilling them at 4°C for a few min and not with CO_2. The observation intervals have been chosen based on the observation that copulation lasts between 1 and 2 h. The experiment is carried out in the laboratory at a natural day-night regime and in six or more replicates. Four mating combinations are recorded: AA, BB, AB and BA, where the first letter of the mating combination always stands for the male, the second letter for the female. The data obtained are used for the calculation of the Sexual Isolation Index (I), and the male (Mm) and female (Mf) mating propensity (Merrel 1950).

$$I = \frac{AB + BA}{AA + BB} \quad ; \quad Mm \text{ (of A males)} = \frac{AA + AB}{BA + BB} \quad ; \quad Mf \text{ (of A females)} = \frac{AA + BA}{AB + BB}$$

A significant deviation of I, Mm or Mf from the value of 1 indicates the existence of a difference between populations A and B. The significance of an observed deviation can be tested with Chi^2.

Variables, Design, and Analysis of the Experiments

The following variables should be standardized before tests are carried out: Age of test flies, observation intervals and marking procedures. The following approach is recommended: 1. Define the optimal age of the test flies by carrying out mating tests with increasing age of both sexes; 2. Standardize the observation interval by measuring the average length of time needed to complete mating; 3. Investigate potential negative side-effects of the genetic or artificial marker on the mating

activity of the adults (i.e., compare mating within a given population using marked and unmarked individuals).

Possible Modifications and Expansion of the Technique

This test is part of a series of tests applied to investigate the evolution of reproductive barriers between populations. The comprehensive test series should follow the outline of isolating mechanisms described by Mayr (1963). Diurnal rhythms of sexual activity of both sexes in both test populations may also be compared with the same data gathered during the experiments, and any alterations of the behavioral patterns may be detected and monitored.

Limitations

The technique is simple and does not require sophisticated equipment.

Selected References

Ehrman, L. 1965. Direct observations of sexual isolation between allopatric and between sympatric strains of the different Drosophila paulistorum races. Evolution 19: 459-64.

Elens, A., J. van den Haute, and J. Delcour. 1973. Sexual vigor and sexual selection in Drosophila melanogaster. Evolution 27: 549-57.

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MATING ACTIVITY AND MATING COMPATIBILITY IN MELON FLY, DACUS CUCURBITAE, POPULATIONS

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and
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Description and Objectives

When the melon fly is treated to function in sterile-release programs, the behavior and effectiveness of the treated adult may be changed compared with the untreated counterpart. We therefore developed a technique to isolate the effect of the normal increase in the mating activity of melon flies over time and of mating compatibility (term here used to include synchrony and other factors that may cause reproductive disconformancy) between normal and treated populations. The test is designed to determine the changes in behavior that cause the reduced mating effectiveness of treated flies. Parameters for mating activity and mating compatibility for populations of untreated insects can be established and comparisons made with populations of treated insects from the same rearing generations.

Materials and Methods

The sexes are segregated 1-2 days after adult emergence while the flies are immobilized by exposure to 7-8°C. Subsequently, the females are maintained in 25x25x25-cm screen cages (50/cage), and the male flies (50/cage) are maintained in 1-liter waxed cardboard containers from which they can be transferred easily. The afternoon (ca. 2 PM) of the day of a test, 50 ♂ are combined with 50 ♀ in the screen cages. Since melon flies mate in the evening, the cages are maintained at ambient temperature and light until 1 h after complete darkness, when they are moved to a refrigerated room (at 7-8°C) or completely immersed in hot water (ca. 80°C) to immobilize pairs in copula. The total number of mated pairs is determined. Peak mating activity is considered to have been reached when the mean number of flies mating does not increase in 2 consecutive mating periods (a mating period is defined as one evening as an adult).

To determine mating compatibility, the pupae from one population are coated with dye so that the ptilina of the emerging adults are marked. The sexes are separated as before, and 100 of each sex from each of the two populations are maintained separately for 9-16 days at ambient temperature (sugar, water and hydrolyzed protein are provided). Mating tests are made after this period of sexual development by releasing 100♂ and 100 ♀ of the two test populations in the afternoon into a 2x2x2-m outdoor cage. When mating activity begins in the evening, mating pairs are captured in individual vials and subsequently classified by mating categories.

Variables, Design, and Analysis of the Experiments

The data are transformed (arcsin) and subjected to analysis of variance. The data for mating activity over time are analyzed by regression analysis. Also, populations are examined by analysis of covariance to determine whether the rates of the development of activity over time are different. The data for the peak mating activity are

EGG PRODUCTION, SPERM DEPLETION, AND SEXUAL COMPETITIVENESS IN DACUS OLEAE

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Description and Objectives

Fecundity, fertility, and sexual competitiveness are among the major biological attributes on which the survival of a population depends. For this reason they are subjects of quality control tests comparing artificially reared and wild insects. Rather simple direct measurements and ratio tests are usually applied to monitor differences. Drastic changes due to colonization are often detected. They are related to decreased genetic variability and the physiological effects of conditions established in laboratory rearing systems.

Materials and Methods

Wild larvae are collected daily from infested fruits placed in screen-bottomed boxes. They are compared with artificially reared insects that are produced simultaneously. Females are placed individually in small 15x15x15-cm wooden (two sides screen, one glass door) or glass tube cages (8x10 cm) with the two openings sealed with tulle or plexiglass. Males are introduced for short periods (3-4 last h of photophase) for mating, and then removed (unless the continuous presence of males is being studied). Adult diet, protein syrup or powder, is provided in small cups and water through dental cotton in small plastic bottles. For oviposition, paraffin domes or fresh olives are provided daily. In tests of sexual competitiveness, flies of various types (lab, wild, untreated, sterilized) are placed in 30x30x30-cm wooden cages (three sides screen, one glass). In order to identify the categories, some flies are marked on the notum by a small spot of oil paint. To accomplish this, the flies are first immobilized in glass tubes placed in water with ice cubes, and then placed on paraffin paper on ice. Ratios of 1:1:1:1 and 1:1:2:2 (wild ♂: wild ♀ : lab ♂: lab ♀) of untreated flies are tested for mating and egg hatch of untreated flies. Egg hatch is checked either in the olives or on moist filter paper where eggs removed from olives are placed for incubation. Paraffin domes, as well as olives, can be used for oviposition; however, wild flies are reluctant to oviposit into paraffin domes. In sterility tests, higher ratios of sterile to normal insects can be used (e.g., 4:4:1:1).

Variables, Design, and Analysis of the Experiments

In egg-production studies the genetic makeup, age, sexual maturation, sexual activity, body size, adult and larval food, oviposition substrate and environmental conditions (temperature, humidity, light) are variables that should be standardized. As far as the oviposition substrate is concerned, it appears that the natural substrate of the population should be utilized (olives, from the area of origin). If this proves difficult, or for comparison, paraffin domes can be used for both wild and lab flies or just the lab flies. Egg hatch data are used for the sperm depletion studies and thus sperm mortality or rate of discharge cannot be studied. In competitiveness tests, sexual maturity of all flies should be equal upon mixing. For example, in D. oleae the female mates less often than the male and thus all females could be mated by lab males, which mature earlier than wild flies, in situations where both types are mixed upon emergence.

Possible Modifications and Expansion of the Technique

One modification would be the provision of natural food (honeydew, pollen). The method(s) could also be applied under natural conditions in field cages.

Limitations

The technique has low efficiency when olives are used as oviposition substrates. The method is a simple one, but requires skillful identification and study of factors affecting reproduction.

Selected References

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MEASURING SEXUAL MATURATION IN DACUS OLEAE

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Description and Objectives

In recent years, it has been found that artificially reared (AR) olive fruit flies mate and oviposit at a younger age than the wild (W) ones. A study of the phenomenon, which probably occurs due to selection, revealed extensive differences which could produce some degree of sexual isolation if the two populations were mixed at a young age. The technique described allows the assessment of rate of maturation and quantification of population differences.

Materials and Methods

Pupae of the categories of flies to be tested are selected on the fourth or fifth day of pupation. They are held at 25±1°C and 60±5% RH under a 12-h photophase at 1200-1500 lux. On the day of emergence, flies are placed in groups of 60 pairs in 42x25x25 cm plexiglass cages (4-6 replicates) or in individual pairs in small glass-tube cages (40-50 replicates). The small cages measure 8x10 cm, have an upper opening sealed by nylon, and their bottoms are sealed by thick paper with a hole in the center which serves as cage door. Males and females of different ages are used in the experiments: a) females 1-day-old with sexually mature males (4- and 10-days old for the AR and W males, respectively, b) sexually mature females (5- and 10-days old for the AR and W females, respectively) with males 1-day-old, and c) 1-day-old males and females. Flies are carefully watched, beginning the day of emergence, during the last 4 h of the photophase. Observations are made every 15 min. It has been found that under a 12-h photophase no mating ever occurs before the last 4 h (Zervas, unpublished). When insects are kept in groups, mating couples are removed carefully from the cage. Females are offered green olives and paraffin domes (the latter only for AR flies) daily for oviposition. A certain number of females, usually 10 of each fly type, are also dissected and checked for presence of sperm and eggs. Insects, especially those from studies with individual pairs, can be reused for other studies such as mating-duration, mating frequency etc.

Variables, Design, and Analysis of the Experiments

The percentage of mated females, or females with mature eggs, are plotted against time to obtain sexual maturation curves.

Factors that need standardization are photoperiod, light intensity, temperature, humidity, density of insects in the cage, synchronization of eclosion, adult diet, age and irradiation dose.

Possible Modifications and Expansion of the Technique

The technique can also be applied under field cage conditions which provide a natural situation for the study of sexual maturation.

Limitations

No special equipment or skill are required.

Selected References

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MONITORING PRODUCTION

INTRODUCTION

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Since our respective pest control programs are dependent on fruit fly colonies, we must be able to culture these insects continuously and economically, without sacrificing their essential behavior capabilities. We therefore automatically evaluate our rearing systems, directly and intuitively, in terms of insect yields and overall efficiency. However, precise monitoring of the processes and products is often necessary to ensure mass production of a dependable supply of a specified quality of insects. This requires: (1) a thorough understanding of every fruit fly mass rearing operation, (2) determination of the effects of these procedures on each developmental stage, (3) analysis of the biological consequences of colonization on the founder population, and (4) a realistic assessment of the need for modifying production technology or altering the colonized strain.

Our mass production efforts necessarily include both the management of physical resources and conscientious maintenance of essential biological characteristics of colonies. We routinely monitor our facilities, equipment, materials, and operations according to a set of standardized procedures or insectary protocols. Temperature, relative humidity, light quality, and air exchange are programmed; traffic patterns are established; and maintenance schedules are implemented. Equipment must function as specified and be compatible with suitable raw materials. Finally, personnel are trained to evaluate alternative supplies, provide adequate handling and storage, perform insect rearing operations, ensure synchrony of tasks, and sustain the system.

The first section illustrates several procedures that we can use to monitor the biological aspects of production by determining age-specific survivorship (yields) and relevant physical characteristics (size, mass, color, biochemical composition, microbial load, etc.) from representative sample cohorts. The article by Boller et al. explains the refinement of these data into detailed life tables that indicate viability (survival to reproductive age), fertility (capability of reproducing), fecundity (number of offspring produced), and rate of development by ontogenetic stage. Tsitsipis and Manoukas, Tanaka, and Leppla incorporate additional phenotypic characteristics of the colony to provide a quantitative developmental life history-A complimentary or supplementary approach, illustrated by Galun, Greany and Viñas, and Manoukas and Tsiropoulos is to periodically determine ranges of tolerance to environmental stress (nutritional or toxic chemicals, pathogens, temperature, etc.). Ultimately, a combination of these methods is used to indicate potentially deleterious fluctuations in fruit fly colonies and ensure continuity of the rearing system.

The section "Measuring Adaptation" points out that the ideal, biologically stable culture will yield a dependable supply of insects, but they will have diverged genetically from the founder population. If we intend to release laboratory adapted flies back into their natural habitat, they must be reasonably functional (i.e.,

competitive) by realizing most aspects of the ecological niche of the target population. Adequate fitness may be ensured only by monitoring parameters indicative of divergence and comparing successive generations of colonized flies with the standard target population. Bush and Berlocher each present techniques for measuring the genetic variation of fruit fly populations, whereas Agee, Meats, and Leppla and Turner suggest methods for measuring changes in thresholds and ranges of response to discrete environmental stimuli. Combinations of these parameters will provide a quantitative basis for formulating judgements concerning the effects of mass rearing operations on the flies and the extent of associated genetic divergence. However, a realistic quality control evaluation will depend on the prudent selection and accurate monitoring of these parameters that adequately define the colony.

Even though our entire pest management schemes are dependent on successful insectaries and the subsequent utilization of competitive strains, quality control procedures are rarely elaborate. The brief articles in this section serve to illustrate that mass rearing of fruit flies still involves a nebulous, advanced "trouble shooting" effort. Based on individual experience we anticipate problems, identify sensitive stages, and establish precautionary measures for handling the associated procedures. Monitoring is precise and involved only when specific operations cannot be allowed to vary beyond defined limits. The following articles were solicited to provide general descriptions of colonization systems, identify operations known to be critical, and indicate practical approaches for ensuring that incipient problems do not curtail production or jeopardize performance.

LIFE TABLE MEASUREMENTS FOR MONITORING THE PRODUCTION OF RHAGOLETIS CERASI IN SWITZERLAND

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Description and Objectives

Significant deviations observed in comparative performance tests with laboratory reared and wild standard strains of Rhagoletis cerasi are assumed to be caused by the laboratory environment. Where the observed deficiencies are not reversible (caused by conditioning) and have a definite genetic basis we are dealing with a selection of laboratory adapted genotypes. Hence, we have to identify those critical stages of the life cycle where increased mortality indicates strong selection pressure on a wild population that has been transferred to the laboratory and passed through a series of bottlenecks before it reaches the F_1 adult stage (Boller and Chambers 1977). Of special importance is a thorough systems analysis of the adult stage where selection directly affects important quality traits connected with sexual and reproductive behavior. Such an analysis is part of a continuous effort to improve the principal features of the mass rearing process. However, a simpler method - the partial life table approach - is used to routinely monitor the occurrence and magnitude of bottlenecks while the population is passing from stage to stage through an entire life cycle. In R. cerasi such partial life tables are established once a year or whenever the rearing methods are modified.

Material and Methods

Life tables have been developed and used widely by ecologists as a method of systematical bookkeeping of mortality factors occurring in populations (Deevey 1947). The various approaches, technical details and methods of analysis have been described by Southwood (1966). We follow the development of a given number of eggs through all consecutive stages according to the flow diagram shown in Fig. 1. The data sheet used in our laboratory and the graphical display of the life table data are given in Fig. 2.

Variables, Design, and Analysis of the Experiments

Ten replicates per strain are assessed and produce the basic data for each partial life table. All replicates are prepared the same day and held under identical conditions. The experimental units and procedures are the same as those used in mass rearing (Katsoyannos et al. 1977). We standardize the following variables in order to be able to compare series of tables established at different times: temperature, humidity, light intensity, photoperiod, amount of larval diet, and larval density. Pupal weights are measured 10 days after pupation. Female density is kept constant at 1 female/dome. Statistical analysis follows normal procedures outlined by Southwood (1966).

The influence of selection can be evaluated by comparing two important life tables. The first one covers the very first step when wild adults (P generation) are subjected to laboratory propagation producing F_1 individuals. The second table covers an entire generation of a laboratory adapted strain (at least the 5th generation reared under laboratory conditions). The laboratory adapted strain will probably have reached the upper limits of its reproductive potential under the given circumstances and can be used as internal standard for measuring the initial impact of the rearing procedures on wild material brought to the laboratory. The bigger the differences between these two

tables the bigger the probability that the strain has undergone major alterations that could affect its overall quality.

Possible Modification and Expansion of the Technique

The expansion of the partial life table is unlimited and can lead to very sophisticated systems analysis. Additional data can be gathered to explain the causes of mortality or deficiencies observed during the individual stages such as the influence of larval density on pupal weight and yield, the influence of nutritional and physical characteristics of larval diet, etc. The introduction of a "diet worth factor" (Bucher and Bracken 1976) for the evaluation of diet performance can increase the information output. Causes of reduced fertility and fecundity can be identified by monitoring important traits of sexual and reproductive behavior. With these additional data complete life tables and indices of population trends can be established providing the basis for key-factor analyses (Morris 1959, Southwood 1966).

Limitations

The establishment of a sensitive program requires a sound knowledge of the species' behavior, physiology and general biology. Once a suitable protocol has been developed the individual parameters can be measured by any person familiar with the rearing procedures.

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examined by analysis of variance to determine whether a treatment has affected the mating propensity.

The mating compatibility test is designed to determine whether treatment has an effect on time of mating, i.e., whether the mating periods of treated and normal flies are compatible in time. With careful control of the test conditions and timing, data indicating mating activities, such as preference, frequency, site, duration, etc., can be collected.

More detailed information on biological and physical variables that affect mating propensity and mating compatibility in melon fly populations has been published elsewhere (see references).

Possible Modifications and Expansion of the Technique

The technique used to examine mating propensity can be adapted to improve the laboratory strain. Given appropriate facilities, the large-cage tests could be conducted under controlled conditions. Altered light cycle would allow control of experimental timing.

Limitations

The mating propensity and mating compatibility tests require working in the late evening, which may be inconvenient and which limits time available for conducting the tests.

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TESTING THE INFLUENCE OF MARKING ON MATING IN C.CAPITATA

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Description and Objectives

Marking with fluorescent powders is widely used in sterile releases of Mediterranean fruit flies, as a means of separating sterile from wild flies in trap catches. Trapping is an integral part of any sterile release program as it provides progressive estimates of the sterile to wild overflooding ratios attained, which are a measure of the program's success. Testing the influence of marking on the mating competitiveness of Mediterranean fruit fly males was part of a series of tests conducted at our laboratory to evaluate the effect of handling techniques on the quality of flies intended for sterile releases (Serghiou 1977). The criterion used for quantitatively measuring differences in competitiveness was the relative participation in mating of marked versus unmarked males. In these tests marked males were combined with unmarked males and females in a 1:1:1 ratio.

Materials and Methods

Methods for rearing the flies have been described (Serghiou and Balock 1975, Serghiou 1977). The fluorescent powders evaluated were: Tinopal SFG^{R}, a brightening agent produced by Ciba-Geigy and found promising by Schroeder et al. (1972) for marking tephritids, and Rocket Red, a compound of the Day-Glo^{R} series (Switzer Brothers Inc., Cleveland, Ohio 44103). Both were tested at 1.5 g of powder/litre of pupae. Pupae were mixed with the powders 1-2 days before eclosion by hand shaking a preweighed quantity of powder with the corresponding volume of pupae in plastic cups for sufficient time until observation confirmed that all pupae were marked. Upon emergence, marked and unmarked flies were sexed and kept separately in small plastic cages to attain sexual maturity. The flies were provided a diet consisting of a mixture of enzymatic yeast hydrolysate and sugar (3:1). Water was supplied separately. Tests were conducted 4 and 11 days after emergence by combining the flies in a ratio of 300 marked males:300 unmarked males:300 unmarked females in organdy cages measuring 2.1x2x2.1 m. Tests were conducted in the laboratory with artificial illumination. Males were released in the cages before releasing females. When mating commenced (minutes after mixing the sexes) pairs in copula were collected and examined to determine whether marked or unmarked males participated in mating. Examination was made by crushing the head of the male on filter paper with a blunt rod that had been dipped in acetone (Steiner 1965). This procedure transferred the dye in the ptilinal suture to the paper where it could be seen under UV light as a bright spot. In each test the first 200 mating pairs were examined; usually these were collected within 1-3 h from the initiation of a test.

Variables, Design, and Analysis of the Experiments

It is important to exclude or minimize the possibility of mating before the initiation of tests. In our experiments we achieved this by separating sexes soon after eclosion. Holbrook et al. (1970) observed by dissecting spermathecae that some mating (less than 10%) took place within 48 h of emergence when flies in paper bags were kept in a room that had a normal daily light cycle. To prevent this early mating, they kept the flies in complete darkness until sexing them 2 days after emergence.

These authors also advise that containers should be removed after the release of males because females would congregate on the containers, even those that had held males 24 h previously. It is also important to use flies that are sexually mature and have a full mating response. In our tests we used flies that were at least 4 days old. We carried out an analysis of variance on our results after transforming the data to percentages and found that marking did not have any adverse effect on male mating competitiveness.

Modification and Expansion of the Technique

The technique described above can also be carried out in field cages and can be expanded to study the effect of marking on the mating response of both sexes. Indeed, Holbrook et al. (1970) conducted such a test in which they combined in field cages equal numbers of marked and unmarked males and females and recorded the mating frequencies of the four possible combinations: unmarked male x unmarked female; unmarked male x marked female; marked male x unmarked female; marked male x marked female. The percentage frequencies in each mating combination after arcsin transformation were analyzed using Duncan's multiple range test. By the null hypothesis, 25% of the mated pairs would be expected in each of the four possible mating combinations.

Limitations

This low technology method requires no special skills and apart from a UV lamp no special equipment. Experience in distinguishing marked from unmarked flies under a UV lamp is very easily gained.

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MEASURING MATING ACTIVITIES IN FIELD CAGES: TECHNICAL PROBLEMS AND POSSIBLE SOLUTIONS

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Description and Objectives

Field cages are used frequently in quality control because they are the important intermediate step between laboratory tests conducted under defined experimental conditions and the actual field tests. In our own studies we have encountered various problems of general nature with field cages. They have, however, very rarely been discussed in fruit fly publications despite the fact that they occur everywhere and can jeopardize the experiments if they are not adequately solved. Here we describe some of the major problems we had in early tests with Rhagoletis cerasi and list possible solutions that might be of interest to colleagues planning similar studies. Added are a few ideas that have not been proven yet but could be incorporated in future designs of field cages and field cage experiments.

Materials and Methods

Shape and size of cages. Each entomologist works with field cages that differ in size and shape due to the fact that they are, in general, tailor-made by local manufacturers using locally available material. However, flexible cages that can be moved and stored easily seem to be the rule, whereas permanent installations are less frequently used. Our five cages are of cubical shape and of a size that allows an average person to reach the ceiling easily (2x2x2 m). Everybody working with cubical cages knows that fruit flies tend to concentrate in the upper corners facing the sun, whereas we would like to see a more homogeneous distribution. We are now experimenting with overhead shading to reduce that particular problem. The best solutions I have seen are cylindrical or hemispherical cages that eliminate the corner-effect. Also, we found that a flexible height (vertical dimension of the cage 3 m) offers a definite advantage because objects of different sizes (e.g., trees, models, etc.) can be enclosed if needed.

Material. Our cages are made of heavy duty plastic screening used for insectaries that are weatherproof and resist even strong winds. However, we observed that the plastic material produces a bright glare when the cages are exposed to direct sunlight. We are also studying now whether overhead shading could solve that problem. I have seen permanent installations with galvanized wire screening. Idea: experiments in temperate zones often suffer from frequent rainfall that interferes with the experimental schedule. A semi-permanent type of field cage, constructed with wire screening and of cylindrical shape, could be used in the field as long as weather conditions are adequate, and then carried to a greenhouse where tests could be conducted on rainy days or during seasons when outside temperatures are too low.

High temperatures. One of our five cages contains equipment that measures and records temperature and RH. We found that the temperature inside the field cages increases several degrees above ambient temperature when the cages are exposed to direct sunlight. The temperatures at noon (the optimal period for mating tests with R. cerasi) might rise to between 35-40°C and hence produce unrealistic conditions. We have not solved this problem, as ventilation of the cages is poor. Overhead shading might again reduce temperatures but also eliminate needed direct sunlight. At present we conduct the 2-h experiments in the morning when inside temperature ranges between 25 and 30°C and keep the flies several days before the experiments under a light regime that provides an artificial noon 4 h earlier than in nature.

Loss of experimental flies. The major problem we faced at the very beginning of our field cage studies was the tremendous loss of flies when we installed the cages over grass-covered ground. Covering the grass with a plastic sheet was not satisfactory as many flies still disappeared in the open gaps between the plastic floor and the cage walls. Ants and spiders also killed substantial numbers of flies. The best solution was the construction of a solid floor inside the cage that keeps the cage clean, keeps predators away and allows easy servicing of the cages by the personnel as flies walking on the floor can be detected easily. Two wooden boards (1x2 m) are fitted into the cage and are laid on a wooden frame that lifts the boards about 5 cm above ground. The boards are painted a neutral light gray color that does not interfere with the flies' behavior but allows easy detection of the insects resting on the floor. A transparent plastic sheet, 8 cm longer and wider than the cage floor, is laid over the floor and provides a perfectly tight connection between the floor and the cage wall (Fig. 1). The plastic also facilitates cage cleaning. In permanent installations I have seen concrete floors that also solve the problem of escape. Escapes through the entrance (zipper) are reduced to a minimum through orientation of the entrances away from the sun (in our case north).

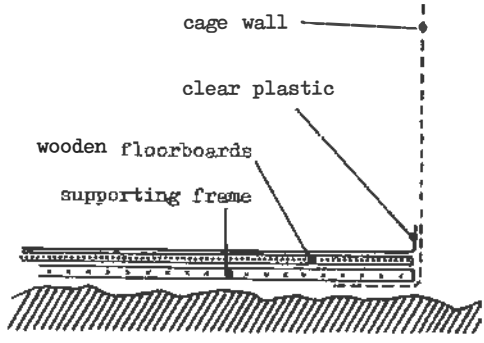

Fig. 1 Details of field cage floor

Marking of the flies. Whenever two or more different strains are included in the same test, flies have to be marked in order to identify the strains participating in mating. Fluorescent pigments (either in the form of powders or aerosol sprays) are widely used as markers, and often one strain is left unmarked. It should be pointed out that there is increasing evidence that fluorescent pigments used as markers increase mating and other activities and could therefore influence the data (Boller et al. 1977, Holbrook et al. 1970, Serghiou, pers. comm.). It is therefore recommended that all flies involved in the test be marked. So far we have no indication that fluorescent yellow and red produce different effects.

Time and replication. Field cage tests are time-consuming and require considerable preparation. Furthermore they are difficult to repeat in time and space due to changing environmental conditions. The statistical analysis of the data therefore calls for an adequate number of replications carried out at the same time. One person can handle 3-4 replications of our standard test (100 males and females of 2 strains = 400 flies per cage; mating pairs collected in 15-min intervals for 2 h). Experimental designs that contain additional observations or increased numbers of flies require more persons or a reduction of the number of replications.

Possible Modification and Expansion of the Technique

The use of field cages is virtually unlimited and offers interesting possibilities in all those cases where direct field observations in orchards are not feasible. Their advantage is that at least some of the experimental variables (such as the experimental flies) can be standardized and that laboratory tests can be repeated and verified under semi-field conditions.

Limitations

Field cage tests have serious limitations. They do not exactly reproduce field conditions as they are still cages that limit certain orientation and flight patterns. The corner-effect of cubical cages introduces a bias that has to be taken into account. The limited replicability of field cage tests in northern climates could possibly be improved by combining tests conducted outdoors and in greenhouses.

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Sexual Physiology

INTRODUCTION

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Physiological assessments provide information on quality traits that are either pre-copulatory or post-copulatory in nature. Those that are post-copulatory are non-behavioral and directly affect the impact of the released fly on the native target population. Examination of pre-copulatory physiology can indicate variations in processes that may detrimentally affect behavioral capability.

In the area of pre-copulatory physiology, a number of important parameters will influence the outcome of insect suppression programs. Some of the questions that arise are: Do mass produced and treated and native insects attain sexual maturity at the same time? Are their mating and other rhythms similar? Are temperature thresholds for essential behaviors appropriate? Are their acoustical, visual and tactile signals similar in both time of emission and nature? Are there differences in visual capability between laboratory reared and native insects? Has colonization resulted in adaptive selection of strains different in their needs for and responses to nutritional factors? Thus, the laboratory reared insects might not be equally competitive in all ecological situations and should be evaluated in this regard before release programs are initiated. These pre-copulatory aspects have been discussed in previous chapters of this book.

Once the pre-copulatory aspects of physiology are established, a number of post-copulatory questions arise: If the native female is normally monogamous, will mating with a released male elicit this response? If the native female is normally polygamous, will a released male elicit the same behavior as a native male?

These considerations lead us to consider the problem of sperm supply in the males, sperm transfer, sperm displacement, or sperm precedence in addition to changes in the sexual receptivity and ovipositional behavior in the female. In adult dipteran males, spermatogenesis (sperm production) continues throughout most of the adult period. Irradiation or chemosterilant treatments are well known to inhibit spermatogenesis by killing gonial cells. Thus, we can expect a released male to produce less sperm. Normally after sterilizing doses, only post-meiotic cells (spermatids and sperm) will mature and be available for insemination. Therefore, an adult male will be capable of fewer inseminations and the major question is how important is this for the species in question.

In addition, we can consider whether polygamous females will utilize sperm from treated males as they would sperm from untreated males. Very often the eggs laid subsequent to a double mating are fertilized mainly by the sperm of the second mating. This phenomenon of sperm displacement or sperm precedence would be of little consequence in a control program providing that sperm from both laboratory reared/treated and native males are utilized equally in a second mating. However, this is not always the case.

Sperm displacement is by no means rare. Parker (1970) and other authors have shown that in at least 20 insect species tested, 16 exhibit sperm displacement. In addition, Prout and Bundgaard (1977) found significant differences amongst untreated genetic strains in sperm displacement ability.

A detailed answer to all of these questions is perhaps not required before preliminary assessments of autocidal techniques in the field are initiated. However, only with these potential problems in mind can we be alert to observe and quantify the many possible alterations in the performance of released insects in a field environment. The short papers in this section describe a variety of tests and methods devised to quantitate different aspects of sexual physiology of laboratory reared and native fruit flies. Success in autocidal programs will depend to a large extent on our ability to assure that there is no sexual isolation between released and native insects. Economic feasibility in autocidal programs depends not merely on the fact that some of the released insects mate with some of the native insects but that they do so in proportion to their relative numbers and elicit as closely as possible the physiological and behavioral responses conducive to maximum population reduction.

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MEASURING MATING FREQUENCY AND ABILITY TO INSEMINATE IN CERATITIS CAPITATA

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Description and Objectives

The sexual behavior of the Mediterranean fruit fly may change under laboratory conditions. Crowding and the presence of readily accessible mates tend to produce flies that show decreased sexual aggression (Fye and LaBrecque 1966; Rössler 1975a, 1975b).

Sperm transfer of laboratory reared males is crucial for the success of the sterile insect technique. The method described here compares the ability of the laboratory reared and field-collected males and females to transfer and to receive sperm. The test involves no-choice homogamic and heterogamic crosses and is divided into two sub-tests which are carried out consecutively: 1. Progress of insemination within the homogamic and the heterogamic populations; 2. Level of insemination within a defined population during a limited time period (= mating frequency).

Materials and Methods

Sub-test One. Field-collected and laboratory reared males and females are sexed after eclosion. They are then placed in groups of equal numbers of males and females (35 individuals in our tests) in 1-liter, ventilated plastic cages and provided with water and food (a mixture of one part yeast hydrolyzate and three parts sucrose). Tests of nine or more groups of each of the following combinations of adults are initiated simultaneously: 1. Field or laboratory homogamic populations; 2. Reciprocal heterogamic (field x laboratory) populations. They are then kept under regular laboratory conditions ($25^{o}\pm2^{o}C$, 70% RH and continuous illumination). Single groups of each of the four combinations are sacrificed at 24-h intervals and the spermathecae of the females checked for the presence of active sperm. The females are anaesthetized with CO_2, placed in a drop of Insect Ringer Solution on a microscope slide, and their genitalia exposed by pulling the tip of the abdomen with fine forceps. The spermathecae, which are brown-black in appearance, are crushed and checked for sperm. The percentage of inseminated females is plotted against time. The data describe the cumulative level of insemination in a given population over a period of time. The test defines three consecutive periods in the experimental population: 1. Premating period; 2. Period of rapid insemination; 3. Period of saturation.

Sub-test Two. This test is carried out with virgin males and females at stage two (rapid insemination ability), as defined in Sub-test One. Groups of homogamic and reciprocal heterogamic crosses, each consisting of equal numbers of males and females, are tested (we use 25 adults of each sex). Each cross is carried out in five or more replicates in 1-liter plastic cages provided with food and water. Populations are sacrificed after 24 h and the spermathecae checked for active sperm. The percentage of inseminated females in the two homogamic and two reciprocal heterogamic crosses may be compared using the Chi^2 test.

Variables, Design, and Analysis of the Experiment

Standardization of the laboratory rearing conditions of both strains is required. The first sub-test defines the correct age of the adults to be used in the second

sub-test. All experimental populations are initiated simultaneously and no standardization of the conditions is necessary during the test period. Conditions should however enable mating of the adults. The following comparative data are obtained in these tests: 1. Premating periods of males and females of field and laboratory origin; 2. Progress of insemination in homogamic and reciprocal heterogamic crosses, measured by the slope of the insemination curve in the second period of Sub-test One; 3. Percentage of receptive females in homogamic and heterogamic crosses; 4. Insemination propensity of laboratory and field collected males and females (Sub-test Two), and detection of possible pre-zygotic isolation mechanisms.

Limitations

The technology used in these tests is very simple and requires only a stereoscopic light microscope and elementary skill in dissecting flies.

Modifications and expansion of the technique

The tests described are carried out under laboratory conditions. The "field" populations in these tests had usually been reared in the laboratory for a few generations prior to the tests. It is possible to carry out the whole test with original field populations under actual field conditions.

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Fig. 1 Flow Diagram for Obtaining Life Table Data in Rhagoletis cerasi Production

Item	Replicates	Activity	Information obtained	Used for Life table
200 eggs on moist filter paper	1	Incubation at 25°C	No. of larvae hatched	+
200 eggs on 20 g of liquid larval diet	10	Store at standard laboratory conditions; check daily after 10th day for pupae formed	No. of pupae	+
			Larval development and peak of pupation	
Pupae obtained	10	Calibrate and weigh 10 days after pupation	Pupal size	
		Store pupae at 5°C for 5 months		
		Sort out dead pupae	Pupal mortality	
		Incubate at 25°C and check daily after 16th day	Emergence rate Emergence pattern	+
Adults (pooled)		Determine sex ratio	Sex ratio No. of females	+
10 males + 10 females in standard oviposition cage	5	Collect eggs daily for 10 consecutive days	Sexual maturation Preoviposition period Fecundity/10 days	
		Check mortality daily	Survival rate	
20 females of the P, F_5 and F_x generation held individually in mini-cages	20	Collect the eggs daily for 4 days	No. of reproducing females	+
		Compare fecundity of reproducing females with mean fecundity of F_5 females	Calculation of 'full' female equivalents	+

Partial Life Table

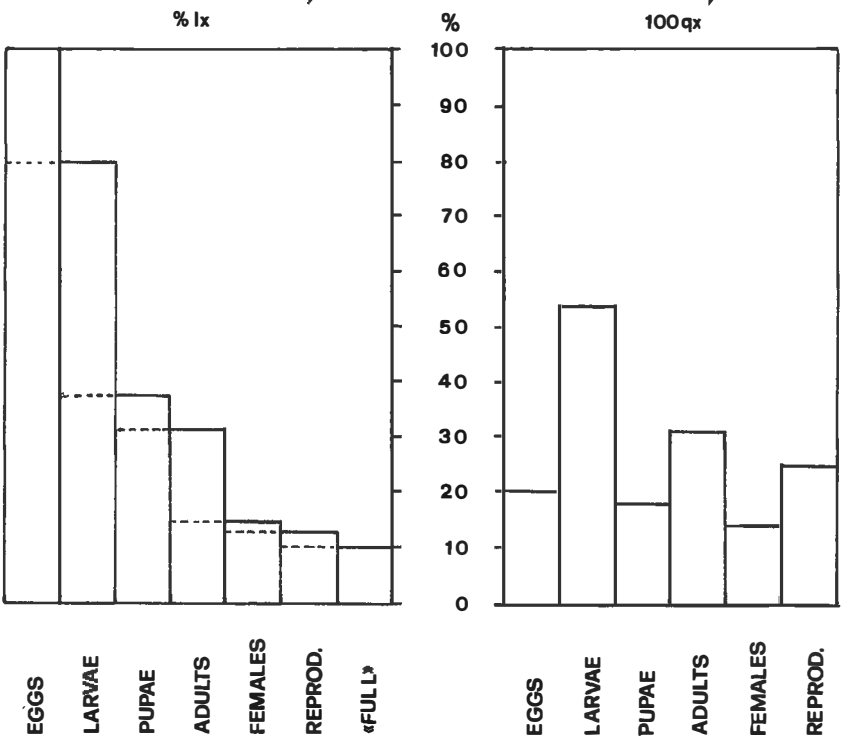
Complete Life Table

Fig. 2 Example of a life table for Rhagoletis cerasi Date: 6/3/76

Strain: Hellikon/CH Generation: P → F_1 Rep.No. 7 (10)

Life Stage(x)	lx abs. no. entering	% lx relative	dx no. dead during stage x	100qx dx as % of lx mortality	
EGGS	200	100	40	20.0	
LARVAE (= eggs hatched)	160	80	85	53.1	
PUPAE	75	37.5	13	17.3	
ADULTS	62	31			
Females	29	14.5	4	13.8	] 31.0
Reproducing	25	12.5	5	25.0	
Reduced * fecundity (20%) = 'full females'	20	10.0			

*] compared with mean fecundity of F_5



MONITORING THE PRODUCTION OF THE "DEMOKRITOS" OLIVE FLY FACILITY IN GREECE

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Description and Objectives

In research, the use of homogeneous experimental material and standardized conditions is a basic requirement. In mass rearing, where the objective is producing insects for the SIT or production of parasites, predators, etc., an additional requirement is the programmed supply of good quality insects (Huettel 1976). Large fluctuations in the production rate could jeopardize the success of large scale projects. The problems arising in the mass production of fruit flies were discussed by Steiner and Mitchell (1966). Monitoring of the various steps in the rearing procedure through detailed bookkeeping should allow tracing and correction of problems in the control of production rate. Such steps include adult maintenance (survival, fecundity and fertility), egg incubation, adult and larval diets, larval and pupal development, pupal handling and recovery (number and size), and adult emergence.

Other specific quality parameters of the mass produced flies should be checked applying appropriate techniques described in this manual.

Materials and Methods

The methodology pertaining to the mass rearing of the olive fly has been described elsewhere (Tsitsipis 1975, 1977a, 1977b). Monitoring of the production facility is achieved by conducting tests of egg fertility and adult emergence, as well as keeping records of the various aspects of the procedure, as will be described in the next part. To check the egg fertility take samples of about 300 eggs 24 h after collection just before their placement on the larval diet and incubate them in a petri dish on filter paper impregnated with 0.3% propionic acid. Record egg hatchability after 3 days. For monitoring adult emergence, take a sample of about 300 pupae shortly before adult emergence (9th day after pupation) and record the adults that emerge over a period of 4-5 days.

Variables, Design,and Analysis of the Data

The following variables are important for production efficiency and constancy of the rearing system and require standardization: ambient conditions (temperature, relative humidity, photoperiod, and light intensity), hygienic procedures, fly density in the cages, adult diet and water supply, oviposition substrate renewal (domes), fly age, distribution of colony cages of various age, dead fly removal, adult and larval diet preparation, and egg incubation. A detailed daily record of the following attributes and activities is to be kept in tabular form: temperature, relative humidity, photoperiod, number of adult cages, cage initiation, and termination, adequacy of food and water supply in the cages, dome renewal, moist sponges in the domes, dead fly removal, total number of eggs collected (volumetrically measured), per cent egg hatch, egg surface sterilization, number of larval diet trays prepared, number of trays seeded with eggs, check of larval diet microbial contamination, total number of pupae collected (volumetrically measured), mean pupal weight (weight of the 300 pupae used for adult emergence check), mean per cent adult emergence, mean larval and pupal developmental periods, and insectary floor cleaning. Additionally, on each numbered cage write the dates of its initiation and those for dome exchange. On the larval diet

trays write the date of the diet preparation and the date of the collection of the eggs seeded on the diet. A checklist of larval diet ingredients for use during its preparation is also recommended.

Bookkeeping procedures such as these will provide the data necessary to assist in tracing problems in the mass rearing system when the expected production is not attained. These data could lead toward taking the appropriate corrective measures.

Possible Modification and Expansion of the Technique

The effect of incubating eggs in large numbers and of pupal storage can be checked by taking additional samples of eggs and pupae immediately after egg collection and pupation, respectively.

Another test concerning optimization of larval diet efficiency deals with checking larval survival and growth on the 5th day after egg hatch (Manoukas and Tsiropoulos 1977). Knowing the productive capacity of the larval diet and the number and weight of the larvae, decisions can be made whether supplementation of the diet with additional nutrients during the last part of larval development is needed (Manoukas 1977).

Limitations

No special skills or equipment are required, however, reporting constancy is absolutely necessary.

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MONITORING THE PRODUCTION OF THE HONOLULU FRUIT FLY FACILITY

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Description and Objectives

Over the past 10 years, Mediterranean fruit flies, oriental fruit flies and melon flies numbering nearly two billion have been produced in our rearing facility. Although production levels ranged up to 30 million per week, few rearing failures were encountered during this period, largely due to careful monitoring of procedures during each step of the rearing operation. In any rearing facility problems do occur occasionally, but rearing failures caused by improper rearing conditions or procedures can usually be pinpointed and corrected if procedures are standardized and monitored. From our experience with tropical fruit flies, genetic breakdown or disease rarely are the causes of production failures. Too often when laboratory reared flies perform poorly in tests (particularly in field studies) the quality of the insect is questioned and not the execution or interpretation of the test.

This report describes some of the procedures and factors that may be critical in maintaining a continuous production of adults, eggs, larvae, and pupae of Hawaiian tephritids of high quality.

Materials and Methods

Adults. When adults are stocked in cages (100,000 adults/1.8 m^3) by spreading pupae over the floor, two 60x91-cm fiberglass screens are suspended perpendicular to and touching the floor. Emerging adults walk up these screens and thus avoid milling over each other. Although diet may be presumed to be adequate based on quantity and nutritional quality, physical defects such as crust-forming of protein hydrolysate may also affect adult nutrition and should be periodically checked. The preoviposition period, 4, 10, and 10 days for the medfly, melon fly and oriental fruit fly, respectively, must have elapsed before practical egg collection can be initiated. Usually, adult medflies are discarded at 14 days of age, while melon flies and oriental fruit flies are kept up to 40 days.

Eggs. Eggs collected by allowing them to be laid through cloth to drop into water should be incubated for 24 h at 27°C before seeding onto the larval diet; eggs collected with an egging receptacle can be seeded soon after a 20-h collection. The developmental periods of larvae from both schedules will be the same. Simple periodic checks of egg hatch can help in assessing or predicting culture failure.

Larvae. Diet temperature during the first two days after egg set should not be lower than 24°C; otherwise low pupal recovery can result. Diet temperature is influenced by tray configuration and spacing between trays. At 27°C and 50% RH, the diet temperature from trays spaced ca. 2.54 cm and 5.6 cm apart can drop 3° and 5°C below ambient, respectively. If cultures are covered with polyethylene sheets or muslin cloth, a diet temperature of near ambient can be maintained. Temperatures will rise to ca. 32°C four days after egg set in diets of wheat mill-feed with seeding rates of 25, 16, and 18 eggs/g of diet for medfly, melon fly and oriental fruit fly, respectively, in 2.54-cm spaced stackable traps. This temperature can be maintained by transferring the cultures from 27°C to 20°C. Then, when the mature larvae leave the

diet the temperature begins to drop. During this period the diet becomes dry. If lost moisture is not replenished a high rate of pupation occurs within the diet. Also, larval size is reduced and the period of larval exit from the diet is prolonged. In a moist diet, more than 90% of mature oriental fruit fly larvae will exit the diet in 3 days as compared with 5 days in a relatively dry diet. Generally, those larval diets containing bulking agents that require less than 10% water (total wt.) to give a suitable diet consistency remain moist throughout the larval period. Water is sprayed on the surface of the diet to replenish moisture during the 4th to 7th days after egg set. Avoid large drops or streams of water as this will destroy "breathing channels" and can smother larvae that are, by this time, feeding near the bottom of the tray. Our system is designed so that mature larvae leave the diet and drop into a water container positioned at the bottom of the larval cabinet. Mortality can result if larvae are held for more than 24 h in water of a temperature of 20°C (8 h in 27°C water). Larvae become inactive shortly after they enter the water. However, on certain days when large numbers of larvae exit from the diet the layer of larvae in the container of water may reach the water surface and larvae near the top will remain active. The water level is, therefore, checked twice per day to prevent pupal mortality which could result if larvae are allowed to remain active for more than 5 h. When mixed in moist vermiculite at a load rate of 1.5 liters of larvae to 6 liters of vermiculite and held at 20°C, mature larvae will pupate within 8 h. When they are held at 27°C instead of 20°C, pupation time is extended and pupal mortality results. Mortality is characterized by pupae turning dark-colored and the puparium soon becoming hollow. Also, increasing the larval density or allowing larvae to pupate in dry vermiculite can prolong pupation.

In Hawaii, wheat mill-feed is the cheapest bulking material that is readily available. All varieties used in the larval diet at a pH of 4.5 produce high larval yields except the variety "soft wheat". For some unknown reason soft wheat-based diet performs as well as the other varieties only when the pH of the prepared diet is 5.5 or higher, instead of 4.5.

Pupae. We prevent mechanical injury by sifting pupae no earlier than 2 days after pupation. Late sifting (5 or more days after pupation) can cause pupal mortality, particularly when the pupation medium is quite moist. During the last 1-2 days before eclosion pupae can generate enough metabolic heat to raise the diet temperature to 40°C if held 3.8 cm or more deep. Approximately 1 cm is the optimum depth of a layer or stored pupae. We do not use pupae from low-recovery cultures for stocking adult breeding cages.

Sanitation and Waste Disposal. All rearing equipment is washed with hot water after use, although no problems with disease have been encountered in 20 years of rearing operations. Disposal of spent medium may pose a problem for large-scale rearing programs. Utilization of rearing waste as a source of energy (methane) to satisfy the heating requirements of the rearing facility is showing great promise in a current study.

LIFE HISTORY MEASUREMENTS OF ANASTREPHA SUSPENSA

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Description and Objectives

Life history measurements are used to ensure the adequacy and continuity of laboratory rearing of Anastrepha suspensa. They can also be used to quantify "bottlenecks" and identify the causes of inadvertent selection during initial establishment and subsequent colonization (Leppla et al. 1976). Survival, reproductive contribution, and specific phenotypic traits of each developmental stage indicate environmental deficiencies and/or genetic divergence from previous generations. Thus, undesirable changes are avoided by providing essential requirements and eliminating causal factors.

Materials and Methods

Caribbean fruit fly larvae are reared according to standard procedures (Kamasaki et al. 1970, Burditt et al. 1974) and ca. 1000 pairs of adults are maintained in each 31x31x31-cm wire screen cage (Greany et al. 1976). This production system is routinely monitored by removing adequate samples of all developmental stages at appropriate intervals (table 1).

Variables, Design, and Analysis of the Experiments

For adults, records are kept of the date of eclosion, total number recovered, sex ratio, and duration of the preoviposition period. Sexually mature females are isolated from the colony at ca. 10 days postemergence, maintained in individual cages to ascertain the number that contribute fertile eggs, and examined for transferred sperm. The total number of eggs derived from all females in each cage is determined volumetrically. Rates of larval development are estimated by recording dates of larval establishment and pupation. Again, yields are derived by volumetrically determining the number of pupae recovered per sampled container; at least 100 pupae are weighed in batches of 10 to calculate the variation in mass. Behavioral traits are used to further characterize the populations and identify causes of undesirable selection and nongenetic influences. The extent of sampling depends on colony size and required accuracy; however, once standardized, measurements and analyses are conducted in the colony environment. Analysis of variance is used for statistical comparisons, but direct inspection of the data is adequate for many evaluations.

Possible Modifications and Expansion of the Technique

Survival, rates of development, and yields of each stage can be used to generate life tables and calculate the biotic potential of the colony (Birch 1948). Parameters involved are the net reproductive rate (R_o); number of female offspring produced by each female of the initial cohort; mean generation length (T); intrinsic rate of natural increase (r); number of female offspring produced per female per time interval; finite rate of increase (λ); and number of times the population multiplies per unit of time. R_o is the sum of the products of age-specific survival and mortality ($l_x m_x$) for the entire reproductive interval. Since $r = \log_e R_o/T$, unequal developmental rates among populations would confound meaningful comparisons. Therefore,

Table 1. Life history monitoring chart with morphological stages and developmental processes that occur in production and guidelines for sampling.

PRODUCTION		SAMPLING
Pairs of Flies	day 10 →	Ten females from each cage (no. eggs/ isolated female and mated condition)
preoviposition (7 days)		
Eggs	day 10 →	Number determined volumetrically (100/collection for % hatch on 3d day postoviposition)
larval development (8 days)		
Larvae	day 21 →	One rearing unit for every 10 (% pupation at specified age)
pupation (4 days)		
Pupae	day 21 →	Number determined volumetrically (100/collection for mass and % eclosion)
maturation (12 days)		
Flies	day 33 →	Adequate no. for behavioral tests

sampling intervals must be equal (similar age distributions) if this method is used to provide indexes of the productivity and overall condition of different populations. Generally, oviposition rates are determined at equivalent intervals, and (antilog_e r) is calculated for the comparisons. An electronic calculator can be programmed to derive all of these parameters from the same set of data.

Limitations

The life history of a colony identifies quality relative to production characteristics but not actual performance (Leppla et al. 1977). Techniques for generating life tables are relatively unsophisticated; however, continuity of sampling is essential. Precision is lost if analyses are not identical with respect to time of day, environmental conditions, sources, methods, etc. Except for the addition of an accurate balance and calculator, duplicate production equipment can be used for all of the quality control determinations.

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QUANTITATION OF FEEDING RESPONSES

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Description and Objectives

Huettel (1976) analyzed the major traits that should be bioassayed for the success of laboratory colonies. One of these is survival. The flies must feed for several days before reaching sexual maturity. Survival during this period is critical to their success later, and it is therefore an important component of quality. Quantitative data on food intake and chemosensory acuity will help to evaluate the ability to locate and identify the food. The adult fly requires water and carbohydrate as an energy source in order to survive. Proteinaceous food is needed to achieve complete ovarian development. Hagen (1953) has shown, by using chemically defined media, that a carbohydrate, amino acids, vitamins and certain minerals are essential for survival and ovarian development.

Information on energy intake and acuity of discrimination of essential food components was obtained in the medfly, using the following methods:

1. Measurements of consumption of sucrose solutions.
2. Intensity discrimination between sugar solutions.
3. Tarsal reflex - proboscis extension.
4. Electrical discharge of stimulated labellar sensilla.
5. Aggregation response to protein hydrolysate.

The most intensive studies in this field were conducted with the blowfly, and only one paper has been published concerning feeding behavior of medfly (Gothilf et al. 1971). However, Mrs. H. Fuchs (1974) has completed her M.Sc. thesis in this field and Mr. Y. Nizan is in the middle of his Ph.D. studies, on chemoreception of the medfly. Since their work has not been published yet, some of their data are described here, including more technical details.

Materials, Methods, and Variables

1. Consumption of sugar solutions. The method recommended is the one originally suggested by Dethier and Rhoades (1954), with the modifications made by Gothilf et al. (1971) for the medfly. A group of 25 females is introduced to a 2-pint jar covered with netting. A J-shaped 2-ml volumetric pipette is filled with a 4% sucrose solution and offered to the flies. At 24-h intervals the pipette is filled to the original mark by means of a calibrated syringe and the amount of fluid taken by the flies is read directly from the syringe. Flies eat much less on the first day, consume relatively larger quantities on the second day, and later maintain a more or less constant intake for several days. A typical intake for 24 h/fly at the age of 3-4 days and in sound physiological state should be 15-20 mm^3, provided the flies were kept at 28°C and constant light (Fuchs 1974). In addition to temperature, light and age, precautions should be taken in handling the flies. CO_2 anaesthesia considerably decreases sugar uptake, and the decrease is measurable even 4 days later. A glass aspirator for handling the flies for feeding experiments is recommended.

2. Intensity discrimination between sugar solutions. The method used is essentially identical to the one mentioned above, except that two J-shaped pipettes are introduced into each jar containing 25 flies. The flies are given a choice between two sucrose solutions and the minimum concentration difference required for a choice is

determined. The threshold of sucrose discrimination is determined in a similar manner, except that one pipette contains sugar solution and the other water only. The lowest concentration that is consumed more than the water is considered to be the threshold of discrimination. Fuchs (1974) found that flies in good physiological state show maximal discrimination of sucrose at a concentration of about 0.125 M. At this range a change of 10% in the concentration is detected by the fly (Weber fraction equals 0.1). The threshold of discrimination of sucrose is about 10^{-4} M.

3. Tarsal reflex - proboscis extension. One of the most intensively studied behavioral systems is the response of the proboscis of the blow fly to chemical stimuli. Stimulation of Phormia tarsi with sugar leads to proboscis extension. The tarsal chemoreceptors of the medfly are not as sensitive as those of the blowfly; therefore acceptance threshold can be measured best by stimulation of labellar receptors. The proboscis is readily extended and attempts at sucking are made by the fly when certain sugar solutions are applied, even to a single labellar hair. The procedure employed for determining labellar acceptance threshold is by the ascending method of presentation (Dethier 1952). Each fly is attached by the wings and dorsum to a wooden stick fixed under a stereomicroscope. Stimulants are applied via micropipette. If flies are deprived of sugar for 24 h, and are given water freely, about 30% detect a concentration of 10^{-3} M of sucrose, and 10^{-2} M is required to stimulate 50% of the flies. If the flies are fed sugar ad lib, 1 M sucrose is needed to cause proboscis extension in 50% of the flies. The variability between flies is quite large and a sample of at least 100 flies is needed in order to obtain a representative threshold (Nizan, unpublished). The precautions are the same as in the previous experiments. Heating from the stereoscopic lamp should be minimized as flies try to detach themselves if heated.

4. Electrical discharge of stimulated labellar sensilla. The most commonly used method for monitoring electrophysiological sensory output of gustatory receptors is the tip recording. The side-wall recording technique is superior, but requires much more skill and time and has not been attempted with the medfly. To record from the tip of a chemosensitive hair, an electrode is inserted into the body of the fly (thorax or head), and the recording electrode in the form of a micro-glass pipette (containing .1 M NaCl and the stimulating sugar) is lowered over the hair (large or intermediate hairs). The disadvantages of this technique are discussed by Dethier (1974). The head of the fly has to be squashed gently with forceps to prevent movements of the proboscis. The amplifying-recording systems that were found suitable for the medfly were described in detail by Gothilf et al. (1971). The threshold of sensitivity to sucrose varied greatly in the labellar hairs of the medfly. In some, sugar spikes were recorded in response to 0.03 M sucrose, whereas in others equivocal recording was obtained only at 0.125 M (Gothilf et al. 1971). This is very common in this peripheral sensory system. Even in the much-studied blowfly, with every precaution taken to eliminate artifacts of recording as a source of variability, and all biological variables of the fly standardized - there still appears to be inconsistency in the response of the contact chemoreceptors of the labellum (Dethier 1974). Frequency of discharge increases with increase of concentration up to 0.5 M sucrose and then levels off. It is therefore proposed to evaluate the responsiveness of the medfly to sucrose stimulation by measuring its response to 0.5 M sucrose. At this concentration 150-200 spikes/sec during the first quarter of the second after onset of stimulation are expected from flies in good physiological state.

Females should be 2-4 days old and given free access to water and sucrose. We observed in one set of experiments in a highly inbred small colony a great decrease in response to sugar and salt stimuli and had to replace our colony. During the recording, a high humidity has to be maintained around the fly and the recording electrode in order to preserve the viability of the preparation and to cut down evaporation from the recording electrode.

5. Aggregation response to protein hydrolysate. Female houseflies feed avidly on filter paper impregnated with protein hydrolysate. A large cluster of flies aggregates on the treated paper within minutes after its introduction into a cage (Robbins et al. 1965). Medflies seem to ignore protein hydrolysate introduced in this manner, and therefore Galun and Gothilf (unpublished) developed the following assay for evaluating medfly appetite for protein: 2.5% enzymatic hydrolysate of casein is dissolved in hot water containing 3% agar and is poured into petri dishes 3.5 cm diam. Control dishes contain agar alone. One treated dish and one control are introduced into a net cage (30x30x30 cm) containing about 300 flies of both sexes. The flies aggregate slowly and feed on the agar-hydrolysate. At the age of 3-5 days about 25 flies are found on the treated dish one-h after the introduction of the dish. Four counts at one h intervals should give a cumulative count of about 100. Unlike houseflies, male medflies also aggregate on protein hydrolysis.

Flies should be fed only sugar-water until tested. Illumination should be strong, otherwise flies show very little activity. Temperature should be maintained at 28°C because even at 25°C very little aggregation is observed. A wet cotton pad should be placed on top of the cage, offering the female an oviposition medium, otherwise many congregate on the control agar plate, trying to oviposit into it.

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MEASURING TOLERANCE OF ANASTREPHA SUSPENSA LARVAE TO TO DELETERIOUS HOST PLANT ALLELOCHEMICS

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Description and Objectives

In most fruit fly rearing and release programs (e.g., the sterile insect technique), it is principally the mating competitiveness of the released flies that is of concern. However, other control techniques can be envisioned in which the survival of the progeny of the released flies in the field would also be important. Such would be the case in "genetic engineering" programs such as the introduction of conditional lethal mutants into a wild population (Pal and LaChance 1974, Whitten and Foster 1975). Programs of this type require that the laboratory reared (LR) flies do not lose their ability to successfully develop in the host fruit, some of which may contain deleterious allelochemics (non-nutritional compounds produced by one organism that affect another species (Whittaker 1970)). For example, we recently found that certain citrus peel oil constituents are toxic to the eggs and larvae of Anastrepha suspensa and that the wild flies are more resistant to these agents than are flies of our laboratory strain.

The objective of these tests is to measure the relative susceptibility of wild and LR fruit fly larvae to allelochemics isolated from host plants and incorporated into their diet.

Materials and Methods

Eggs are obtained from wild and LR insects and are incubated in a favorable environment until eclosion. Then the larvae are placed at a prescribed rate (e.g., 10 per 10-ml of diet) on artificial medium containing fruit tissue with suspected allelochemic activity. If the active compound is known, it should be added to the medium at varying rates so as to provide a concentration range. Records are kept of the developmental status of the larvae on a daily basis (1st, 2nd, or 3rd instar; pupa; and living or dead). Duration of the pupal period is measured, as well as pupal weights on the day of formation. Note is made of the percent emergence of flies from the puparia and of the sex ratio of emerging flies. Each test is replicated 5-10 times, with an equivalent number of control replicates.

Variables, Design, and Analysis of the Experiments

Application of newly-hatched larvae rather than eggs to diet avoids interreplicate variation in initial numbers of larvae. Chemical purity of the compound being tested is important. Data are most appropriately analyzed by using probit analysis techniques and by comparison of LD_{50} values.

Possible Modifications and Expansion of the Techniques

This technique may be used to assist in isolation and identification of suspected allelochemics from selected plant parts by incorporating the tissue (or extract thereof) into the medium. Control programs requiring insects with the ability to survive in fruit in the field may be benefited by routinely incorporating host plant allelochemics into the larval rearing medium to help maintain selection pressure in favor of retention of wild-type characteristics. Also, the procedures may be useful in identifying suspected toxic or growth-retarding dietary constituents.

Limitations

No special skills or equipment are required for these tests; however, the usual chemical techniques are required for isolation and identification of any allelochemics that may be discovered.

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NUTRITION AND QUALITY OF MASS PRODUCED OLIVE FLIES

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Description and Objectives

Most work on the dietetics of the olive fruit fly, Dacus oleae, and other insects has been empirical. Yet it is well established that the quality traits of a given organism can be expressed only when its nutritional needs for growth, reproduction and other special activities are satisfied. Unfortunately, no reliable technique has yet been found for the quantitative determination of D. oleae nutrient intake. However, among several parameters studied, pupal weight seems to express best the nutrient intake of a given stock of this insect. A technique for producing pupae of specific weights and presumably of specific quality is by the use of specified larval densities in a standardized larval diet (Manoukas and Tsiropoulos 1977). Adults emerged from these pupae are then utilized in tests of the quality of performance of certain traits (Tsiropoulos and Manoukas 1977).

Materials and Methods

In tests conducted in our laboratory, eggs 48±8 h old from a given stock of D. oleae flies maintained at the "Demokritos" insectary (Tsitsipis 1975) were used. Egg densities of 5 to 100 eggs/g diet were tested. Description of the procedure is given by Manoukas (1975a) and Manoukas and Tsiropoulos (1977). Evaluation of the diets was done by standard chemical procedures (AOAC 1955). The adults obtained from the various larval densities tested were kept in cages described by Hagen et al. (1963) to evaluate survival, egg production, egg hatchability and adult competitiveness (Tsiropoulos and Manoukas 1977).

Variables, Design, and Analysis of the Experiments

The following variables have been identified and require standardization: age and viability of eggs, on composition of larval diets in terms of the major nutrients, such as proteins, free amino acids, lipids and ash (Manoukas 1975b, 1977), and non-nutritional parameters such as texture, pH and preservatives (Manoukas 1975a, Manoukas and Mazomenos 1977). Adult nutrition also requires standardization. The following is a suggested approach for the production of pupae of a certain weight and evaluation of fly quality of a given stock: 1. Determine the major nutrients of the diet and standardize non-nutritional properties. Information on the chemical composition of the olive fruit (Manoukas et al. 1973) and the composition of the fly body or eggs (Manoukas 1972, Tsiropoulos 1977) may be useful for improving olive fly larval and adult nutrition. 2. Define the optimum age and density of eggs according to the quality traits to be studied. Density studies showed that it is necessary to establish stocks of flies in our insectary according to the specific purpose or biological qualities required, such as egg production (breeding stock), field releases, or hosts for parasite development. 3. Estimate the number of neonate larvae, survival at the desired stage(s), and pupation. Weigh the pupae and count the adults. Determine the "diet worth factor" (Bucher and Bracken 1976) if you have a standard size of pupae. This factor for the olive fly is 62%. However, for some purposes an index that considers pupal size describes best the practical value of a diet. Thus the "diet worth factor" for the olive fly is similar for densities of 10 and 20 eggs/g diet but pupal weight is significantly different (Manoukas and Tsiropoulos 1977). In addition,

nutritional balances and biochemical determinations may be required. Utilization and efficiency of the major nutrients should be calculated. (Information on this approach will be provided to any one interested.) 4. Use an appropriate adult diet when evaluating quality traits.

Possible Modifications and Expansion of the Technique

The technique for obtaining pupae of different weights by changing larval density can be applied in many insects of economic importance and is especially useful for the tephritids.

Limitations

The standard equipment of a nutritional or biochemical laboratory is essential. Genetic studies are required to evaluate the quality traits obtained. Contaminants (inhibitors) produced by larval cultures reared at particularly high densities are detrimental to insect quality.

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THE USE OF GEL ELECTROPHORESIS TO MONITOR GENETIC VARIATION AND MAINTAIN QUALITY IN MASS REARED FRUIT FLIES

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Description and Objectives

Phenotypic changes that affect the quality of mass reared insects fall into two broad categories; those that are environmentally induced and those that occur at the genetic level. If the former can be recognized early enough, conditions of mass rearing may be altered to correct the problem. However, many changes that lower the quality of mass reared insects may be too subtle to be detected by conventional means. Frequently a problem may not be perceived until the insect fails to perform satisfactorily in the field. By this time, the domesticated strain may have undergone irreversible genetic changes that render it useless.

An early warning system is therefore needed that is capable of monitoring laboratory populations for potentially harmful genetic changes. Relatively simple methods are now available for routinely screening genetic loci for changes that might affect the quality of mass-reared insects.

Gel electrophoresis has been used recently to monitor genetic changes in factory reared screwworm flies and to establish the genetic basis for the recent loss of competitive ability in the mass reared flies (Bush et al. 1976, Bush and Neck 1976). A survey of several loci revealed that genetic variation decreased with colonization time and several alleles were eventually lost permanently from the colony. The most dramatic change occurred at the α-glycerophosphate dehydrogenase locus. This enzyme serves a key role in energy flow during flight. An allele that is very rare in natural Central Texas populations rapidly became fixed, or nearly so, in each strain reared under factory conditions. This process was found to be related to a loss of competitive ability in nature. While wild flies are active throughout the day, factory flies appear later, usually around noon. Mating in the wild flies is, therefore, probably completed before factory flies become competent for sexual activity. Appropriate biochemical studies on purified enzymes demonstrated that the allelic form found in the factory functioned best at high ambient temperatures while the normal allele functioned over a wide temperature range comparable to that encountered in nature. Domestication had selected a fly that performed well at the high temperatures maintained in the factory but not in nature.

Gel electrophoresis is now being used routinely in the screwworm mass rearing program to genetically monitor ecologically and behaviorally significant loci at the biochemical level. By using this technique in a quality control program, the effects of altering rearing conditions on gene frequencies can be studied and new strains with specific genotypes synthesized at appropriate times to meet changing environmental conditions.

Materials and Methods

One-dimensional gel electrophoresis is a technique now widely used to rapidly establish gene frequencies for specific soluble enzymatic and non-enzymatic proteins of the cytoplasm in natural and laboratory populations. The various principles and methods for enzyme staining and electrophoresis have been presented by Harris and Hopkinson (1976)

and Gordon (1975), and techniques specifically developed for Tephritidae are reviewed by Bush and Huettel (1972), Bateman (1976), and Berlocher (1976). Critical reviews on the adaptive function of various proteins, and the various ways genetic data can be utilized, are given by Lewontin (1974), Selander and Johnson (1975), Wagner and Selander (1975), Nei (1975) and Hedrick et al. (1976).

Separations of genetic variants in proteins are carried out on crude extracts of the whole fly or larva homogenized in a drop of buffer. Enzyme molecules, which carry net positive or negative electric charges according to the pH of the buffer held in the matrix of the supporting gel, will migrate in an electric field. When a sample of an extract is placed in a gel, proteins can be separated by zone electrophoresis. The speed and distance that an enzyme migrates is related to the size of its net charge and the strength of the electric field. The location of enzymes in the gel can be identified by using specific staining techniques. Generally, only one or two enzymes can be stained on a single gel.

Many proteins exist in natural populations in two or more molecular forms called isoenzymes which differ by a single amino acid substitution. If these substitutions involve amino acids that alter net charge, the multimolecular forms will migrate at different rates. These electrophoretically detectable isoenzymes are called electromorphs. Due to the nature of protein synthesis individuals that are homozygous or heterozygous for each electromorph can be identified by direct visual inspection of gels.

Resolution can be enhanced and the number of detectable electromorphs increased by using the variable gel-sieving method of Johnson (1975, 1977a,b) and Singh et al. (1976).

Two-dimensional gel electrophoresis. A more sophisticated technique introduced to study genetic variation in natural and domesticated insect populations is the high resolution two-dimensional gel electrophoresis technique of O'Farrell (1975). As in conventional one-dimensional electrophoresis proteins differing in only a single charge can be resolved. However, unlike one-dimensional electrophoresis, all classes of proteins, even those at extremely small concentrations, are visible on a single gel without the need for specific staining procedures for each protein on separate gels. This is accomplished by special autoradiography techniques (Laskey and Mills 1975).

The advantage of the O'Farrell technique is that a very large number of electromorphs (ca. 1000-1500) can be characterized simultaneously in a single individual on one gel (O'Farrell 1975, Gurdon et al. 1975, Ames and Nikaido 1976).

Limitations

The use of gel-electrophoresis requires a minimum of biochemical expertise and equipment, and some knowledge of enzymology and gene action is essential. Anyone attempting to apply this approach to quality control programs should have a firm grasp of population genetics and be well versed in the ecology and behavior of the target organism. Furthermore, unless adequate biological information is available on what constitutes normal behavior in nature, it may be difficult to interpret the meaning of observed genetic changes occurring during domestication. The same can be said for any method used to measure any trait whether it be at the phenotypic or genetic level.

The fact that the O'Farrell technique resolves several hundred proteins on a single gel makes it very difficult to analyze each gel for genetic variants (e.g., a position change). A small number of samples can be handled manually, but recording total genetic variation is laborious, time-consuming, and subject to error. If the technique is to be used for population genetics, or in quality control programs, gene frequencies for each locus must be established in a short period of time.

A computer scanning technique for scanning and analyzing individual gels for position changes which reflect charge changes of specific polymorphic proteins is now under development. This approach requires a wedding of the two-dimensional electrophoretic

techniques with computer scanning methods. The methods used in electrophoresis, therefore, require some modification to meet the limitations imposed by the computer scanning equipment and analytic techniques. Details of these methods will be made available upon request.

Finally, it is imperative to undertake a thorough survey of genetic variation in natural populations, particularly in species with several host races and geographically isolated populations. This information will not only help to establish the status of these populations, but it will indicate the level of normal variation in wild populations that must be maintained in the mass reared colony.

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TESTING THE HERITABILITY OF PHENOTYPIC QUALITY TRAITS

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Description and Objectives

There is a growing awareness among entomologists that insects produced in mass cultures often differ substantially from wild insects of the same species. The problem which faces many entomologists is: How does one prevent these changes from occurring in laboratory reared insects? The answer depends a great deal on whether the change is basically due to environmental factors, or to selection (genetic change). If genetic change is found to be unimportant, then improvements in diet and other environmental factors need to be made. If on the other hand there is a large genetic component to the observed change, then the selective forces must be identified, and if possible, reversed. Heritability is a simple measure of the amount of trait variation that has a genetic basis; establishing the heritability of a trait is thus an essential step in achieving quality control.

The Concept of Heritability

Heritability is defined as the proportion of total variation in a trait that can be ascribed to genetic variation; heritability thus ranges, by definition, between 1 and 0. For any given trait, total phenotypic variation is caused by the interaction of genetic and environmental variation. Since biologists usually express variation by the statistical term variance, the relationship between genetic and phenotypic variation can be precisely expressed by the equation

$$V_P = V_G + V_E$$

where V_P = total phenotypic variance, V_G = genetic variance, and V_E = environmentally induced variance. In these terms, heritability is given by

$$h^2 = \frac{V_G}{V_P}$$

where h^2 denotes heritability. (Because of established convention, h^2 stands for heritability itself, and not its square.) Since the total phenotypic variation in a trait is easily measured, it would seem that heritability could be most easily estimated by experimentally reducing either V_E or V_G to 0. Unfortunately, such direct manipulations are usually impossible. Only in Drosophila can a population of genetically identical organisms be produced (thus reducing V_G to 0), and the elimination of environmental variation is in practice extremely difficult.

However, these difficulties can be avoided by approaching the problem in another way. If a trait has a high heritability, then genetically related individuals should be more similar than unrelated individuals, assuming that all individuals are exposed to the same environmental variation. Thus, heritability can be measured by determining the extent to which a trait is correlated in two sets of related individuals, for example parents and offspring. An example of such a correlation will be explained below.

Requirements for the Analysis

The requirements for the correlation technique are:

1. That single pair matings of the species under study can be made.

2. That sufficient progeny (3 or 4 as a minimum; 10 - 20 preferable) from each pair mating (cross) can be reared to adulthood for testing.

Design and Analysis of an Experiment

As an example, assume that a laboratory strain of a particular insect produces insects which are consistently smaller than wild individuals, and that we wish to know the heritability of size in this insect. In the following discussion wing length will be used as an estimator of size, although any measurement which reliably reflects to total size can be used. The first stage of the experiment is to establish pairs of insects (one male, one unmated female) in individual cages, after first measuring and recording the wing lengths of each (see Fig. 1). These are two ways in which to establish the pairs: pairs can either be chosen at random, or they can be established assortatively (large males with large females, small males with small females). Although there are some advantages to the latter course, both techniques work. The relative merits of each approach are discussed by Falconer (1964).

In the second stage, the pairs of insects are provided with oviposition sites or devices, and a set of progeny (F_1's) from each pair is reared to maturity. These individuals are then measured, and the wing lengths recorded.

Finally, the wing lengths of the parents are averaged (to produce a midparent value for each cross), the F_1 wing lengths from each group of progeny are averaged, and these two data are recorded for each cross. The F_1 values for each cross are then regressed against the midparent values, and the slope of the regression line established. Heritability is then given by the simple expression

$$h^2 = b$$

where b is the regression slope.

As with any regression, confidence in the results is increased by increasing the number of points, or in this case, the number of crosses used. In practical terms, it is good to have at least 15 pairs while 30 or more is preferable. While fairly good estimates of h^2 can be obtained using only 3 or 4 progeny from a cross, 10 or 20 progeny yields greater accuracy, especially when h^2 is low.

Possible Modification and Expansion of the Technique

A number of other types of correlations between different types of relatives (full-sibs, half-sibs, progeny vs. single parent) can be used to estimate h^2; for a discussion, see Falconer (1964). As an example, consider how the heritability of mating propensity would be estimated. In many tephritid flies, mating propensity, as measured by Boller, Remund and Katsoyannos, is probably largely due to male mating propensity. On the other hand, females as well as males undoubtedly contribute genetically to the mating propensity of their sons.

The experiment could proceed as follows. Unmated females and males are mixed in a large cage, and pairs are removed as they form (the elapsed time before the pair forms is recorded for each pair). These mated pairs are placed in cages, and a set of progeny reared from each, as in the previous example. Once these flies reach maturity, the mating propensity of the males are determined in the same way. It is very important, though, that the males are not mated against their own sisters, but against females drawn randomly from the original population. Average mating propensity

for each set of F_1's are calculated, and are plotted against the paternal mating propensity. The only difference between this analysis and the previous one is that h^2 is not exactly equal to b, but is probably closer to

$$h^2 = 2b$$

because of the unknown maternal genetic contribution (Falconer 1964).

Limitations

The single greatest limitation with the heritability estimation procedure described here is that it focuses attention on single traits. Selection, on the other hand, seldom affects single traits; selection for one feature may affect another. This has great significance for quality control programs. Suppose, for example, that mating propensity in a particular insect is found to have the fairly high heritability of .65. This implies the possibility of selecting for a strain of insects which mate faster than the wild males, which might be desirable for a sterile male release program. Should such a strain be selected? The answer depends a great deal on whether such selection would also affect longevity, flight ability, on any other component of overall fitness. Possible correlated selection between such traits must be investigated before a successful selection program can be carried out. Heritability estimates and selection programs will undoubtedly be important in the future of insect mass culture, but they must be carefully planned if they are to be successful.

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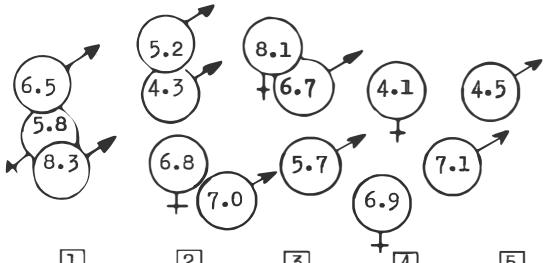
Figure 1. Testing the heritability of quality control traits

Step 1.

Pairs (one male, one unmated female) of insects are drawn from a variable population. In this example, pairs are established assortatively based on their wing lengths (see text).

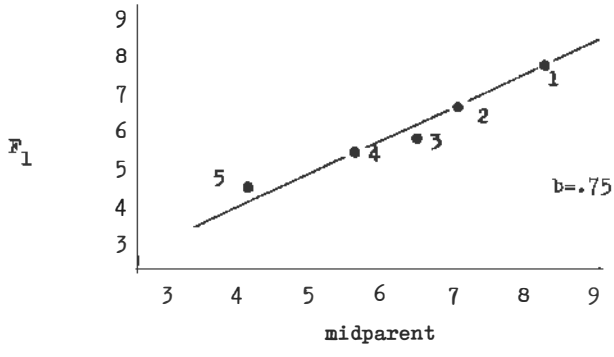
Variable population numbers denote wing length in millimeters



Cages for each pair mating (cross)	1	2	3	4	5
♀ wing lengths	8.10	6.90	6.80	5.80	4.10
♂ wing lengths	8.30	7.00	6.70	5.70	4.30
average (midparent) wing length	8.20	6.95	6.75	5.75	4.20
	⇩	⇩	⇩	⇩	⇩
Step 2.					
Sets of progeny (F_1's) are reared from each cross, and the wing lengths are recorded	6.7	7.1	6.5	5.5	4.5
	8.2	6.8	6.1	6.0	3.9
	8.4	6.9	5.2	6.4	4.9
	7.5		6.7	5.3	5.6
	7.8			5.8	
average F_1 wing length from each cross	7.72	6.93	6.13	5.8	4.7

Step 3.

The average F_1 wing lengths are regressed against the midparent values	mid P(x)	8.20	6.95	6.75	5.75	4.20
	F_1 (y)	7.72	6.93	5.80	5.80	4.70



Conclusion: The slope of the regression line is equal to the heritability (h^2=b=.75). This indicates that variation in wing lengths is largely determined by genetic variation. An actual estimate of h^2 for wing length in Drosophila gave a value of .577 (Falconer 1964), which is still fairly high.

MEASURING QUALITY OF INSECT VISION WITH ELECTRORETINOGRAM

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Description and Objectives

Caribbean fruit flies, *Anastrepha suspensa*, the screwworm fly, *Cochliomyia hominivorax*, the apple maggot fly, *Rhagoletis pomonella*, and other species of laboratory reared insects have displayed subnormal visual sensitivities. Some laboratory reared Caribbean fruit flies were 1/10th as sensitive as the wild fly. The electroretinogram (ERG) method was adopted to detect visual differences between wild and colonized populations and to routinely monitor colonies. Maintenance of adequate visual sensitivity in colonized populations increases the probability that released insects will accomplish their intended functions in the field.

Materials and Methods

Both sexes of laboratory reared and wild pupae and adults of the Caribbean fruit fly, for example, are held at 22±2°C, 50±10% RH, photoperiod of 14L:10D, with suitable food in 15-cm^3 cages until 2-3 days posteclosion when they are tested. At least 5 specimens of each sex and strain are tested for visual sensitivity using the ERG technique (Agee 1977). An electrode is implanted into each eye of the insect, and one eye is exposed to the test light. Neutral density filters are adjusted until the "criterion" 200 μv ERG response is obtained. Then the filter factor is recorded and averages and ranges of filter factors are compared for each test population. The time required to test a single insect is as follows: 2-5 minutes for preparation, 20-25 minutes for the insect to become dark adapted, and 1 minute to determine the visual sensitivity. An experienced technician can test 16-20 insects per day with a single test system. The level of visual sensitivity for a population can be determined by randomly sampling only 5 insects.

Variables, Design, and Analysis of the Experiments

The technique is one of few that easily and rapidly tests the physiological threshold of a critical behavioral input. Diet, gene pool, treatment, and perhaps other factors such as handling procedures influence the visual sensitivity of insects. For each species, the optimum dark-adaptation period is defined and standardized; the optimum depth for the electrode placement is determined (usually 30-50 μm); and if possible, sexually immature insects are used. This allows the evaluation of insects before they attain reproductive age permitting possible modifications of culture or release plan (Agee and Park 1975).

Possible Modifications and Expansion of the Technique

Three to 4 systems (microscope, micromanipulators, and vision analyzers) could be centered around one biological amplifier and oscilloscope to increase to 80 specimens the number of insects that a single technician could test in one day.

Limitations

Complete instrumentation costs $ 2,500 to $ 3,000 and a technician with considerable manual skill is required to perform the techniques and operate the instruments. However, college or electronics training are not necessary.

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MONITORING SPATIAL AND TEMPORAL ASPECTS OF VISUAL ACUITY IN LABORATORY REARED FRUIT FLIES

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Description and Objectives

A loss of acuity in bright and/or dusk illumination may affect the behaviour and therefore the effectiveness of released laboratory stocks. Simple routine methods of monitoring aspects of visual acuity in the laboratory are therefore most desirable.

Materials and Methods

The standard method of measuring visual acuity is to observe the optomotor response to regular arrays of moving black and white stripes. Insects either move bodily in the direction of movement of the stripes or they turn their heads in the same direction.

Visual acuity can be determined from the optomotor response by fixing the insect with sticky tape at the centre of a glass cylinder (20-40 cm diam) so that only its head can move. A striped paper cylinder is fitted around the outside of the glass cylinder and rotated. If this elicits a response (head turning in direction of stripes) finer and finer sets of stripes should be tested until no response occurs. The finest stripes that will elicit a response indicate the visual acuity, which is defined as the reciprocal of the angle that two similar stripes of such a set subtend to the eye.

Visual acuity should be tested at least under bright illumination (20,000 lux) and dusk illumination (10 lux). Both the black and the white stripes in any one set should have the same width so that 50% of each set is black and 50% is white; each set will therefore have the same reflectance. If a very fine nylon fibre (about 2 cm long) is glued across the back of the head, the angle of turn can be measured with a protractor mounted beneath. Repeat tests on any one individual should always start with the head facing forwards. The angle of turn will decrease as finer stripes are tested so it is most convenient to define acuity in terms of the finest stripes that elicit a certain minimum of response (e.g., 0.5°). Alternative methods of measuring visual acuity of either immobilized insects (as above) or of ones in tethered flight are referred to by Burtt and Catton (1966).

The temporal aspect of acuity is best measured in terms of "flicker-fusion frequency". The ability to distinguish a succession of images from one another is most important to a flying insect. The flicker-fusion frequency of the eye can be used as an index of this ability.

The apparatus required is one for observing the electroretinogram or ERG (see Agee's article in this publication). When light is experienced as a flash the ERG has a definite form which varies with species and method of recording (Swihart 1972). A series of flashes emitted by a stroboscope will elicit a series of ERG fluctuations if the speed of flicker is slow enough. As the speed of flicker is increased the ERG amplitude decreases until it is flat. The highest frequency at which this occurs is the flicker-fusion frequency and its value will vary with the intensity of light used. Intensities should therefore be standardized as above.

Variables, Design, and Analysis of the Experiments

Many factors can affect visual physiology such as wavelength of light used, dark adaptation and diurnal rhythms. Tests should be standardized with these phenomena in mind. Burtt and Catton (1966) discuss aspects of design and analysis.

Possible Modifications and Expansion of Technique

The optomotor response can be used as a method of assessing the relative attractiveness of various visual stimuli. Natural objects such as trees and fruit, or visual lures such as chequer-board or other patterns in various colours can be photographed and the resulting transparencies projected onto a screen. The image can be moved by shifting the slide in the projector and the optomotor response of flying or non-flying insects can be tested. The brightness of the image can be standardized by adjusting the voltage supplied to the projector lamp. The light energy reaching the insect can be checked with a thermopile light meter.

Limitations

The simplest methods given above involve no expensive apparatus and require very little time.

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MONITORING RESPONSES TO CERTAIN CLIMATIC VARIABLES

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Description and Objectives

The response of laboratory flies to certain climatic variables should be monitored so that field releases can be avoided at times when successful survival and dispersion is not favoured. Other tests can establish what climatic conditions will permit successful puparial emergence and maturation of adults and what period must elapse before insects released as pupae can mate with target populations. Suitable tests can also check the effects of various modifications of the culture regime, including methods of temperature acclimation.

Materials and Methods

For determination of temperature thresholds for cold-torpor and tethered flight see Meats (1973a, 1973b, 1974). For free flight thresholds see Cockbain (1961). For survival at extreme temperatures see Meats (1976). For determination of maturation state see Drew (1969), Meats and Khoo (1976). The last reference also shows how, in the case of D. tryoni, it is possible to use the day-degree relationship to predict both (a) the conditions necessary for successful maturation and (b) the rate and hence the duration of the maturation process, in terms of daily field maximum and minimum temperatures.

Variables, Design, and Analysis of the Experiments

Many physiological attributes are significantly affected by thermal history and consequent acclimation. For example, the thresholds for torpor, survival and tethered flight in D. tryoni can be varied by several degrees depending upon previous treatment (Meats 1976). For comparative purposes, therefore, tests should be done on insects having a similar acclimation history. Another important consideration is the way in which insects are introduced to test conditions. A sudden transfer to an extreme temperature (either high or low) can indicate an LD_{50} at that temperature that may be only about half that indicated by a test involving a gradual approach (typical of the field) to the same temperature (Meats and Fitt 1977). Analogous phenomena are reported in many of the references cited in the Materials and Methods section.

Expansion of the Technique

Survival limits at both high and low extremes of temperature as determined by single-exposure experiments may not give a true indication of tolerance of daily exposures to the kind of temperature extremes found in the field. The effect of daily doses of temperature extremes, typical of those that occur in the field, can be tested in cabinets capable of environmental simulation (Meats and Fitt 1977). Once the relation between single and repeated doses is established it is then possible for single-dose tests to be used for monitoring.

Limitations

Many tests can be done with quite simple equipment but the full range requires the use of environment cabinets in which controlled fluctuations of temperature and humidity can be produced.

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RESPIROMETRIC MONITORING OF ANASTREPHA SUSPENSA COLONIES

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Description and Objectives

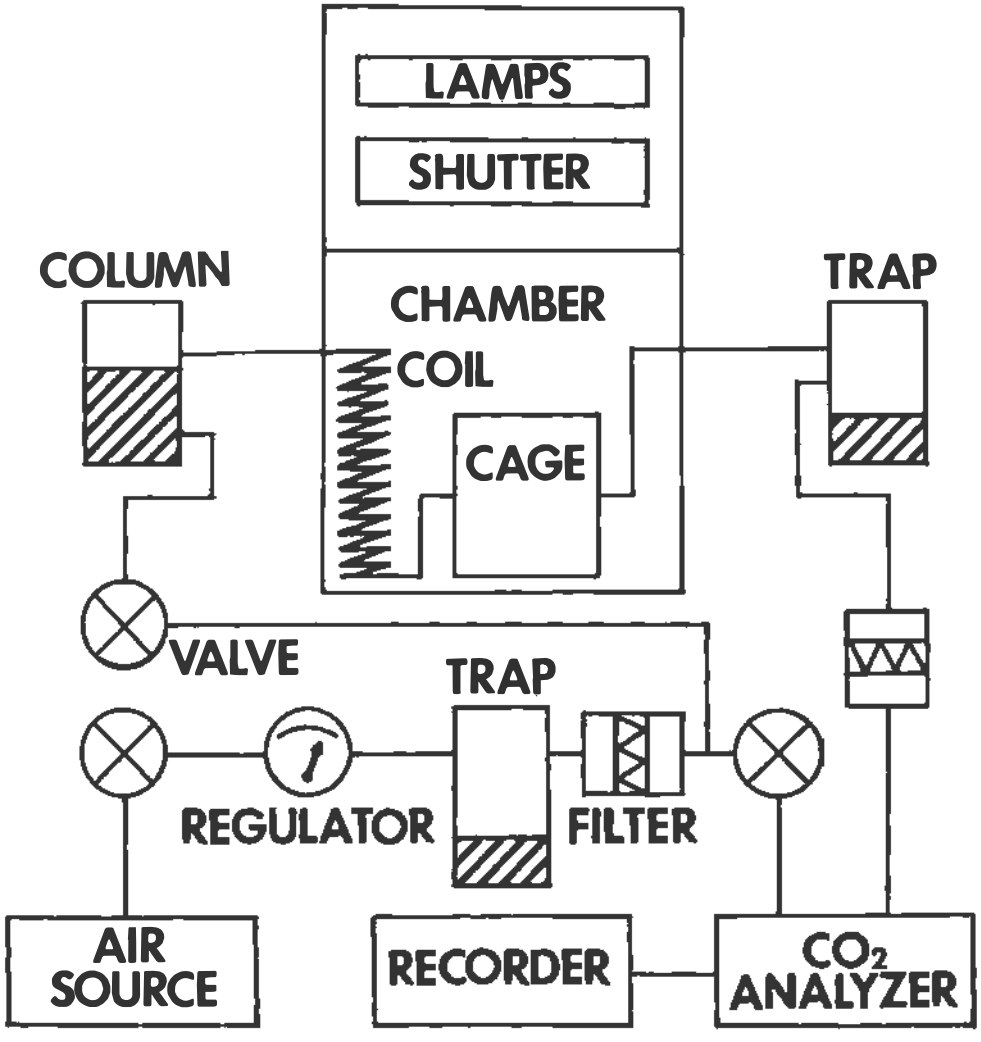
Both the physical and metabolic aspects of insect behavior involve respiration; therefore the quantity and periodicity of carbon dioxide produced by caged populations of adult Caribbean fruit flies, Anastrepha suspensa, provide a precise index of overall activity (Leppla et al. 1976). Furthermore, respiration rates can be monitored directly by infrared gas analysis without performing intermediate volumetric determinations or interpreting mechanical transductions (Hamilton 1959, Street and Bruce 1976, Turner et al. 1977). The resulting measurements are exceptionally quantitative, objective, repeatable, and accurate; however actual quality evaluations depend on comparisons between wild and laboratory strains (external standards), on comparisons of colony samples over time (internal standards), and on the purpose for which the insects are colonized.

Materials and Methods

The simplified CO_2 monitoring system is composed of devices that regulate, filter, hydrate, and meter the air supplied to an insect cage maintained in a bioclimatic chamber (fig. 1). Air provided by a compressor must be filtered for particles and vapors. However, only a low pressure regulator and needle valve are required if CO_2-free bottled air is used. Air from either source is passed through a water column and heated to 27±0.5°C in a 400x1.2-cm diam heat exchange coil after it enters the chamber. The 758-liter test chamber, a plywood box covered with fiber board on the internal and external surfaces to ensure thermal and sound insulation, houses the 12-liter insect cage. This cubical cage is fabricated from 6 mm-thick plexiglass and has air inlet and outlet ports in the center of two opposing sides. It also has a 3 mm-thick glass lid that transmits 350-nm and longer wavelengths of ultraviolet radiation. The lid is held in place with springs, and insects are introduced through a 3.8-cm diam stoppered hole located near the top of the cage. CO_2-laden air from the sealed cage is refiltered to remove contaminants before it enters the Mine Safety Corp. model 200 infrared gas analyzer. The analyzer output voltage is printed as a continuous line (1 min-response time) on a strip chart.

Insects are maintained during the pupal stage and for 10 days post-emergence in an environment identical to the test environment. Temperature, RH, and photoperiod are cycled gradually to achieve 27±1°C, 60±2% RH, and 217 lux diurnal conditions and 24±1°C, 78±2% RH, and 2.17 lux nocturnal conditions. Then these preconditioned flies are transferred to the provisioned test cage and monitored for three consecutive days. Data from the second 24 h are used to analyze behavioral responses in terms of total activity (CO_2 discharged), thresholds (changes relative to light intensity), startle (immediate changes during phase transitions), and general patterns (shapes of the 24 h-curves).

Fig. 1 CO_2 Monitoring System



Variables, Design, and Analysis of the Experiments

At the beginning of each trial, equipment is cleaned, the photoperiod clock and shutter cams are inspected, environmental monitoring instruments are serviced (temperature, RH, light, and time), and the airflow rate is calibrated. Next, the electrical circuits, CO_2 detector, and recorder are checked and activated. Gas of known CO_2 content is used to calibrate the detector. Records are kept of the source, species, stage, sex, number, age, and mass of the test populations. Generally, optimum results are obtained by using 100 insects (ca. 10 mg per fly) and an air exchange rate of 30-50 cc per min or a similar proportion. Tests are replicated as statistically appropriate, and data are analyzed manually or by computer (Turner et al. 1973).

Possible Modifications and Expansion of the Technique

The insect cage and CO_2 detection system may be altered to accommodate different insects and test populations, however, optimum ages, sexes, densities, etc. must be predetermined. Also, respirometric monitoring should be conducted in concert with behavioral measurements such as the occurrence of mating, oviposition, and egg hatch. Respiration can be determined chemically in terms of oxygen uptake or CO_2 output, and alternative instrumentation may be substituted for the infrared gas analyzer.

Limitations

An initial investment of ca. $5,000 and engineering expertise are required. Thereafter, the operator must be trained in equipment maintenance. The CO_2 monitoring system quantifies locomotor behavior under confined conditions and identifies changes, but does not indicate potential impact of these changes on field performance.

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IMPLEMENTATION OF QUALITY CONTROL

A PROPOSED SYSTEM FOR IMPLEMENTING QUALITY CONTROL IN A PEST MANAGEMENT PROGRAM

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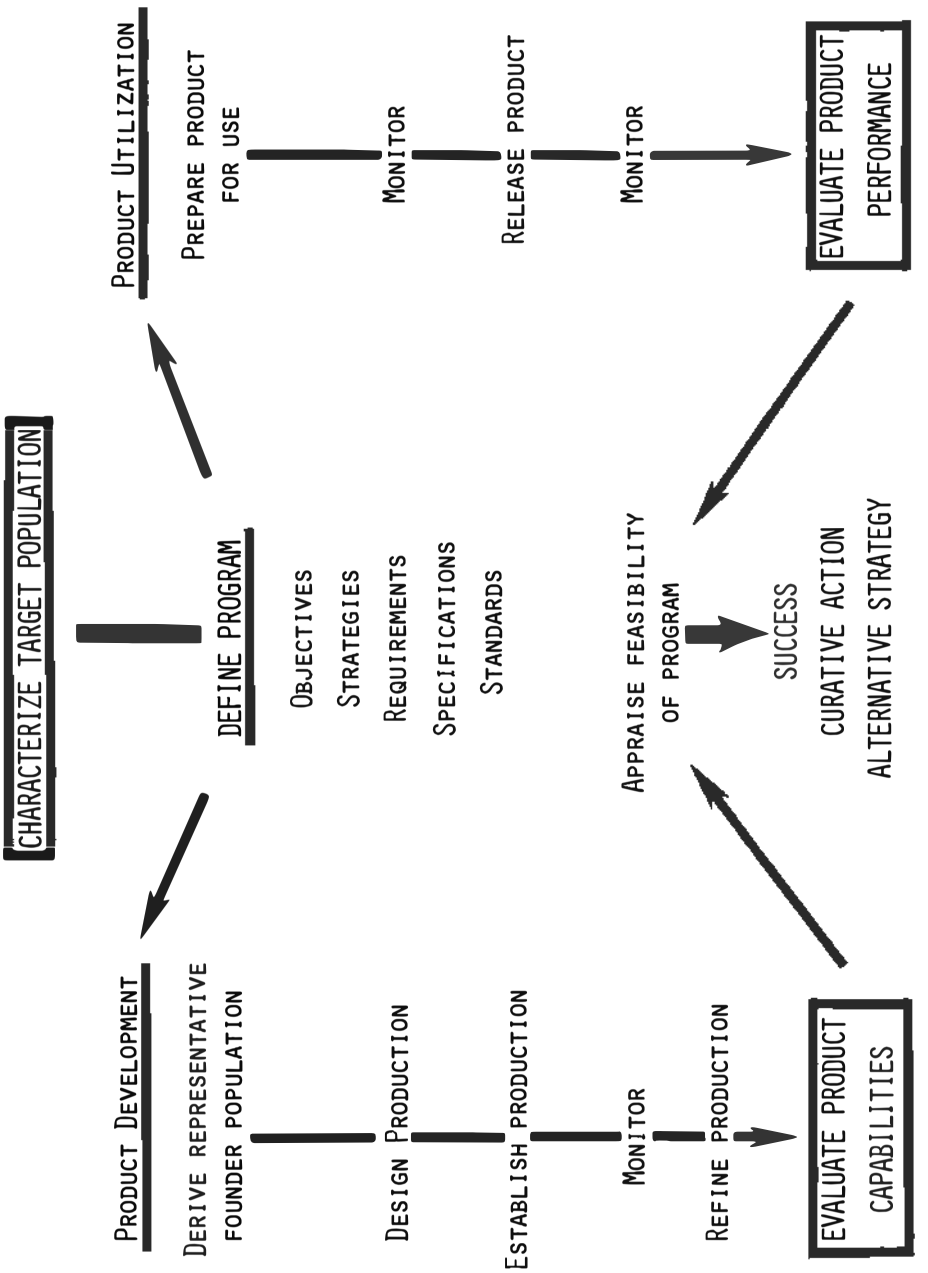
The many contributions to this idea book will undoubtedly lead to a basic question: In practice, what steps should be taken to plan and implement a quality control program? Although it is not possible to provide a scheme that describes specific techniques that can be applied without alteration to every situation, we attempt to present a general summary of what we believe is a feasible approach.

The proposed quality control system (Fig. 1) identifies steps that should be included and indicates a probable order of implementation. However, the contingencies of individual programs will dictate priorities for emphasizing specific operations. This plan includes three interacting components that are defined by their respective areas of primary responsibility: 1. Program analysis (thorough characterization of the target population, precise definition of the biological objectives and requirements, establishment of specifications and standards, and realistic assessment of the overall feasibility of the pest management strategy); 2. Production (derivation of a suitable founder population, establishment of the production line and monitoring its operational efficiency); and 3. Product utilization (preparation of insects for release, distribution, and monitoring of performance). Each of these program components and their incorporated elements involves standardization of both physical and associated biological aspects. Routine operations are relatively stable but continuous adjustment is required to compensate for changes in the colony. Thus, quality control is accomplished by selectively characterizing and evaluating the colonized insects after production and after utilization according to the same fundamental criteria that are used to describe the target.

Analysis and characterization of the wild target population from the behavioral, ecological and genetical points of view is not only the critical first step in developing an effective pest management strategy; it is also a continuing process that provides the standard of comparison for appraising the entire program. Sound knowledge of the target species is essential for definition of the objectives (in terms of both the pest management program and its rearing component) and for the establishment of standards (specifications of the required attributes of the mass reared insects) for all monitoring activities of the quality control program. The establishment of a priority list of important quality components will help to single out those aspects deserving comprehensive, reduced, or only marginal investigations.

Fig. 1. Scheme for implementing quality control in operational pest management programs



The production colony is established with representative samples from the target population after adequate rearing technology has been developed and tested. Whether the founder population should be derived from peripheral or central locations in the geographical distribution of the target population is still a matter of conjecture; however, in either case care should be taken to ensure genetic heterozygosity. Attention should be focused on those environmental factors that interfere with key behavioral traits of the adult stage. These factors generate the first and probably most important selective bottleneck in the rearing program. During mass propagation of the insects, development is monitored routinely with sensitive methods at various points in the production line to provide early warning, should the rearing process start to drift toward the specified limits. However, monitoring of the production line with routine methods provides only a general indication of the overall physiological condition of the colony, and usually does not identify specific deficiencies.

Post-production characterization and evaluation of the colony is essential for judging the severity of undesirable selection, as selection is inevitable. If the mass reared insects are generally competent (deficient in only a minimum of manageable traits) and the nature of deficiencies can be quantified, then strain development by selection and/or hybridisation is sufficient corrective action. We recall existing controversies over the question of whether wild material should be introduced periodically into laboratory strains or whether new strains should be established periodically with new founder populations. Whatever the approach to solving an existing problem, refinement of the production process will have to be balanced against economic feasibility relative to the original program objectives.

Product utilization is the operation covering preparation of insects for release (treatments, handling, distribution, and monitoring of their performance and ultimate use in the field). Most of the technical contributions to this book describe performance tests that indicate functional changes in the insects and identify the concomitant intrinsic and extrinsic causes. Monitoring of pre-release and post-release performance provides crucial information for evaluating whether the insects are adequate for the achievement of the objectives. If it becomes evident that the goals cannot be achieved then cost/benefit relationships will largely determine whether corrective actions in the rearing and handling procedures are justified. Otherwise, alternative control strategies have to be considered.

Each pest management program will face distinctive problems and conditions, thus implementation of this protocol will vary. It may, in fact, vary within each program as it progresses. Therefore, its application cannot be rigidly prescribed a priori. Appropriate techniques can be selected from the options presented in this publication by addressing a series of pertinent questions to each program phase concerning the role and importance of each quality trait (Tab. 1). Limits are then specified for these traits, controls are established by selecting suitable tests, and quality is ensured by implementing systematic feedback between production and utilization phases. (Virtually any of the tests can give interpretations meaningful to production and adaptation; therefore, only those considered to be most applicable are categorized in the table.)

Ultimately, a systems analysis that accounts for all primary variables contributing to the efficient production of a high quality product yields the most effective program. The quality control effort is an integral component of this overall pest management strategy, and is expended in concert with analysis, production, and utilization components. The initial target population is characterized, and the product is evaluated for adaptedness and behavioral performance after colonization and after release. The challenge is to implement the most accurate and efficient techniques for ensuring that the colonized insects function in their intended role in nature.

Table 1. Selection of tests for implementing quality control: In each program phase, question the nature and importance of each quality trait and select the most appropriate tests.

PHASE AND QUESTION	QUALITY TRAIT AND TEST
TARGET CHARACTERIZATION	**ADAPTATION AND PRODUCTION CONTINUITY**
Adaptation - limits of primary characteristics	Isoenzyme analysis Classical genetics ERG Acoustic analysis Actographics Behavioral analysis Life history analysis
Habitat - essential relationships	
Motility - characteristic patterns	
Reproduction - essential characteristics	
	HABITAT INTERACTION
	Shape and color response Host response Climatic potential Temporal adjustment
COLONIZATION AND PRODUCTION	
Adaptation and Continuity - acceptable compromises	
Habitat - retained or reinforced relationships	
Motility - effects on propensity and capability	
Reproduction - essential traits retained	
	MOTILITY
	Flight propensity Flight capability Distribution patterns Locomotion rhythms Release-recapture
UTILIZATION	
Continuity - adequate numbers	
Habitat - appropriate interaction	
Motility - adequate performance	
Reproduction - achieve objective	
	REPRODUCTION
	Mating propensity Mating compatibility Mate sorting Pheromone production Pheromone response Sperm production Sperm utilization Mating inhibition Fertility inhibition