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**Contributions to the 4th workshop  
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“Quality control  
of mass reared arthropods”**

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## Preface

The IOBC global working group "Quality control in mass reared arthropods" was established in 1981 with the aim to advocate quality as well as quantity in the mass rearing of insects. At that time the ideas of quality control in insects were almost revolutionary to biological science because quality had been developed for industrial goods only and had never been considered in insect rearing. High quantity rearing of insects for the sterile male technique revealed the weaknesses of factory production without any defined way of assuring quality. The first quality control procedures for insects were developed for pest organisms belonging to the Diptera and Lepidoptera. After a first period of methods development for performance tests and control, a second period was initiated to refine and complete quality control systems and to implement them into existing production units. Today, models of total quality control strategies exist for a few pest insects but not yet for entomophagous arthropods used for biological control.

Obviously, there is an increasing general concern today about quality and colony maintenance in mass reared parasitoids and predators used for inundative release against pests. Production systems of entomophagous insects are based on phytophagous hosts or prey and an adequate host plant, thus entailing the monitoring of interactions on three trophic levels. The complexity of such rearing methods requires not only a clear definition of quality parameters for each level but also the necessity of a systems based approach which will aid in a better quantification of the relevant parameter.

Quality assessment and control in entomophagous arthropods is not only an important tool for inundative releases in biological control but also for inoculative release. The reasons for deviations in laboratory populations are manifold and there is an inherent risk in every rearing system to end up with an organism that is not well suited to its intended purpose.

The workshop, held in Vancouver, Canada, in June 1988, gave entomologists and producers of entomophagous insects the opportunity to profit from the experience of their colleagues working with mass reared pest insects. Moreover, it brought together a variety of specialists who are faced daily with questions on insect rearing in industrialized and developing countries. The aim was to profit from each other, to exchange knowledge and ideas and to intensify the contact between scientists and rearing managers.

The publication of some of the papers presented at the workshop in the present volume was made possible by the generous financial support of IOBC and other bodies interested in the further development of quality control in reared insects.

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Chairman of the IOBC-working group  
"Quality control in mass reared arthropods"

## Quality assessment and control in entomophagous insects used for biological control<sup>1</sup>

By F. BIGLER

### Abstract

The necessity for quality assessment and control in entomophagous insects and mites depends on a variety of factors that can be summarized in four main topics 1. the genetic structure of a population and the inheritance of attributes, 2. the physical rearing conditions, 3. the rearing diet (host, prey) and 4. the objective of release. The four topics are discussed with regard to their relative impact on the performance of a natural enemy. The interrelationships among the factors are presented in a diagram. The overall quality is divided into major components and single attributes and their importance is discussed. When we try to assess the quality, the problem we are concerned with is to know what, why and how to measure. The next step is the very difficult interpretation of laboratory results and their relation to the field performance. Examples of successful prediction of the field performance based on laboratory assessment of single attributes are presented. The quality control requirements relative to the rearing systems of three successfully applied parasitoids are discussed using a partition of the overall quality into the major components. The need for quality control increases with decreasing natural rearing conditions. In conclusion, each particular situation has to be analysed carefully to determine significant parameters relevant to the field performance.

### 1 Introduction

Rearing insects and predatory mites for biological control purposes can have a profound effect on the genetic structure of a population which in turn will influence both the rearing success and the efficacy in the field. In contrast to the lasting consequences of genetic changes, the colony may undergo nonpermanent adaptation to rearing conditions that can be restored (MACKAUER 1976). Preservation of the natural attributes of the insect colony is therefore one of the particular objectives in insect rearing for release in biological control programmes (REMINGTON 1968; MACKAUER 1980). This often is in contrast to maximum efficiency of production due to economic constraints. Artificial conditions improve the rearing success and the turnover time but they may impair the colony's quality. However, essential attributes, relevant to the quality of a population, are not easily identified and assessed. This is not primarily a methodological question but rather a problem to clearly define characteristics that distinguish an effective insect from a poor one. It is still a matter of controversy whether measuring single quality traits is useful for the elucidation of the overall quality in terms of laboratory and field performance. BOLLER (1979), BOLLER et al. (1981) and CHAMBERS et al. (1983) developed a quality control system for fruit flies that help production managers to identify early deterioration of a mass reared population. This control system includes measurements of single quality components assessed in the laboratory as well as in field tests that allow the final judgement of the performance. This example shows that such a complex problem needs a variety of approaches and must be analysed step by step in order to understand and manage the whole system. The development of computer models could play a major role in determining the main

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components of the overall quality. The need for quality control in a rearing procedure depends on the scale and time of rearing, the objective of release, the rearing conditions, the source population and the replacement of the lab colony (MACKAUER 1980; LEPPA et al. 1983; JOSLYN 1984; WAAGE et al. 1985; VAN LENTEREN and WOETS 1988). The decision and urgency for quality control in a rearing programme should be based on the evaluation of these factors after a thorough analysis.

## 2 Criteria for quality control in rearing procedures

The establishment and maintenance of an insect colony for biological control purposes raises many technical questions. Generally, the responsible staff is not asking first for quality control. The urgency to develop a quality control system is not the same for all biocontrol organisms because it depends largely on the purpose for which insects are produced and their specific rearing characteristics. Some of the most important factors that determine the necessity of quality control are summarized in fig. 1. Four main characteristics can be distinguished: 1. the genetic structure of the population, 2. the rearing diet, 3. the physical rearing conditions and 4. the release programme.

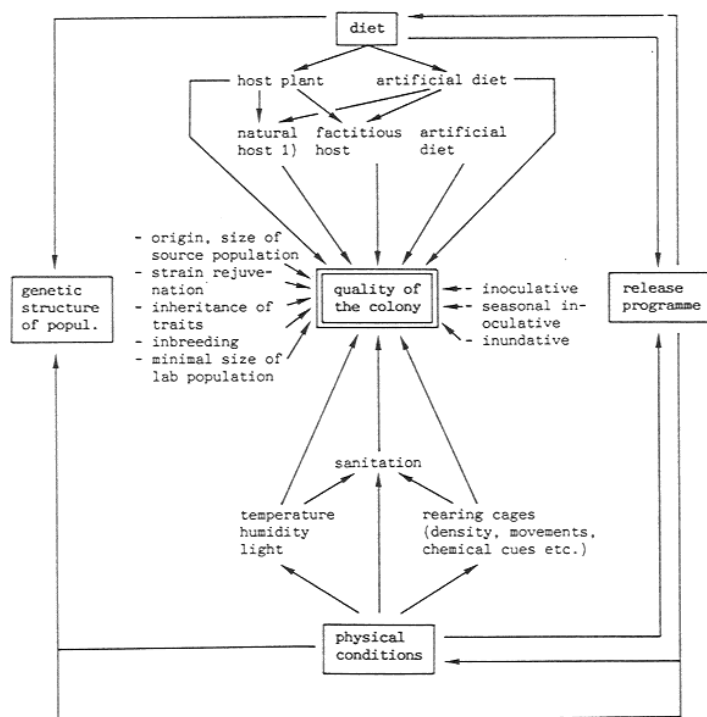


Fig. 1. Schematic presentation of the relationships between the rearing system and its main components, the genetics of the population, the release programme and their impact on the colony's quality. 1) host includes also prey



## 2.1 Genetic considerations

The genetic structure of the sampled and introduced population of predators or parasitoids is known in only a few cases (WILSON 1965; REMINGTON 1968; MESSENGER and VAN DEN BOSCH 1971; McDONALD 1976; GONZALEZ et al. 1979; BARTLETT 1984; JOSLYN 1984). The introduced population may deviate genetically from the natural population(s) because of limited sampling in time and space and because of selection processes during shipment and/or quarantine. A substantial reduction in genetic variability may occur before the source colony is established. If the colony shows some evidence to be effective against a pest at an early stage of introduction, and if it must be propagated further under artificial conditions for an uncertain number of generations, it is advisable to assess some quality components (see e.g. MACKAUER 1976; VAN LENTEREN and WOETS 1988). This basic information may be used as a reference for comparing the quality and the genetics of further generations reared in the laboratory. The degree of changes in genetic patterns, behaviour and vigour can be quantified only if there is a standard to compare them with. Standards must be defined genetically, but too often knowledge is lacking or very poor at the beginning of the colonization and later it may be useless to establish the genetic profile of the population because changes may have already occurred. However, the colony may be used as an internal standard (HUETTEL 1976; CHAMBERS 1977) after a number of generations if there is clear evidence that it still functions in its intended role. Even if genetic drift does not result in a loss of quality, it should be considered as an early warning signal. Examples are known where deterioration of a colony was observed as early as 5 to 6 generations after initiation under artificial rearing conditions (JONES et al. 1978; BIGLER et al. 1988; VAN BERGEIJK et al., in press). Therefore, it is crucial to eliminate "bottlenecks" in the earliest colonization steps. Whether or not a colony can be replaced depends on the efforts and costs. Periodic and complete replacement may be a very efficient method to prevent quality problems but on the other hand it runs the same risks of genetic deviation as with the first colonization if the rearing system remains unchanged. The effect of partial replacement depends on the proportion of new individuals to the number in the existing colony, the genetic diversity, the heredity of attributes and the selection processes under the prevailing rearing conditions. Partial rejuvenation remains uncertain as long as these questions are not answered. The genetic structure of a laboratory population is influenced further by the number of generations reproduced in the laboratory and the size of the colony. BARTLETT (1985) and VAN LENTEREN (1986) are comparing the differences between field and laboratory conditions affecting the genetic variability in laboratory colonization.

## 2.2 Diet considerations

The subsystem of the host (including prey) and host plant determines strongly the necessity for quality control. The schematic presentation of possible subsystems of host plant, host and parasitoid/predator exemplifies the variety of possible rearing procedures (fig. 1). A multitude of behavioural, morphological and physiological attributes may be altered by rearing the host on artificial diets or on different host plants. The rate of population increase of the entomophagous insect may be decreased indirectly by the suboptimal physiological conditions of the natural host plant(s) which produce hosts of poor quality (NEUENSCHWANDER et al. 1989) and consequently inferior parasitoids or predators. Optimal rearing of all organisms on the three trophic levels of each production system in entomophagous insects is often difficult and expensive and is one of the sources for failures in biological control. In fact, all organisms in the complete production procedure would need a minimal quality assessment and control. Effects of nutritional factors of different diets on quality attributes in three predatory mites are discussed by DICKE et al. (1989). Changes in behavioural traits that are crucial in the sequences from

habitat finding to host acceptance may be altered through artificial host systems. A recent example demonstrates the role of host plant/host relationships in the attraction of the parasitoid *Epidinocarsis lopezi* to the habitat of its host, the cassava mealybug, *Phenacoccus manihoti* (NADEL and VAN ALPHEN 1987). I share the opinion of NOLDUS (1989) who summarizes the complex situation of insect rearing and its relation to semiochemicals in entomophagous organisms when he concludes that control of behavioural traits becomes more important in future mass rearing systems which are based on artificial rearing. As an example he stresses the progress of the in vitro rearing of *Trichogramma*.

### 2.3 Physical conditions

The physical conditions of the rearing system may have an impact on the genetic structure of the population by selection processes due to the laboratory environment. Economic aspects of the production demand that a high turnover occurs. This is achieved by applying optimal climatic conditions and using small rearing cages with high insect and host densities. Thus, the inherent density dependent behaviour, often a relevant attribute of a species for successful pest control, may be changed. The physical conditions may also cause obvious or cryptic hygienic problems with diseases and other microorganisms which may act upon the quality of the insects.

### 2.4 Relation between quality and the release programme

The release programme itself does not directly influence the quality of the insect's colony but it surely dictates the rearing parameters and has, therefore, an indirect impact on the quality. The performance requirements of a parasitoid or predator may vary according to the release purpose and programme. The value, in terms of behaviour and adaptability of the released natural enemy, depends not only on the method of biocontrol (inoculative, seasonal inoculative, inundative) but also on the structure of the ecosystem, the diversity of the crop, the pest structure and density, the season, etc. The production intensity and the rearing system are chosen according to the system parameters of the release programme.

The schematic diagram in fig. 1 does not claim to be complete but it may help to analyse the subsystems and components of a rearing system in order to elucidate the most critical points.

## 3 The relative importance of quality components and single attributes

BOLLER and CHAMBERS (1977) divided the overall quality of fruit flies reared for sterile insect release programmes into major quality components, traits and measurable parameters. The question remains whether laboratory assessed traits or attributes have a predictive value for the performance of an insect in the field. MACKAUER and VAN DEN BOSCH (1973) and MESSENGER et al. (1976) concluded that it is hardly possible to identify attributes which will "precisely" characterize an effective biocontrol agent for a particular situation. The first problem is the clear definition of *what* attribute are to be measured. Although, we know that the main attributes of natural enemy performance include growth rate relative to the pest, searching behaviour, dispersal, density dependence, pest specificity, etc. it is impossible to precisely assign the value of these characters to the field performance. However, there is evidence that, if we are able to define traits which may be important in a particular case, we will increase the chance to get a more precise answer. Obviously, a conclusive answer will never be reached by laboratory evaluations alone but the result must be validated in the field. When we know what attributes to assess, we have to ask the question *why* do we want to quantify this particular trait and not other ones. Reviewing

the literature, one would conclude that too often those characters are measured which are easy to assess from the standpoint of labour and available methodology. Evidently, the chance of such approaches to be successful is very low. When the attribute(s) are precisely defined and we know why we want to measure it we have to ask *how* we can do it. The development of laboratory and field methods may be very time consuming and the validation of laboratory results in the field may fail. Then the question must be raised whether the methods are not adequate or whether the parameter is not relevant to the performance. FORCE and MESSENGER (1968) studied the temperature tolerances and competitive interactions in the laboratory of three introduced parasitoides released against the spotted alfalfa aphid and compared the results to their effectiveness in the field. They found a close relationship between the laboratory results based on temperature tolerance, reproductive potential and host selection and the parasitoid's efficiency in the release areas. They concluded that laboratory analysis of certain features of a host-parasitoid system is feasible and useful. BIGLER et al. (1988) compared the locomotory activity of several strains of *Trichogramma maidis* in the laboratory with their field efficiency in parasitizing eggs of their natural host, the European corn borer. A clear relationship between locomotion and parasitism was demonstrated. Although, we assume that other traits, not related to locomotion, may exhibit a great influence on the performance, we have defined and assessed one feature that is most probably relevant to the parasitoid's searching ability.

A schematic presentation of a possible division of the overall quality of biocontrol agents into major quality components is shown in fig. 2. Each attribute can be split up into single traits and measurable parameters. More details are discussed by BOLLER and CHAMBERS (1977). Biological control is divided in inoculative, seasonal inoculative and inundative release. The major quality components consist of complex physiological and

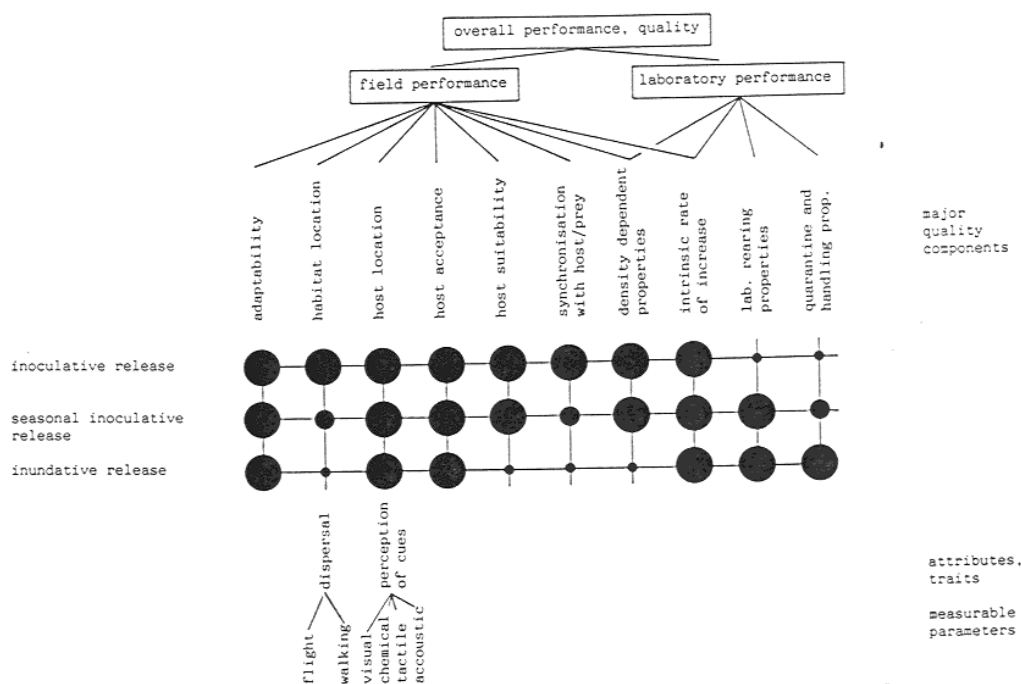


Fig. 2. Relative importance of quality components in relation to the objective of arthropods used in biological control (●, ●, ● = low to high importance)

ecological properties that are not all of equal importance and depend on the use and the release programme. The relative importance of each attribute has to be evaluated for each particular control programme and there is no doubt that all kinds of general statements would lead to misinterpretations. Thorough knowledge of ecological, physiological and biological properties of each specific pest-enemy relationship is needed before evaluating major components.

Adaptability of the released insects to the physical and ecological environment in the release area is probably one of the most important attributes regardless of the release programme (see MESSENGER and VAN DEN BOSCH 1971). In the sequence of host searching, the habitat location may be very important in inoculative release but not in inundative applications where the natural enemy is released in the habitat (crop) with the target pest. By searching for its host, it must locate the microhabitat by dispersal and perception of specific cues. Flying and/or walking are important parameters for dispersal, depending upon the insect species. The host may be perceived by visual, chemical, tactile or acoustical cues or by a combination of one or more of them. Rearing natural enemies on factitious hosts or on artificial diets under unnatural conditions runs the fundamental risk of changing the acceptance and suitability of their natural hosts. Host acceptance is of central importance in all cases of biological control. Host suitability, however, may be considered of minor significance in inundative release programmes as long as the defense mechanisms of the host can be overcome and the host is still killed by the natural enemy. NEUFFER (1987) and VAN BERGEIJK et al. (in press) investigated the impact of factitious hosts on host acceptance and suitability in the egg parasitoid *Trichogramma maidis*. They demonstrated a steady change in both attributes with increasing numbers of generations reared on factitious hosts. The significance of density dependent behaviour varies with the release programme, but it is also a function of the tolerable pest population in a particular crop. The density of a natural enemy can easily be adjusted to the population density of the pest in inundative releases but not in inoculative programmes. Density dependent behaviour is, in addition, a major component that influences the laboratory performance. A particular innate density behaviour may favour or impede the rearing success. The intrinsic rate of population increase may be a crucial factor in rearing success and field performance. There might be other major quality components not listed in fig. 2 which are of great importance.

The main point to be considered when analysing the relevant attributes is: 1. be aware that each situation is unique 2. ask clearly which attribute(s) is important and why, 3. ask how it can be quantified and validated in the field and 4. recognize that all predictive methods have their limitations. Thus, a quality evaluation system consists of laboratory, semi-field and field methods to give clear answers to clearly formulated questions.

#### 4 Examples of quality requirements according to the rearing systems

Three examples of successful biological control agents are chosen to discuss the possible requirements in quality control according to the objective and the rearing system (table).

The parasitoid *Epidinocarsis lopezi*, was established in 1988 on more than 1.5 million km<sup>2</sup> in 18 African countries and its efficiency is well documented (NEUENSCHWANDER et al. 1989). The rearing system is well adapted to the parasitoid's natural requirements and the widespread control achieved within a few years proves its performance. Quality parameters were not directly assessed but care was taken with the very sophisticated rearing system to rear a healthy, vigorous insect. In general, quality control procedures are of minor value in the cases of inoculative release with *E. lopezi*.

The parasitoid, *Encarsia formosa*, has been applied on 2361 ha of greenhouses against the greenhouse whitefly in 1985 (VAN LENTEREN and WOETS 1988). The basic characteristics of the rearing system indicate that *E. formosa* is reared under conditions similar to

those in commercial greenhouses. Although, for economic reasons, there are some differences between rearing under laboratory conditions and those of normal greenhouse conditions (for more details see VAN LENTEREN and WOETS 1988), there is clear evidence from the successful application that the parasitoids retained an excellent performance in the greenhouse. We can assume from the high success rate that the almost natural rearing conditions are a good prerequisite for maintaining high quality with this species. Nevertheless, a minimum check of some important quality components shouldn't be neglected because minor shifts in the behaviour of the population cannot be excluded. *E. formosa* is being produced in a few specialised insectaries and is shipped long distances, to be used for seasonal inoculative release with the expectation of a relatively fast control of the target organism. These circumstances require a very sophisticated product control system at the end of the production process after the shipment is received and prior to the parasitoid's release. For commercial products like *E. formosa*, a uniform product quality and greenhouse performance are absolutely necessary even after long transports of the organisms.

The egg-parasitoid *Trichogramma maidis* is applied against the European corn borer in Switzerland and other European countries. Inundative releases are made during the first half of the egg-laying period of the pest insect. During the second half of the period, eggs are destroyed by progeny of the first release. Thus, the method used is basically inundative but to some extent seasonally inoculative as well. An immediate effect of the parasitoids is crucial for success. A mass rearing of *T. maidis* is conducted under extremely artificial conditions as shown in the table. However, after release in the corn field, the parasitoids must be able to face the harsh conditions that may prevail. The temperatures in the rearing rooms fluctuate within optimal limits whereas in the field they may vary from 10°C to 35°C within 24 hours. Therefore, adaptability to physical conditions is very important. Because the parasitoids are released in the corn field, they don't need to locate the habitat, but they must search immediately and efficiently for the egg-masses of the corn borer. For high rates of parasitization they must have a high locomotor activity (BIGLER et al. 1988) and probably must perceive several chemical cues. Once a female has located a corn borer egg-mass, it must recognize it as its host and accept it for oviposition. Moreover, the host should be suitable for the parasite's development. NEUFFER (1987) and VAN BERGEIJK et al. (in press) have shown that host acceptance and suitability decrease with increasing numbers of generations reared on factitious hosts. Synchronization with the host and density dependent properties are not important features because both are regulated within the system. A minimum rate of increase is needed for efficient mass production and for a rapid population increase in the field. Rearing and handling properties must meet commercial requirements such as methods of storage, packing, shipment, etc. without loss of their field performance. Based on laboratory and field measurements between 1980 and 1982, BIGLER et al. (1982) have shown that the field performance of *T. maidis* was inferior after the constant propagation in the rearing system summarized in the table. As a consequence, the rearing system has been improved by maintaining a basic population of *T. maidis* consisting of at least 100 000 individuals under semi-natural conditions, i.e., in a large greenhouse or field insectary with fluctuating physical conditions (15–30°C, 50–90% rel humidity). Corn borer egg-masses are provided on corn plants 3–4 m away from the release point (BIGLER et al. 1989). Thus, the adult parasitoids are forced to disperse and to search for their natural host on the plant. A portion of this strain is periodically propagated on the factitious host for a maximum of 6 generations and stored in diapause for several months. A continuous rearing of *T. maidis* on its natural host and on corn plants prevents the strain from undergoing quality deterioration.

These three examples show that quality control requirements depend primarily on the objective of biocontrol and the rearing system. Based on the general rule that performance tests have increasing importance with decreasing natural rearing conditions, each production system should be analysed for impacts on quality components and adjusted to satisfy

Examples of successful use of parasitoids in three biological control programmes and the requirements of quality control in the respective rearing systems

main characteristics		Examples of biocontrol programmes	
crop		mainly tomato, cucumber (greenhouse)	maize (field crop)
continent		Europe (The Netherlands)	Europe (Switzerland)
pest		<i>Trialeurodes vaporariorum</i> (greenhouse whitefly)	<i>Ostrinia nubilalis</i> (European corn borer)
parasitoid		<i>Encarsia formosa</i> (Hym., Aphelinidae)	<i>Trichogramma maidis</i> (Hym., Trichogrammatidae)
host range		monophagous	oligophagous
objective, programme		seasonal inoculative release	inundative release
rearing system			
physical conditions		greenhouse	laboratory (little variability)
rearing host		natural	factitious
host plant		tobacco (unnatural)	none
host density		high	extremely high
parasitoid density		high	extremely high
rearing cages		large (2 × 2 × 4 m)	very small (25 × 30 × 3 cm)
strain replacement		yes (partly)	no
source of information		NEUENSCHWANDER et al. 1989	BIGLER et al. 1989
requirements in quality control			
adaptability		+	++
habitat location		+	-
host location		+	++
host acceptance		-	++
host suitability		-	++
synchronisation with host		-	-
density dependent properties		++	-
rate of increase		+	++
rearing and handling properties		+	++

Quality control requirements, - not necessary, + occasionally necessary, ++ regularly necessary.

the insect's natural requirement as closely as possible. The investment of time and money in such rearing systems make it worth while to maintain a high level of quality of the biocontrol agents.

## 5 Conclusions

When dealing with natural enemies we often do not know what particular attributes make an insect successful. But, we have to be aware that in all phases of a biocontrol project, the agent being reared undergoes genetic and adaptive changes which may influence its efficiency. Thus, each step in establishing and maintaining a population must be analysed carefully to prevent as much deterioration as possible. To discover such changes, we must be able to quantify certain characteristics and to compare them with known standards functioning in the field. To define the most important performance components we must know the main constraints of the rearing system on the innate behavioural characteristics of the particular biocontrol agent. Consequently, laboratory and field methods have to be developed, combined and validated. At present, very few cases are known where quality control procedures are applied in biocontrol programmes. Because of the lacking experience, scientists and production managers should concentrate their efforts on a few organisms which can be used as models for the establishment of basic information. I am convinced that future plant protection systems will include laboratory reared organisms to a greater extent than they do today. Quality standards will be required for marketable organisms. Users and growers will require uniform products of a given standard. Therefore, product quality control of an organism as defined by LEPLA and FISHER (1989) includes different steps starting with thorough control procedures in the rearing plant and ending with a final check based on simple, reliable and rapid tests just before their release. Promoting and coordinating all efforts aimed at developing quality control systems for insects and mites in general, but especially for those used in biological and biotechnical pest control, is the goal of the "IOBC Global Working Group on Quality Control of Mass-reared Arthropods".

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## Zusammenfassung

### *Zur Qualitätsbeurteilung und -überwachung entomophager Insekten im Rahmen der biologischen Schädlingsbekämpfung*

Die Notwendigkeit von Qualitätsüberwachungen bei entomophagen Insekten und Milben hängt von vielen Faktoren ab, die in vier Hauptgruppen zusammengefaßt werden können: 1. Die genetische Struktur der Population und die Vererbung einzelner Eigenschaften, 2. die physikalischen Zuchtbedingungen, 3. die Nahrung (Wirte, Beute) und 4. das Ziel und die Methode der Anwendung von Parasitoiden und Räubern. Die vier Hauptthemen werden im Hinblick auf ihre Bedeutung für die Vitalität der gezüchteten Nützlinge diskutiert. Die gegenseitigen Beziehungen zwischen den Hauptgruppen sind schematisch dargestellt. Die Gesamtqualität (Nutzleistung und Zuchteigenschaften) wird in Hauptkomponenten und einzelne meßbare Qualitätsmerkmale unterteilt, und ihre Bedeutung ist in Abhängigkeit der jeweiligen Zielsetzung der biologischen Methode besprochen. Damit Qualitätsüberwachung erfolgreich wird, muß man sich genau im klaren sein, welche Parameter warum und wie gemessen werden sollen. Ergebnisse von Labormethoden sind nur sinnvoll, wenn sie mit Felddaten erhärtet werden können. Es werden Beispiele gezeigt, bei denen die Nutzleistung im Feld aufgrund von Labormessungen erfolgreich im voraus ermittelt wurde. Die Bedürfnisse der Qualitätsüberwachung werden anhand von drei erfolgreich eingesetzten Parasitoiden gezeigt. In jedem Fall sind die Haupteigenschaften je nach den Bedürfnissen und Anforderungen gewichtet. Die Beispiele verdeutlichen, daß der Bedarf an Qualitätsüberwachung im allgemeinen steigt, je künstlicher die Zuchtbedingungen im Vergleich zum Einsatzgebiet der Nützlinge sind. Damit Parameter gefunden werden, die bezüglich der Nutzleistung aussagekräftig sind, muß jedes einzelne Zuchtsystem sorgfältig analysiert werden.

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## The impact of poor quality of mass-reared Mediterranean fruit flies on the sterile insect technique used for eradication<sup>1</sup>

By C. O. CALKINS and T. R. ASHLEY

### Abstract

The formula proposed by E. F. KNIPLING for eradication of an insect population using the sterile insect technique was applied to a target population of Mediterranean fruit flies using releases of sterile flies possessing varying levels of behavioral quality. The density of sterile flies in the large medfly eradication program in Central America was based upon 2000 pupae/ha. No adjustments were made for poor eclosion, flight ability or mating propensity. If such adjustments were made, the cost of the program would have to be increased substantially or the size of the release area must be reduced. The actual cost figures for producing flies of poor quality are explained.

A major concern of entomologists and program directors of fruit fly eradication programs involved in the release of sterile males is that the flies produced are adequate to fulfill their mission; that is, they compete well with wild males and are successful in mating with wild females.

The ability of entomologists to evaluate and quantify the potential or real effectiveness of mass-reared, sterilized flies in interactions with wild flies has not existed until the last few years. Until then, the effectiveness of the flies was determined by whether the sterile insect technique (SIT) worked. If SIT failed, managers were not able to specify what went wrong or where and when the problem occurred. They only speculated that the flies must have been of low quality. It was not until the pioneering work of BOLLER and CHAMBERS (1977) on quantifying the behavioral quality of mass-reared fruit flies that these flies could be evaluated and the reasons for their inadequacies identified and corrected.

What remains to be shown is what impact poor quality flies have on an eradication effort using the SIT. If flies are totally incompetent or are totally competent in their interactions with wild populations, there is no problem determining the impact. However, when flies produced in a factory are neither totally incompetent nor competent, their impact on the field population is very difficult to quantify. This paper attempts to show the type of impact that flies with differing levels of competency might have on wild populations.

The premise developed by KNIPLING (1979) for eradication of an insect population by the sterile insect technique involves overflooding a population, which in this case has a theoretical five-fold rate of increase, so as to achieve a density of 9 times as many sterile insects as there are fertile ones. His formula (table 1) reveals that such a population would theoretically be eradicated within five generations. Several assumptions are made when using this formula: e.g., all sterile males are equally competitive with wild males, and sterile males have the same temporal and spatial access to the wild females as do wild males.

The biotic potential (i.e., surviving daughters per female) for medfly is considerably

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Table 1. Dynamics of an insect population having a growth rate of five when subjected to the release of a constant number of effective sterile insects each generation, beginning with a 9:1 ratio of sterile to fertile insects (Knipling 1979)

Generation	Fertile Females in population	S:F ratio	Fertilized Females in population
1	1,000,000	9:1	100,000
2	500,000	18:1	26,316
3	131,580	68:1	1,907
4	9,535	942:1	10
5	50	180,000:1	0

higher than five. A reasonable rate of increase might be closer to 12. This is based upon the maximum number of eggs a female could lay in her lifetime, ca. 280 (HANNA 1947) less 10% for time spent searching for hosts, provided there were adequate host fruit available, a 10% survival rate to the adult stage that results in ca. 25 flies in the  $F^1$  generation,  $F_2$  of which are females.

The growth rate of endemic Mediterranean fruit flies (*Ceratitis capitata* [Wied.]) populations probably reaches its maximum only when an almost infinite number of susceptible hosts is available and when conditions are agreeable for pupal and adult survival and adult activity. This situation occurs in Guatemala and southern Mexico during the dry season (November to June) when coffee berries are ripening.

If KNIPLING's high overflooding ratio of 9 sterile to 1 fertile male was used for medfly when the potential population growth rate is 12, control is not achieved (table 2). Instead of a decrease in the population during each generation, the population actually increases but at a much slower rate than normal. Obviously, a greater overflooding ratio of sterile to fertile medflies is needed.

HOOPER (1978), after examining the literature of SIT programs to eradicate fruit flies, indicated a minimum sterile to fertile ratio of 100:1 was needed. Using this overflooding ratio in the formula, the population of medflies would be eradicated in three generations (table 3).

As mentioned previously, assumptions are made regarding the competitiveness of flies. However, we know from experience that mass-reared flies are not always as competent in performing their intended mission as one would like (BOLLER et al. 1981). The quality of these flies can be affected by conditions during the rearing process: irradiation, handling and shipping, and release into the field. In addition, detrimental genetic aberrations sometimes appear in the domesticated fly colony. Tests have been developed to quantify behaviors and abilities essential to the reproductive life of male fruit flies (BOLLER et al. 1981; CHAMBERS et al. 1982). The important components used in this paper for illustrative purposes are adult eclosion rate, flight ability and mating competitiveness.

BRAZZEL et al. (1988) developed specifications and tolerances for quality control tests of flies when leaving the factory and of flies after shipment to the release or distribution site. The minimum requirements established for eclosion, flight ability and mating competitiveness are 75, 60 and 50% respectively. When these figures are put into KNIPLING's formula with a population growth rate of 12, the actual ratio of effective overflooding is reduced

Table 2. Dynamics of a Mediterranean fruit fly populations in which SIT was and was not utilized

Each population had a growth rate of 12 and was subjected each generation to the release of a constant number of effective sterile insects beginning at a 9:1 ratio of sterile to fertile flies

Generation	No SIT	SIT Utilized			Percent fertile matings
	Uncontrolled fertile population	Fertile females in population	S:F ratio	Fertilized females in population	
1	1,000,000	1,000,000	9.0:1	100,000	10.0
2	12,000,000	1,200,000	7.5:1	141,176	11.8
3	144,000,000	1,694,118	5.3:1	268,908	15.9
4	1,726,000,000	3,226,891	2.8:1	849,181	26.3
5	20,736,000,000	10,190,182	0.9:1	5,360,035	52.6

Table 3. Dynamics of a Mediterranean fruit fly population with a growth rate of 12 when subjected to the release of a constant number of sterile insects each generation, beginning with a sterile to fertile ratio of 100:1

Generation	Fertile females in population	S:F ratio	Fertilized females in population	Percent fertile matings
1	1,000,000	100:1	9,901	0.99
2	118,812	842:1	141	0.12
3	1,692	59,102:1	0	0.002

significantly, from 100:1 to 23:1. Although this prolongs the time necessary to eradicate the population, eradication still occurs theoretically in five generations (table 4).

To illustrate the effects of flies of varying quality on the actual overflooding ratios, we will examine actual data accumulated from two separate eradication programs of medfly sterile releases, and from an experimental population during development of these tests. The first program involved flies produced by the Guatemala Moscamed Commission at the San Miguel Petapa factory, where ARS has had a pilot test designed to implement a technical and managerial system for quality control of mass-reared medflies. Because the distribution point is only ca. 20 km from the factory, quality control data at the factory door are assumed to be similar to those at the release site. The eclosion rate, flight ability and mating competitiveness averaged 92.8, 91.1 and 64.2 % during a 4-month period in 1985. At those rates, the actual overflooding ratio of effective flies released in the program would average 54:1 if the ratio were intended to be 100:1. Theoretically, eradication of the population would occur in four generations (table 5).

Table 4. Dynamics of a Mediterranean fruit fly population having a growth rate of 12, when subjected to the release of a constant number of sterile males each generation, beginning with a 100:1 overflooding ratio, but having this ratio corrected for minimum quality specifications for effective flies (23%) at the release site

Generation	Fertile females in population	S:F ratio	Fertilized females in population	Percent fertile matings
1	1,000,000	23:1	42,553	4.17
2	500,000	46:1	10,638	2.13
3	127,660	180:1	705	0.55
4	8,463	2,717:1	3	0.04
5	36	638,889:1	0	

Table 5. Dynamics of a Mediterranean fruit fly population having a growth rate of 12, when subjected to the release of a constant number of sterile males each generation, beginning with a 100:1 overflooding ratio

Ratio corrected for actual percentage (54%) of effective flies produced at San Miguel Petapa, Guatemala

Generation	Fertile females in population	S:F ratio	Fertilized females in population	Percent fertile matings
1	1,000,000	54:1	18,182	1.8
2	218,184	248:1	876	0.4
3	10,515	5,137:1	2	0.02
4	24	2,250,000:1	0	

The second program involved eradication of the medfly in Miami, Florida in 1985, by the use of bait sprays followed by release of sterile flies for a period equivalent to three field generations. ARS was asked to install a system of quality control tests to evaluate flies that were reared and sterilized in Honolulu, Hawaii, and packaged and shipped to Miami for release (CALKINS et al. 1988). In Miami, the eclosion rate, flight ability and mating competitiveness averaged 90, 80 and 75 % respectively. The actual percentage of effective flies released was 54, which in an overflooding ratio of 100:1 would reduce the actual ratio to 54:1 (table 6).

The quality control information from the experimental population was derived from the work of BOLLER et al. (1981). We used eclosion rate (76.8), percent fliers (59.6), and the mean mating index (41.1) for strain B in table 1 of their publication to illustrate what effect

Table 6. Dynamics of a Mediterranean fruit fly population having a growth rate of 12, when subjected to the release of a constant number of sterile males each generation, beginning with a 100:1 pupal overflooding ratio

Ratio corrected for actual percentage (54 %) of effective flies produced at Honolulu, Hawaii and shipped to Miami, Florida for release

Generation	Fertile females in population	S:F ratio	Fertilized females in population	Percent fertile matings
1	1,000,000	54:1	18,182	1.8
2	218,184	248:1	876	0.4
3	10,515	5,137:1	2	0.02
4	24	2,250,000:1	0	

a poorer strain of fly could have on an eradication program. The data indicated that only 17 % of the pupae used, actually resulted in effective flies. Their use in an eradication program would still have a suppressing effect on the wild population but it would take six generations to achieve eradication (table 7) at a purportive 100:1 overflooding ratio.

Table 7. Dynamics of a Mediterranean fruit fly population having a growth rate of 12, when subjected to the release of a constant number of sterile males each generation, beginning with a 100:1 pupal overflooding ratio

Ratio corrected for actual percentage (17 %) of effective flies from strain B (BOLLER et al. 1981, table 1)

Generation	Fertile females in population	S:F ratio	Fertilized females in population	Percent fertile matings
1	1,000,000	54:1	55,556	5.6
2	666,672	25:1	25,641	3.8
3	307,695	55:1	5,594	1.8
4	67,133	253:1	264	0.4
5	3,172	5,360:1	1	0.02
6	12	1,416,667	0	

There has been speculation that genetic resistance to SIT could develop in a species. For this to happen, the wild populations must be exposed to the sterile-released insects for a sufficient number of generations for selection in the wild population to manifest itself, provided that a single gene was not responsible for resistance. Two factors could be

involved, increased number of matings by wild females or increased discrimination in mate choice. ITÔ and KAWAMOTO (1979) indicated that increased discrimination in choosing a mate by wild females would be the most logical means of selection. However, changes or shifts in the temporal and spatial mating interactions in the wild population could result in a form of resistance to SIT. The more generations of wild flies exposed to sterile flies the greater chance there is for this type of resistance to develop. Therefore, a high overflooding ratio may be necessary to reduce the number of generations necessary for eradication so as to prevent the development of such resistance.

One should not confuse incompatibility between sterile and wild flies with resistance. If changes occur in the mass-reared colony during or after initial colonization and if these changes prevent the released insects from mating with wild females, this is a form of incompatibility. It develops, not as a result of selection in the wild population when exposed to the control mechanism, but rather is due to a change in the factory population because of selection for the rearing regime or genetic drift. Quality control tests to measure compatibility in pheromone responses and mating interactions with wild individuals are necessary at frequent intervals to assure that incompatibility is not developing in the factory strain.

In large medfly eradication programs, actual population levels are not determined precisely. The bait sprays are designed to reduce the population density to some low level and to remove mated females from the population. Inundative releases of sterile flies are then assumed to overflood the remaining flies at some ratio. This ratio is then determined by placing traps in the release area and determining the ratio of marked and unmarked flies trapped.

In the large medfly eradication program underway in southern Mexico and Guatemala, bait sprays are applied to reduce the wild population. These applications of bait sprays are followed by releases of sterile flies for final eradication. The target density of released flies in Guatemala is 2000 pupae per hectare (L. WHITE, pers. comm.). Quantities are determined by volumetric measure of pupae placed into emergence cages or paper bags. Approximately 600 million flies are released each week over an area of ca. 3000 km<sup>2</sup>. The cost of producing the flies in Guatemala is \$62.01 (Feb. 1986) per million flies. The cost of releasing the flies by air is \$9.94 per million flies. Thus, the total weekly cost of rearing and releasing sterile flies is \$43,170 over a 3,000 km<sup>2</sup> area. Of the flies released in Guatemala, 80% are reared at the Metapa, Mexico facility and 20% at the San Miguel Petapa, Guatemala facility.

In order to maintain a level of 2000 effective flies/ha, the number released based on quality control data from the San Miguel Petapa rearing facility would have to be increased to 3,704. This would increase the cost of treatment to \$79,944/week, almost double or reduce the treated area to 1,626 km<sup>2</sup>. If the quality of the flies released only meets the minimum standards (table 4), the cost would increase to \$187,696 weekly or 4.3 times the original to maintain the level of 2,000 effective flies/ha or would reduce the treated area to 698 km<sup>2</sup>.

It is obvious that the relatively minor expense of maintaining an effective quality control system in an eradication program is a worthy investment. It is not enough to maintain the flies at minimum levels. It is necessary to produce the largest, healthiest, most vigorous and competitive flies possible to keep the costs of a large program within reason and to assure the maximum probability of success.

If compensation for low quality is not made by increasing the numbers of released flies, the number of generations needed to eradicate the wild flies increases as previously shown in tables 4, 5, 6 and 7. If we agree that a single generation of wild medflies in the field probably takes about 40 days to complete, and we determine what percentage of effective flies are being released and using KNIPLING's formula, we can determine about how many generations (in days) would be needed to eradicate the target population. If you want to see

cost figures that will get your attention, determine the cost per day of rearing flies and multiply the number of extra days needed to eradicate the target population because poor flies are being released. An example of these cost figures are shown in table 8.

If the added expense, because of the extra time needed to achieve eradication, is combined with the increased risk of the target population developing resistance to the sterile release technique by being exposed to the sterile flies for a longer time, the problem becomes great enough to seriously damage or defeat the program.

Table 8. The cost of extending an eradication program on the reduction of the treated area because of the release of poor quality Mediterranean fruit flies

Example	No. gen. to eradicate	Weeks to eradicate	Cost to treat 3000 km <sup>2</sup> for required no. gen.	No. km <sup>2</sup> to treat with 600 million flies to eradicate in 3 gen.
(U.S. dollars)				
1986				
Table 3	3	17.1	746,057	3000
Tables 5&6	4	22.9	986,743	1620
Table 4	5	28.6	1,233,428	690
Table 7	6	34.3	1,480,114	510

### Zusammenfassung

Zum Einfluß der Qualität von Mittelmeerfruchtfliegen aus Massenzuchten auf den Erfolg der sterilen Insekten-Technik

Die von E. F. KNIPLING vorgeschlagene Formel, um die Ausrottung einer Insektenpopulation mittels der Sterilen-Insekten-Technik zu berechnen, wurde auf eine Zielpopulation von Mittelmeerfruchtfliegen angewendet, in die sterile Fliegen mit unterschiedlichen Verhaltensqualitäten eingebracht wurden. Die Dichte der sterilen Fliegen im großen Ausrottungsprogramm der Mittelmeerfruchtfliege in Zentralamerika basierte auf einer Puppenzahl von 2000 Puppen/ha. Es wurden dabei keine Korrekturen für geringere Schlüpfprozente, reduzierte Flugfähigkeit oder verminderte Paarungsbereitschaft einbezogen. Wären solche Korrekturen vorgenommen worden, hätten sich die Kosten für das Ausrottungsprogramm erheblich erhöht und die zu behandelnden Flächen hätten reduziert werden müssen. Die aktuellen Kosten, die bei Auslassen von Fliegen minderer Qualität entstehen, werden erläutert.

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## Quality requirements in natural enemies used for inoculative release: Practical experience from a successful biological control programme<sup>1</sup>

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and EDWINA MADOJEMU

### Abstract

During the past six years, the South-American encyrtid wasp *Epidinocarsis lopezi* (De Santis) was released from the ground and from the air in about 100 areas in Africa for the biological control of the cassava mealybug (CM) *Phenacoccus manihoti* Mat.-Ferr. It was established in all release sites and, in 1988, occurred in 18 African countries over 1.5 mio. km<sup>2</sup>. Its efficiency in permanently reducing CM populations has been documented.

*E. lopezi* was reared on its original host plant, cassava, and on the original insect host, the CM. Rearing methods for plants, CM, and *E. lopezi* were developed in the course of the actual production of parasitoids for release. Continuous production without bottlenecks was assured by >50 rearing units with potted plants and by large mechanized cages with hydroponic cultures, where cassava, insect host and parasitoid were reared in the same unit. Timing of parasitoid infestation and inoculum size were improved through in-depth biological and ecological studies. Host instar preference, host feeding and other nutritional requirements, mutilation of hosts, low reproductive capacity, superparasitism, and developmental time were taken into account for efficient rearing, storage, and transport.

The successful establishment of *E. lopezi*, its spread, and efficiency in Africa attest to the production of sufficient numbers of wasps of high quality. Insect and plant studies, technology development, and careful supervision contributed to good quality which, in inoculative releases, takes precedence over high numbers.

### 1 Introduction

In the early 1970s, the mealybug *Phenacoccus manihoti* Matile-Ferrero (Hom., Pseudococcidae) (CM) was accidentally introduced from South America to Africa where it spread and became the major cassava pest (review by NEUENSCHWANDER and HERREN 1988). A large scale biological control project was undertaken by the Africa-wide Biological Control Programme (ABCP) of the International Institute of Tropical Agriculture (IITA) in collaboration with numerous national and international agencies (HERREN 1987).

Following an extended search in South American and quarantine at the CIBC International in London, the solitary and host specific wasp *Epidinocarsis lopezi* (De Santis) (Hym., Encyrtidae) was imported into Africa, reared and first released in Nigeria in 1981 (HERREN and LEMA 1982). By mid 1988 it had been successfully established in 18 African countries and had spread over an area of 1.5 mio. km<sup>2</sup> (HERREN et al. 1987; NEUENSCHWANDER and HERREN 1988, unpubl. results). CM populations declined after the release and have remained low since (HAMMOND et al. 1987; NEUENSCHWANDER and HAMMOND 1988). Surveys of subsistence farms (unpubl. results), exclusion experiments (NEUENSCHWANDER et al. 1986), and a computer simulation model (GUTIERREZ et al. 1988) also documented the efficiency of *E. lopezi* in preventing most CM outbreaks.

In its first phase, the ABCP focussed on the production of sufficient numbers of

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beneficials for safely maintaining the cultures for study and release. As successive generations of *E. lopezi* were reared, questions of how to maintain their quality arose. Improvements in rearing were first based on knowledge from other insectaries (FINNEY and FISHER 1964; HAGEN 1964; WAAGE et al. 1985), particularly on other encyrtid mealybug parasitoids (SMITH and ARMITAGE 1931; CLAUSEN 1942; AVIDOV et al. 1967; VIGGIANI 1975; BARTLETT 1978). Soon, the successful establishment spawned a large number of in-depth studies on *E. lopezi* (review in NEUENSCHWANDER and HERREN 1988), making this species one of the best known encyrtids.

The present text describes how these results were taken into account in the insectary and describes the stimulatory interplay between purely scientific knowledge and technical advances. It presents new applied research, unique rearing technologies, and all aspects of insectary work which allowed the programme to satisfy the need for high quality insects. The contribution of the different factors to the successful production, storage, and release of *E. lopezi* is discussed.

## 2 Material and methods

### 2.1 Rearing of cassava plants

In order to develop a stable culture of potted cassava at IITA, a series of small experiments was performed in a glasshouse where temperatures fluctuated between 27 and 35 °C with up to 60 000 lux. Cassava stems of different lengths were planted in pots of different sizes, different soil types were tested, and the possibility to re-utilize infested plants from the insectary by cutting them back was explored. Stems were infested with CM crawlers, then disinfested by treating them with dimethoate 0.1 % (LEUSCHNER et al. 1980) or 52 °C hot water (BAKER 1962), or planting them horizontally, i.e. covered with soil. The residual effect of an acaricide, used against cassava green mites (CGM) [*Mononychellus tanajoa* (Bondar), Acari, Tetranychidae], on CM and *E. lopezi* was also evaluated (details in NEUENSCHWANDER et al. 1984).

To determine the nutritional requirements and optimum environmental conditions for cassava grown in nutrient solutions, cuttings were placed in plastic covered bottles containing a nutrient solution [MURASHIGE and KOOG (1962) or FORNO et al. (1979) plant salt mixtures] diluted by a factor of two. The bottles were either aerated, aerated by adding CO<sub>2</sub> (MAHON et al. 1977), or not aerated, and the plants were measured after four weeks. Later, different fungicides were tried out.

For presprouting large numbers of cuttings for hydroponic culture, disinfested cuttings (with dimethoate) were quickly dipped into a solution of 2000 ppm rooting hormone (indolbutyric acid) (SYKES and HARNEY 1974) to promote the formation of roots. The next day, the cuttings were densely packed in plastic trays under a plastic cover. Four times during the day they were inundated for a few minutes to a depth of about 10 cm with a salt solution [Luwassa®, Introhydro, Bern, Switzerland, containing N (15 %), P<sub>2</sub>O<sub>5</sub> (7 %), K<sub>2</sub>O (22 %), MgO (5 %), Fe (0.1 %), and traces of B, Cu, Mn, Zn, Co, Ni and V], to which the fungicide Roval® (Rhône-Poulenc, Lyon, France) was added. This solution was pumped into the trays from a storage tank by a pump controlled over a timer. After 1–1.5 weeks, the cuttings, which had short roots and newly formed sprouts, were ready for use in the large scale rearing units. Three cassava varieties were tested and their performance compared with the one in soil.

In 1988, every week, about 1200 cuttings were planted in soil in 600 l pots and 1500 cuttings were prepared for the hydroponic cultures. Plants in pots were used for infestation with CM after 4–6 weeks, depending on the season. No phytosanitary treatments were made, but plants attacked by CGM or whiteflies (*Bemisia* sp., Hom., Aleurodidae) were rogued. A total of 3 ha of land per year, half of it under fallow, was needed to produce this planting material. For the entire green house operation from harvesting of the cuttings to planting, watering, and the transfer to the CM cages, six technicians were employed at IITA.

### 2.2 Mealybug production

To investigate the success of an infestation of cassava with CM depending on the plants' condition, CM egg masses were placed on equally sized plants, planted in pots of different sizes under different irrigation schedules. After five weeks, CM on the 12 plants of each of the eight treatments were counted and related to the number of green and dry leaves (details in NEUENSCHWANDER et al. 1984).

Based on observations concerning CM crawler emergence and survival, a crawler collecting device was constructed (fig. in HAUG et al. 1987). It consisted of 12 ventilated jars covered with screw caps.

Through an opening, screened with a fabric of 0.25 mm mesh width, freshly hatched crawlers moved upward into a collection vial, while older instars or any parasitoids were retained. Up to 1 mio. crawlers were collected daily, their number being calculated from their weight, i.e. 300 crawlers per mg. Using a salt-shaker like vial, the crawlers were immediately sprinkled over the plants of the large scale hydroponic cultures. For small scale use, CM infested leaves were cut and placed on the potted plants for infestation. These CM cultures were maintained in wooden cages (44 cm × 45 cm × 58 cm) with fine screen sides and glass tops on 30 cm tall plants in the glass house under a lath shelter, where temperatures occasionally surpassed 40°C.

In 1988, about 75 cages with four pots with two CM infested plants each were maintained. Every week, plants from 20 cages, which had stayed in the glasshouse for two weeks, were brought to the predator and parasitoid rearing rooms. Eight were used for the production of *E. lopezi* alone. The entire CM rearing required one technician.

### 2.3 Parasitoid culture

At IITA, a total of three exotic CM parasitoids and up to four exotic predators were reared in closed insectary rooms with air-conditioning and air fans. Before temperatures rose above 32°C, air-conditioning was switched on, which brought temperatures to a minimum of 25°C. When no air-conditioning was needed, a strong fan brought in fresh air, while air was removed at the far end of the room. Without air circulation, the air had a stale taste and efficient rearing of *E. lopezi* proved impossible. In 1988, the rearing of *E. lopezi* alone required up to 54 cages. Two CM infested plants per cage were inoculated with 50–70 *E. lopezi*. Tabs were kept on the daily manipulations of each cage (adding/removal of plants or parasitoids) and cages were replaced when unwanted indigenous hyperparasitoids (NEUENSCHWANDER et al. 1987) were found repeatedly. Old plants were cut, and the CM infested stems and leaves were kept for emergence of *E. lopezi* from the last mummies (= hardened CM, containing a parasitoid pupa). This work was executed by three technicians and one assistant.

Rearing of *E. lopezi* proved to be almost impossible on cassava plants which had collapsed under the CM attack and were drying out. This was partly explained by an observation in a screen house on plants in randomly mixed trays, four with heavily infested, yellowish plants, and five with vigorously growing, green plants, from which all *E. lopezi* adults were removed with an aspirator and sexed.

### 2.4 Large scale plant growth chambers

Based on hydroponic cultures, a new mechanized rearing system was developed from the existing Ruthner system (RESH 1978). Eight stainless steel cages with a ground surface of 2 m × 2 m and a height of 4 m were installed pairwise in four rooms. In each cage, nine trays are mounted on a chain rotating in a paternoster system around a centrally mounted light source (fig. in HAUG et al. 1987). 600 cassava cuttings are inserted through covers of the trays. When a tray reaches the filling position, the chain stops moving and a programmable amount of nutrient solution from a central tank is pumped into the tray. At the same time, the preceding tray is emptied at the draining point. Between the filling and the draining station, the roots are submerged in ca. 5 mm of nutrient solution. After drainage, only a small amount of liquid remains in the tray during its 30 min trip around the light source and the humid roots are exposed to the air. The nutrient solution, a ready made salt mixture (Luwassa®, 1 g/l) to which the fungicide Roval® (1 g/l) is added, is replenished daily with water. After 7 days, when it is discarded, it contains still 10.2 % of the original K, 2.8 % of the phosphates, and 15.6 % of the nitrates. Different light intensities are obtained by individual switching of eight high pressure sodium lamps (250 Watts each) and four 40 Watt daylight fluorescent tubes, enclosed in a glassbox with heat extraction. With full illumination, the maximum irradiance at plant levels is 34 000 lux, but it drops to ca 350 lux as the plants move to the top above the light source. Relative humidity drops linearly, from 94 % without lights, by 10 % for each additional 10 000 lux (corresponding to two Na-lamps), which in turn increases average temperature by 2.8°C. Temperatures depend on the cooling of the room.

In 1988, temperatures ranged daily between 26 and 33°C, while the relative humidities were maintained at 50–90 % RH, if necessary by means of a humidifier. Rearing of *E. lopezi* in these cages was managed as follows: The plants were infested with only about 60 000 CM crawlers, i.e. 100 per plant, when the sprouts were 5–10 cm long. After 1 week, a few leaves with third instar CM were placed on each tray and two days later, 1000 wasps were released in the cage. When their offspring, produced on the third instars, emerged, the initial infestation with crawlers had reached the third instar, ready for stinging. New crawlers were added when needed. Operating the mechanized cages required one assistant, an engineer half time, and three technicians.

## 2.5 Collection, storage, and transport

Collection of adult *E. lopezi* from the cages was done by means of aspirators, at a rate of about 300 wasps/man-hour. From the mechanized cages, insects were collected, mostly from the glass cage surrounding the lights, with small battery operated aspiratory which caused a negligible mortality of below 5%. The 20-ml plastic vials, used for storage and transport, contained a strip of filter paper with a drop of diluted honey.

Since storage conditions during transport were known to be critical (HERREN et al. 1987), new experiments with larger storage vials and different foods were performed. Survival of hundreds of freshly emerged *E. lopezi* adults per 1 l jar with screen windows was compared at 15 and 27°C for different food sources. Similarly, 182 100-ml-plastic boxes with 10 freshly emerged females and 10 males each were installed with a filter paper wetted from outside, and supplied with either sugar in grains, sugar water, honey, wheat® (Beltsville Bee Diet, Frenchtown N. J., USA), or CM on a piece of cassava stem, with or without these foods. Survival was monitored daily. From an identical set, females were removed at different times, offered CM third instars in a plastic dish on a cassava leaf for exactly 30 min under constant observation. All stung CM were dissected to count *E. lopezi* eggs. Afterwards, each female was dissected and the mature eggs in the ovarioles were counted.

## 3 Results and discussion

### 3.1 Production of host plants

At IITA, all rearing of CM and its parasitoids was done on the original euphorbiaceae host cassava, *Manihot esculenta* Crantz. Laboratories in temperate zones have occasionally used another euphorbiaceae, poinsettia *Euphorbia pulcherrima* (A. PANIS, pers. comm.) as host for CM. In fact, the CM attacks numerous other plant species, but only under high population pressure.

Disinfection of cuttings (table 1) with dimethoate, as is commonly recommended (LEUSCHNER et al. 1980), was used for large scale treatments. Because of the survival of some crawlers and the slight phytotoxic effect, this treatment is, however, not ideal for

Table 1. Disinfection of cassava cuttings planted in a glasshouse, Ibadan 1983  
30 cuttings infested with CM crawlers per treatment, evaluated after 4 weeks

Treatment	% cuttings dead	with CM infestation	No. of leaves ( $\pm$ S.E.)
Vertical planting, control	0	63.3	13.1 $\pm$ 0.9
Horizontal planting, control	0	40.0	12.7 $\pm$ 0.9
Disinfection with dimethoate 5 min	0	3.3	9.6 $\pm$ 0.5
Disinfection with 52 °C water			
15 min	6.7	0	9.7 $\pm$ 0.7
30 min	50.0	0	7.0 $\pm$ 0.6
60 min	60.0	0	5.7 $\pm$ 0.9

some special requirements. When the cuttings were buried, which has no ill effects on plant growth, disinfection was not satisfactory. Hot water treatment of the cuttings for 15 min offered the best alternative. In order to diminish the negative effect on plant growth, as shown by the lower production of leaves, treatment time was later reduced to 5 min.

Survival of cassava cuttings with two, three, and five nodes was 37, 55, and 89% respectively, i.e. about 18% per node. Though cassava can be grown from much smaller cuttings (SYKES and HARNEY 1972) and even in tissue culture (NG 1984), the relatively low price of cassava cuttings led us to adopt five node cuttings as a standard, resulting in a very high survival rate. In 0.2, 1.0, and 2.6 l pots, the surviving plants averaged 8.4, 13.9, and 17.0 leaves after seven weeks. It was decided that for the wooden rearing cages the

intermediate pot size was preferable. Any effort to recycle used plants from the insectary to save planting material was abandoned when 60 heavily infested plants from the insectary, cut back to the wood, did not resprout and died. Out of 60 lightly infested plants, only 46.7% resprouted, while all uninfested plants survived a cutback.

Very rich forest soil with a pH of 7.8 and a high Ca content (19.4 ME/100 g) gave very poor root development resulting in weak cassava plants. By contrast, sandy loams with a pH of 6.1 and a Ca content of 5.2 ME/100 g were preferable. Such soils also facilitated good drainage which proved to be critical especially during the rainy season. Whenever soils were too poor, as shown by yellowing of the leaves, a minimal addition of NPK fertilizer brought plants back to full vigour.

When CM infested cassava plants were treated against the CGM (table 2), mortality of the CM was minimal on the second and third instar CM which are attacked by *E. lopezi*, and somewhat higher on the first instars. Later parasitization rates by *E. lopezi* were

Table 2. Influence of the acaricide Kelthane on cassava mealybug and its parasitoid *E. lopezi*  
Number of individuals tested in brackets

	Control	Kelthane	
		0.6 ml/l	1.2 ml/l
% mortality cassava mealybug			
first instar	4.4 (204)	5.4 (221)	6.7 (164)
second instar	2.2 (136)	0.6 (154)	1.6 (186)
third instar	1.1 (93)	1.6 (64)	0.0 (123)
% parasitism <sup>1</sup> by <i>E. lopezi</i>	17.4	14.2	11.3

<sup>1</sup> Based on second and third instar mealybugs.

significantly reduced by one third ( $\chi^2 = 4.2$ ) by the acaricide treatment. Consequently, efforts were made to keep cassava in the glasshouses clean by timely roguing of infested plants only. Other pests and the common cassava mosaic virus disease and bacterial blight never proved a problem on the vigorously growing plants, even if susceptible varieties were used.

Handling of soil and some of the associated technical and sanitation problems were overcome by hydroponic cultures. Aerated hydroponic cultures also gave faster plant growth than potted plants, with plants in aerated nutrient solution producing 9.8 leaves within 4 weeks, whereas plants in non-aerated medium had 4.1 leaves, and those in soil had 2.9 leaves (mean S.E. = 1.9, N = 32). Aeration increased dry weight 2.5 times and helped reduce fungal root diseases, which are a special problem in hydroponic cultures where all plants share the same liquid. Later, cuttings were therefore always treated with fungicides, e.g. copper sulphate, in a dip, and organic fungicides were added to all nutrient solutions. Adding CO<sub>2</sub> did not improve growth.

A test of different varieties in hydroponic culture in mechanized cages gave no clear winner but proved that all varieties grew significantly better in hydroponic culture than in soil. Thus, Odungbo produced 29.8 leaves in five weeks, TMS 91934 28.0, and TMS 30572 26.6 leaves, as compared to 15.8, 16.4, and 14.9 leaves, respectively, in soil ( $\pm 1.2$  S.E., N = 9).

### 3.2 Production of cassava mealybugs

For the maintenance cultures of *E. lopezi* in wooden cages, mixtures of CM instars were acceptable. But for the timing of large production units, it was important to obtain large numbers of crawlers. Crawlers have the advantage that they can be collected easily, counted by weight, and are free of parasitoid larvae.

Field observations and life table studies indicated that CM was more abundant on stressed plants (NWANZE et al. 1979; SCHULTHESS 1987). An experiment on potted plants of equal age, which were stressed to different degrees as shown by their size and the number of dried leaves, confirmed these observations. The number of green leaves ( $X_1$ ) was negatively correlated with the mean number of CM per plant ( $Y$ ) ( $Y = 327 - 2.176 X_1$  with  $t = 2.24$  n.s.), while the percentage of dry leaves ( $X_2$ , calculated as arc sine  $\sqrt{p}$ ) was positively correlated ( $Y = 197 + 2.725 X_2$ , with a significant  $t = 3.20$  for  $N = 8$ ). For rearing CM in the crawler production device, weak plants were therefore utilized up to their collapse.

One hydroponic unit was used for CM production alone. In a life table study, differences between four tested varieties [IITA varieties TMS 30572, 4(2)1425, and 50395, and the local variety Odungbo] were small and non significant. On the average 10.5 % of the 500 crawlers per plant settled, 69.9 % of all settled first instars reached maturity on the average after 23 days, and went on to lay eggs for another 19.8 ( $\pm 1.8$  S.E.) days. Among the 180 tagged females, 24.4 % wandered off their marked site and were lost. The remaining 136 females laid 362.9 ( $\pm 35.0$  S.E.) eggs. These results are in good agreement with laboratory data on potted plants (SCHULTHESS 1987), demonstrating the viability of hydroponic cultures for CM production.

### 3.3 Rearing *Epidinocarsis lopezi*

#### 3.3.1 Host density

Enough hosts had to be offered for two reasons: 1. to avoid superparasitism, which in the laboratory occurs frequently, though only one parasitoid larva survives (IZIQUEL 1985) and supernumerary larvae are encapsulated (SULLIVAN and NEUENSCHWANDER 1988; NENON et al. 1988). 2. to overcome loss by host feeding and mutilation (NEUENSCHWANDER and MADOJEMU 1986; LÖHR et al. 1988).

However, very high host densities are of no benefit either: 1. Host plants in the laboratory easily collapse, which makes them unsuitable for the parasitoid. It was observed that the sex ratio among adults in an open screenhouse was 1.4 % females ( $N = 282$ ) on heavily infested, collapsing plants, 22.4 % ( $N = 76$ ) on infested green plants, and 79.2 % ( $N = 24$ ) on the screen of the roof, suggesting that females prefer green plants and disperse more easily than males. Similarly, it was observed that females are attracted to the odor of infested cassava plants and not to CM per se (NADEL and VAN ALPHEN 1987), indicating the importance of green cassava plants for this parasitoid. 2. The reproductive capacity of *E. lopezi* is low (IZIQUEL 1985; LÖHR et al. 1988). 3. Observations of *E. lopezi* females in cages indicate that females avoid large CM colonies with abundant honeydew. 4. If plants are kept green they have to be replaced less often, no debris of dry leaves, where mummies often hide, accumulates in the cages, and maintenance is facilitated.

#### 3.3.2 Host quality

Mixed colonies containing all instars were preferred for rearing for the following reasons: Second instars are preferred for host feeding, third instars for reproduction, while sex ratio is strongly male biased on the younger stages (KRAAIJEVELD and VAN ALPHEN 1986; NEUENSCHWANDER and MADOJEMU 1986; LÖHR et al. 1988). Ovipositing females are less preferred. Thus new production of hosts for late emerging *E. lopezi* larvae from small CM (LÖHR et al. 1988) is assured.

### 3.3.3 Production

In the cages, the inoculum size was relatively large, i.e. 50–70 *E. lopezi* per two plants. In the large mechanized rearing units with 600 plants, an inoculum of only 1000 wasps was introduced to few CM hosts, while a large host population was building up during 2 weeks when the first wasp generation developed.

When wasps were removed weekly for a large scale aerial release programme in 1988, average production from the wooden cages in the third week after inoculation was 211.5 *E. lopezi* ( $\pm 16.7$  S.E.,  $N = 39$ ) per pot with two plants, i.e. 105.7 wasps per plant. Up to 1292 wasps were recovered from one cage in one week. In the fourth and subsequent weeks, *E. lopezi* production on the same plants declined rapidly. Most plants stayed in the insectary for four weeks, in addition to the six weeks in the glasshouse for plant growth and CM production.

In the hydroponic units a production cycle lasted on the average 65.6 days ( $\pm 4.6$  S.E.,  $N = 9$ ), in addition to the 10 days the cuttings spent in the presprouting unit. Harvested production averaged 40 800 ( $\pm 3754$  S.E.) wasps per unit or 68.0 wasps per plant. But even during the most intensive use of *E. lopezi*, not all wasps which emerged in the cages and hydroponic units could be recovered and died.

In view of the complicated interactions between parasitoids, hosts, and host plant, a demographic analysis of mass rearing of *E. lopezi*, corresponding to the one done for fruit flies (CAREY and VARGAS 1985), is not (yet) possible.

### 3.3.4 Storage

Under simulated transport conditions as well as during the actual releases, only about 50 % of all *E. lopezi* adults survived 4 days at 6 °C in the release vials, but all survivors showed normal reproduction (HERREN et al. 1987). If larger storage units were used, *E. lopezi* in a dark refrigerator at 10 °C survived 6.2 days (maximum life span 114 days,  $N = 410$ ). In an illuminated refrigerator at 16 °C, i.e. just above the lower thermal threshold (LEMA and HERREN 1982), survival averaged 4.4 days without food (maximum life span 9 days,  $N = 760$ ) and 5.4 days with sugar (max. 48 days,  $N = 1450$ ), as compared to 1.3 days (max. 3 days,  $N = 592$ ) without and 4.3 days (max. 17 days,  $N = 311$ ) with sugar at 28 °C.

Under less crowded conditions, at 28 °C, the mean survival was better (table 3). Survival curves were highly skewed with very few adults surviving sometimes very long periods and standard deviations increasing with the mean. Male and female survivals, with one exception, were identical. When fed sugar, females survived on the average 1 day longer than males. Sugar increased longevity as compared to no food or when hosts were offered. When only CM were offered, replacing the plant support daily and keeping CM from wandering around seemed to increase wasp survival. With other foods offered, this influence waned.

Almost all females contained ripe eggs in their ovarioles. Upon emergence, 11.6 eggs ( $\pm 0.9$  S.E.,  $N = 27$ ) were counted. But even after being fed only sugar for 25–40 days, females still had 6.2 eggs ( $\pm 3.8$  S.E.,  $N = 14$ ) and resorbed only few eggs. Protein rich food, like wheat, did not improve egg production. Almost all females were able to oviposit within the first 30 min after being taken from the storage containers.

Though the physiological implications from these preliminary experiments are not yet completely clear, the practical conclusions are fairly simple: *E. lopezi* cannot be stored in reasonable numbers beyond a few days, though cold treatment considerably prolongs the life span of a few individuals. Wasps which survive storage and transport are capable of ovipositing immediately. Since sugar grains are impractical for transport, paper strips with sugar water or honey provide still the best solution.



Table 3. Mean and maximum longevity of *Epidinocarsis lopezi* adults in days ( $\pm$  standard error), provided with different food sources at 28 °C

Food		Longevity		S.E.	Number
		mean	maximum		
Nil		2.02	7	0.05	600
Foods only	Honeydew	4.82	13	0.15	460
	Wheat	5.10	22	0.17	440
	Honey	6.33	17	0.16	600
	Sugar water	9.57	44 <sup>1</sup>	0.36	340
	Sugar grains	12.54	52 <sup>2</sup>	0.35	700
CM only	Old stems <sup>3</sup>	2.94	9	0.11	300
	New stems <sup>4</sup>	5.22	14	0.18	300
CM plus	Honeydew <sup>3</sup>	4.71	12	0.23	160
	Honeydew <sup>4</sup>	5.03	14	0.19	300
	Wheat <sup>4</sup>	5.63	15	0.20	400
	Honey <sup>3</sup>	6.69	23	0.25	380
	Honey <sup>4</sup>	6.52	15	0.24	300
	Sugar grains <sup>3</sup>	5.13	16	0.23	200

<sup>1</sup> Maximum for male only 24 days. – <sup>2</sup> Maximum for male only 41 days. – <sup>3</sup> Cassava stems not replaced, CM therefore often wandering around. – <sup>4</sup> Cassava stems replaced daily, CM therefore calmly fixed.

### 3.3.5 Releases

The following number of living wasps were released (table 4). By the time of release outside Nigeria, mortalities were sometimes as low as 25 %, averaged 40–50 %, and went as high as 75 %, with exceptional 90 % in remote areas after 4–6 days of transport. In most sites, several hundred to several thousand *E. lopezi* adults with sex ratios of around 50 % females were released, the lowest (but still successful) release involving only 60 adults (HERREN et al. 1987).

Table 4. Numbers of live *Epidinocarsis lopezi* released in Africa between 1981 and June 1988, produced in the insectarium at IITA, Ibadan, Nigeria

Year	Countries	No. release sites <sup>1</sup>	No. wasps released	Release method
1981	Nigeria	1	1850	ground
1982	Nigeria, Congo, Zaire	6	7550	ground
1983	Nigeria <sup>2</sup> , Congo, Zaire	6	14140	ground
1984	Nigeria, Guinea-Bissau, Senegal, Gambia, Ghana, Zaire <sup>2</sup> , Togo, Zambia	19	12545	ground
1985	Rwanda, Zambia, Senegal, Guinea-Bissau, Zaire, Malawi, Sierra Leone	15	40220	ground
	Ghana	2	4000	aerial <sup>3</sup>
1986	Gabon, Malawi, Zambia, Gabon <sup>2</sup> , Rwanda	16	29400	ground
	Côte d'Ivoire, Zambia	12	77000	aerial <sup>3</sup>
1987	Côte d'Ivoire, Togo, Sierra Leone, Malawi, Rwanda, Zaire	18	119600	ground
	Zambia	14	91000	aerial <sup>3</sup>
1988	Burundi, Tanzania, Sierra Leone, C.A.R.	6	17700	ground
	C.A.R.	12	78000	aerial <sup>3</sup>

<sup>1</sup> One release site comprises several fields within an area of 8000 km<sup>2</sup>. Releases were sometimes repeated the next year. – <sup>2</sup> Local insectaries were supplied for subsequent release. – <sup>3</sup> In most cases, survival of *E. lopezi* was checked in one vial.

### 3.3.6 What quality requirements?

Anywhere in the long production line from inoculation of the plants in the insectary to the release in the field, insects can be involuntarily selected out for qualities which help them survive in the laboratory but are deleterious in the field. At numerous occasions, populations can shrink to low levels causing bottlenecks in production and unwanted genetic drift (BARTLETT 1985; WAAGE et al. 1985). Which of the many factors involved in producing *E. lopezi* for inoculative release then is important for maintaining the good quality of the stock which is so obvious from the successful release and field monitoring programme?

Rearing *E. lopezi* on its original host insect and on the original host plant under near natural conditions might have been the single most important contribution to the maintenance of its quality. The use of cassava as a host plant in the insectary required development and research which was not readily available in literature, similar to the effort that went into the development of commercial insectaries (SMITH and ARMITAGE 1931; DE BACH and WHITE 1960).

Careful technical manipulations and constant supervision of all insectary activities, with a good planning for a constant supply of healthy host plants and plentiful CM at the right stage were essential. The rather low esteem of insectary work among academics in entomology and other disciplines is the result of a low output of highly focussed scientific papers, a general problem in applied ecology (SLOBODKIN 1988). It overlooks the fact that insectaries provide unanticipated challenges which are often solved through research only at a later stage. With *E. lopezi*, e.g., many of the observed interactions between the wasp and the plant and between adult wasps, especially under crowded conditions, are not yet understood.

Good sized and numerous rearing units provided the necessary flexibility of the operation to avoid bottlenecks in production. Technological improvements and, particularly, hydroponic cultures further helped to assure a sufficient supply of host plants and insect hosts. Simpler and cheaper hydroponic units with a core of rockwool (HAUG et al. 1987), as used in many glasshouses, are now being developed. Hydroponics of some kind also offer the chance to rear plants, pest insects, and their beneficials in the same unit, thereby avoiding transport and manipulations.

Host instar composition, host densities, inoculum size, and the timing of the operations were developed alongside experiments elucidating the biology of *E. lopezi*. These studies eventually involved 15 different factors in a complicated flow diagram from host habitat finding to mating of the offspring (LÖHR et al. 1988) including behavioural parameters which are favoured for quality control (BOLLER and CHAMBERS 1977). They demonstrated why it is so difficult to produce large numbers of *E. lopezi*. We have, however, no proof that studying and taking into account biological parameters helped in the success of the programme.

In the absence of selection programmes (HOY 1985) and genetic engineering, the limits for quality control in the insectary producing parasitoids for inoculative releases remains the original quality of the insects obtained in explorations and after their passage through the quarantine insectary. These activities are often the limiting factors in a biological control programme, needing better funding in order to provide and maintain the necessary numbers and quality of beneficials.

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### Zusammenfassung

#### Qualitätsanforderungen an freizulassende natürliche Feinde zur biologischen Schädlingsbekämpfung: Praktische Erfahrungen aus einem erfolgreichen Programm

Während der letzten sechs Jahre wurde der aus Südamerika stammende Parasitoid, *Epidinocarsis lopezi* (De Santis) (Hym., Encyrtidae) in ungefähr 100 Gebieten Afrikas zur biologischen Bekämpfung von *Phenacoccus manihoti* Mat.-Ferr. (Hom., Pseudococcidae) freigesetzt. Der Parasitoid konnte in allen Gebieten erfolgreich eingebracht werden und trat 1988 in 18 afrikanischen Ländern in einem Gebiet von 1,5 Mio. km<sup>2</sup> auf. Es konnte nachgewiesen werden, daß er die Schädlingspopulation dauerhaft reduziert.

*E. lopezi* wurde auf seiner ursprünglichen Wirtspflanze, Cassava, und seinem ursprünglichen Wirt, *P. manihoti*, gezüchtet. Die Zuchtmethoden für die Pflanzen, *P. manihoti* und *E. lopezi* wurden im Rahmen der laufenden Parasitoidenproduktion entwickelt. Eine Dauerzucht ohne Engpässe konnte bei der Verwendung von mehr als 50 Käfigen mit getopften Pflanzen sichergestellt werden und ebenso bei der Verwendung von großen, mechanisierten Käfigen mit Hydrokultur, in denen Cassava, die Wirtsinsekten und die Parasitoide gleichzeitig gezüchtet wurden. Die Wahl des Zeitpunktes zum Freisetzen der Parasitoide und die Menge der freigelassenen Insekten konnte durch grundlegende biologische und ökologische Studien verbessert werden. Die Bevorzugung eines bestimmten Wirtsstadiums, das Fressen der Wirtstiere und andere Nahrungsansprüche, Verstümmelung der Wirtstiere, geringe Reproduktionsrate, Superparasitismus und die Entwicklungsdauer wurden bei der Entwicklung einer effektiven Zuchtmethode, der Lagerung und des Transportes mit berücksichtigt.

Die erfolgreiche Einbringung von *E. lopezi*, seine Verbreitung und Wirksamkeit in Afrika zeigen, daß eine ausreichende Anzahl an Parasitoiden hoher Qualität ausgebracht wurde. Untersuchungen an Insekten und Pflanzen, Entwicklung der Technologie und die sorgfältige Überwachung tragen zu einer guten Qualität bei, welche bei der Einbringung von natürlichen Feinden wichtiger ist, als eine große Anzahl Insekten freizusetzen.

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## Semiochemicals, foraging behaviour and quality of entomophagous insects for biological control<sup>1</sup>

By L. P. J. J. NOLDUS

### Abstract

In this paper the question is addressed whether or not responses to semiochemicals are relevant to the quality of entomophagous insects for inundative biological pest control. Four approaches to answer this question are distinguished: 1. behavioural manipulation with semiochemicals, 2. use of natural intraspecific variation in responses to semiochemicals, 3. artificial selection for intraspecific variation in responses to semiochemicals, and 4. application of simulation models of foraging behaviour. In addition, the possible impact of mass-rearing methods on responses to semiochemicals is discussed. Special attention is paid to *Trichogramma* egg parasitoids: evidence for the use of semiochemicals by *Trichogramma* spp., as well as current mass-rearing and quality control methods are briefly reviewed. From the possible impact of mass rearing on responses to semiochemicals, recommendations for mass rearing, quality control and further research are inferred.

### 1 Quality of entomophagous insects

The selection of a suitable natural enemy and an appropriate rearing method are essential steps in any biological control program. The overall quality of a mass-reared entomophagous insect is determined by the performance in its intended role, i.e. reduction of pest numbers below the economic threshold after release into the field (HUETTEL 1976; BOLLER and CHAMBERS 1977; MOORE et al. 1985; BIGLER 1989). Overall quality encompasses a number of traits related to the species to be controlled, the crop, the climate, and the release strategy to be applied. Many authors have listed desirable traits of natural enemies (e.g., MESSENGER et al. 1976; BOLLER and CHAMBERS 1977; ROSEN 1985; VAN LENTEREN 1986; MINKENBERG and VAN LENTEREN 1986; PAK 1988). For inundative and seasonal inoculative biological control these include: adaptability to climatic extremes and various habitats, searching efficiency, host specificity, host discrimination, host utilization (ability to kill a host and/or use it for reproduction), reproductive capacity, and lack of negative side effects. Each of these traits might conceivably be affected by mass-rearing procedures, including storage and other aspects of production, which may subsequently lead to altered performance in the field.

Among the traits relevant to quality may be the response of a natural enemy to semiochemicals originating from host habitat (crop) or host. In this paper I examine to what extent responses to semiochemicals are relevant to quality of entomophagous insects for biological control, and how responses to such substances can be influenced by rearing methods. Examples will mainly be drawn from the literature on parasitic wasps, in particular the genus *Trichogramma*. However, the general considerations apply equally well to predatory insects. Therefore, the words parasitoid and host can also be read as predator and prey, respectively, unless explicit reference is made to a guild of parasitoids. The term natural enemy refers to entomophagous arthropods throughout this paper.

<sup>1</sup> Paper presented at the Symposium on "Quality control in mass-reared arthropods" of the IOBC global working group, held June 26-30, 1988 in Vancouver, Canada.

## 2 Semiochemicals in the foraging behaviour of parasitoids

### 2.1 Terminology and scope

The term semiochemicals refers to the chemicals involved in chemical communication between animals (LAW and REGNIER 1971). Pertinent to the present discussion are pheromones (involved in intraspecific interactions), kairomones (involved in interspecific interactions and evoking a response favourable to the receiver) and synomones (interspecific, but with response favourable to both emitter and receiver) (NORDLUND and LEWIS 1976; NORDLUND 1981). For a recent discussion of terminology of these information-conveying chemicals, see DICKE and SABELIS (1988).

That semiochemicals play a role in various phases of the foraging behaviour of entomophagous arthropods is a well-documented phenomenon. There is no need to summarize the large amount of evidence published on this topic, as several comprehensive recent reviews are available (VINSON 1984a, b, 1985, 1986; LEWIS and NORDLUND 1985; KAINOH 1987). Here, we are concerned with those responses to semiochemicals that contribute to the quality of a natural enemy as a biological control agent. The next question is whether or not these responses are affected by mass-rearing procedures.

This review is restricted to stimuli mediating foraging for hosts or prey. Sex pheromones have been identified for parasitoids (ELLER et al. 1984; MOHAMED and COPPEL 1987) but will not further be discussed in this paper.

### 2.2 Semiochemicals for *Trichogramma* spp.

#### 2.2.1 *Trichogramma* spp. as biological control agents

Egg parasitoids of the genus *Trichogramma* are the most widely used entomophagous insects in the biological control of insect pests worldwide (KING et al. 1985). *Trichogramma* spp. are presently reared for commercial purposes in at least 20 different countries, with the Soviet Union and China leading in acreages of application (COULSON et al. 1982; RIDGWAY and MORRISON 1985; GUSEV and LEBEDEV 1988). Control is mostly attempted through inundative releases, against at least 28 different herbivorous pest species on some 20 different crops (HASSAN 1988; VOEGELÉ et al. 1988). Considerable variability in efficacy of mass releases exists, however, and may be due to the quality of the wasps. In most cases, relative inefficiency of mass-reared *Trichogramma* is compensated for by increasing the frequency of releases or the numbers of released wasps. However, such compensatory measures do not solve the underlying problem, and therefore a critical discussion of mass rearing and quality control in *Trichogramma* seems appropriate.

#### 2.2.2 Foraging behaviour of *Trichogramma*

For foraging *Trichogramma* spp., three levels of resource distribution are relevant: the host habitat, host aggregations within the habitat, and individual hosts (i.e. host eggs). Due to the minute size of adult *Trichogramma* wasps (0.5–1 mm) and their inability to fly upwind in any but the slowest winds, movement between potential host habitats and localization of host aggregations may be passive rather than active in most cases (KELLER and LEWIS 1985; KELLER et al. 1985). Their small size also hampers direct observation of foraging behaviour in the field, and therefore most of the evidence reviewed below originates from laboratory experiments. So far, plant odours, host sex pheromones, host scales, egg odours, and contact chemicals associated with host eggs, have been identified or suggested as semiochemicals for *Trichogramma* spp. These groups of cues will briefly be reviewed below.

## 2.2.3 Plant odours

Several authors have documented differential rates of parasitism of one host species by *Trichogramma* on different plant species. For example, RABB and BRADLEY (1968) compared parasitism of *Manduca sexta* eggs by *T. minutum* on three solanaceous plants and found parasitism on tomato and jimsonweed but not on tobacco. A similar difference between parasitization rates by *Trichogramma* spp. on tomato and tobacco has been observed by MARTIN et al. (1981) for *H. virescens*. BAR et al. (1979) found parasitism of *Heliothis armigera* by *T. semifumatum* on tomato, but not on cotton. In a study of *Choristoneura fumiferana* on balsam fir, higher percentages of egg parasitism by *T. minutum* were observed with increasing proportions of non-budworm-host trees in a stand (KEMP and SIMMONS 1978). Effects of polycultures vs. monocultures on parasitism by *Trichogramma* spp. appear ambiguous: ALTIERI et al. (1981) reported higher rates of parasitism in soybean/corn intercropping plots vs. soybean monocultures, and NORDLUND et al. (1984) found that interplanting corn with tomatoes and beans increased parasitism over corn grown in monoculture. However, ANDOW and RISCH (1987) observed higher parasitism rates in corn monocultures vs. polycultures of corn/bean/squash and corn/clover.

Some of these authors suggested that olfactory cues are used in host-habitat selection by *Trichogramma*. However, this can not be concluded from such indirect evidence, because the behavioural mechanism is unknown and many other factors may have caused the observed differences. For example, structures on the leaf surface may trap *Trichogramma* (RABB and BRADLEY 1968) or reduce walking speed and thus lead to lower searching success (TREACY et al. 1985, 1986; KELLER 1987).

There is also direct evidence on the role of plant odours in *Trichogramma* foraging (table 1). ALTIERI et al. (1981, 1982) were able to increase rates of parasitism by *Trichogramma* spp. by spraying plants with an extract of *Amaranthus* sp. The mechanism causing enhanced parasitism after the addition of such plant chemicals was not examined. NORDLUND et al. (1985a, b, 1987) showed that the higher rates of parasitism found in tomato (NORDLUND et al. 1984) might be explained by tomato volatiles: in the laboratory, parasitism rates of *H. zea* eggs by *T. pretiosum* could be increased by application of tomato extract and parasitoids showed a positive response to tomato odour in a Y-tube olfactometer. Recently, KAISER (1988) documented arrestment of *T. maidis* by the odour of corn leaves in a four-armed airflow olfactometer. An opposite effect of plant odours has also been recorded. BAR et al. (1979) found that *T. semifumatum* was repelled by the odour of

Table 1. Evidence for plant odours that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Source	Response	Reference
<i>T. semifumatum</i>	cotton	repellence in olfactometer	BAR et al. (1979)
<i>Trichogramma</i> sp.	<i>Amaranthus</i> sp.	increased parasitism	ALTIERI et al. (1981)
<i>T. pretiosum</i>	corn		
<i>T. pretiosum</i>	<i>Amaranthus</i> sp.	increased parasitism	ALTIERI et al. (1982)
<i>T. pretiosum</i>	tomato	preference in Y-tube olfactometer <sup>1</sup> , increased parasitism	NORDLUND et al. (1985a, 1985b)
<i>T. sp. p. buesi</i>	cotton	repellence in T-tube olfactometer <sup>1</sup>	CABELLO and VARGAS (1985)
<i>T. maidis</i>	corn	preference in 4-arm olfactometer <sup>1</sup>	KAISER (1988)

<sup>1</sup> Tested against clean air



cotton in an olfactometer. Repellency by cotton odour has also been recorded for *T. sp. p. buesi* (CABELLO and VARGAS 1985).

One could envisage parasitoids being repelled by the odour of toxic plants, and thus being protected from oviposition in unsuitable host insects. Such odours would then serve as kairomones for the parasitoids. However, I have not found any evidence for this.

#### 2.2.4 Host sex pheromones

Various pieces of direct and indirect evidence indicate that within a habitat *Trichogramma* spp. are able to distinguish between host-infested and uninfested areas. In this phase, foraging behaviour seems to be mediated by odours originating from adult female host insects (table 2). The first observation of this phenomenon was made by LEWIS et al. (1982), who established increased rates of parasitism by *Trichogramma* spp. in cotton plots treated with a synthetic sex pheromone blend of *Heliothis zea*. In an airflow olfactometer *T. pretiosum* responded to the odour released by calling virgin moths (NOLDUS 1988). The same has been found for the noctuid *Mamestra brassicae* and *T. evanescens* (NOLDUS and VAN LENTEREN 1985a). Wind tunnel experiments showed that both *Trichogramma* spp. are not attracted to the pheromone source; the odour suppresses phototactic upward flight and leads to arrestment of the parasitoid (NOLDUS et al. 1988a, b). A recent study by KAISER (1988) indicates that *T. maidis* is arrested by the sex pheromone of its host *Ostrinia nubilalis*. For *Pieris brassicae*, a host of *T. evanescens*, no female sex pheromone is known, but virgin female butterflies release volatiles that attract wasps in an olfactometer, while mated butterflies do not elicit a response in the parasitoids (NOLDUS and VAN LENTEREN 1985a).

Other authors used a "diffusion olfactometer" to study olfactory orientation of *Trichogramma* and recorded responses to adult host odours (FERREIRA et al. 1979; BOURARACH and HAWLITZKY 1984; ZAKI 1985). These results remain inconclusive, however, because large groups of insects were tested simultaneously, behavioural observations were not made, and only small proportions of wasps "responded".

Table 2. Evidence for adult host odours that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Source	Response	Reference
<i>T. pretiosum</i>	<i>Heliothis zea</i>	host sex pheromone gland, synthetic sex attractant	increased parasitism, preference in 4-arm olfactometer <sup>1</sup> , arrestment, suppressed flight	LEWIS et al. (1982), NOLDUS (1988), NOLDUS et al. (1988a)
<i>T. evanescens</i>	<i>Mamestra brassicae</i>	host sex pheromone gland	preference in 4-arm olfactometer <sup>1</sup> , arrestment, suppressed flight	NOLDUS and VAN LENTEREN (1985a), NOLDUS et al. (1988a, b)
<i>T. cordubensis</i>	<i>Heliothis armigera</i> , <i>Earias insulana</i>	adult female moth	preference in T-tube olfactometer	CABELLO and VARGAS (1985)
<i>T. sp. p. buesi</i>	<i>Ephestia kuehniella</i>	adult female moth	preference in T-tube olfactometer	CABELLO and VARGAS (1985)

<sup>1</sup> Tested against clean air.

## 2.2.5 Host scales

The response to contact kairomones present in scales originating from the body of adult host insects is a well-documented aspect of *Trichogramma* foraging behaviour. LAING (1937) first demonstrated that contact of *T. evanescens* with "traces" of *S. cerealella* or *M. brassicae* led to increased residence times and higher host-finding rates in contaminated areas. Decades later, LEWIS et al. (1971) found that traces left by ovipositing *H. zea* or *Plodia interpunctella* moths increased parasitism by *T. evanescens*. Body scales of the moths contained the kairomone (LEWIS et al. 1972), and a hexane extract of these scales sprayed over plants could increase rates of parasitism by *T. evanescens* and *T. achaeae* in laboratory, greenhouse and field (LEWIS et al. 1972, 1975a). Inhibition of flight and klinokinesis are the major components leading to intensified searching in contaminated areas and enhanced host finding (BEEVERS et al. 1981; MORRISON and LEWIS 1981; GARDNER and VAN LENTEREN 1986). The response of *Trichogramma* to host scales appears to be a general phenomenon, as it has been described for several *Trichogramma*-host combinations (table 3).

Chemical analysis of *H. zea* scale extracts yielded tricosane as the most active component for *T. evanescens* (JONES et al. 1973). GUELDNER et al. (1984) identified a number of organic acids in the scales of *H. zea* which were attributed a minor kairomonal role for *T. pretiosum*. Recently, SHU and JONES (1988) isolated three dimethylnonatriacontanes from the hexane extract of scales of *O. nubilalis*. The relative contribution of each of these components to the kairomonal effect of the scales for *T. nubilale* remains to be elucidated.

Table 3. Evidence for host scales that contain semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Response	Reference
<i>T. evanescens</i>	<i>Sitotroga cerealella</i> , <i>Mamestra brassicae</i>	arrestment	LAING (1937)
<i>T. evanescens</i>	<i>Heliothis zea</i> , <i>Plodia</i> <i>interpunctella</i>	increased parasitism	LEWIS et al. (1971, 1972), JONES et al. (1973)
<i>T. achaeae</i>	<i>Heliothis zea</i>	increased parasitism	LEWIS et al. (1975a)
<i>T. pretiosum</i>	<i>Heliothis zea</i>	increased parasitism	LEWIS et al. (1975b) GUELDNER et al. (1984)
<i>T. evanescens</i> <sup>1</sup>	<i>Mamestra brassicae</i>	arrestment	SMITS (1982), NOLDUS and VAN LENTEREN (1985b)
<i>T. evanescens</i> <sup>1</sup>	<i>Pieris</i> <i>brassicae</i>	arrestment	NOLDUS and VAN LENTEREN (1985b), GARDNER and VAN LENTEREN (1986)
<i>T. evanescens</i> <sup>1</sup>	<i>Pieris rapae</i>	arrestment	NOLDUS and VAN LENTEREN (1985b)
<i>T. exiguum</i> , <i>T. maltbyi</i> , <i>T. minutum</i> , <i>T. sp. nr. pretiosum</i>	<i>Heliothis zea</i> , <i>Manduca sexta</i> , <i>Ostrinia nubilalis</i>	arrestment	THOMSON and STINNER (1989)
<i>T. minutum</i>	<i>Choristoneura</i> <i>fumiferana</i>	arrestment	ZABORSKI et al. (1987)
<i>T. nubilale</i>	<i>Ostrinia nubilalis</i>	arrestment	SHU and JONES (1988)

<sup>1</sup> Presently named *T. maidis* [strain no. 11 in PAK and VAN HEININGEN (1985)].

#### 2.2.6 Host egg odours

No conclusive evidence exists about the possible involvement of odours originating from eggs in host finding by *Trichogramma* spp. LAING (1937) mentioned that *T. evanescens* did not react to the presence of eggs of *Sitotroga cerealella* or *Mamestra brassicae* from a distance of less than 5 mm if those eggs were invisible for the parasitoid. FERREIRA et al. (1979) reported attraction of eight *Trichogramma* spp. to the eggs of *Ephesia kuehniella* in a diffusion olfactometer. However, due to the lack of air flow, their method does not allow conclusions with regard to orientation behaviour. The same applies to the results of FERREIRA-ANUNCIADA and PINTUREAU (1981) and BOURARACH and HAWLITZKY (1984).

#### 2.2.7 Contact chemicals associated with host eggs

Upon contact with a potential host, *Trichogramma* wasps engage in a specific examination behaviour, i.e. an evaluation of various host characteristics (host recognition), after which the object is accepted (host acceptance) or rejected for oviposition. Besides physical properties, such as perceived host size (SALT 1935; SCHMIDT and SMITH 1985; PAK and DE JONG 1987), semiochemicals play a role in host recognition (table 4). The secretion from the accessory gland of *H. zea*, present as a coating on the egg, contains a kairomone that mediates host recognition by *T. pretiosum* (NORDLUND et al. 1987). A similar effect was obtained with an egg wash of *Pieris brassicae*, inducing host acceptance in *T. maidis* (PAK and DE JONG 1987). Applied on a leaf surface, this material led to intensified search behaviour and arrestment of *T. evanescens* (= *T. maidis*) (NOLDUS and VAN LENTEREN 1985b). In some cases, the chemicals present on the host egg elicit rejection rather than acceptance, as shown for *T. buesi* when offered eggs of *P. brassicae* (PAK and DE JONG 1987). The results of TAYLOR (1969) for *T. semifumatum* and *Estigmene acrea* point at a similar effect. In these cases the term synomone is more appropriate than kairomone (PAK and DE JONG 1987). Host-recognition mediators are probably proteinaceous and of very low volatility (STRAND and VINSON 1983; NORDLUND et al. 1987; VINSON et al. 1988a). *T. evanescens* did not respond to an egg wash of *P. brassicae* in an olfactometer (NOLDUS and VAN LENTEREN 1983).

During host examination *Trichogramma* wasps also discriminate between parasitized and unparasitized hosts. This ability was first demonstrated by SALT (1934), who later proved that external as well as internal chemical markers mediate host discrimination (SALT 1937). Host discrimination is now considered to be a general phenomenon among parasitic insects (VAN LENTEREN 1981). Like in most parasitoids, host discrimination appears to function only intraspecifically in *Trichogramma* (ABLES et al. 1981). It aids *Trichogramma* in the avoidance of superparasitism, although superparasitism can be an adaptive strategy under certain circumstances (VAN DIJKEN and WAAGE 1987). The external marking pheromones employed by *Trichogramma* spp. appear to be of low, though distinct volatility (SALT 1937). To date, no information exists on their chemical nature.

### 3 Semiochemicals and quality of entomophagous insects for biological control

#### 3.1 Introduction

The ability of an insect to respond to a certain semiochemical has presumably evolved under and is maintained by natural selection. Does this mean that this ability is relevant for the animal's quality in the context of biological control? Present-day agro-ecosystems are often very different from the natural context in which these responses evolved, especially with regard to seasonal inoculative or inundative biological control. Therefore, many

Table 4. Evidence for contact chemicals associated with host eggs that function as semiochemicals for *Trichogramma* spp.

Parasitoid	Host	Source	Active component(s)	Response	Reference
<i>T. evanescens</i> <sup>1</sup>	<i>Pieris brassicae</i>	host accessory gland	not known	host recognition, arrestment	NOLDUS and VAN LENTEREN (1985b), PAK and DE JONG (1987)
<i>T. buesi</i>	<i>Pieris brassicae</i>	host accessory gland	not known	host rejection	PAK and DE JONG (1987)
<i>T. buesi</i> , <i>T. maidis</i> <sup>2</sup>	<i>Mamestra brassicae</i>	host accessory gland	not known	host acceptance	PAK and DE JONG (1987) <sup>3</sup>
<i>T. pretiosum</i>	<i>Heliothis zea</i>	conspecific female wasp	not known	host discrimination	ABLES et al. (1981)
<i>T. dendrolimi</i>	not relevant	—	leucine, phenylalanine, isoleucine, histidine	oviposition into artificial medium	WU and QIN (1982)
<i>T. pretiosum</i> , <i>T. minutum</i>	<i>Heliothis virescens</i>	egg contents	KCl, MgSO <sub>4</sub>	oviposition into artificial medium	NETTLES et al. (1982)

<sup>1</sup> Presently named *T. maidis* [strain no. 11 in PAK and VAN HEININGEN (1985)]. — <sup>2</sup> Previously referred to as *T. evanescens* (SMITS [1982], NOLDUS and VAN LENTEREN [1985a, b], GARDNER and VAN LENTEREN [1986]). — <sup>3</sup> Indirect evidence.

natural responses may be superfluous, and others that are uncommon in natural populations may be advantageous. I distinguish four approaches to investigate whether responses to semiochemicals are relevant for quality, and if so, which responses are relevant. These are: 1. manipulation of behaviour with semiochemicals, 2. use of natural intraspecific variation in responses to semiochemicals, 3. artificial selection for intraspecific variation in responses to semiochemicals, and 4. application of simulation models of foraging behaviour, with varying responses to semiochemicals. Each approach has its own merits and limitations, and they should be regarded as complementary rather than as alternatives.

### 3.2 Manipulation of behaviour with semiochemicals

Behavioural manipulation has been suggested by several authors as a means to improve the quality of inundatively released natural enemies (e.g., VINSON 1977; HASKELL et al. 1981; GREANY et al. 1984; WALL 1984; LEWIS and NORDLUND 1985; COPPEL 1986; POWELL 1986; VAN LENTEREN 1987), but has rarely reached beyond the realms of speculation. Modification of behaviour can be brought about prior to or after release in the field. If manipulation leads to a change in performance then responses to semiochemicals may be relevant to quality. However, such evidence only proves that the insects can use the chemical cues, and that they did use them in a particular experimental setting with a resulting increase in foraging success. It does not provide direct proof that insects will also use these cues in a setting where the cues are not artificially added to the environment, and that responses to such cues can be a limiting factor for searching efficiency. A recent study on the winter moth and its tachinid fly parasitoid *Cyzenis albicans* showed that attack rates on apple trees by feral flies could be increased by an application of oak foliage extract

(ROLAND et al. 1988). This corroborated the results of wind tunnel experiments, which had indicated the presence of an attractive odour in oak leaves.

Most experimental work on the possibilities of behavioural manipulation of parasitoids outside the laboratory has been done with *Trichogramma* spp. by W. J. LEWIS and co-operators in Tifton (Georgia, USA). They have been able to show that chemical stimuli which evoke behavioural responses in the laboratory can be employed to increase rates of parasitism by *Trichogramma* spp. in greenhouse and field settings. Confinement of released parasitoids to the target area, i.e. prevention of unwanted dispersal, has been the key phrase throughout these series of experiments (LEWIS and NORDLUND 1985; LEWIS et al. 1985).

As mentioned above, plant extracts can be used to increase parasitism (ALTIERI et al. 1981, 1982), although the mechanism is not clear. Results on employment of kairomones show a more direct link between behaviour in the laboratory and field performance. Initially, a "blanket treatment" of moth scale extract resulted in increased egg parasitism and seemed a very promising employment strategy for enhancing *Trichogramma*'s field performance (LEWIS et al. 1972, 1975a, b). However, with increasing plot size, this turned out to be true only at high host densities; at low or medium densities a homogeneous treatment led to arrestment of wasps in host-free areas and a resulting decrease of parasitization efficiency. The application of diatomaceous earth particles impregnated with kairomone extract around host oviposition sites gave the desired effect of enhanced local search without reduced movement at larger scale (LEWIS et al. 1979). This, however, was a very unpractical and labour-intensive method, and certainly not feasible for large-scale application. By augmenting the host density with sterilized moth eggs at the start of the season, the problem of low density could be circumvented and an easier kairomone application pattern could be employed (NORDLUND et al. 1981; GROSS et al. 1981a, 1984). However, this still did not yield a commercially feasible situation. From the correlation between high activity of adult moths and *Trichogramma* performance (LEWIS et al. 1979), LEWIS et al. (1985) inferred that volatile cues might be critical for consistent benefit of released wasps independent of host density. This was supported by the finding that a synthetic sex pheromone blend of *H. zea* increased rates of parasitism by *Trichogramma* spp. in cotton plots (LEWIS et al. 1982). The behavioural mechanism underlying this effect is much like the response to contact kairomones: *Trichogramma* is not attracted by host sex pheromone but the odour arrests the parasitoids and suppresses upward (phototactic) flight (NOLDUS 1988; NOLDUS et al. 1988a).

Besides treating parasitoids behaviour in situ, a pre-release experience of parasitoids with contact kairomones from host scales can suppress the tendency of naive wasps to disperse, and lead to increased confinement to the target area and higher parasitism rates (GROSS et al. 1975).

### 3.3 Natural intraspecific variation

Variation between populations, biotypes or strains of a species is a well-established phenomenon (MACKAUER 1976). Races or strains of a species may differ greatly with respect to any of their quality components and related behavioural traits. The response to certain semiochemicals can be among those properties, but field testing of strains differing in that respect is necessary to determine the relevance for quality. Obviously, this applies to all potential quality traits. But it is also virtually impossible to find strains that differ in such a small number of traits that variation in performance can reliably be attributed to one of those traits. The possibility of covariance between the trait under study and another trait, that has not been measured, but which is actually responsible for an observed variation in performance, can hardly ever be ruled out. So a correlation between variation in responses to semiochemicals in the lab and performance in the field does not prove that this particular response is a quality trait. This also applies to variation obtained through

selective breeding and certainly for the study of interspecific variation. Obviously, with each extra source of variation, the number of field releases necessary before a conclusive judgement can be made increases exponentially.

As far as *Trichogramma* is concerned, intraspecific variation was first described more than fifty years ago. However, much of the early work is hard to interpret due to the dubious taxonomic status of many of the races, ecotypes and strains mentioned in papers (QUEDNAU 1960; NAGARKATTI and NAGARAJA 1977). A good example are reports on differences in fecundity, temperature sensitivity and host preference between a "yellow race" and a "grey race" of *T. minutum* (FLANDERS 1930b, c; HARLAND and ATTECK 1933; LUND 1934). Later these races received the status of separate species. During the last decades the taxonomy of the genus *Trichogramma* has made progress (VOEGELÉ 1988), but still many problems exist. Also, various studies on variation between strains of *Trichogramma* spp. have recently been published (SMITH and HUBBES 1986; HUO et al. 1988; OUYANG et al. 1988; PAK 1988), including a comparative study between laboratory behaviour and field performance (PAK and VAN HEININGEN 1985). To date, no evidence on intraspecific variation in responses to semiochemicals has been published.

### 3.4 Artificial selection on intraspecific variation

Selective breeding of natural enemies has been used mostly for genetic improvement, e.g. the enhancement of temperature tolerance, pesticide tolerance, etc. (MESSENGER et al. 1976; HOY 1985; VINSON 1986). However, it can also be applied for the evaluation of potential quality traits. If a genetic basis for the variability in responses to certain semiochemicals within a population is proven, lines with diverging responses to certain cues can be selected. Next, results of tests of such lines in the field should indicate whether such a selection should be incorporated into mass-rearing procedures, and whether the particular response should be monitored during quality control. To date, examples of such an approach are very rare. A recent study by PRÉVOST and LEWIS (1988) indicates a genetic basis for responses to host odours in the larval parasitoid *Microplitis croceipes*, but selection has not yet been attempted.

Selective breeding in *Trichogramma* spp. has been the subject of several studies. URQUIJO (1946, 1950) reported improved fecundity and parasitization activity of *T. minutum* after selection during many generations. However, as far as the second trait is concerned, due to the lack of direct observations it is not clear what was exactly selected for. The absence of a control (non-selected line) further decreases the value of these accounts. BRENIÈRE (1965) obtained positive results with selection for fecundity in *T. australicum*, and RAM and SHARMA (1977) also succeeded to increase fecundity, but not sex ratio, in *T. fasciatum*. Fecundity, longevity and sex ratio are not affected by the age of the mother wasps at oviposition (DAVIS and BURBUTIS 1974). ASHLEY et al. (1974) selected *T. pretiosum* for improved heat tolerance and locomotory activity. Further reports on selective breeding involve flight propensity (STEEL 1981), heat tolerance (LOPEZ and MORRISON 1980) and insecticide resistance (HSIU et al. 1988). Recently, CHASSAIN and BOULÉTREAU (1987) showed that variation in the manner in which *T. maidis* distributes its eggs among host eggs within a cluster is genetically based. The relationship between this trait and quality was not examined.

These accounts show that variation in components of foraging behaviour of *Trichogramma* spp. has a genetic basis. The basis for behavioural variation in responses to semiochemicals has yet to be explored.

### 3.5 Application of simulation models

Simulation models of natural enemy foraging behaviour and population dynamics may aid in identifying the importance of responses to semiochemicals. An example is a study of the predatory mite *Phytoseiulus persimilis* and the two-spotted spider mite *Tetranychus urticae*, where a model for local predator-prey dynamics could only be validated after incorporation of arrestment of *P. persimilis* by kairomones left by *T. urticae* (SABELIS and VAN DER MEER 1986). System analysis can provide an estimate of the relevance of various responses to the animal's performance as a biological control agent.

For *Trichogramma* spp., simulation models have been used to investigate various aspects related to biological control. Models have been developed for the maximization of the output of fit mated females in a mass rearing, with special reference to aspects as development rate, sex ratio and clutch sizes (GOODENOUGH et al. 1983; WAAGE and LANE 1984; WAAGE and NG 1984; TERYTZE and MENTSCHER 1987), and for the efficacy of inundative releases, with emphasis on the role of the total plant leaf surface (KNIPLING and MCGUIRE 1968; NEED and BURBUTIS 1979; KANOUR and BURBUTIS 1984; CHIANG et al. 1986), host spatial distribution (HASSELL 1982), or initial host density (VAN HAMBURG and HASSELL 1984). Although non-random distribution of parasitism in the field after release has been described (ALLEN and GONZALEZ 1974), all these models, in so far as they deal with foraging behaviour, assume that parasitoids search at random, independent of the spatial scale. However, the probability of encounters with host egg clusters is likely to be a function of cluster size (VAN DER SCHAAF et al. 1984). Also in YANO's (1978) specific model for individual searching behaviour of *T. dendrolimi*, only success-motivated area-restricted search after oviposition is included and semiochemicals are not mentioned. This in spite of ample laboratory evidence of responses of *Trichogramma* to host scales and other cues (section 2.2).

According to GOODENOUGH and WITZ (1985), the main reason for the exclusion of small-scale foraging components from models for host-parasitoid dynamics in the field is the fact that *Trichogramma*'s searching behaviour can hardly be studied under field conditions. But one can still simulate different foraging strategies and test model predictions against observed patterns of parasitism in the field. Very recently, a model has been presented for the distribution of parasitism by *Trichogramma* in the field as a function of the distance from the release point (CHERNYSHEV et al. 1988). Input consists of search parameters such as linear speed and turning angle, based on laboratory measurements. The model succeeds in predicting parasitism on egg cards placed in the field, but underestimates the parasitism of naturally laid eggs. Based on this discrepancy the authors suggest that the response to host kairomones must be an important component in *Trichogramma*'s foraging behaviour in the field.

### 3.6 Are responses to semiochemicals relevant for quality of *Trichogramma*?

So far I have made an inventory of all responses to semiochemicals reported for *Trichogramma* spp. and I have reviewed existing evidence on the relevance of such responses for the quality of biological control agents. There is no doubt that *Trichogramma* parasitoids use semiochemical cues in their foraging behaviour in the field. Under certain circumstances it has been possible to enhance the performance of mass-released insects by artificial employment of such semiochemicals. Apparently, searching efficiency at various spatial scales, including retention in target areas, is a key to success and is mediated in part by various chemical cues. This makes adjustment to semiochemicals related to the agroecosystem in which *Trichogramma* has to function an important attribute of effective parasitoids. Wasps should preferably respond to kairomones originating from the pest species to be controlled, and not be repelled by odours from the crop on which it occurs.

However, apart from information derived from manipulative experiments, the contribution to quality of responses to semi-chemicals is still ill-defined. Studies on naturally occurring or artificially selected intraspecific variation, as well as simulation models of *Trichogramma*'s foraging behaviour have hardly addressed responses to semi-chemicals so far.

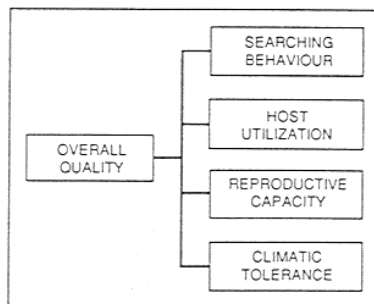


Fig. 1. Diagram of major quality components for *Trichogramma* spp.

With the information presently available we can identify searching behaviour, host utilization, reproductive capacity and climatic tolerance as major quality components for *Trichogramma* wasps (fig. 1). Searching behaviour, where semi-chemicals are involved, has been worked out in more detail in fig. 2. It is divided here into three behavioural traits: host-plant acceptance, host-finding capacity and host handling. Measurable parameters include the response to plant odours, host sex pheromones, host scales, host accessory gland secretions and marking pheromones. The diagram further contains parameters related to responses to the physical environment (plant structure, leaf surface) as well as to the vigour of parasitoids (flight ability, walking speed and turning rate, handling time). Handling time consists of drumming, drilling and oviposition time (PAK et al. 1986). If considerable variation in such responses is observed, they should be screened during the selection of potential control agents (GROSS 1981; PAK 1988), as well as during quality control programs.

Obviously, if manipulative employment of semi-chemicals becomes part of inundative biological control programs, behavioural traits related to semi-chemicals become even more important for quality of *Trichogramma*. However, so far, success has depended on

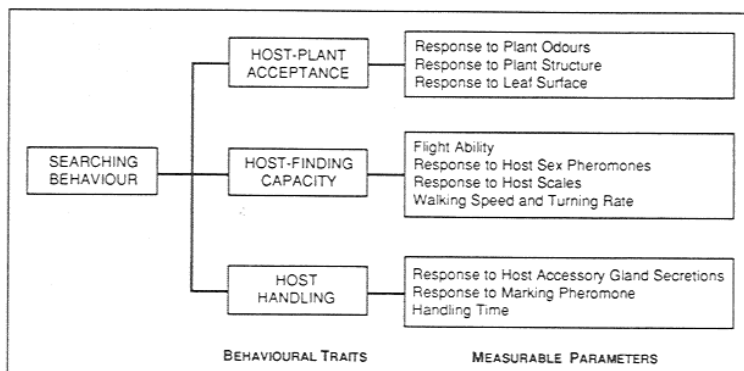


Fig. 2. Diagram of behavioural traits and measurable parameters related to searching behaviour of *Trichogramma* spp.



very elaborate application patterns (GROSS 1981), which are neither practical nor feasible (GARDNER and VAN LENTEREN 1986).

#### 4 Mass rearing of *Trichogramma*

Rearing *Trichogramma* on lepidopteran eggs depends on a continuous, dependable supply of acceptable host eggs. Natural host species can be used for mass rearing, such as *Heliothis* spp. (LEWIS et al. 1976; MORRISON 1985), *Ostrinia nubilalis* (BURBUTIS and GOLDSTEIN 1983) or *Trichoplusia* spp. (MORRISON 1985). However, most commercial mass rearings of *Trichogramma* spp. are based on an easily, cheaply and more dependably reared factitious host. Most commonly used are the Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephesia* (= *Anagasta*) *kuehniella* (FLANDERS 1930a; MORRISON et al. 1976; BIGLER 1986; HASSAN et al. 1984). In Asia, most mass rearings are based on eggs of the oak silkworm, *Antheraea pernyi*, the eri silkworm, *Philosamia cynthia ricini*, or the rice meal moth, *Corcyra cephalonica* (COULSON et al. 1982; LI 1982; CADAPAN 1988).

The desire to increase the efficiency of mass rearing has encouraged research into in vitro rearing of *Trichogramma* spp. (THOMPSON 1986; VINSON et al. 1988b). A suitable artificial medium for continuous rearing should possess a nutritional value allowing immature development of the parasitoids leading to emergence of fit adults, and it should be acceptable for oviposition by adult wasps. These two obstacles have been overcome during the past 15 years by research groups in the United States, China and France (table 5). In China, the method now approaches practical utilization.

Table 5. Published reports on advances of in vitro rearing of *Trichogramma*

Country	References
U.S.A.	RAJENDRAM and HAGEN (1974); HOFFMAN et al. (1975); RAJENDRAM (1978a, b); NETTLES et al. (1982, 1983, 1985); MORRISON et al. (1983); STRAND and VINSON (1985); XIE et al. (1986a, b, 1988); IRIE et al. (1987); VINSON et al. (1988b)
China	GUAN et al. (1978), HUBEI RESEARCH GROUP (1979); LIU et al. (1979); WU et al. (1980, 1982); GAO et al. (1982); LIU and WU (1982); WU and QIN (1982); DAI et al. (1988); LI et al. (1988); QIN and WU (1988)
France	GRENIER and BONNOT (1988)

Low-temperature storage techniques in mass rearing of *Trichogramma* offer ways to gradually build up a stock of host and/or parasitoid material, in order to have a sufficient number of parasitoids available at the start of the release season. Further, year-round rearing implies more efficient use of rearing equipment. Different kinds of low-temperature storage should be distinguished: storage of unparasitized host eggs and long- or short-term storage of parasitized eggs. Low-temperature storage of unparasitized eggs was already mentioned by MOKRZECKI and BRAGINA (1916), but has only recently been investigated in detail. Various storage programs have been proposed, with storage in liquid nitrogen or at temperatures between -10 and +5 °C, for periods up to 2-8 months, and have been described for most of the common factitious hosts, i.e. *E. kuehniella* (VOEGELÉ et al. 1974), *S. cerealella* (GENNADIEV et al. 1985; MORRISON 1988) and silkworms (COULSON et al. 1982; LI 1982; HU and XU 1988; WANG et al. 1988). Low-temperature storage of parasitized host eggs, via induced diapause of *Trichogramma*, was already suggested by SALT (1940) as a means to solve long-distance transportation problems. PETERSON (1931) already mentioned the successful storage of *T. minutum* at 4.4 °C for 6 months. Recently, this technique has received renewed interest, and a storage method for

*T. maidis* has been reported yielding 89% emergence after 9 months storage at 3°C (VOEGELÉ et al. 1986). Short-term storage of parasitized host eggs at a lower temperature reduces the development rate and can be used to program the emergence of adult wasps. STINNER et al. (1974a) were the first to develop such a system for *Trichogramma*. This technique has subsequently been incorporated into schemes for mass rearing and release of *Trichogramma* (MORRISON et al. 1978; BOUSE and MORRISON 1985).

## 5 Impact of mass rearing on responses to semiochemicals

### 5.1 Introduction

Information is accumulating on how different rearing methods can affect responses to semiochemicals. A response is a change in behaviour as the result of a stimulus. A change in response can indicate that: 1. the insect can no longer perceive the stimulus, 2. the response threshold has changed, 3. dose-response relations have changed, 4. the behaviour upon perception changes in a different manner (change of motor pattern), 5. the response relative to another stimulus has changed (change of preference). Unless explicit reference to one of these aspects is made, no distinction between them is made in the following sections. Aspects of mass rearing with a potential impact on quality include the rearing substrate (natural or factitious host, artificial medium), host diet, presence or absence of host kairomones and food for adults, and abiotic conditions such as temperature, humidity and light. In theory, all the quality components can be affected. This includes searching behaviour, where responses to semiochemicals are involved.

Continuous rearing of natural enemies outside the environment in which they have to perform can lead to adverse genotypic and/or phenotypic changes. Components of the insect's quality that have a genetic basis can undergo selection in an unwanted direction, or may change because of lack of directed selection (MACKAUER 1976; BARTLETT 1984, 1985). Further, rearing conditions can exert non-genetic influences, either through the physiology of the developing larvae or by directly affecting the adults. Many behavioural traits depend on or are modified by the experience of the adult insect, which is also common in parasitoids (VET 1983, 1988). Behavioural plasticity (of which learning is but one example) with respect to responses to semiochemicals may be regarded as a quality trait in itself, which may be affected by mass rearing.

### 5.2 Genetic effects

A rearing environment where substantial searching activity of natural enemies is not required for host finding and where semiochemicals originating from the plant-host complex in which they are to be released are absent, may lead to changes or disappearance of responses to such cues. Such changes may not occur immediately; for example, WESELOH (1987) did not find such an effect after rearing *Cotesia melanoscela* in the laboratory for 25 generations.

Genetic changes can be detected by comparison of the quality of the colony in the course of time. Thus, ASHLEY et al. (1973) found a decrease in field performance of a strain of *T. pretiosum* in the course of four years of laboratory rearing. Alternatively, a simultaneous comparison between laboratory-reared and wild insects can be made (NAGARKATTI and NAGARAJA 1978; NAGARKATTI 1979; SOUTHARD et al. 1982; HAWLITZKY and BOULAY 1988). THOMSON and STINNER (1988) found no difference between lab-reared and field-collected *T. exiguum* in response to *H. zea* scales. I have made such a comparison with regard to the olfactory responses of *T. pretiosum* to the sex pheromone of its host *H. zea*. Exposing adult female wasps to a synthetic sex pheromone blend in a wind tunnel leads to prolonged searching times on a platform and a higher incidence of landing vs.

upward phototactic flight (NOLDUS et al. 1988a). Furthermore, the proportion of insects landing on the platform increases with increasing dosage of the olfactory stimulus (NOLDUS 1989, fig. 3). These experiments were carried out with a strain of *T. pretiosum* that had been reared in the laboratory on eggs of *H. zea* for 15 years without infusion of field individuals. These parasitoids had probably not been exposed to host sex pheromone for more than 450 generations, as the eggs were always thoroughly rinsed with sodium hypochlorite before exposure to parasitoids. Moreover, there had certainly been no selection for responses to host-searching cues, as rearing occurred on egg cards in test tubes, and hardly any searching activity of the wasps was required. How would this laboratory strain compare with *T. pretiosum* parasitizing *H. zea* in the field, with respect to the response to host sex pheromone in the wind tunnel? To investigate this, parasitoids were collected from cotton fields near Tifton (Georgia, USA) by applying a *H. zea* egg to ca. 100 plants for 24 hours. After that, eggs were recollected and incubated. The emerging progeny crossed successfully with the laboratory strain of *T. pretiosum*. After laboratory rearing for 10 generations, both strains, the "old" laboratory strain and the "new" strain were tested in the wind tunnel according to the procedure described by NOLDUS et al. (1988a). The old strain showed the same response as in previous experiments, with respect to the intercept as well as the slope of the dose-response curve (fig. 3). The new strain showed an almost identical dose response, although the intercept was higher, indicating an overall higher propensity to land on the platform and a weaker phototactic response. However, the strength of the response to the odour is reflected by the slope, not the intercept of the line. Rearing the new strain in the laboratory for 10 generations may already have been long enough to cause genetic changes, but yet these results indicate that in spite of rearing *T. pretiosum* for a large number of generations in the absence of host sex pheromone, the dose response to this sex pheromone is still similar to the one of a recently initiated lab strain.

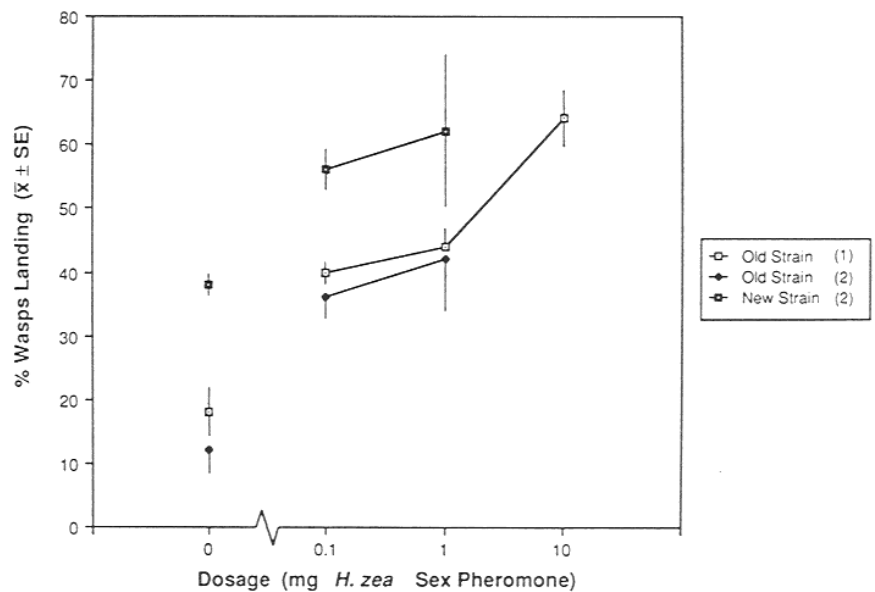


Fig. 3. Response of *Trichogramma pretiosum* females to the sex pheromone of *Heliothis zea*: proportion of wasps landing on a platform in a wind tunnel as a function of pheromone dosage. 1. NOLDUS (1989); 2. Comparison of a 15-year old and a recently initiated laboratory strain. See NOLDUS et al. (1988a) for details on experimental procedures

Detecting a change in quality in a laboratory culture by itself says nothing about what caused the change. Furthermore, a deterioration in field performance does not directly indicate which quality trait has been affected. Explicit cause-effect studies are required to answer those questions. For example, a study by STEIN (1960) suggested that the rearing host may influence the field performance of mass-released *Trichogramma* spp. Later work supported this notion (LEWIS et al. 1976; BIGLER et al. 1982). However, effects of rearing host species have been found for development rate, morphology, size, fecundity, longevity, sex ratio, locomotory capacity, searching capacity and host preference (NOLDUS 1989).

Rearing parasitoids on host species other than the target pest species may influence the host preference of the emerging wasps (KING et al. 1985). As host recognition and acceptance are mediated by a complex of physical as well as chemical factors, changes in host preference may be caused by selection of parasitoids with altered responses to host recognition chemicals. ZABORSKI et al. (1987) found that the rearing host had no influence on the response of *T. minutum* to the scales of *C. fumiferana*. Other effects of the rearing host may involve semiochemicals, such as "searching capacity" as measured (but not clearly defined) by STINNER et al. (1974b) and host preference. Interpreting changes in host preference is complicated by the fact that differences in egg surface chemicals are associated with differences in size and shape, and other physical characteristics. Increased acceptance by *Trichogramma* spp. of a host species after rearing on it have been found by FLANDERS (1935), SALT (1940), TAYLOR and STERN (1971), KAISER et al. (1989) and KAISER and PHAM-DELÈGUE (1988) but none of these authors mentioned a chemical basis. In three species of phytoseiid mites, the rearing prey altered the response to prey odours but the preference was left intact (DICKE 1988b).

In vitro rearing of parasitoids requires chemical stimulants to induce oviposition into the medium by the adult wasps. These have recently been identified for *Trichogramma* spp. (NETTLES et al. 1982; WU and QIN 1982, table 4) as well as for a larval parasitoid (TILDEN and FERKOVICH 1988). Rearing insects on such media may lead to selection for parasitoids which no longer recognize and accept the target host in the field. Rearing predators on a (semi)artificial diet can also have severe effects on their responses to semiochemicals. This phenomenon has been studied in most detail in predatory mites (DICKE 1988a; DICKE et al. 1989).

### 5.3 Non-genetic effects

Preimaginal conditioning is a rare phenomenon in parasitic insects (VET 1988) and has never been proven for *Trichogramma*. However, several accounts on adult learning in relation to responses to semiochemicals exist. In *T. pretiosum* and *T. achaeae* a pre-release exposure to host scales has been shown to suppress dispersal and lead to higher parasitization success (GROSS et al. 1975). Similar effects were found of a pre-release oviposition experience (GROSS et al. 1981b; NORDLUND et al. 1981). In the case of oviposition experience, semiochemicals may be involved in the learning process, but this has never explicitly been investigated. THOMSON and STINNER (1989) found that the response of *T. pretiosum* to host scales decreased after contact with scales but increased after an oviposition experience. This effect was not further tested in relation to parasitization success. They also found that preferences for the scales of a certain host species were not modified by experience with either scales or host eggs. The same has been found for *T. evanescens* and *P. brassicae* (GARDNER and VAN LENTEREN 1986). Effects of experience on responses to semiochemicals by *Trichogramma* spp. cannot be generalized. For example, oviposition by *T. maidis* in an egg of *O. nubilalis* on corn increased the response to odour of corn as well as to odour of corn in combination with eggs and sex pheromone of *O. nubilalis* in an airflow olfactometer (KAISER 1988). In contrast, in experiments with *M. brassicae* and cabbage, an oviposition experience in a complete plant-host complex had a very strong

effect on behaviour of *T. evanescens* in a wind tunnel (increased walking speed, longer search paths and increased retention on a platform) regardless of the odour offered, so that the response to host sex pheromone was no longer expressed (NOLDUS et al. 1988b). An oviposition experience can also cause an immediate increase of host acceptance, as has been shown for *T. maidis* (KAISER et al. 1987, 1989; KAISER and PHAM-DELÈGUE 1988). But again, it is not known to what extent semiochemicals are involved here.

The diet on which the rearing host is fed may have a profound effect on the foraging behaviour of emerging parasitoids. For example, rearing the larval parasitoids *Microplitis croceipes* or *M. demolitor* on *H. zea* larvae fed on an artificial diet rather than on the host plant cowpea, leads to a significant reduction of oriented flights towards *H. zea* larvae on cowpea by the emerged wasps (DROST et al. 1988; HÉRARD et al. 1988b, c).

Chilling parasitized hosts in order to program the emergence of the parasitoids can influence the subsequent responses of these adult wasps to host odours. This effect has recently been shown for the larval parasitoid *M. demolitor* by HÉRARD et al. (1988a), who found that chilling pupae not only negatively affected the reproductive performance of emerging adults, but also rendered most adult females unresponsive to volatile semiochemicals. A similar effect might occur in *Trichogramma* as a result of low-temperature storage of parasitized host eggs. However, this has not yet been investigated.

Finally, low humidity during rearing might lead to irreversible damage to a wasp's receptor system, as has been found in tephritid fruit flies (STÄDLER et al. 1987).

## 6 Quality control of *Trichogramma*

### 6.1 Current quality control methods

Crucial in biological control programs using *Trichogramma* is the selection of a suitable species/strain for release (PAK 1988). Next, an appropriate rearing method has to be chosen. Obviously, arguments at this point stem from economic as well as quality considerations (MARSTON and ERTLE 1973; CHAMBERS 1977). Based on knowledge of the many aspects of mass rearing that can affect the quality of released *Trichogramma* spp., a decision with regard to rearing host (or medium) and other rearing conditions can be made. Several authors have stressed the importance of a broad genetic base of a mass rearing, to be ensured by a large founder population and regular replacement with fresh field material (MACKAUER 1976; MORRISON and KING 1977; KING and MORRISON 1984; VAN LENTEREN 1986). Once a particular rearing method has been selected, the quality of the produced wasps has to be monitored.

In most current mass rearings of *Trichogramma*, main emphasis has been put on production control, rather than on product control (sensu CHAMBERS and ASHLEY 1984), i.e. quality control measures mainly deal with maximizing output of mated female wasps (TERTYZE and MENTSCHER 1987). Often, the only parameters regularly monitored are percentage parasitism, percentage emergence, sex ratio, fecundity and longevity (KING and MORRISON 1984; GENNADIEV 1985; MORRISON 1985). However, these production-oriented parameters may not relate at all to field performance. For example, while fecundity may be important in the context of inoculative control (COULSON et al. 1982), searching capacity rather than fecundity may be a limiting factor for field performance of inundatively released *Trichogramma* spp. in many cases, depending on the distribution of host eggs in the field (HIROSE et al. 1976; VAN DER SCHAAF et al. 1984).

Although quality control for *Trichogramma* is still in its infancy in many respects, progress is being made. The importance of locomotory capacity in *Trichogramma* has been recognized and selection for this trait forms a standard component of several *Trichogramma* mass rearings (MORRISON and KING 1977; BIGLER et al. 1988). In China, mass rearing also includes selection for flight capacity (COULSON et al. 1982), a procedure which

has recently also been adopted in some western systems (BIGLER 1986; BIGLER *et al.* 1987). Some rearing systems include alternation of rearing host (BIGLER 1986; BIGLER *et al.* 1987; HASSAN, pers. comm.), selection for temperature tolerance (HASSAN, pers. comm.), or provision of additional food for adult wasps (COULSON *et al.* 1982).

## 6.2 Recommendations for quality control of *Trichogramma*

Due to the diversity of agro-ecosystems in which *Trichogramma* spp. are used for biological control, generalizations with regard to quality are hard to make. Quality should be viewed in the context of a particular crop-pest system, with factors such as plant architecture, leaf surface characteristics, host-egg distribution, plant- and host-related semiochemicals, taken into consideration.

Quality of *Trichogramma* obviously includes more than fecundity, longevity and sex ratio. A general awareness of the importance of behavioural characteristics for quality is growing, and should now be reflected in the design of mass-rearing methods as well as in the selection of quality control procedures. However, maximization of a quality trait is not synonymous with optimization. For example, *Trichogramma* wasps should have sufficient locomotory and flight capacities, but a too strong innate propensity of flight may lead to rapid dispersal from the target area. The desired level of a particular trait should be verified by means of comparative field experiments to determine the contribution of the trait to overall performance, as has recently been done for locomotory capacity (BIGLER *et al.* 1988).

Behavioural traits that are not under continuous selective pressure in the rearing have to be monitored to assess possible changes in the course of time. This includes especially the various components of host finding and selection under field conditions (NEUFFER 1982; VOEGELÉ *et al.* 1986). However, product control requires a specified quality standard (CHAMBERS and ASHLEY 1984), and what is the standard for *Trichogramma*? The development of theoretical models for rearing as well as the implementation of behavioural traits in quality control is still hampered by our limited knowledge of *Trichogramma*'s foraging behaviour under natural circumstances (KELLER *et al.* 1985) and of the relative importance of genetic vs. learned aspects of its behaviour (WAAGE *et al.* 1985). As far as semiochemicals are concerned, the review presented above shows that a fair amount of information exists on the responses of *Trichogramma* spp. to plant or host cues under experimental conditions, but that these responses have rarely been related to quality. This neglected area certainly deserves closer attention. Simple bio-assays for behavioural studies in the laboratory – which exist for the measurement of host preference (VAN DIJKEN *et al.* 1986; WÄCKERS *et al.* 1987) – should be developed for responses to semiochemicals, e.g. the response to host scales. However, eventually, the step to the field has to be made to assess the relevance of responses to semiochemicals for overall quality of *Trichogramma*. Behavioural manipulation of *Trichogramma* with semiochemicals will have drastic implications for quality control but, for the time being, VINSON's (1986) statement that this is "...an exciting potential rather than a reality" is still valid. Basic research on the role of these cues in the behavioural ecology of *Trichogramma* is still a pressing necessity.

In vitro rearing of *Trichogramma* has not yet developed to such a level that quality control comes in the picture (THOMPSON 1986; QIN and WU 1988). So far, researchers have mainly dealt with morphological aspects of the emerging wasps or whether or not they are fecund (STRAND and VINSON 1985). Future mass-rearing systems based on artificial rearing may involve even less inherent selection for behavioural quality than the present ones. In that case, quality control based on behavioural traits becomes even more important.

In conclusion, to be truly successful with semiochemicals and natural enemies we must 1. know which traits of the natural enemy are desirable, 2. know the mechanism(s) by which semiochemicals influence those traits, 3. have the ability to measure and manipulate

those traits, and 4. have the technology to manipulate those traits prior to and/or after release. Such information and technology may require considerable research investment, but without it we will be operating in the dark with regard to application of semiochemicals as well as to what problems are caused by rearing under unnatural conditions.

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### Zusammenfassung

*Über die Bedeutung von Signalstoffen, Verhaltensweisen bei der Nahrungssuche und Qualität von entomophagen Insekten für die biologische Schädlingsbekämpfung*

Es wird der Frage nachgegangen, ob im Rahmen der biologischen Schädlingsbekämpfung Reaktionen auf Signalstoffe relevant sind für die Qualität von entomophagen Insekten. Es wurden vier Aspekte unterschieden, um diese Frage zu beantworten: 1. Verhaltensmanipulation mittels Signalstoffen, 2. Nutzung der natürlichen intraspezifischen Variation in der Reaktion auf Signalstoffe, 3. künstliche Selektion hinsichtlich intraspezifischer Reaktionen auf Signalstoffe und 4. Anwendung von Simulationsmodellen auf Verhaltensweisen bei der Nahrungssuche. Weiterhin wird der mögliche Einfluß von Massenzuchtmethoden auf die Reaktionen auf Signalstoffe diskutiert. Der Eiparasitoid *Trichogramma* steht im Mittelpunkt dieser Betrachtungen: Es werden Hinweise für die Benutzung von Signalstoffen bei *Trichogramma* spp. sowie Zucht- und Qualitätskontrollmethoden besprochen. Aufgrund des möglichen Einflusses der Massenzucht auf die Reaktionen auf Signalstoffe werden Empfehlungen für die Massenzucht, Qualitätskontrolle und weitere Forschung gegeben.

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## Total quality control in insect mass production for insect pest management<sup>1</sup>

By N. C. LEPPLA and W. R. FISHER

### Abstract

Total quality control in insect mass production involves eight generic elements: Management, Research, Methods Development, Material, Production, Utilization, Personnel and Quality Control. Management determines policy, performs planning and administration and controls program design as it evolves. Research is the source of new technologies and Methods Development creates new operational procedures. Material encompasses purchasing, establishing specifications and standards, assigning responsibility for quality materials and verifying compliance, and storage. The components of production are facilities, equipment, operations, and production control. Utilization is the treatment, handling and distribution of finished insect products. Management delegates responsibility for the selection, training, motivation and health and safety of employees to personnel. Quality control impinges on all program elements with primary emphasis on process and product control. This element provides information management must have to optimize the entire pest management program.

### 1 Introduction

The concept of total quality control has evolved to encompass the entire structure and associated mechanisms for developing and improving product quality and productivity throughout an organization (FEIGENBAUM 1983). It is composed of applications and standards for products and services, associated specifications, production materials and processes, field performance or effectiveness, and product efficacy (cost effectiveness and environmental impact). In terms of insect mass production for insect pest management (IPM), LEPPLA et al. (1977) originally classified these elements under three broad categories: 1. Program Analysis, 2. Production, and 3. Product Utilization. Program analysis consists of a thorough characterization of the target population, precise definition of the biological objectives and requirements, establishment of specifications and standards, and realistic assessment to the overall feasibility of the IPM program. Management also appraises program success and orchestrates improvement or adopts an alternative IPM strategy. Production includes derivation of a suitable founder population, establishment of the production line and monitoring its operational efficiency. Product utilization is the preparation of insects for release, their distribution and monitoring their performance. Although continuously overlooked, this entire system is based on characterization of the parent (target) population as a means of evaluating the consequences of production and utilization.

### 2 Elements of total quality control

Total quality control provides a means of effectively organizing and managing the complex elements inherent to insect mass production and utilization. In this context, paraphrasing

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FEIGENBAUM (1983), control is the delegation of responsibility and authority while retaining the means of assuring satisfactory results. The American National Standards Institute, American Society of Quality Control (ANSI/ASQC) Standard Z-1.15-1979 lists seven generic elements of a total quality system: 1. Policy, planning and administration; 2. Design assurance and design change control; 3. Control of purchased material; 4. Production quality control; 5. User contact and field performance; 6. Corrective action; and 7. Employee selection, training and motivation (ANONYMOUS 1979; BESTERFIELD 1986). These elements have been adapted and expanded into a total quality control system for insect pest management programs based on mass produced insects (fig. 1).

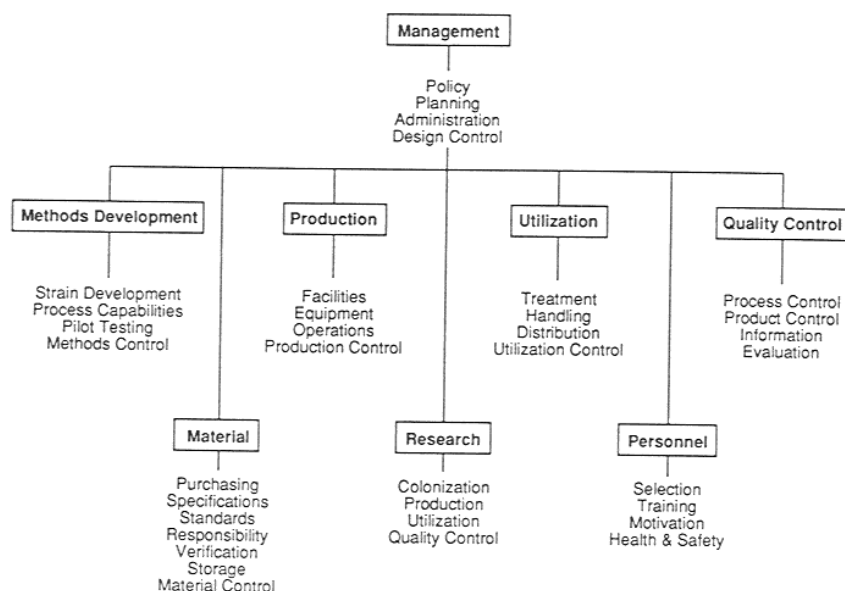


Fig. 1. A total quality control system composed of generic elements for insect mass production in support of insect pest management. The type of insect being reared and required organizational structure determine which elements are emphasized. Interactions among elements are orchestrated by management, although each element has a degree of internal control.

The first element, Management, includes organization and implementation of the entire pest management program, and determination of its applicability. Initially, quality control standards and tests are developed within the system to evaluate process and product function and tolerances. All aspects of the program, including personnel, are reviewed to assure safety and eliminate potential sources of reduced insect quality. Feedback and diagnostic systems are established to improve the insects and, if necessary, correct deficiencies in their performance. If the program remains efficacious, it continues to improve through the incorporation of pertinent quality control information (LEPPLA 1986; LEPPLA and ASHLEY 1988).

Methods Development constantly improves the program's design by incorporating new technologies, whether invented or transferred. It bridges the gap between research and implementation. Strain development, one of the primary areas of responsibility, depends on the specific purpose, required specifications and rearing capability or process parameters for a candidate species. Then appropriate specimens are collected, established and maintained according to optimum protocols. Process capabilities are determined for every

production and utilization operation, alternative designs are reviewed and pilot tests are conducted. As an IPM program evolves, management controls design changes based on these studies.

Material control emphasizes the reception and storage of materials that conform to specified quality and economy. This can involve both incoming materials and those produced in one area of an organization and transferred to another internal location. Material control is essential but often overlooked or poorly orchestrated. It necessitates clear delineation of the requirements for quality materials and the assurance of a dependable supply. Appropriate standards must be applied to all materials. It is not reasonable, for instance, to perform exhaustive chemical analysis on dietary ingredients while ignoring water quality and storage temperatures. A rating system based on product testing is used to determine vendor capability and award contracts. Thereafter, the vendor accepts primary responsibility for satisfying the contract and certifying material quality. Periodic inspection and testing assures compliance.

Production control is regulation of the consistency, reliability and timeliness of production output (CHAMBERS and ASHLEY 1984). Personnel and machines are employed to perform required mental and manual operations. Mass production of high quality insects depends on the appropriate use of suitable facilities and equipment, and on the accurate performance of rearing operations. These rearing operations include reception of quality materials, larval diet preparation, adult colony maintenance, egg collection and treatment, larval rearing, pupae handling, maintenance and quality control (LEPPLA 1984). A typical production system may be based on a contract that specifies 250 million male pupae per week with a given average ( $\pm 1-3$  SD) and range weight and score on subsequent adult performance tests (i.e. eclosion, flight, orientation, mating, and longevity). To achieve these goals, individual processes comprising operations, such as rearing larvae in trays of diet, must remain under control. This requires the monitoring of diet quality and quantity, larval density and distribution, sanitation, and the physical environment. Performance of these operations is monitored directly but their processes are controlled by measuring larval yields, size and uniformity along the production line. More difficult parameters, such as the occurrence of pathogens, can be monitored as needed to trouble shoot the system and solve intermittent problems. Thus, if the rearing products achieve acceptable standards, the underlying processes are under control.

Research is the source of new technologies for insect colonization, production, utilization and quality control. Implementation, a management function, is accomplished in cooperation with appropriate technical units but Research provides the information and technical expertise. As a hypothetical example, Research may discover a temperature-dependent, sex-linked male sterility factor that eliminates the need for irradiating pupae. Management instructs Methods Development to investigate the potential for developing large scale systems for maintaining the strain and inducing sterility in the males for release. Adjustments may have to be made in Production and Utilization, and Quality Control must evaluate the consequences of the new methods on insect performance. This evaluation may depend on new ways to monitor sterility that also are provided by Research. If the system can accommodate the new technology and it is cost effective, the improvement becomes operational.

Utilization is monitored in terms of products that are preserved or potentially enhanced. For example, the ill effects of sterilization can be moderated by precisely timing and controlling dosages. Orientation and retention in the release area can be enhanced by exposing the insects to environmental cues prior to treatment, handling, and distribution. Careful deployment can increase dispersal and the opportunity to encounter suitable habitats. This preservation and enhancement of quality becomes increasingly important as utilization is mechanized. In the event corrective action is necessary, it can be more positively viewed as optimization by means of quality control. The selection and training

of personnel has only recently been recognized as a critical element in IPM programs based on mass produced insects. Management has increased its level of responsibility for identifying the duties and responsibilities of every employee by requiring accurate job descriptions and clear lines of authority. Every employee must understand the consequences of their performance, establish individual and group goals, and accept their role in achievement of the overall mission (to mass produce insects and deploy them effectively in the IPM program). Management therefore exercises great care in selecting employees and providing them with a work environment conducive to quality performance. However, performance criteria change as a program evolves. It has traditionally taken a different mentality during the building phase than it has to maintain a relatively stable program. Builders act as entrepreneurs, pragmatically solving major problems while leaving the seemingly endless details to those who follow. Conversely, maintainers fill the gaps, correct errors of expediency, patiently improve each process, and continue to stabilize the program. Both mentalities are essential but they require different kinds of managerial support and work environments. In each case, the combination of materials, equipment, instructions and supervision must meet requirements for quality performance. Individual accountability, incentives and recognition also must be incorporated. Employee selection, training, motivation, and health and safety may be more important than all other elements because highly qualified and dedicated personnel can often achieve success in an otherwise marginal program.

Quality control in insect mass production depends on the consistent performance of rearing operations at specified levels of excellence, and rearing process and product quality (fig. 2). Production control is the assurance that insect rearing and associated operations are performed. Performance of rearing operations is controlled by directly monitoring procedures, equipment and environments. This involves the use of schedules, check sheets and other means of assuring the completion of each step in rearing and maintenance. Each rearing operation is composed of successive processes that yield increasingly more mature insects. Process control is the regulation of these rearing processes by monitoring

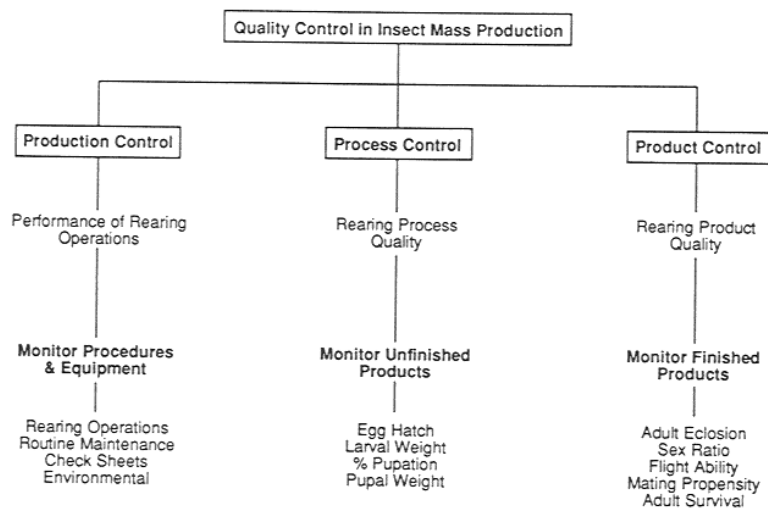


Fig. 2. The three primary subdivisions of quality control that occur in insect mass production. Production control assures the performance of rearing operations by monitoring related procedures and physical environments. Process control directly links every procedure and rearing environment with a specific stage of insect development. Product control evaluates the final insect stage that is produced and indirectly relates its quality to the rearing system.

unfinished products and comparing them with established specifications and standards. Product control is the assurance that adult insects are appropriate for treatment, handling, and transfer to utilization. Distribution and regulation of the target population are the final operations.

### 3 Optimization via total quality control

Total quality control not only defines but also makes it possible to optimize an entire pest management program. It provides direction in the persistent search for ways to perfect all of the associated science and technology. Each element, operation and procedure in the program is organized and evaluated relative to all others in an interactive matrix. Emphasis is placed on improving the less effective or efficient by assigning responsibility to interdisciplinary adhoc work units. The effectiveness of these units depends on the rapid classification of problems through accurate troubleshooting networks. Problems may involve anything from simple oversights to recurrent shortcomings that require further research. If personnel cause a deficiency, accurate position descriptions within the total quality control system assist in evaluation, training and restaffing. As a consequence of this optimization system, resources are allocated in ways that are likely to yield significant results.

A typical optimization problem is maximizing the yield of acceptable pupae from a rearing unit, the larval rearing container. Variables include the initial rearing procedures (Research), dietary ingredients and rearing containers (Material), mass rearing processes (Methods Development and Production), and the procedures for monitoring larval development (Quality Control). Each of these elements is represented by specialists who focus on every aspect of the rearing unit. Although closely related, this is not the same problem as maximizing the productivity of a production batch or shift. The larval rearing unit in this case is the individual container. It represents a finite amount of food and habitat that is maintained to support development of high quality larvae. Yields might be improved by redistributing or reconstituting the supporting resources, or by changing the procedures for handling eggs. Perhaps a better container could be provided or the rate of development altered by improving the holding environment. The strain might be managed by periodic infusion or replacement to yield more "reearable" larvae. Eventually a compromise is realized in which efficiency of the larval rearing unit is acceptable within the overall production system and further optimization efforts are unwarranted.

An example of optimizing a larval rearing unit was developed using the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), (FISHER 1983). Fall armyworm eggs were derived from a colony that had been maintained without infusing wild stock for more than six years at the Insect Attractants, Behavior, and Basic Biology Research Laboratory. Larvae were reared on artificial diet in 6-liter plastic containers with polystyrene grids (LEPPLA 1985). Paper toweling containing ca 400 eggs in masses was glued to the inside of each container lid. Four containers were established each week day and maintained at  $26 \pm 2^\circ\text{C}$  and  $50 \pm 5\%$  RH with a 14h photophase ( $n = 184$  containers). The egg sheets were removed after three days to determine egg hatch, which varied from ca 25% to 100%. After 21 days, pupae were removed and counted. To determine usability, 25 pupae were randomly selected from each container, sexed, held for five days and weighed. Pupal weights were plotted on mean and range control charts as the average results from all four containers for each production day (CHAMBERS and ASHLEY 1984).

An operating characteristic curve was prepared using the control chart for mean pupal weight of females (JURAN et al. 1974). It was used to determine the sensitivity of the sampling procedure to changes in production processes. An operating characteristic curve is based on the z-distribution, representing the probability of a sample mean of a certain size being within established control limits:



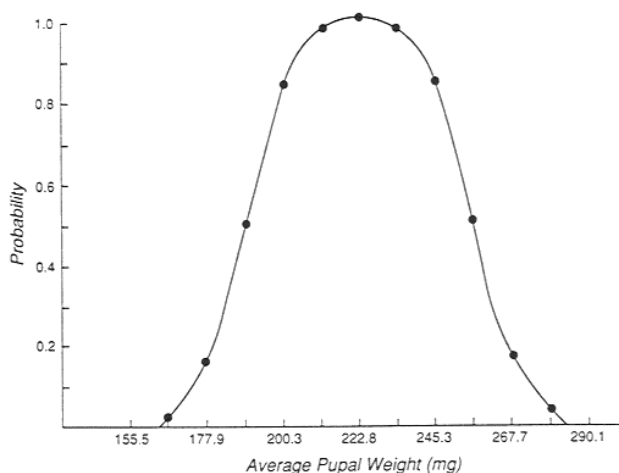


Fig. 4. An operating characteristic curve for mean weight of fall armyworm pupae. Data were transferred from a mean control chart based on a sample size of 5. The area under the curve is the probability of not detecting a change. Therefore, as a sample mean diverges from the expected mean, the area decreases and the probability of detecting a change increases.

Probable causes must be systematically evaluated before appropriate action can be taken when an insect mass rearing process is out-of-control. A dichotomously-branching, diagnostic chart can be used to accomplish this evaluation efficiently (fig. 5). In the example of usable pupae per rearing unit, a significant reduction in yield triggers a review of processes associated with reproduction, oviposition, fertility, larval density, contamination and routine procedures. All process variables that directly or indirectly affect insect quality are monitored, including events such as equipment service, power outages, visitors, new lots of materials, preparation of dilutions and employees on leave. Performance of routine procedures is noted on check sheets.

#### 4 Conclusion

The total quality control system for insect mass production in support of IPM programs has evolved from a relatively simplistic organizational structure based on production and utilization controlled by management to a minimal set of all-encompassing, interactive elements. This evolution has occurred through a process of interfacing major program components with the "Generic Guidelines for Quality Systems" of the American Society for Quality Control. Management gave rise to Quality Control and Personnel, and Production and Utilization yielded independent units for Methods Development and Material. Research continues to provide sources of new technology for Management's implementation. The system is dynamic and adaptable, so some programs will elevate subunits, such as strain development or personnel training, while others will combine entire elements. Material, for example, has traditionally been an operation within Production. Although all programs are unique, they share these adaptable organizational elements and technologies.

Total quality control addresses the major managerial issues of program effectiveness, efficiency, and success. Effectiveness requires that the right things be done. This may require further research or just monitoring to assure performance. Efficiency is concerned

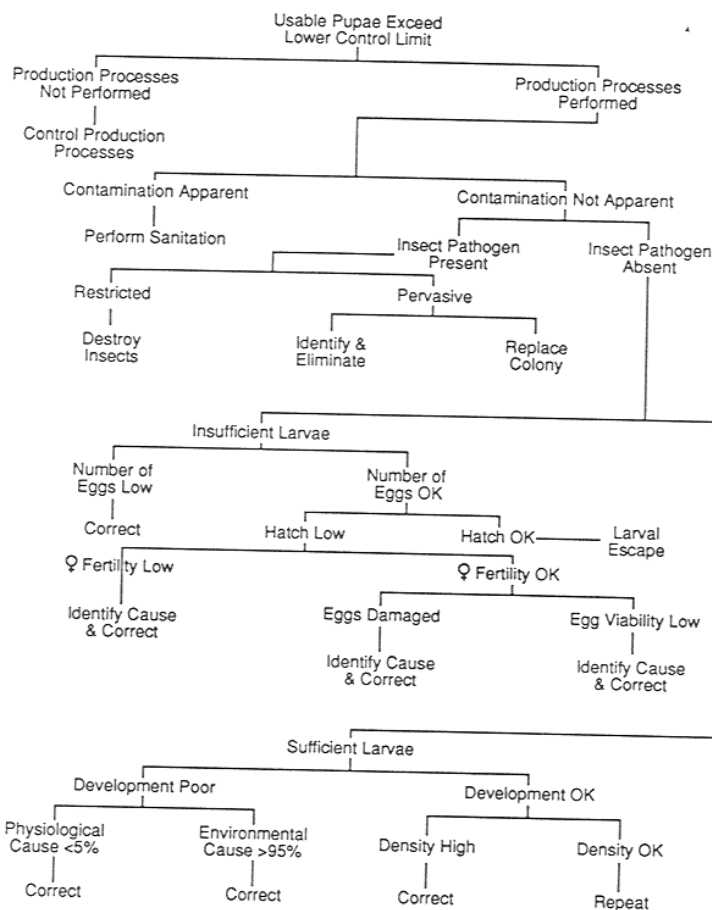


Fig. 5. A diagnostic chart for solving problems encountered in mass rearing insect larvae. This troubleshooting guide was developed using the fall armyworm. Routine production control is eliminated first and then all rearing operations are scrutinized

with doing things well in a cost effective manner. A continuous effort is made to improve production processes and preserve product quality. Management needs to know if the program is successful, if progress is being made and, if so, at what rate. To accurately determine success, shared objectives must be universally understood throughout the program. FEIGENBAUM (1983) defined a quality system to be an agreed on operating work structure, documented in effective technical and managerial procedures, for guiding the coordinated actions of the people, machinery and information to assure program success. It requires an appropriate level of control maintained through continuous feedback. As a general principle, the level of control is a function of the magnitude versus the probability of risk. Feedback can be both quantitative and qualitative, in the form of information or advice, based on observations, reports, meetings and perhaps outside review. However, information is expensive, so it is important to separate "need to know from nice to know".

The application of total quality control to foster communication networks and assign responsibility is probably its most important purpose. Its structure helps to identify the



interdependence among elements that represent work units. Management requires Methods Development to respond to continuous needs for the best available technology. Material is critical to both Production and Utilization. Research gives Management new technologies for implementation by personnel in every unit. Quality control information is required by management to formulate effective decisions. Strengths and weaknesses are readily identified and emphasis shifted accordingly. What emerges is a team with individual assignments and responsibilities for cooperation in achieving the program's mission.

#### Acknowledgements

We thank T. R. ASHLEY for his opinions and expert advice concerning the total quality control concepts; also he and V. CHEW checked the statistics. C. O. CALKINS, D. A. DAME, and D. A. TAYLOR provided helpful reviews of the manuscript. Application of total quality control concepts to insect mass production evolved from reviews of screwworm, Mediterranean fruit fly, and boll weevil pest management programs and from quality control workshops of the International Organization for Biological Control of Noxious Animals and Plants, Global Working Group on Quality Control of Mass-Reared Arthropods.

#### Zusammenfassung

##### *Umfassende Qualitätsüberwachung bei in Massen gezüchteten Insekten für die Schädlingsbekämpfung*

Eine umfassende Qualitätsüberwachung erfolgt auf verschiedenen Stufen der Massenzucht und schließt acht Hauptkomponenten ein: Organisation und Koordination, Forschung, Methodenentwicklung, Ausrüstung, Zuchtverfahren, Lagerung und Transport, Personalausbildung, Kontrolle des Produktionsablaufs und des Endproduktes. Das gesamte Zucht- und Kontrollprogramm wird von einem verantwortlichen Leiter koordiniert, der laufend auf allen Stufen die Erfüllung der Qualitätsnormen überwacht. Die Forschung erarbeitet neue Technologien, die zu routinemäßigen Verfahren weiterentwickelt oder in bestehenden Prüfmethoden genutzt werden können. Die Anforderungen an die gezüchteten Organismen sind laufend zu überprüfen und möglicherweise neu zu definieren. Zudem müssen Qualitätsstandards festgelegt, Stichprobenpläne angepaßt und die Einflüsse der Lagerung und der Transporte auf die Organismen untersucht werden. Die Qualitätskomponenten des Produktionsablaufs umfassen die Überwachung der Maschinen und Zuchteinrichtungen sowie deren optimale Nutzung und deren Einflüsse auf das Produkt. Vor und während der Anwendung der Organismen müssen die Manipulationen (Verpackung, Transport, Applikation usw.) so überwacht werden, daß die Qualitätsnormen des fertigen Produktes erfüllt werden. Weil die Massenzucht von Insekten mit hoher Qualität große Anforderungen an das Personal stellt, müssen qualifizierte Kräfte damit betraut und durch ständige Schulung weitergebildet werden. Qualitätskontrolle greift in alle Produktions- und Anwendungsabläufe bei in Massen gezüchteten Insekten ein, wobei aber der Schwerpunkt bei den biologischen Parametern liegt, welche die „Innere Qualität“ der Organismen bestimmen. Diese Elemente liefern die nötige Information über mögliche Qualitätsänderungen, so daß rechtzeitige Anpassungen, unter Berücksichtigung des gesamten Programms, vorgenommen werden können.

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## Quality control of mass-reared arthropods: Nutritional effects on performance of predatory mites<sup>1</sup>

By M. DICKE, MARIJKE DE JONG, M. P. T. ALERS, F. C. T. STELDER, R. WUNDERINK and J. POST

### Abstract

Financial aspects of a mass-rearing programme of beneficial arthropods may affect decisions on the rearing materials, among which host/prey species and their host plants. These decisions may severely influence the beneficial's quality because of effects of nutritional history on the arthropod's performance. This may be manifest in obvious characteristics such as rates of development and oviposition but also in characteristics that, although overlooked so far, may also decisively affect predator quality.

This paper presents nutritional effects on performance characteristics of predatory mites (Acarina, Phytoseiidae): 1. diapause induction, 2. response to volatile kairomones and 3. food utilization. These effects have become manifest by accident and it is expected that more effects can be foreseen.

Current data, although not indicating an unsurmountable decline in predator performance, should make one cautious in using novel diets or in using only one species as food for polyphagous predators.

### 1 Introduction

Mass rearing of beneficial arthropods for use in biological control programmes aims at low costs and high yields. In addition to high yields, arthropod quality is an important factor governing mass rearing success (MORRISON and KING 1977).

In papers advocating quality control, much attention has been given to the occurrence of genetic changes in mass reared populations (MACKAUER 1976; BOLLER and CHAMBERS 1977; VAN LENTEREN 1986; VAN LENTEREN and WOETS 1988). However, phenotypic variation in biological characteristics (e.g. OVERMEER and VAN ZON 1983; VET 1984; SLANSKY and SCRIBER 1985; BERNAYS 1986; KLOWDEN 1986; KLOWDEN et al. 1987; STÄDLER et al. 1987) has only occasionally been mentioned in the context of quality control in mass-reared arthropods (HOUSE 1977; BIGLER et al. 1982; VAN LENTEREN 1986; but see contributions by BIGLER 1989 and NOLDUS 1989). Rearing conditions that may affect phenotypic variation are e.g. humidity and host/prey species. In developing a mass-rearing programme, exactly such components are modified with the aim of economizing production.

In most commercial mass-rearing programmes the host plant used is different from the crop species in which biological control is to be carried out and occasionally host/prey species are different from the pest species to be controlled (VAN LENTEREN and WOETS 1988). Moreover, usually one prey/host species is used in mass rearing. Continuous mass rearing of parasitic wasps may be affected by using a single host species for several years (BIGLER et al. 1982). Because predators, in contrast to parasitic wasps, need several prey individuals to complete development, it may be expected that performance of polyphagous predators is also sensitive to rearing on only one prey species: for instance, using only one prey species that is deficient in one or more nutrients, may cause specific hunger (DETHIER

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1976) or suboptimal quality (SLANSKY and SCRIBER 1985). This paper deals with nutritional aspects of performance of predatory mites.

## 2 Predatory mites

Predatory mites are used in biological control of many pest species (HELLE and SABELIS 1985) and several phytoseiid species are mass-reared (VAN LENTEREN and WOETS 1988). When observing the outcome of mass-rearing programmes, generally only obvious characteristics (e.g. development rate, oviposition rate, sex ratio) are taken into account. However, nutritional effects on predator performance may also be manifest for many less obvious characteristics that may be very important for predator performance. The most important aspects of predatory-mite biology, in the context of biological control, are given below.

Predatory mites (Acarina, Phytoseiidae) alternate long-range dispersal on air currents with local search for food. Departure for an aerial voyage is based on chemical information about prey availability (SABELIS and DICKE 1985; SABELIS and JANSSEN, in press). After landing volatile and non-volatile infochemicals (*sensu* DICKE and SABELIS 1988) may affect prey searching decisions (SABELIS and DICKE 1985). After the subsequent location of a prey patch, a prey colony in a patch and prey individuals in a colony (cf. SABELIS and DICKE 1985), decisions have to be made on acceptance/rejection of the encountered prey individuals. Such decisions are affected by gut fullness (SABELIS 1981, 1986, 1989), by composition of prey supply (DICKE 1988b; DICKE et al. 1988) and by infochemicals (SABELIS and DICKE 1985).

Phytoseiid mites have high food conversion efficiencies: e.g. *P. persimilis* females convert 70% of ingested food into predator eggs (SABELIS 1981). The rate of egg production is positively correlated with gut fullness. Also development rate is dependent on gut fullness (SABELIS 1985). Thus, the intrinsic rate of population increase ( $r_m$ ), which depends on rates of development and reproduction, is severely affected by rate and efficiency of food conversion.

Several species of predatory mites exterminate prey locally (e.g. SABELIS and VAN DER MEER 1986). This is the effect of a high predator  $r_m$  that is effectuated at the cost of prey mortality. Moreover, in developing a population-dynamical model it became obvious that suppression of predator dispersal is also essential for incidence of prey extermination (SABELIS and VAN DER MEER 1986). Infochemicals appeared to mediate suppression of predator dispersal (SABELIS et al. 1984; SABELIS and DICKE 1985; SABELIS and JANSSEN, in press).

Female predatory mites enter reproductive diapause in temperate zones at the end of the season (OVERMEER 1985). The most conspicuous characteristic of diapausing females is that they do not produce eggs. Environmental factors affecting diapause induction are photoperiod (temperature dependent) and thermoperiod (OVERMEER 1985; VAN HOUTEN et al. 1987, 1988). There is a clear correlation between geographical latitude and critical photophase (OVERMEER 1985).

Predatory mites may feed on a variety of food sources. Some species, such as *Phytoseiulus persimilis* Athias-Henriot only feed on spider mites, which themselves may feed on many different plant species. Other phytoseiid species such as *A. potentillae* may feed on a wide range of unrelated prey species, not restricted to the Acari. Also plant tissues may be included in the diet of some phytoseiid species (PORRES et al. 1977; OVERMEER 1981). As a result, prey and/or host plants different from those encountered by the predators after release in agricultural plots may be used in a mass rearing programme.

In this paper we provide evidence for dietary effects on several biological characteristics in predatory mites: response to kairomones, ability to enter diapause and predation rate.

This concerns data on laboratory populations that are much smaller than commercial mass-rearing populations. No data are available for the latter yet, but the dietary effects discovered so far should alert anyone maintaining a large scale mass rearing.

### 3 Materials and methods

#### 3.1 Predators

*Amblyseius potentillae* (Garman) was collected from apple trees in Serooskerke, The Netherlands, in 1974 (McMURTRY et al. 1976) and has been reared on *T. urticae* since then. In 1980 a subpopulation was used to start a culture on *Vicia faba* pollen (OVERMEER 1981).

*Typhlodromus pyri* Scheuten was collected from chestnut, *Aesculus hippocastanum* L. in Amsterdam, The Netherlands, in 1978 (OVERMEER 1981). Also of this predator subpopulations have been reared on *T. urticae* or *V. faba* pollen. We received predators from both subpopulations of *A. potentillae* and *T. pyri* in 1982 from W. P. J. OVERMEER and A. Q. VAN ZON, and reared them on their respective diets in our laboratory.

*Amblyseius swirskii* Athias-Henriot was collected from *Citrus* spp. in Bet Dagan, Israel, in 1970 (M. WYSOKI, pers. comm.) and has been reared on *Carpobrotus edulis* L. pollen since then. We received predators from this population in 1984 from M. WYSOKI and have reared part of them on *T. urticae* and part on *V. faba* pollen. *Amblyseius swirskii* is morphologically very similar to *A. potentillae* (ATHIAS-HENRIOT 1962), but did not interbreed with our *A. potentillae* population (DICKE, unpubl. data 1987).

All rearings occurred at 25–26°C, 60 ± 10 r.h. (for more details about rearing conditions, see DICKE and GROENEVELD 1986).

For all three species a subpopulation of the predators reared on *V. faba* pollen was reared on a mixture of this pollen and crystalline  $\beta$ -carotene (1 mg carotene/5 mg pollen). In the remainder of this paper different predator populations are indicated by the suffix (Tu), (Vf) or (Vfc) for being reared on *T. urticae*, *V. faba* pollen or *V. faba* pollen supplemented with  $\beta$ -carotene respectively.

#### 3.2 Methods

Olfactometer: Individual predators were observed in a Y-tube olfactometer to study the response to volatile kairomones. For details see SABELIS and VAN DE BAAN (1983) and DICKE and GROENEVELD (1986).

Predation rates: Young adult female predators in the oviposition phase (c. 3–4 days since their final moult) were placed on plastic or leaf discs on wet cotton wool with one prey type available. Consumed and drowned prey were replaced at regular intervals (30 min for low densities, 2 h for high densities). Predation rates were determined during 6 h after an initial period of 8 h during which the predator's gut content had reached steady state (RABBINGE 1976; SABELIS 1981, 1985; DICKE et al. 1988). Experimental conditions were 26 ± 1°C, 60 ± 10% r.h. For more details of the method see DICKE et al. (1988).

Diapause induction: This characteristic was studied at 17 ± 1°C, LD 8:16, 60 ± 10% r.h., 400 lux. For details of the method see OVERMEER and VAN ZON (1983), DICKE (1988a) and DICKE et al. (1989b).

Gut emptying rate: To determine the rate of gut emptying young adult female predators in the oviposition phase were starved during 48 h. Thus, no eggs were developing in predators used in the experiment. Subsequently, the females were offered an ample food supply during 4 h, after which they were transferred to an empty rearing unit. After different times of starvation at 26 ± 1°C, 60 ± 10% r.h., a sample of predators was collected, anaesthetized with ethylether vapour and weighed individually on a Cahn electrobalance. After weighing the predators were discarded.

### 3 Biological characteristics affected by predator diet

#### 3.1 The importance of dietary carotenoids for being able to enter reproductive diapause

The ability of *A. potentillae* to enter reproductive diapause is affected by the rearing diet: availability of carotenoids with provitamin A function is essential (VAN ZON et al. 1981; VEERMAN et al. 1983; VAN HOUTEN et al. 1987, 1988). These nutrients are not (sufficiently)

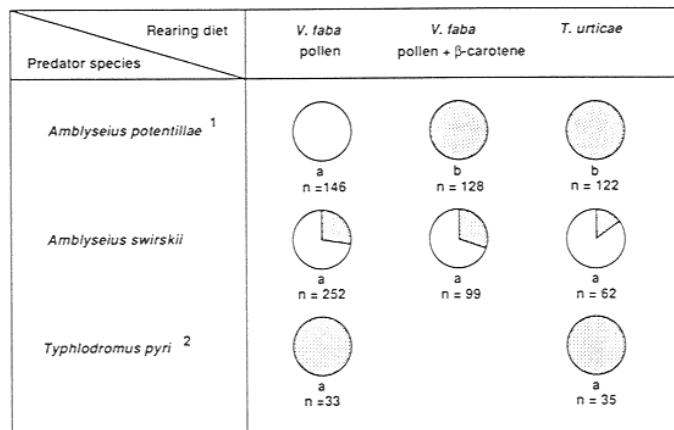


Fig. 1. Diapause induction at 17°C, LD 8:16 in three phytoseiid species reared on different diets. Black parts of circle indicate percentage diapause. Different letters in a row indicate significant differences ( $\alpha = 0.05$ , G-test, SOKAL and ROHLF 1981). <sup>1</sup> Data of OVERMEER and VAN ZON (1983). – <sup>2</sup> Data of DICKE (1988a)

available to the predator in *V. faba* pollen, but they are in several other pollens and in phytophagous prey, e.g. spider mites and rust mites (OVERMEER and VAN ZON 1983; DICKE et al. 1989) (fig. 1).

In contrast, no effect of rearing diet on diapause induction has been recorded for the phytoseiid *T. pyri* (DICKE 1988a). Females of this species enter reproductive diapause both when reared on spider mites or when reared on *V. faba* pollen (fig. 1).

Field observations on *A. swirskii* had indicated that diapause was absent in this species in Israel (WYSOKI and SWIRSKI 1971). This was corroborated by current laboratory experiments: diapause induction incidence was low and no effect of rearing diet was observed (fig. 1).

Thus, in contrast to *A. potentillae*, no evidence is available which indicates that diapause induction in *A. swirskii* and *T. pyri* is affected by predator diet: carotenoids do not seem to be important nutrients for *T. pyri* and *A. swirskii* with respect to diapause induction.

### 3.2 Response to volatile kairomones as affected by dietary carotenoids

The response of predatory mites to volatile kairomones (*sensu* DICKE and SABELIS 1988) is not a fixed characteristic. The response may depend on predator developmental stage, satiation level and prey species offered (SABELIS and VAN DE BAAN 1983; DONG and CHANT 1986). Moreover, the response may also be affected by the predator's rearing diet (DICKE and GROENEVELD 1986; DICKE et al. 1986; DICKE 1988a). When reared on *V. faba* pollen *A. potentillae* and *T. pyri* respond to volatile kairomones of a larger number of prey species than when reared on the spider mite *T. urticae* (table 1). The "extra" prey species, *T. urticae* and *A. schlechtendali*, are less preferred prey species (DICKE and GROENEVELD 1986; DICKE 1988a; DICKE and DE JONG 1988; DICKE et al. 1988). For both predator species the diet-dependent difference in behavioural response could be accounted for by a carotenoid deficiency in *V. faba* pollen: after addition of  $\beta$ -carotene (*A. potentillae* and *T. pyri*) or vitamin A (*A. potentillae*) to *V. faba* pollen predators responded in a way similar to conspecifics reared on the carotenoid-rich *T. urticae* (DICKE et al. 1986; DICKE 1988a). DICKE et al. (1986) explained the behaviour of carotenoid-deficient predators by stressing that these increase their long-term fitness (survival during winter and subsequent repro-

Table 1. Response of predatory mites towards volatile prey kairomones in a Y-tube olfactometer. Prey infested leaves were offered vs. clean leaves. For each predator-prey combination the percentage of predators walking into the arm with infested leaves is indicated. Between brackets total number of predators observed is indicated. Predators that did not walk towards the end of either arm are not included in the table

Predator species and predator diet	Prey species <sup>1</sup>		
	<i>P. ulmi</i>	<i>T. urticae</i>	<i>A. schlechtendali</i>
<i>T. pyri</i> (Tu)	64 (56) <sup>2</sup>	53 (40)	56 (45)
<i>T. pyri</i> (Vf)	61 (109)*	64 (86)**	75 (40)***
<i>T. pyri</i> (Vfc)	59 (133)*	52 (64)	
<i>A. potentillae</i> (Tu)	78 (40)***	53 (40)	54 (63)
<i>A. potentillae</i> (Vf)	73 (52)***	75 (59)***	78 (49)***
<i>A. potentillae</i> (Vfc)	75 (40)***	50 (40)	53 (40)
<i>A. swirskii</i> (Tu)		51 (55)	
<i>A. swirskii</i> (Vf)		71 (83)***	

<sup>1</sup> *P. ulmi*: 250 spider mites on apple leaves. *T. urticae*: 2700 spider mites on Lima bean leaves. *A. schlechtendali*: 120,000 rust mites on apple leaves.

<sup>2</sup> Asterisks indicate level of significance (sign test): \* 0.01 < P < 0.05; \*\* 0.001 < P < 0.01; \*\*\* P < 0.001.

duction in spring) when, under conditions where the preferred prey species is not available, they forage for inferior prey that can nonetheless relieve the carotenoid lack. This explanation does not hold for *T. pyri* that does not need carotenoids for diapause induction (fig. 1). Yet the differential behaviour towards kairomones by *T. pyri* (Vf) and *T. pyri* (Tu) suggests that this predator species needs carotenoids for another vital physiological process.

These data raise the question whether in *A. potentillae* carotenoids are only needed for diapause induction or also for other processes. To study this, one ideally would need a non-diapause strain of this predator species. Unfortunately, such a strain was not available to us. The second best alternative is to investigate a non-diapausing species that is closely related to *A. potentillae*. *Amblyseius swirskii* is such a species (ATHIAS-HENRIOT 1962; WYSOKI and SWIRSKI 1971, this paper). Our present investigation shows that *A. swirskii*'s response to the volatile *T. urticae* kairomone is identical to that of *A. potentillae* (table 1): dietary  $\beta$ -carotene affects the behavioural response of this species in the same way as in *A. potentillae*. This indicates that also *A. swirskii* (Vf) is in need of carotenoids. Thus, in addition to its function in diapause induction in *A. potentillae*, carotenoids presumably also have an unknown function in all three predator species investigated.

### 3.3 Predation rates of "vegetarian" and "carnivorous" predatory mites

Predation rates of young ovipositing females of *A. potentillae* reared on *T. urticae* or *V. faba* pollen have been measured for the same predator population over several years. Data for high *P. ulmi* densities (> 3 spider-mite larvae per cm<sup>2</sup>), at which maximum predation rates are attained (RABBINGE 1976; DICKE et al. 1988), are similar except for that of *A. potentillae* (Vf) in 1986 when the observed predation rate was approximately one third of that for the other experiments (fig. 2). First indications for a reduced predation rate in *A. potentillae* (Vf) had been noticed already in 1985 (data not shown). The low predation rate recorded for *A. potentillae* (Vf) was not specific for *P. ulmi* larvae as prey but also occurred when using *A. schlechtendali* adults as prey (fig. 3) or *T. urticae* larvae in 1986 (fig. 4). In all cases a large difference exists between predators reared on *T. urticae* and predators reared on *V. faba* pollen. This occurred over a range of prey densities, e.g. see fig. 5 for data for *P.*

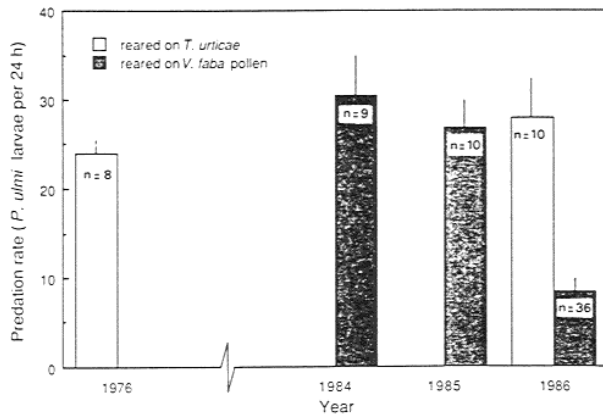


Fig. 2. Maximum predation rates (mean and standard error) on *P. ulmi* larvae, recorded over a range of years for one population of *A. potentillae* reared on *T. urticae* or *V. faba* pollen. Prey densities:  $> 3$  *P. ulmi* larvae per  $\text{cm}^2$ . (Data for 1976 from RABBINGE 1976). For history of laboratory rearing see "Materials and methods"

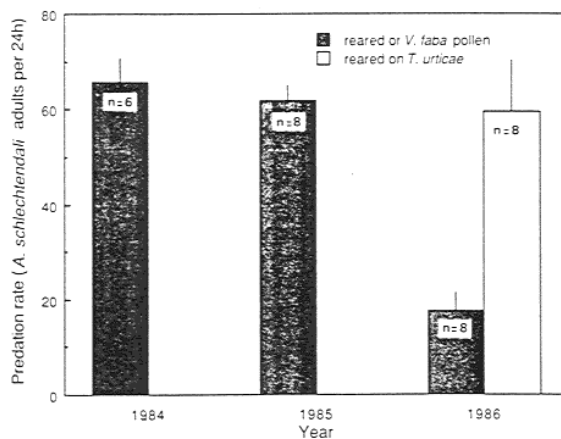


Fig. 3. Maximum predation rates (mean and standard error) on *A. schlehtendali* adults, recorded over several years for one population of *A. potentillae* reared on *T. urticae* or *V. faba* pollen. Prey density: 10 *A. schlehtendali* adults per  $\text{cm}^2$ . For history of laboratory rearing see "Materials and methods"

*ulmi* larvae as prey. This effect is not caused by a difference in carotenoid content of rearing diet because predation rates of *A. potentillae* (Vf) did not differ significantly from predation rates of *A. potentillae* (Vf) (fig. 6A).

To investigate whether the difference is caused by genetic changes in the population reared on *V. faba* pollen we tested *A. potentillae* (Vf), that were fed *T. urticae* during 2 days before the experiment or since the protonymphal stage (c. 6–8 days prior to the experiment). The results with regard to *T. urticae* or *P. ulmi* larvae (fig. 6A and 6B) show that feeding on *T. urticae* prior to the experiment resulted in higher predation rates that do not differ from those measured for *A. potentillae* (Tu). Thus, the low predation rates of predators reared on a vegetarian diet increase after feeding on prey for several days. This means that the difference in maximum predation rate between *A. potentillae* (Vf) and *A.*



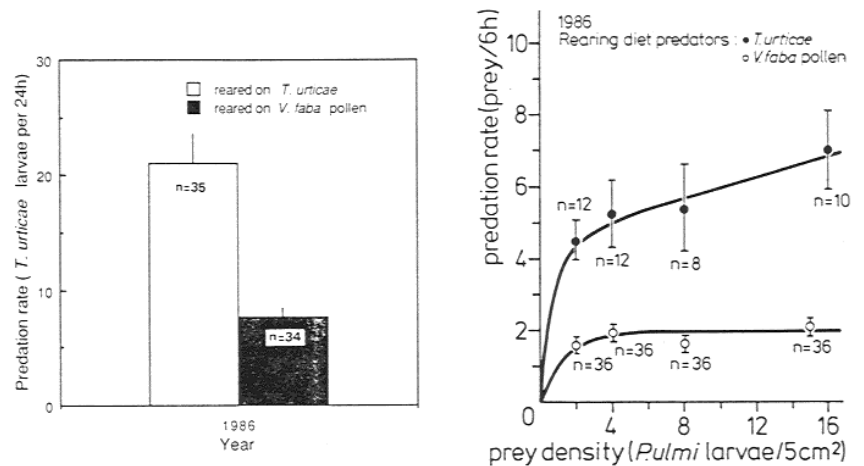


Fig. 4 (left). Maximum predation rates (mean and standard error) on *T. urticae* larvae, recorded over several years for one population of *A. potentillae* reared on *T. urticae* or *V. faba* pollen. Prey density: 3 *T. urticae* larvae per cm<sup>2</sup>. For history of laboratory rearing see "Materials and methods". – Fig. 5 (right). Functional response on *P. ulmi* larvae of *A. potentillae* reared on *T. urticae* or *V. faba* pollen. For history of laboratory rearing see "Materials and methods"

*potentillae* (Tu) is not the direct result of a genetic difference. However, a genetic difference regarding the mechanism causing the lower predation rate after prolonged feeding on a diet of *V. faba* pollen, cannot be excluded yet.

To investigate the underlying mechanism it should be realized that predation rate is the product of encounter rate (dependent on prey density, prey and predator walking speed, prey and predator activity) and success ratio, i.e. percentage of contacts with prey that result in predation (dependent on e.g. prey vigour, prey cuticle thickness and relative gut fullness of predator). Each of these two main components of predation rate may have been affected by predator rearing diet. For instance: 1. *Amblyseius potentillae* (Vf) may have a lower walking speed and/or activity than *A. potentillae* (Tu); 2. vegetarian predators may need time for synthesis of enzymes used for digesting animal food; 3. vegetarian predators may digest pollen more efficiently (for review of effects of food intake on enzyme synthesis, see CHAPMAN 1985); 4. predators reared on *V. faba* pollen may no longer recognize prey individuals after contact (cf. BLANEY et al. 1986); or 5. vegetarian predators may be less vigorous. These hypothetic events affect encounter rate (1), gut fullness and thereby success ratio (2 and 3) or the overall relationship between success ratio and gut fullness (4 and 5). In a preliminary analysis of the underlying mechanism of the lower predation rate we investigated behaviour of starved (24 h at 26 °C) predators until satiation (defined to occur after 10 unsuccessful encounters with prey; SABELIS 1981) towards 3 prey types: *T. urticae* larvae, *P. ulmi* larvae or *A. schlechtendali* adults. The data (table 2) show that encounter rates are similar for predators reared on either diet. This suggests that it is the success ratio which is affected by rearing diet. *Amblyseius potentillae* (Vf) had a lower success ratio than *A. potentillae* (Tu) and consumed less prey until satiation. In comparison to *A. potentillae* (Tu) mean number of unsuccessful encounters by *A. potentillae* (Vf) before the first predation were higher both for *T. urticae* and for *A. schlechtendali* as prey. These data can be explained in two ways: a. predators reared on *V. faba* pollen have an overall lower success ratio and thus reach the 10-unsuccessful-encounter criterion sooner (see 4 and 5, above); or b. predators reared on *V. faba* pollen have a higher relative gut

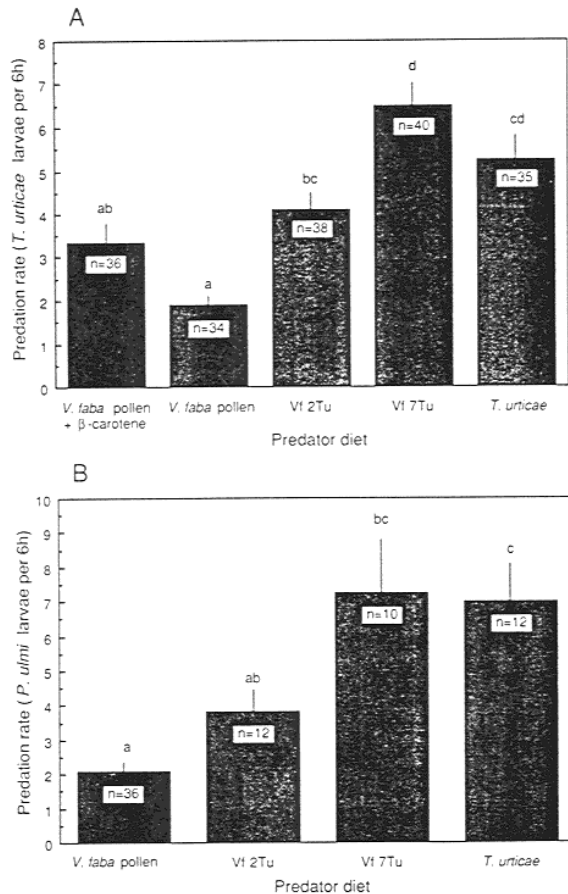


Fig. 6. Maximum predation rates (mean and standard error) for *A. potentillae* differing in short-term and long-term feeding history. A: Prey = *T. urticae* larvae; B: Prey = *P. ulmi*. Vi 2Tu = predators reared on *V. faba* pollen and provided with *T. urticae* during two days before the experiment; Vi 7Tu = predators reared on *V. faba* pollen and provided with *T. urticae* since protonymphal stage (ca. 7 days before experiment). Different numbers indicate significant differences (ANOVA and subsequent Tukey test,  $\alpha = 0.05$ )

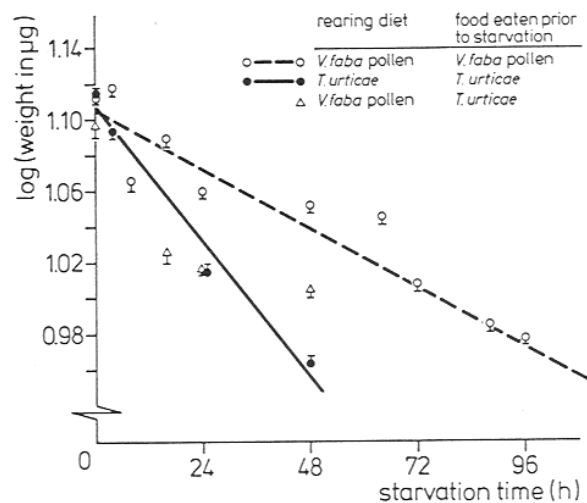
content after 24 h of starvation than conspecifics reared on *T. urticae* and thereby have a lower success ratio and eat less prey until satiation (see 2 and 3, above).

To discriminate between these two possibilities, we measured predator weights during starvation at 26 °C. *Amblyseius potentillae* (Tu) that had fed on *T. urticae* during 4 h prior to starvation lost weight more quickly than *A. potentillae* (Vf) that had fed on pollen prior to starvation (fig. 7). During the first 24 h of starvation *A. potentillae* (Vf) that had fed on *T. urticae* lost weight as quickly as *A. potentillae* (Tu) (fig. 7; slopes of regression lines  $-0.0035 \pm 0.0008$  [s.e.] and  $-0.0032 \pm 0.0004$  [s.e.] respectively). This indicates that pollen-reared predators still digest *T. urticae* as fast as do *T. urticae*-reared predators (thus hypothetical event [2] mentioned above appears to be invalid). In contrast, *A. potentillae* (Vf) appear to empty pollen from their gut much more slowly (slope of regression line  $-0.0013 \pm 0.0001$  [s.e.], fig. 7). Since oviposition rate of *A. potentillae* (Vf) has not been

Table 2. Behavioural components of young *A. potentillae* females in the oviposition phase, as affected by rearing diet<sup>1</sup>

Behavioural characteristic	Prey species Rearing diet predators	<i>A. schlechtendali</i>		<i>T. urticae</i>		<i>P. ulmi</i>	
		<i>V. faba</i>	<i>T. urticae</i>	<i>V. faba</i>	<i>T. urticae</i>	<i>V. faba</i>	<i>T. urticae</i>
Number of contacts with prey before first predation		1.1 ± 1.3a (n=9)	1.0 ± 0.9a (n=8)	2.6 ± 2.1a (n=22)	1.5 ± 1.2b (n=22)	14.6 ± 8.8a (n=9)	1.7 ± 1.5b (n=9)
Number of predations until satiation		13.9 ± 2.3a (n=9)	23.3 ± 10.1b (n=8)	3.8 ± 2.2a (n=19)	8.0 ± 3.7b (n=16)	nd <sup>2</sup>	nd
Success ratio		0.68a	0.77b	0.47a	0.57b	nd	nd
Encounter rate/min (Predation time excluded)		2.9 ± 1.1a (n=9)	3.2 ± 1.4a (n=8)	1.5 ± 0.4a (n=19)	1.3 ± 0.4a (n=16)	1.4 ± 0.7a (n=9)	1.3 ± 0.6a (n=9)

<sup>1</sup> Mean ± s.d. are given. For each prey species, different letters behind the values of a parameter for predators from different rearing history indicate significant differences ( $\alpha=0.05$ ). Statistical tests used: Number of contacts 1st success: test based on geometrical distribution (SNEDECOR and COCHRAN 1967); Number of predations until satiation and Encounter rate: Mann-Whitney-U-test; Success ratio: U-test for two fractions in two binomial distributions (ZIPP 1974). - <sup>2</sup> not determined.

Fig. 7. Effect of starvation on gut content of *A. potentillae* reared on *T. urticae* or *V. faba* pollen. (Mean and standard error)

impaired ( $2.5 \pm 0.5$  [s.d.] eggs/day at 26 °C in 1986/1987 [ $n = 32$ ], compared to 2.0 eggs per day at 25 °C according to OVERMEER 1981) these data suggest that utilization efficiency of pollen has increased over time (cf. hypothetical event [3], above). This may have been caused by a genetic change, but also by non-genetic changes such as e.g. acquisition of microorganisms (e.g. DADD 1985). Non-genetic changes seem to be more likely because an

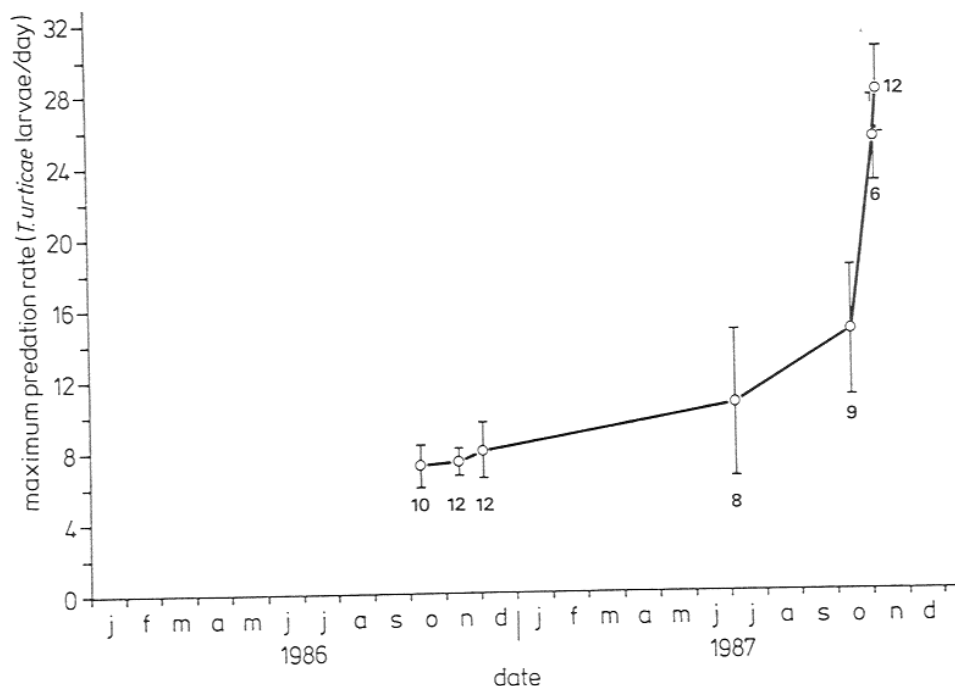


Fig. 8. Changes in maximum predation rate of *A. potentillae* (Vf) on *T. urticae* larvae over time. Prey density 15 larvae per 5 cm<sup>2</sup>. Mean and standard error are indicated. Numbers refer to number of predators observed

increase of maximum predation rate by *A. potentillae* (Vf) towards the previous level has recently been observed (fig. 8): In November 1987 the predation rate of *A. potentillae* (Vf) was similar to that of *A. potentillae* (Tu) in November 1986 (fig. 4) which is assumed to have been also the level of *A. potentillae* (Vf)'s predation rate before the decline (cf. fig. 2 for *P. ulmi* as prey). The time scale over which the increase in predation rate is observed is similar to that of the decrease in predation rate in 1985 (observed for *P. ulmi* larvae as prey).

#### 4 Consequences for mass rearing

All above-mentioned dietary effects concern essential components of predator performance. Yet their discovery is dependent on more intensive examination than is usually carried out in mass rearings. How serious are the observed effects for application of mass-reared phytoseiid mites in a biological control programme?

The effects on diapause induction and response to volatile kairomones are inherent to nutrient availability in rearing diet. The response to an increased number of kairomones by *A. potentillae* (Vf) is a valuable trait in the context of release strategies, because it diminishes the probability of predator dispersal upon release in the field (SABELIS and AFMAN 1984; SABELIS and JANSSEN, in press). This effect disappears after feeding on a carotenoid source, just as is the case for the effect on diapause induction. Thus, the effects of carotenoid-deficiency of *V. faba* pollen do not seem to hamper predator performance, but rather improve it. However, current data suggest that carotenoids also affect other, as

yet unknown, biological characteristics. Until these characteristics have been identified no definitive inferences can be made on the overall effect of carotenoid-deficiency. For instance, carotenoids with provitamin A function have been reported to affect development rate (HOUSE 1977) and cellular differentiation (EICHELE 1987; PETKOVICH et al. 1987).

Effects of pollen as a rearing diet on pollen digestion have become manifest in *A. potentillae* only after several years of using this rearing diet. The effects on predation rate fade away during some days after transfer to animal prey. Therefore, also this effect of rearing diet does not seem to affect predator performance in the field seriously.

Thus, current data indicate that rearing diet can affect biological characteristics, some of which are unknown as yet for the case of *V. faba* pollen and predatory mites. As a consequence, when making changes in rearing diet of predatory mites, one may expect changes in biological characteristics to occur. The severeness of these changes is unpredictable.

All dietary effects mentioned in this paper have been discovered by accident. This should make one careful in application of unnatural rearing diets and presumably also in application of a single food species for a polyphagous arthropod. Other effects may have gone unnoticed to date, e.g. change in sex ratio, susceptibility to diseases or changes in responsiveness to infochemicals.

So far, we have only dealt with changes in rearing diet of the predators. Moreover, the rearing diet of the prey, which is much more often subject to modifications in mass-rearing programmes than the prey species itself (VAN LENTEREN and WOETS 1988), may also affect natural enemies of phytophages, directly or indirectly (BOETHEL and EIKENBARY 1986). Since the effects of plants on natural enemies of herbivores have generally been neglected (PRICE et al. 1980) and since nutrients of the first trophic level may reach the third level many dietary effects of plants on predatory mites may have remained unnoticed. Future investigations on dietary effects on predator performance should also include these aspects. Among the effects that may be expected are repercussions of sequestration of secondary plant compounds such as intoxication, increased or decreased susceptibility to diseases (e.g. FERRON 1985; DUFFEY et al. 1986; see MORAES and McMURTRY [1986, 1987] for examples for predatory mites) and conditioning to plant-related infochemicals (e.g. VET 1984, 1988).

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#### Zusammenfassung

##### *Qualitätskontrolle bei Arthropoden in Massenzucht: Einfluß der Nahrung auf die Leistung von Raubmilben*

Finanzielle Gesichtspunkte eines Massenzucht-Programms von Nutzarthropoden können Entscheidungen über Zuchteinrichtungen und -materialien, wie zum Beispiel die Wahl der Wirt/Beute-Arten und deren Wirtspflanzen, stark beeinflussen. Solche Entscheidungen können sich in der Folge auf die Qualität der Nutzorganismen auswirken, weil deren Leistung von den Nahrungsbedingungen abhängt. Dies kann sich in leicht meßbaren Eigenschaften äußern, wie zum Beispiel in der Entwicklungsrate und der Eiablage. Es kann aber auch Eigenschaften betreffen, die, obwohl sie gern übersehen werden, die Qualität von Nutzarthropoden entscheidend beeinträchtigen.

Die vorliegende Arbeit zeigt Einflüsse der Ernährung auf Eigenschaften bei Raubmilben, die Leistung und Verhalten charakterisieren: 1. Diapauseinduktion, 2. Reaktion auf flüchtige Kairomone und 3. Beuteverzehr. Die ernährungsbedingten Einflüsse wurden zufällig entdeckt, und es muß angenommen werden, daß noch andere Veränderungen vorliegen. Die dargelegten Resultate zeigen eine verminderte Leistung der Raubmilben, und sie weisen darauf hin, daß bei der Verwendung neuer Diäten oder bei Fütterung mit nur einer Beutetierart bei polyphagen Räubern Vorsicht geboten ist.

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## The relationship of research to total quality control with special reference to sterile insect technique<sup>1</sup>

By D. A. DAME

### Abstract

Research is but one of the elements of total quality control in insect mass rearing programs, but the pre-operational research phase is usually very extensive and is likely to provide documentation and experience for virtually all of the elements of quality control. The duration of the research phases is largely program-dependent, whereas the scope of research subject matter is generally similar from program to program. The relationship of pre-operational research in release programs to program decision-making and the establishment of quality control methodology is outlined in this report.

### 1 Introduction

CHAMBERS (1977) and BOLLER and CHAMBERS (1977) characterized quality control (QC) technology as it applies to the process of insect mass production and the performance of the product, and CHAMBERS and ASHLEY (1984) have more recently addressed the three major QC components: process control, production control and product control. LEPPLA (1989) has helped us to conceptualize these QC components by focusing on the many primary elements that comprise total QC: methods development, material, production, utilization, personnel, quality control and research. Research is but one of the elements of total quality control, but the pre-operational research phase is usually very extensive and is likely to provide documentation and experience related to the establishment of virtually all of the QC elements.

The duration of the pre-operational research phase is largely program-dependent, whereas the scope of research subject matter is generally similar from program to program (table). The subsequent operational research phase, established to support the operational program, usually includes both long term program-oriented research and short term methods development investigations, which are directed at problem solving and pressing technological deficiencies. During the operational phase of the program, long term and perhaps more basic type research can provide important information regarding program enhancement, efficiency and assessment.

### 2 Relationship between pre-operational research and QC

The scope of studies conducted prior to embarking on an operational program naturally includes many factors that are not specifically oriented to QC. However, most of the findings are relevant to QC. For example, studies on the seasonal variation in target population distribution and density yield data that is critical to assessment not only of the

<sup>1</sup> Paper presented at the Symposium on "Quality control in mass-reared arthropods" of the IOBC global working group, held June 26-30, 1988 in Vancouver, Canada.

## Relationship of pre-operational research to quality control

Research often essential during pre-operational phase (QC related)	Assessments to develop information for operational decisions (independent of QC)
Colonization Gene pool Mating/Survival Replenishment	Target isolation Dispersal Barrier Cost
Rearing Selection/Adaptation Competitiveness QC control parameters	Cost Release only Release & IPM IPM only
Handling Collection Storage Distribution Release	Environmental impact Rearing facility Released organisms
Field effectiveness Stage Season Dispersal Release rate Survival	End point determination Monitoring Recovery interval Simulation models
Sterilization Treatment mode Stage Somatic protection Permanence of sterility Required sterility level Fail-safe quarantine	Program continuity Staff recruitment Oversight body
Genetic sexing Multiple stocks Recombinant purge Reduced fecundity	Limiting factors Multiple species Cryptic species Commodity export Other pests/diseases
Computer models Data/managements analysis Monitor/predict production Project release rate Project distribution Simulate IPM impact Predict end point	Operational factors Labor force Regional cooperation Funding logistics Political support

impact of the released insects but also of their quality. Similarly, studies on colonization, rearing, handling, sterilization (in sterile insect technique, SIT, programs), sexing and computer modeling are closely related to the development and utilization of methodology for production, process and product control. The following sections discuss several essential subject areas that are common to the development of most release programs and are relevant to the establishment of QC.

## 2.1 Colonization

Colonization methodology naturally varies with insect species and group. Variability in the behavioral traits of field-collected founders largely determines not only the ease of colonization but also the numbers required to obtain a representative gene pool. Colonization usually must be repeated at intervals in order to replenish the original genome,

incorporate new genome into the stock, or replace the strain. The knowledge obtained during these pre-operational events provides a basis for establishing the QC methodology to assess the colonization process during the operational phase.

## 2.2 Rearing assessment

Investigations leading to the development of optimal rearing and maintenance techniques and the determination of appropriate nutritional and environmental conditions provide information for an objective selection of the most useful quality indicators. In the pre-operational phase the establishment of control charts for these parameters provides the essential basis for monitoring variability in the production, the processes and the products during the operational phase.

Suboptimal colonization and/or rearing methodology may inhibit both the colonization process and the development of a satisfactory strain. For example, one founder stock of tsetse flies produced weak individuals and a slowly developing colony when suboptimal maintenance methods were utilized. But a similar founder stock from the same location, initiated and maintained under optimal conditions, produced a rapidly developing colony with flies that performed as well as their indigenous counterparts when tested in the field two years after colonization (DAME et al. 1975).

The potential for strain adaptation during the initial generations is very high (MACKAUER 1976). Factors that inhibit development, survival or expression of behavioral traits tend to create genetic bottlenecks and narrow the genome. The resulting adaptation then alters the characteristics of the strain and may affect field performance. One of the earlier examples of this type of negative impact was observed when mosquitoes that had been forced to mate in the confinement of small cages during the colonization process failed to compete with unselected stocks for indigenous mates when released in the expanses of the natural habitat (DAME et al. 1964).

A wide variety of factors, many of which are related directly to rearing methodology, contribute to decreased competitiveness of mass-produced insects. The establishment of an enforceable program to assure that supplies and materials meet established specifications and standards should be recognized as an essential activity during the pre-operational phase. Whether a rearing deficiency be of nutritional, behavioral, environmental or pathological origin, the ability to monitor indicator parameters to detect performance shortcomings should be developed during the pre-operational research phase of the program.

## 2.3 Handling

Of the many sources of quality reduction, the effect of handling is the most obvious and usually receives the most attention. Handling effects can occur at almost any production or post-production stage; common examples include atmospheric or physical disturbance of the pupae or the stress of adult confinement. Handling stresses often occur during collection and storage of the product and in the process of distribution and release. Methods developed to stockpile large quantities of insects for subsequent use are particularly suspect as sources of product deterioration.

One of the major pre-operational research objectives is to develop methods that minimize these effects and to design techniques that enhance their detection and quantify their impact on the product. These QC-related commitments subsequently play a significant role in the operational phase.

#### 2.4 Field effectiveness

Regardless of the overall objectives of the release program, research to determine the appropriate stage for release, the optimum time of year to initiate operational releases, the potential release requirements, the dispersal capacity of the released population, and the range of post-release survival rates is an important part of the pre-operational effort. These studies provide biodata, on both the product and the indigenous population, that are essential for immediate and long term assessment of the quality of the released organism. They also provide pre-operational quantification of the suitability of the specific methods employed. Again, this activity links directly to the development and use of QC methodology to monitor operational efficiency and impact.

#### 2.5 Sterilization

Reproductive sterilization methodology is normally utilized only in SIT programs. When this is the case, a major portion of the research effort is devoted to assessment of the technique used to inhibit reproduction. The studies involve gathering data to document selection of the treatment agent and stage of development for exposure, as well as methods to protect the insect from somatic damage. Inherent in these investigations is the need to determine the permanence of the induced infertility and the level of infertility required in the released insects. Adoption of methods to assure complete security from inadvertent release of fertile insects and the establishment of fail-safe quarantine measures for the operational facility also can be considered a QC factor.

#### 2.6 Genetic sexing

The ability to separate the sexes of mass-produced arthropods, economically and without reduction in the quality of the released insects, can markedly enhance program acceptability. Under some circumstances the release of both sexes may result in additional damage to the host plant or animal. In the case of disease vectors, their release could provoke an adverse public response regardless of the fact that the ultimate near-term objective is to stem the pathogen transmission completely.

The use of genetic sexing technology provides a method for purging one sex from the release stocks early in the developmental stages, and thus offers the potential for increased rearing space. In some instances, the genetic approach provides a means to improve the quality of the released insects. For example, the genetic approach is a highly desirable alternative with anopheline mosquitoes (KAISER *et al.* 1978) for which the available non-genetic sex separation methods must be applied in the adult stage and cause unacceptable deterioration in quality. In this instance the quality of the final product, the MACHO strain, more than compensated for the extra work involved. It was necessary to devote considerable effort to selection, purification, and strain stabilization studies. The wild genome had to be reinserted into the selected stock, which had been passed through an extremely narrow genetic window. Fecundity of the MACHO stock was substantially reduced in comparison to the unselected stock, although this was readily counterbalanced by maintenance of additional cages for egg production. And, it was necessary to separately maintain susceptible, resistant, and mixed stocks and to carefully monitor the production strain for genetic recombinations that reduce the efficiency of the sexual separation. When the incidence of recombinant individuals reached an unacceptable level, it was necessary to purge the stock or replace it with a pure strain.

Thus, the production process may be more complex when genetic sexing techniques are utilized but the capacity to produce significantly more insects, which are of higher quality when liberated, may provide adequate justification for extra effort. This method also

precludes the release of sterile females which could influence assortative mating or cause other problems associated with oviposition or biting.

## 2.7 Computer models

Data generation during the pre-operational phase may be extensive, requiring the development of data management schemes, data analyses, and the construction of mathematical and descriptive computer models. Programs to prepare and analyze control charts, assess the applicability of various parameters for QC, monitor and predict colony performance and production rates, chart release distribution characteristics, manage inventories and coordinate logistical arrangements are usually developed during the pre-operational phase. These programs are then either directly convertible or provide the basis for construction of similar programs for operational needs (CALKINS and ASHLEY 1989).

Simulation models based on data generated during the pre-operational phase can simplify the management of quality control responsibilities during the operational phase. Colony performance models, field population simulations, and integrated pest management models provide a mechanism for managers to compare operational observations with expectation. They also provide management with helpful tools for allocation of resources, alteration of program directives to meet the specific situations, and prediction of program end points.

## 3 Considerations on the merit of embarking on operational programs

Many of the pre-operational QC developments provide information that is essential in weighing the merits of embarking on an operational program. Other non-QC related information also contributes to the body of knowledge and factors that influence the decision-making process.

### 3.1 QC related

Large operational SIT programs usually require geographical or habitat isolation in order to maximize the probability of success and minimize the likelihood of subsequent re-infestation. The quality monitoring methodology developed in the pre-operational phase should provide the tools necessary to estimate the dispersal of the released organisms, the feasibility and costs of long term maintenance of a production facility, and the likelihood of implementing the barrier concept.

Economic considerations relative to conducting a release program, or integrating releases with other pest management strategies, or utilizing IPM strategies without arthropod releases can be estimated from the simulation models produced in the pre-operational phase. In this regard the indigenous population simulation model and the production simulation model will play a key role in providing cost estimates of program alternatives.

The environmental impact of the program can in some respects be estimated with QC-related data. For example, the impact of the rearing facility on the immediate surroundings can be estimated in part by assessment of effect of the disposal of program-generated wastes and of the utilization of environmentally sensitive local resources. Also, from the assessment of the quality of the released stock and from estimates of the quantities required it will be possible to predict the levels of host-related damage or losses that might occur.

Estimation of the probable end point for an operational program will be assisted by the use of the simulation models, which are based on the pre-operational field assessment of the indigenous target population. By charting these field-related parameters and testing varying possibilities with the models, it should be possible to predict the time interval that

will be required for a given intensity of surveillance to be sure that the indigenous population has been reduced to an acceptable level or eliminated.

Finally, perhaps as important as all of the other considerations discussed, staff continuity should be considered essential when entering the operational phase. Proper technology transfer from the pre-operational to the operational phase can not automatically occur simply by review of the documentation generated in the earlier phase. The QC lessons learned in the pre-operational experience are not all documented for future reference. Even if it were possible to do so, the multiple relationships between observed events and subsequent occurrences probably could not be adequately conveyed to replacement staff. Thus, it seems essential to maintain continuity of concept and experience by direct recruitment of qualified pre-operational staff into the operational phase. Where possible, this admonition applies also to upper level management personnel, who play such an important role in the implementation of the program. Continuity can be further assured by appropriate selection of long-term oversight committee members.

### 3.2 Non-QC related

Factors related to the economics of the commodity at risk, such as existing import and export restrictions or the expected impact of other disease or pest problems, need to be considered but may not be related to QC per se. Similarly, the existence of multiple target species or the likelihood of cryptic species within the target habitat range deserve appropriate consideration.

Other non-QC operational factors that impact on the decision to progress to the operational phase are institutional, political and fiscal. These include an assessment of the stability of cooperating agencies, the strength of the institutional infrastructures, depth of expertise, availability of an acceptable labor force, ability to reabsorb the labor force into the post-program economy, collaboration between affected adjacent regions, political support, long term funding and logistical factors. Although this list is obviously incomplete, the significance of the non-QC related factors should not be underestimated.

## 4 Relationship of QC to program decision making

The fulfillment of pre-operational QC research commitments, coupled with the acceptability of the other decision-related factors, will not necessarily assure a positive administrative decision to initiate the operational phase. Administrators need also to consider the requirements that might arise after successful completion of the program. They need reassurance from multiple sources that the theoretical models and concepts are sound, that the engineering and facility plans are appropriate, that the scheme is realistic, feasible and workable, and that the benefits of the program outweigh any unavoidable adverse impacts.

Also, the pre-operational staff must be committed to dealing with possible biases among administrators and the general public due to inaccurate information. For example, many otherwise well-informed people incorrectly (VON BORSTEL 1960) believe that SIT programs cannot be successful if the target females mate more than once. Proper attention to informational accuracy and completeness can help to forestall such problems.

Furthermore, it can be expected that the responsible administrative authorities will want absolute proof that the proposed program will work in its entirety and that there will always be enough QC data available to support the difficult ongoing process of decision making. There is likely to be an expectation that the pre-operational QC research program has dealt with and solved all the problems, leaving no unknowns to confront the administrator. If adequate time and funds were available for the research, it might be possible to provide such assurances in some instances.

But the more likely scenario is that the cost to provide such proof will far exceed the funds that can be made available for the pre-operational research and development program. Thus, it will still be necessary for administrators to weigh carefully the available and sometimes conflicting information. Also, as complete as the daily QC information may be, even in the best run programs there will be gaps in information and feedback that will sometimes force managers to base important decisions on incomplete documentation. This reality reinforces the soundness of maintaining continuity of staff and experience, practice that should significantly enhance program efficiency.

Research is one of the foundation blocks of QC. In the pre-operational phases it is clear that the research program plays an important role in establishing the basis for all of the other elements, i.e., for total QC. Continued research support is essential during the operational phase also, in order to meet the ongoing demands for improved methodology and to become better prepared for the unexpected.

### Zusammenfassung

#### *Beziehung der Forschung zur gesamten Qualitätskontrolle unter besonderer Berücksichtigung der Sterilen-Insekten-Technik*

Die Forschung bildet nur ein Element der gesamten Qualitätskontrolle innerhalb des Programms der Insekten-Massenzucht, doch ist die präoperative Forschungsphase generell sehr umfassend und geeignet zum Sammeln von Quellenmaterial und Erfahrungen für alle Elemente der Qualitätskontrolle. Die Dauer der Forschungsphase ist stark programmabhängig, während der Umfang der Forschungsgegenstände im allgemeinen von Programm zu Programm ähnlich ist. Die Beziehungen der präoperativen Forschung bei Freilassungsprogrammen zur programmentscheidenden Phase sowie die Schaffung der Methodik der Qualitätskontrolle werden in der vorliegenden Arbeit umrissen.

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