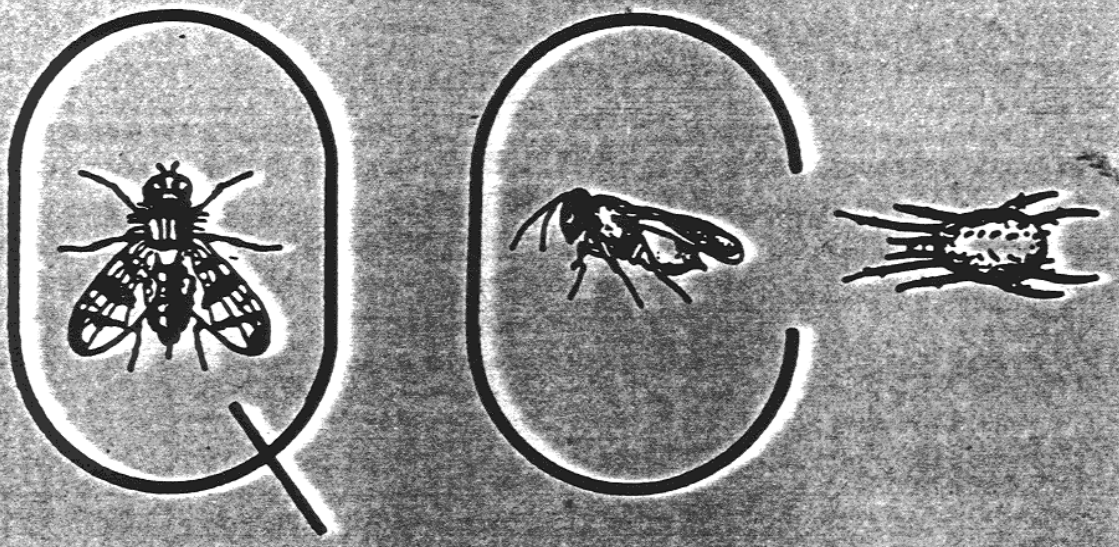
ORGANISATION INTERNATIONALE DE LUTTE BIOLOGIQUE
CONTRE LES ANIMAUX ET LES PLANTES NUISIBLES

INTERNATIONAL ORGANIZATION FOR BIOLOGICAL CONTROL
OF NOXIOUS ANIMALS AND PLANTS



Fifth workshop of the IOBC global working group " Quality control of mass reared arthropods "

Wageningen, NL, March 25-28, 1991

Proceedings

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PREFACE

The 10th anniversary of the IOBC global working group "Quality control of mass reared arthropods" has been celebrated with the 5th workshop held at the Agricultural University of Wageningen, Netherlands. Fifty scientists and production managers of twenty countries attended the meeting. It was unfortunate that only few North American members of the working group were allowed to travel to Europe because of the Gulf War. Thus, many colleagues who belong to the "founders" of the group or who joined the group recently were not present. This resulted in a weak representation of quality control in fruit flies, biting flies and Lepidopterans. Most participants are - as scientist or manager - involved in the production of entomophagous arthropods. Twelve companies, producing beneficials, were present and shared their experience and ideas on quality control for the first time.

The presented papers and the workshop sessions showed that quality control is implemented only partially in a few production systems of beneficial organisms. Though, producers are aware of the necessity, methods and techniques are still lacking for most cases. However, simple and quick methods are urgently needed in the future because governments in Europe and North America may set quality criteria for commercially produced beneficial organisms. The goals of the IOBC working group will therefore be:

1. to develop QC techniques and methods
2. to support producers of organisms for biological control in implementing QC in their mass rearing
3. to advise governments and producers when establishing quality regulations

The results of the workshop session are summarized at the end of this volume. The group was divided into a Trichogramma and Greenhouse subgroup which both aimed at establishing a first draft of guidelines for QC of commercialized beneficials (product control). These preliminary lists will be worked out based on the experience of the producers, and completed at the next workshops.

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

ACKNOWLEDGEMENTS

We gratefully acknowledge the Agricultural University of Wageningen, Department of Entomology, for the organization of the workshop.

We wish to thank the following organizations for financial support:

International Organisation for Biological Control of noxious animals and weeds (IOBC)

United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS)

C O N T E N T S

PAPERS

QUALITY CONTROL OF NATURAL ENEMIES: HOPE OR ILLUSION?

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Abstract

Mainly in two types of biological control programmes development of mass-rearing techniques is necessary: for the ***inundative*** *and the* ***seasonal inoculative*** *release method. Mass-rearing methods are usually developed on an ad hoc basis by small companies, which may result in natural enemies of bad quality and failure of a biological programme. Large scale mass-production programmes are available only for some 15 natural enemy species. The technology to rear natural enemies on "unnatural" hosts and host plants, or on artificial diets, is not far developed yet and seems to be hampered not only by physiological problems but also by ethological and ecological ones (e.g. associative learning of host-habitat cues). Conflicting requirements for natural enemies in mass-rearing programmes and field performance form another obstacle: artificial selection which occurs in the laboratory rearings may lead to a reduced efficiency in the field.*

The problems listed above make good quality-control programmes a necessity. In such programmes not only natural-enemy numbers but also natural-enemy quality (field performance) should be determined. Until very recently, variability in natural enemy performance as a result of associative learning was not taken into account. We are starting to understand the relationship between genotypic variation and phenotypic plasticity. This knowledge can be used to improve quality control programmes. Simple, representative and reliable quality control programmes for natural enemies are not yet available. Their development will, in my opinion, demand at least another decade of fundamental research.

1. Introduction

We apply quality control to mass-reared organisms to maintain the quality of the population. The overall quality of an organism is defined as the performance in its intended role after release

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

into the field. The aim of quality control is then to check whether the overall quality is maintained, but that is too general a statement to be manageble. Characteristics have to be identified which are quantifiable and which are relevant for field performance. This is a straightforward statement, but very difficult to make concrete as was explained during the last quality-control meeting of our working group by e.g. Bigler (1989).

Rather than ensuing a scientific approach on the development of quality control, I would first like to follow a more pragmatic way. The aim of releases of mass-produced natural enemies is to control a pest. In this context **the aim of quality control should be to determine whether a natural enemy is still in a condition to properly control the pest.** Formulated in this way we do not deal with terms like maximal or optimal quality, but something like **acceptable quality**. At the first discussions on quality control which I attended, I usually got the impression that the aim was to keep the quality of the mass reared population identical to that of the once collected field population. This is not only an illusion, it is an unnecessary and expensive goal to pursue. Another important consideration is that quality control is not applied for the sake of the scientist, but as a mere necessity. Leppla and Fisher (1989) formulated this dilemma as: Information is expensive, so it is important to separate "**need** to know from **nice** to know". Characteristics to be measured should be very limited in number, but directly linked to field performance will companies producing natural enemies ever be able to apply quality control on a regular basis. Quality tests can be developed by causal-analytical methods, as well as in an emperical way. Will we, however, develop robust quality control, than understanding of the causal-analytical relationships is essential.

2. Different quality criteria for different types of biological control

Quality criteria we should apply depend on the type of biological control programme in which the mass produced natural enemy will be released. Currently three types of biological control are distinguished (van Lenteren 1986b):

a. The **inoculative** release method (also known as "classical" biological control), where beneficial organisms are collected in an exploration area and introduced in the area where the pest occurs. Only a limited number of beneficials is released, the aim is a long-term control effect. The method is generally applied in forest and orchard ecosystems where a continuous existence of natural enemies can be guaranteed. Two European examples of successful programmes are the introduction of the parasite *Aphelinus mali*, against the apple woolly aphis, *Eriosoma lanigerum* in 1920 in France (Marchal 1921), and of the parasite *Prospaltella perniciosi* against the San Jose Scale *Quadraspidiotus perniciosus* in 1950 in Germany (Klett 1959).

b. The **inundative** release method, where beneficials are collected, mass reared and periodically released in large numbers to obtain an immediate control effect (i.e. use as biotic insecticide). This method is, among others, applied against univoltine pests in annual crops. A well known

European example is the application of the parasitic wasp *Trichogramma evanescens* against the european comborer, *Ostrinia nubilalis*, in Europe (e.g. Hassan 1981).

c. The **seasonal inoculative** release method, where natural enemies are collected, mass reared and periodically released in short-term crops (6-12 months) where multivoltine pests occur. A quite large number of natural enemies is released to obtain both an immediate control effect and also a build-up of a natural enemy population for control later during the same season. This method can be applied when the culture method of a crop prevents control extending over many years, for example in greenhouses where the crop together with the pests and natural enemies are removed at the end of the growing season. The method is essentially different from the inundative method, and rather resembles the inoculative method in that control is obtained for many generations of the pest and control would be permanent if the crop were grown for a much longer period. The seasonal inoculative release method has been developed in Europe during the last two decades and is applied with great commercial success in greenhouses (van Lenteren and Woets 1988). Two well known natural enemies used on a large scale are the spider mite predator *Phytoseiulus persimilis* and the whitefly parasite *Encarsia formosa*.

3. Criteria for pre-introductory evaluation of natural enemies

The type of biological control programme determines the selection criteria for natural enemies (see table 1) and, strongly related to that, the quality control aspects (see e.g. Bigler 1989). The attributes given in table 1 seem necessary for good control and are often listed in articles on evaluation of natural enemies (for reviews see van Lenteren 1980, 1983, 1986b, van Lenteren and Woets 1988). Several of the criteria are not absolute but are only meaningful in comparison to values for other natural enemies, like criteria 5 to 8. Some of the criteria have proven to be reliable (1-6), for others the usefulness still has to be shown (7-8).

Table 1: Criteria for pre-introductory evaluation of natural enemies

criterium	release programme: inoculative	seasonal inoculative	inundative
1. seasonal synchronization with host	+	-	-
2. internal synchronization with host	+	+	-
3. no negative effects	+	+	+
4. adaptation to climate	+	+	+
5. good culture method	-	+	+
6. host specificity	+	+	-
7. high kill rate potential	+	+	-
8. good searching efficiency	+	+	±

4. Obstacles in mass production

Artificial selection forces in mass rearing may lead to problems related to the criteria in table 1 if rearing conditions differ largely from the situation in which natural enemies are to be released. If temperature in the mass rearing differs considerably from the field situation, synchronization problems can be expected. Rearing on different hosts or host plants can create problems with natural enemy quality and the reaction of natural enemies to semiochemicals, etc. I will first list a number of obstacles which might be encountered in mass production programmes and then discuss a few of the less obvious ones.

1. One of the main obstacles seems to be the production of qualitatively good natural enemies at econmical costs (Beirne 1974). But with a decreasing number of pesticides available, and with increasing costs per unit of volume for chemical pesticides (Metcalf 1980) the economical aspect will become of lesser importance.
2. Another obstacle mentioned by Beirne (1974) is the lack of effective techniques for mass producing natural enemies on artificial diets.
3. Mackauer (1972) mentioned a third obstacle: the lack of techniques that prevent selection pressures leading to genetic deterioration of the mass-produced organisms. Through such a deterioration the natural enemy can loose its effectivness (Boller 1972, Boller and Chambers 1977).
4. A rather technical obstacle is cannibalism among predators which makes individual rearing (e.g. *Chrysopa*) or rearing at relatively high prey densities (e.g. *Amblyseius* and *Phytoseiulus*) necessary and may lead to very expensive rearing procedures.
5. The obstacle of behavioural changes as a result of rearing at "unnatural" conditions, or on "unnatural" hosts or on artificial media. Natural enemies may change their reaction to host or host-plant cues as a result of pre-imaginal or imaginal conditioning (Morrison and King 1977, Vet et al. 1990). Below I will addres this problem in more detail.
6. Rearing on unnatural hosts may lead to reduced vigour as the result of an inadequate supply of nutrition (both quantity and/or quality) by the unnatural host (Morrison and King 1977).
7. The same effect can occur when the host is reared on an unnatural diet (even if the host itself remains apparently unaffected)(Morrison and King 1977).
8. The rearings can be infected by pathogens. One of the problems often encountered in insect rearing is the occurrence of pathogens and microbial contaminants leading to high mortality, prolonged development, diminutive adults, wide fluctuations in the quality of insects and direct pathological effects. Goodwin (1984), Shapiro (1984), Sikorowski (1984) and Singh & Moore (1985) give information on the impact of micro-organisms on insect cultures and the measures available to minimize or eliminate the pathogens or contaminations. Further, they treat recognition of diseases and micro-organimsms in insect rearing, and the sources of microbial

contaminants. The most common microbial contaminants encountered in insect fungi, followed by bacteriae, viruses, protozoa and nematodes. The field-collected i are used to start a laboratory colony are a major source for microbial contaminan main source are the dietary ingredients. Desinfection of insects and dietary i adviced to prevent such contaminations. The cause of contamination is usually but elimination of pathogens is difficult (Bartlett 1984a).

After consideration of these obstacles my main conclusion would be to rear the natural enemies under as natural a situation as possible, which is supported by a number of researchers with experience in mass production like e.g. Bigler (1989) and King and Morrisson (1984). The problems which remain, even when rearing is done as natural as possible, are related to obstacles 3-5 and 8.

5. Conflicting requirements concerning performance of natural enemies in a mass rearing and under field conditions

Anyone starting a mass rearing does not only have to overcome the above mentioned obstacles but should also realize the conflicting requirements for natural enemies in a mass production programme and for field performance (table 2). Both, to discover whether one of the obstacles mentioned above play a role and to be able to trace if important aspects of field performance listed in table 2 do not get lost in a mass rearing, stress the need for good quality-control methods.

Table 2: Conflicting requirements concerning performance of natural enemies in a mass rearing and under field conditions

appreciated in mass rearing	important for field performance
1. polyphagy, makes rearing on unnatural host easier	mono-, oligophagy, more specific, greater pest reduction capacity
2. good parasitism at high host densities	good parasitism at low host densities
3. no strong migration as a result of direct or indirect interference	strong migration as a result of direct or indirect interference
4. migration behaviour unneccessary and unwanted, ability to disperse minimal	migration behaviour essential
5. associative learning not appreciated	associative learning appreciated

6. Development of quality control

The problem of quality control in mass-rearing programmes of entomophagous insects can be approached from two sides:

a. measure how well the insect functions in its intended role, if it does not function well enough, trace the cause and improve the rearing method

b. list what changes we can expect when a mass rearing is started, measure these and if the changes are undesired, improve the rearing method.

The disadvantage of the first method is that changes may have occurred that cannot be corrected for because the material has already changed so much that causes and their effect cannot be traced anymore. The disadvantage of the second method is that perhaps too many measurements have to be performed. I think that the second approach is best in so far that possible problems are foreseable and corrections can be made in time.

Bartlett (1984a) approaches the problem from the second viewpoint. He states that many authors have suggested remedial measures for assumed genetic deterioration, but that causes for deterioration are not so easily identified, demand detailed genetic studies and that it is difficult to define and measure detrimental genetic traits. He continuous with:" I believe an unappreciated element of this problem is that the genetic changes taking place when an insect colony is started are natural ones that occur whenever any biological organism goes from one environment to another. These processes have been very well studied as evolutionary events and involve such concepts as colonization, selection, genetic drift, effective population numbers, migration, genetic revolutions, and domestication theory." In two other articles (Bartlett 1984b and 1985) he discusses what happens to genetic variability in the process of domestication, what factors might change variability and which ones might be expected to have little or no effect. In laboratory domestications those insects are selected that have suitable genotypes to survive in this new environment (Spurway 1955). In table 3 the changes are listed that a field population may undergo when introduced into the laboratory. For more details I refer to Bartlett (1985).

Table 3. Factors influencing changes in field populations when introduced into the laboratory.

1. Laboratory populations are kept at constant environments with stable abiotic factors (light, temperature, wind, humidity) and constant biotic factors (food, no predation or parasitism), there is no selection to overcome unexpected stresses. The result is a change of the criteria that determine fitness, and a modification of the whole genetic system (Lerner 1958).
2. There is no interspecific competition in laboratory populations with as result a possible change in genetic variability (Lerner 1958).
3. Laboratory conditions are made suitable for the average, sometimes for the poorest, genotype. No choice of environment is possible as all individuals are confined to the same environment. The result is a possible decrease in genetic variability (Lerner 1958).
4. Density-dependent behaviours (e.g. searching efficiency) may be affected in laboratory situations (Bartlett 1984b).
5. Mate-selection processes may be changed because unmated or previously mated females will have restricted means of escape (Bartlett 1984b).
6. Dispersal characteristics, specifically adult flight behaviour and larval dispersal, may be severely restricted by laboratory conditions (Bush et al. 1976).

Variability in traits is usually abundantly present in natural populations (Prakash 1973), and can remain large even in inbred populations (Yamazaki 1972). But the differences between field and laboratory situations will result in differences in variability. Part of the "open population", where

gene migration can occur and environmental diversity is large, is brought into the laboratory, becomes a "closed population" and all the genetic changes will be made from the limited genetic variation in the original founders (Bartlett 1984b and 1985). The size of the founder population will directly affect how much variation will be taken from the native gene pool. Although there is no agreement on the size of founder populations for starting a mass production, a minimum number of thousand individuals is mentioned in the literature (Bartlett 1985). Founder populations for a number of natural enemies were much smaller (see van Lenteren and Woets 1988 for examples). Fitness characteristics for the field will be different than those for the laboratory (e.g. difference in importance of ability to diapause, or the ability to locate mates (role of pheromones)), so laboratory selection forces may produce a genetic revolution (Mayr 1970) and new balanced gene systems will be selected (Lopez-Fanjul and Hill 1973).

One of the cures often suggested to overcome or correct for genetic revolutions is the regular introduction of wild individuals from the field. But if the rearing conditions remain the same in the laboratory, the introduced wild individuals will be subjected to the same process of genetic revolution. If a genetic differentiation between laboratory and field population has occurred which led to genetic isolation (Oliver 1972) - and positive correlations have been found between the incompatibility of races and the difference between the environments where the races occur (e.g. Jaenson 1978, Jansson 1978), and for the length of time two populations have been isolated - introduction of native individuals seems to be useless if incompatibility is complete. If one wants to introduce wild genes it should be done regularly and from the start of a laboratory rearing onwards. It should not be delayed until problems occur. The risk of introducing native insects are concurrent introduction of parasites, predators or pathogens (Bartlett 1984b)

Another effect of laboratory colonization can be inbreeding: mating of relatives and production of progeny more homozygous than when random mating occurs in large populations. Homozygous individuals often contain harmful traits. The inbreeding coefficient is directly related to the size of the founder population and, because of artificial selection in the laboratory which results in an even smaller population size, the rate of inbreeding will increase and the result is often a definite and rapid effect on the genetic composition of the laboratory population (Bartlett 1984b and 1985). Inbreeding can be prevented by different methods to maintain genetic variability. Joslyn (1984) proposes the following methods.

1. *Precolonization methods*: selection and pooling of founder insects from throughout the range of the species to provide a wide representation of the gene pool, resulting in a greater fitness of the laboratory material.

2. *Postcolonization methods*:

   2a. *Variable laboratory environments* (variation over time and space). Although the concept is simple, putting it into practice is difficult. Consider for example the

investments for rearing facilities with varying temperatures, humidities, and light regimes, the creation of possibilities to choose from diets (hosts), the provision of space for dispersal etc., etc.

2b. Gene infusion: the regular rejuvenation of the gene pool with wild insects.

A fundamental question to this inbreeding problem is: What is the effective population size to keep genetic variation sufficiently large? Joslyn (1984) says that to maintain sufficient heterogeneity, a colony should not decline below the number of founder insects. The larger the colony the better. Very few data are available about effective population size, Joslyn mentions a minimum number of 500 individuals.

The above discussion leads to a number of criteria to be considered before a mass rearing is started. Table 4 presents such a list, which was compiled by Bartlett (1984b).

Quality control is put in a much wider perspective by Chambers and Ashley (1984) and Leppla and Fisher (1989). I strongly advice these articles to all engaged in mass production of beneficials, they present some refreshing and for most entomologists new ideas. These authors approach quality control from the industrial side and consider three elements as essential: product control, process control and production control. Product control rejects faulty products, and production control maintains consistency of production output. Process control tells how the manufacturing processes are performing.

Table 4. Criteria to be considered before starting a mass-rearing programme

1.	the effectve number of parents at the start of a mass rearing is much lower than the number of founder individuals, so start with a large population
2.	compensate for density-dependent phenomena (large cages)
3.	create a proper balance of competition, but no overcrowding
4.	set environmental conditions for the best, not the worst or average genotype, use fluctuating abiotic conditions
5.	maintain seperate laboratory strains and cross them systematically to increase F1 variability
6.	measure frequencies of biochemical and morphological markers in founder populations and monitor changes
7.	develop morphological and biochemical genetic markers for population studies
8.	determine the standards that apply to the intended use of the insects, and then adapt rearing procedures to maximize those values in the domesticated strain.

Elements as production and process control are seldomly used in mass-rearing programmes. Mass rearing, usually done by small private companies, is developed by trial-and-error. Knowledge about mass-rearing techniques is limited in such organizations, and the time (money) for extensive orientation lacks. If success is to be obtained, quality control of the end product is essential, but producers are generally more than happy if they can meet demands. Although most experts on quality control have adopted tools and procedures to regulate the processes of production so that product quality will be insured (Chambers and Ashley 1984), such tools and procedures are not yet

widely used by the smaller private companies, which make up about 95% of the total number of producers. The main cause is that development of product, process and production control requires extra financial investments which are very limited at the start of a mass-production industry. It is a short-term consideration, but it should be regarded as a serious constraint for starting producers. Quality control seems to be developed best when mass-rearing is done in large governmentally supported units. Chambers and Ashley (1984) state that entomologists often concentrate too much on production control, while at best only controlling production processes and products: "We still see quality control as an alarm and inspection system that oversees and intimidates production personnel."

During the IOBC quality control meetings, the production companies are usually very hesitant or openly refusing to discuss their methodology or standards. When we distinguish product control, process control and production control, this problem may be easier to handle. In my opinion commercial producers of natural enemies should protect their production methodology and, therefore, we cannot expect them to discuss process and production control in any detail. I would strongly press the need to have discussions on these aspects within the companies. What remains for discussion here is the aspect of product control. Setting minimum standards and simple methods to control standards is of mutual benefit to producers and researchers. It is of great importance that producers of natural enemies will be considered reliable partners in crop protection. This means that their products should have a certain basic quality. Minimal performance standards and the methods to determine these should therefore be developed and agreed upon. I see this as the first priority of this meeting and the next few years. We are going to miss an enormous opportunity in a Europe without borders, if we do not come to a positive form of cooperation.

Until now I have discussed quality problems related to collecting a founder population, and starting and running a mass production. Even if natural enemies leave the production unit in an excellent condition it does not necessarily mean that they are still in this condition when they are released in the field. Storage, shipment, developmental stage of natural enemy during shipment and release, method and moment of introduction of natural enemies can all cause a decrease of quality. I will not discuss these aspects here, but refer for examples to van Lenteren (1986a, b) and van Lenteren and Woets (1988).

7. Quality and the problem of erratic performance of natural enemies

At the previous quality control meeting I have discussed how, when, what and why natural enemies learn. For a review of this information I refer to Vet (1988). Our ideas have developed since and in coopertion with Lewis's group we have recently written two articles presenting conceptual frameworks for thinking about the variability in responses of foraging natural enemies

(Lewis et al. 1990, Vet et al. 1990).

Response patterns of natural enemies can be influenced by the insect's (1) genetic composition, (2) phenotypic plasticity and (3) physiological condition. Presently, the quest for factors inducing variability in parasite foraging behaviour has centered on the influence of experience. Experience in either pre-adult or adult stages can conceivably modify adult behaviour (e.g. Vet al al. 1990). Studies on genetic sources of variability in parasite behaviour are still very rare (see Lewis et al. 1990 for examples). Apart from interesting theoretical questions (e.g. is plasticity in behaviour adaptive?), there are several important practical considerations that make it necessary to understand the variation in behaviour, because variability in foraging behaviour ultimately results in variability in the effectiveness of natural enemies in controlling populations of insect pests. Understanding the nature of behavioural variablility may mean being able to manipulate it to our benefit.

Intraspecific intrinsic variation in foraging behaviour of natural enemies is a common but overlooked feature of natural enemies. These variations result from adaptations to the variety of foraging circumstances encountered by individuals of the species. Erratic performance of natural enemies limits their use as pest control agents. Variation in host-habitat and host-location ability can be a major source of inconsistent results in biological control. Sources of variation remain poorly understood. Most investigations have focused on extrinsic factors such as the foraging environment. Very limited consideration has been given to intraspecific variation in the natural enemies composition or behavioural state. During the past decade we have shown that foraging responses among individuals of the same natural enemy population can be quite variable, even if they are offered the precise same set of stimuli. Further, the behaviour of individuals is often plastic and can vary considerably depending on the history of that individual. Predictably effective performance of natural enemies can only be obtained by proper matching of the intrinsic condition of the searching individual with the target environment. Bearing this in mind, we first constructed a conceptual model for comprehensively examining the respective roles of such variables (Lewis et al. 1990).

One source of variation is **genetically fixed** and leads to differences among individuals that are adapted for different foraging environments. The fact that strains of parasites that occupy different regions with different climatic conditions are inherently more suited for their respective ecological conditions has well been documented and appreciated (see e.g. Pak 1988). Also, populations of a natural enemy species with longstanding associations with different hosts and habitats are known to differ in their affinity and behaviour relative to those host-habitat situations. At the natural enemy selection phase one should screen for the diversity of genetic traits and ensure that traits of the selected population are correctly attuned to the situation in which they will be released. Determination of traits can be done based on the list of criteria given before. The population should regularly be monitored for the presence of these essential traits.

Another source of foraging variation is the **phenotypic plasticity** that allows individuals to make ongoing modifications of behaviour through learning, which suits them for different host-habitat situations. Only recently have we begun to appreciate the extent to which natural enemies can learn. Many parasite species are able to acquire by experience an increased preference for and ability to forage in a particular environmental situation. There is evidence that a natural enemy may undergo some modification in its foraging traits during the immature stage (preimaginal conditioning, see e.g. van Lenteren 1986a), but variations in foraging as a result of adult experiences are much clearer and abundantly found (adult conditioning).

In order to structure our present knowledge of learning in natural enemies, we have coherently framed previously anecdotal behavioural data in a conceptual model, which is now open to experimental testing (Vet et al. 1990). As stated before, a very crucial factor inducing variability in foraging behaviour in parasitic wasps is the experience gained by the insect. Certain key stimuli evoke absolute responses that do not change, and responses of naive and experienced females are thus the same. These key stimuli act as an "anchor" by which responses to other stimuli are altered in a reliable manner. Observations of a foraging parasite's responses to stimuli involved in host finding lead to the following statements:

1. different stimuli evoke different responses or levels of response
2. strong responses are less variable than weak ones
3. learning can change response levels
4. learning increases originally low responses more than originally high responses
5. host-derived stimuli serve as rewards in associative learning of other stimuli.

The model on variation through learning specifies how the intrinsic variability of a response will depend on the magnitude of the response and predicts when and how learning will modify the insect's behaviour (Vet et al. 1990). The effect of experience on the mean and variability (and thus the predictability) of behavioural responses has interesting implications for the use of natural enemies in biological control. Unpredictable behaviour hampers the development of reliable introduction schemes and lead to the incorrect conclusion that natural enemies are of bad quality, which, in turn, can lead to disinterest in biological control, or in release of exhorbitantly high numbers of animals, resulting in high control costs. Post release migration behaviour of parasites away from the target area is considered a special problem. Increasing the mean and reducing the variability of the response to target stimuli through experience could considerably alleviate this problem. By understanding the sources and mechanisms of learning, we can provide the appropriate level of experience. This may, for example lead to artificial incorporation of semiochemicals in the diet when pre-imaginal learning plays a role. Further, pre-release exposure of the adults to crucial stimuli (e.g. frass or host scales) may be used as training technique to obtain increased parasitism in the field.

A third factor determining variation in foraging behaviour is the natural enemies **physiological state** relative to other needs, such as food and mating. Knowing the rough distribution of such needs over the lifespan of a natural enemy may lead to improved rearing and release techniques. Artificial rearing may result in deterioration of physical and physiological qualities like abnormal or lacking flight reactions to volatile host odours.

Often abnormalities in physical and physiological qualities as well as those in learning abilities, are difficult to trace. They remain unobserved and the reaction is usually that the genetic composition of a natural enemy has changed. Further, the sources of variation are not mutually exclusive but overlap extensively. With appropriate knowledge of the three major sources of intrinsic variation in foraging behaviour we can establish and engineer natural enemies with the best genotypic and phenotypic qualities for their intended application.

The simplicity of our conceptual models - which differentiate between tye influence of the genome, the environment, the physiology and experience on the behaviour - enables us to formulate clear and testable hypotheses bearing on the desired or unavoidable manipulation of natural enemies, interspecific differences in behavioural plasticity and learning mechanisms.

A straightforward conclusion - which was already drawn before based on other reasons - is that, founded on the new information about learning, the host habitat and the host should provide the same cues in mass rearing as in the field, or that the natural enemies should be exposed to these cues before being released in the field. Another important conclusion is that quality control considerations will have to include both the genotypic and phenotypic aspects of subtle but important behavioural traits.

8. Conclusions

The type of biological control programme largely determines the elements of a quality control programme: programmes for seasonal inoculative control will contain more elements than programmes for inundative control. Elements of quality control programmes should be designed to obtain acceptable quality, not per sé the best quality. The number of tests will be smallest if the natural enemies are reared under conditions at which they also have to function in the field: same climate, same host and host plant. The more artificial rearing becomes, the more tests will have to be performed and pre-release training may be necessary.

Small companies starting with production of natural enemies are often rather ignorant about the obstacles and complications related to mass rearing. They are even more ignorant about the development and application of quality control. A special point of concern is the lack of knowledge about the sources of variability of natural enemy behaviour and methods to prevent genetic deterioration of natural enemies. Organizations as the EC and IOBC could play a very positive role by developing training courses in this area to meet the rapidly increasing demand for consistent

biological control.

Cooperation within the field of quality control between state funded research and commercial producers has been a frustrating affair to date. If production and process control are left to the companies, mutually beneficial work should be possible in the area of product control. Will the biological control industry survive and florish, the production of reliable natural enemies meeting a certain quality standard is elementary. In good cooperation we will be able to provide such natural enemies and, in this way, we help solving an important environmental problem.

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QUALITY CONTROL OF MASS-REARED ARTHROPODS : A GENETICAL AND STATISTICAL APPROACH

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ABSTRACT

"Quality Control" (QC) is a well-known concept that has been developped and applied by industrial manufacturers for a considerable time. Concerning mass-reared arthropods, however, this concept must be adapted to living organisms that are known to evolve in response to environmental constraints that they encounter during release in the field. Because these constraints are essentially fluctuating and unpredictable, a statistical approach must be used to estimate the efficiency of the released biocontrol agent with only few of its fundamental biological traits.

Owing to the fact that an optimized strategy would be to release a population which is as much genetically variable as possible, this paper gives a summary of the experimental methods available to measure the genetic variability of biological features in a given population, and to perform artificial selection of these traits. Additionally, a simple well-known statistical method is presented, aiming to provide a starting framework for the screening of the traits involved in the efficiency of an insect to control the pest in the field.

In conclusion, the risks of handling this QC concept are critically discussed and other substituting concepts are proposed.

I. INTRODUCTION

What is the fundamental meaning of the «Quality Control» (QC) concept? According to the dictionary, the words «Quality» and «Control» refer to the following definitions :

«Quality» : «Superiority in kind» (among other definitions). i.e. efficient to perform the function it is devoted to. In the present case : to control a given pest, on a given crop, in a given environment, etc. It already appears difficult to speak about «intrinsic Quality» because most

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen. March 1991.

of the characteristics involved are external (i.e. environmental) factors. Therefore, «Quality» has to be defined in reference to the interactions between the mass-produced insect and the environmental characteristics it is supposed to encounter during the release. Because insects are able to evolve (i.e. to modify their biological characteristics along generations) in response to environmental constraints, «Quality» needs to be analysed from a populational point of view.

«**Control**» : «To check, to verify by evidence of experiments». i.e. to quantify. Thus, QC is also a statistical concept and is indeed considered as such by most of engineers in industry.

QC therefore refers to two complementary fields : Population Genetics and Biostatistics. These two approaches correspond to the main chapters of the present paper. In conclusion, the risks of handling this QC concept is critically discussed and other substituting concepts are proposed.

II. A GENETICAL POINT OF VIEW

Talking about QC from a genetical point of view is likely to generate fruitful hypothesis. How can we define the «Quality» of an insect in interaction with the encountered environment which is essentially fluctuating and unpredictable? A way to tackle the problem is to handle it in terms of genetic variability in the biological characteristics of the biocontrol agent that are implicated in its efficiency to control the pest. The fundamental strategy will then consist in releasing a population which is as genetically variable as possible. This would allows us to increase the chances of having at least part of this population capable of efficiently controling the target (under inundative releases) or generating the following efficient generations (under inoculative releases). This strategy, which is like hunting with buckshot instead of using a large-bore gun, makes a stand against the fact that several authors usually defined «Quality» according to uniform standards (**Boller & Chambers**, 1977 ; **Chambers & Ashley**, 1984 ; **Raulston & King**, 1984 and others). This genetical point of view leads us to forget individuals and to consider only populations : an individual cannot have for certain a better «Quality» than an other one, but, in return, a population (or a strain, or even a species) can utterly be much more efficient as compared to an other one for a given biological control program.

These considerations imply to be able (1) to quantify accurately the genetic variability observed in the biological characteristics of the biocontrol agent and (2) to maintain this genetic variability (if any) in mass-rearing procedures. Moreover, if a significant genetic variation is found, (3) an artificial selection program of the desired biological traits can be performed. These three main points are discussed below.

II.1. EXPERIMENTAL PROCEDURES TO MEASURE THE GENETIC VARIABILITY IN BIOLOGICAL TRAITS

Several general genetics books such as **Falconer** (1972) or **Merrell** (1975) provide the basic information to perfom genetic analysis of biological traits in a given population. Basically, when variability is observed within a population, two main experimental procedures are available to check if some genetic determinisms are involved :

- *Parents/offsprings regression analysis* : The character under study is measured on a set of parents, and on a set of their offspring. Then, a regression analysis is done over the two successive generations to see if the characteristics of the parents are genetically inherited to their progeny. An example of the use of this method, which concerns the analysis of the variation in Handling Time in *Trichogramma maidis* (Hym.; Chalcidoidea) is provided in **Wajnberg** (1989).

This simple procedure can be generalized when the analysed character cannot be quantified by a single value. For example, several quantitative features are sometimes necessary to quantify a single behavioural trait. In such a case, a multivariate regression method (i.e. canonical regression analysis) can be used to study the correlation between parent and offspring generations. Such an example is given in **Wajnberg** (1991).

- *Family analysis* : Each family is founded by a single couple (or a mated female). Then, the trait analysed is measured on several individuals in each family. Finally, the average values are compared with a standard ANOVA procedure. If the null hypothesis is rejected, the trait can thus be considered as a family feature which strongly suggests the existence of a genetic basis of the observed variability. This procedure is sometimes called «isofemale lines methods» (**Parsons**, 1980). Examples are given in **Wajnberg** *et al* (1985) on *Drosophila* larvae suitability to endoparasitoids and in **Wajnberg** *et al* (1989) on the superparasitism intensity in *Trichogramma maidis*.

This method, which differs from the preceding one by the fact that the trait is measured on a single generation, can also be generalized to the multivariate case. Each individual is then characterized by a set of variables and the multivariate mean-vectors corresponding to each family are compared with a standard multivariate analysis of variance (MANOVA, **Smith** *et al*, 1962).

When the species studied are diplo-diploid (which is usually not the case for hymenopterous parasitoids that are haplo-diploid) the heritability of the traits (i.e. the proportion of the total variability observed attributable to additive genetic effect) can be estimated from the statistical parameters obtained by the procedures described above (i.e. from regression coefficient, correlation, etc.) (see **Falconer**, 1972 or **Collins**, 1984 for a review).

These two main approaches are in fact the basic framework from which numerous experimental protocols can be drawn.

II.2. MAINTENANCE OF GENETIC VARIABILITY

Once a genetic variability is observed (with the tools briefly described above) for a given set of fundamental biological traits, we must try to keep it (and/or even to increasing it) during the mass-rearing procedure (**Joslyn**, 1984). In fact, the problem of the maintenance of the genetic variation in (natural or laboratory) populations is a basic question of population genetics (see **Lewontin**, 1974 or **Hartl**, 1980 for a review). Schematically, it can be shown that the amount of genetic variation in a given population can suffer reduction under three main processes (**Falconer**, 1972) :

- *Migration* : Some individuals may leave the population they belong to and thus some alleles may dissappear.

- *Genetic drift or founder effect* : Due to the fact that all individuals of a given generation do not genetically equally contribute to the next one, some alleles may be randomly lost at each generation. This process, as well as inbreeding, will progressively lead to a reduction of the genetic variability within the population. This reduction will be proportional to the number of generations, and inversely proportional to the number of individuals of a generation founding the following one.

- *Directional selection* : Under standard conditions, as it is almost always the case in laboratory mass-rearing, some genotypes are favoured and unfitted individuals usually die. After few generations, the genotypic variance may rapidly be reduced.

These different mechanisms cannot be avoided. For the best, they can be reduced as much as possible. Therefore, in several laboratories, different procedures are usually used on that purpose :

- Wild individuals are periodically trapped in the field and «reinjected» in the mass-reared population to renew the gene pool. This artificial kind of immigration is sometimes called «gene infusion» (**Joclyn**, 1984).

- Fluctuating mass-rearing conditions (in space and/or in time) must be preferred instead of standard conditions. This can be done automatically (i.e. fluctuating photoperiod, thermo-period, etc.) or manually (i.e. for some parasitoids : some rearing generations on a factitious

host and some others on the natural host) (see **Van Bergeijk** *et al*, 1989)).

- Concerning genetic drift, we must be aware that each laboratory generation must not be founded by too few individuals. Moreover, another complementary possibility is to rear insects in parallel independent sub-populations that are pooled just before release. When possible, the insects may be put into diapause during winter in order to reduce as much as possible the number of laboratory generations. Finally, in order to limit inbreeding, some laboratory strains are reared with a regular crossing procedure such as the one presented on Fig. 1. (**Plantevin**, 1975).

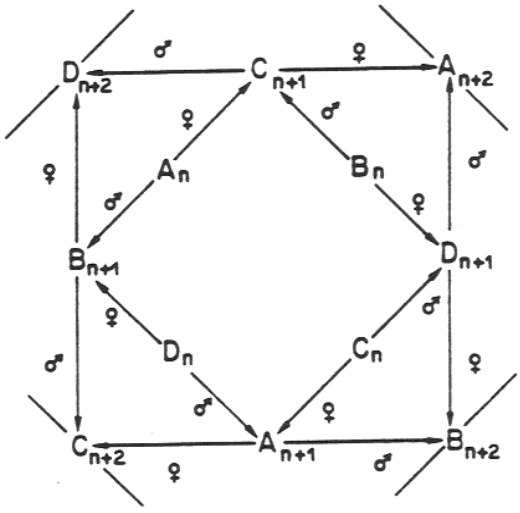

Figure 1 : Example of regular crossing procedure used to limit inbreeding in mass-reared populations. In this graph, the reared population is divided into four sub-populations (A, B, C & D) that are crossed following a regular pattern from generation to generation. Such a procedure can be done with more than four sub-populations. reproduced from **Plantevin** (1975) with permission.

II.3. ARTIFICIAL SELECTION OF BIOLOGICAL TRAITS

When a genetic variability is observed for a given set of biological characteristics, an artificial selection program can be done to improve the efficiency of the agent under field release. Several general texts already deal with theoretical and practical means to artificial selection (see **Li**, 1968 or **Falconer**, 1972 for a review). In short, all of the existing methods can be classified into two categories : in mass selection, the individuals that found the next generation are chosen according to their own phenotype. In family selection, however, the

choice of individuals is based on the mean phenotype of the family which they come from. Both of these methods also enable to select several characters simultaneously by combining each of the (weighted) phenotypic measurements into a single value (with a linear index) which is used to determine the culling level (**Falconer**, 1972).

III. A STATISTICAL POINT OF VIEW

As discussed above, the efficiency of a biocontrol agent is likely to depend on the environmental constraints encountered during the release. Therefore, an ideal way to work on QC would be to get an exhaustive knowledge of these environmental characteristics. This is, of course, not possible. Therefore, statistical tools have to be used to estimate as much accurately as possible the efficiency of a biological control agent with only a few of its fundamental biological traits.

Among the different available methods, the multiple linear regression analysis seems to provide substantial help. Its principle is quite simple and, these days, softwares are available on any kind of computer to make the calculations. The use of this method can lead : (1) to get an estimation of the efficiency of a released population only from a few parameters easy to measure (either biological traits of the agent or environmental features). (2) to identify, among all the parameters used, those that are really implicated in the biocontrol efficiency of the released population, or even to suggest new parameters for further tests. (3) to recognize, among all compared populations (or strains, or species), those that are actually the most efficient in a given environment.

Let's get into some details of the principles of this statistical method : the fundamental aim is to estimate the efficiency (Y) of a released population (expressed as % of parasitism or predation, or as density of the pest remaining in the field) from few simple traits (X_1, X_2, ..., X_p) (e.g. sex ratio, longevity, fecundity, host acceptance, etc.). These characters are recorded on a set of different populations. The model we want to fit to the data is (for the i^{th} population):

$$Y_i = a_0 + a_1X_{1i} + a_2X_{2i} + ... + a_pX_{pi} + e_i$$

where e_i, the error components, are supposed to follow independent normal distributions with a null average and a constant variance over all populations. Let $\hat{Y}$ an estimation of Y

obtained from this model, the procedure leads thus to estimate the coefficient a_0, a_1, a_2, ..., a_p that minimized $\sum_{(i)} (Y_i - \hat{Y}_i)^2$ (least square procedure).

Once the coefficients are computed, an estimation of the efficiency of any population can be obtained from only the traits measured in the laboratory. Moreover, these coefficients are estimated with confidence intervals. Thus, if the different traits measured are not too much correlated between each other, the hypothesis that each of these coefficients are equal to zero can be tested. Owing to the fact that this enables to rapidly know the importance of every measured traits, this procedure allows to test any kind of parameters, and to retain only those that are significant. Finally, from the study of the residual values ($Y-\hat{Y}$) (i.e. the incapacity to estimate correctly with this method the biological control efficiency) for each tested population, it becomes possible to identify the populations that present the higher efficiency, as compared to the global information contained in the data. An accurate analysis of these populations could lead to new hypotheses suggesting to study other biological traits that were not previously taken into account.

This simple statistical method is in fact a basic approach from which other analyses have to be developped (i.e. to include threshold effects and any kind of limiting factors).

IV. CONCLUSION

From both a genetical and a statistical point of view, the «Quality» of an insect appears to be difficult to define and to quantify. And, as a devil's advocate, I think that we have to be careful when handling this concept because :

- QC concept often seems to be too rigid or mechanistic. The use of this concept inclines to wrongly consider an insect as a chemical compound (the more you release, the better the control is).

- This concept misleadingly supposes that insects have *a priori* to be phenotypically fixed and optimized.

- This concept does not take into account the variation in the environmental abiotic characteristics the agent is likely to encounter in the field.

- Neither does it take into account the variations in the biotic environmental features (such as the evolution of the host and/or the plant).

- Finally, this concept is wrongly considered by some authors to be typically production oriented in spite of the fact that the final result happens in the field during the release (**King**

& **Morrison**, 1984).

What is it possible to do to keep away from falling into the different traps of this concept?

- We might change it, and thus rather talk about «efficiency» of the released population as it is done here. Then the environmental factors are implicitly taken into account (**Wajnberg & Pizzol**, 1989).

- We might complete it with accurate study of the (genetic) variations involved in the biological traits studied, and not only of the average values.

- We should try to know, as accurately as possible, the environmental characteristics (both biotic and abiotic) that the released population is likely to encounter in the field. An insect can be very efficient against a given pest in a given environment, but totally ineffective against the same pest in an other environment.

- We might continuously look for other biological characters in order to progressively improve our accuracy to estimate the efficiency of the mass-reared insect. This implies an unremitting comparison between the data collected in the laboratory and those collected in the field.

Finally, an *a priori* accurate estimation of an insect efficiency to control a given pest appears to be a tremendous piece of work which could be, in some case, unrealistic.

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THE INHERITANCE OF CLUTCH SIZE REGULATION IN TRICHOGRAMMA SPECIES (HYMENOPTERA: CHALCIDOIDEA: TRICHOGRAMMATIDAE)

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Key words: *Trichogramma*, clutch size, parasitism, genetics.

ABSTRACT

Parasitoid wasps of the genus *Trichogramma* adjust the number of progeny allocated to their insect egg hosts in response to host volume. The sensitivity of this response, and the range of host sizes accepted by the wasps, are important parameters influencing yields during mass rearing and the management of efficacy in the field. Data are presented which demonstrate that some variations in clutch size regulation are inherited. Significant correlations with maternal behaviour could be obtained for both the number of eggs laid per host and discrimination between hosts of different volumes. These results are discussed in terms of their consequences for the maintenance of quality during mass rearing of these parasitoids.

INTRODUCTION

Mass rearing of beneficial arthropods is accompanied by the potential for changing the genetic profile of the population. Selective factors inherent in the rearing process may affect both the behaviour and physiology of the insects or mites produced (Bartlett 1985). These considerations are especially relevant for those methodologies that employ factitious hosts, and relatively crowded and inbred populations of parasitoids or predators (Waage et al. 1985). The rearing of *Trichogramma* spp. for inundative release has required the use of cheaply produced cereal moth eggs as hosts. These eggs are presented to the parasitoids under conditions in which both hosts and wasps are concentrated in a relatively small area (Morrison 1985). In this situation, intraspecific competition is likely and selection of behaviours advantageous in the rearing environment may occur. However, these same behaviours may have deleterious consequences for the efficacy of *Trichogramma* spp. applied in the field.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

MATERIALS AND METHODS

Cultures

Cultures of *Trichogramma dendrolimi* Matsumura were obtained from the Department of Entomology, Wageningen University, The Netherlands. Two geographical strains were used, one originating from Czechoslovakia, the other from Romania (see Pak & van Heiningen 1985). The cultures were maintained on the eggs of *E. kuehniella*, at 25°, 70% RH and 16:8 L:D photoperiod. The eggs of *E. kuehniella* were killed with UV and stored at 8°C before use.

Cultures of *Trichogramma evanescens* Westwood were obtained from the Department of Environmental Biology, University of Guelph, Guelph, Ontario. The cultures were maintained on *Manduca sexta* L. (Sphingidae) eggs obtained from the Carolina Biological Supply Company, Burlington, N. Carolina. Thirty generations were reared at 25°C, 50-60% RH and 16:8 L:D photoperiod. Eggs of *M. sexta* were killed by freezing at -20°C and were stored at -5°C.

In all experiments, unfed, one-day-old, mated wasps were used. The wasps had no prior opportunity for oviposition. Each wasp was used once.

Eggs of *Mamestra brassicae* L. (Noctuidae), obtained from cultures at Wageningen University, were used as hosts for experiments with *T. dendrolimi*. Eggs were collected daily and stored at 4°C. Eggs were used within 24 hours of collection. Eggs of *M. sexta* were used in all experiments with *T. evanescens*. For each experiment, all host eggs were drawn from the same batch to maximize uniformity. Hosts were presented on cardboard cards (1 x 1 cm) and attached using a small amount of acacia gum.

Experiment I

Progeny allocation by the two strains of *T. dendrolimi* parasitizing *M. brassicae* eggs was determined by exposing 20 hosts to a single female for two hours. The experiment was conducted at 20°C and 60% R.H. under fluorescent lighting. At the same time, both male x female and female x male crosses were made between the strains. Successful mating was verified by the production of female hybrids. Hybrid progeny were subsequently exposed to *M. brassicae* eggs to evaluate their progeny allocation behaviour. Four replications with 25 wasps in each treatment group were conducted. All treatments within replications were completed on the same day, using eggs from the same batch to avoid host egg effects. Parasitized eggs were separated and maintained at 20°C in individual gelatin capsules. The number of emergents was determined for each host.

Experiment II

Inheritance of clutch size in *T. evanescens* was evaluated by comparing maternal and daughter responses to eggs of *M. sexta*. Each of forty wasps was exposed for 1 hour to 9 eggs

which were mounted in a 3 x 3 pattern with 2 mm between hosts. Wasps were observed and parasitized hosts were removed and reared separately to ensure against superparasitism. Experiments were conducted under diffuse daylight, at 23-24°C and 40% RH. The number of emergents was determined for each host. A single female was then selected randomly from the progeny of each female. This daughter was then exposed to *M. sexta* eggs under conditions identical to those used for the maternal generation. The number of emergents from each host was determined.

Experiment III

Allocation of progeny by *T. evanescens* in response to differences in exposed volume of *M. sexta* hosts was evaluated. Wasps were presented with 9 hosts for 1 hour. Conditions were as for Experiment II. Two treatments were tested. Fully exposed hosts were mounted on the surface of 1 x 1 cm cardboard pieces. Partially embedded hosts were placed in 1.3-1.4 mm diameter holes punched into similar cardboard squares (see Schmidt & Smith 1985). Wasps were observed ovipositing and parasitized hosts were removed to prevent superparasitism. The number of emergents from each host was subsequently determined.

Experiment IV

A selection experiment was conducted using *T. dendrolimi* from the Czechoslovakian strain. Wasps were selected to minimize or maximize the difference in clutch size allocated to fully exposed and partially embedded hosts. Eggs of *M. brassicae* were used. The hosts were presented on 4 x 1 cm cards. Nine or ten hosts were mounted on the surface. Another nine or ten were placed in holes made in the cards such that only part of the egg surface was exposed. Wasps were allowed to parasitize the eggs freely for 48 h at 25°C.

In the first generation, twenty-five wasps were isolated and individually presented with cards bearing a mixture of surface mounted or embedded hosts. After parasitization, the hosts were removed, separated and incubated individually in gelatine capsules. The number of emergents per parasitized host was determined for each female and host type. Daughters of the female which showed the least difference in allocation to the two host types were selected to found the minimal difference group. Daughters of the female which differed most greatly in the clutch size allocated to the two host types were selected to initiate the maximal difference group.

In each of the subsequent generations, identical methods were used. Each wasp was exposed to a mixture of hosts. Following parasitization, the hosts were separated and incubated individually. The number of emergents was determined for each host type. Daughters of the female showing either the greatest or least difference in progeny allocation to the two host types were chosen to propagate their respective groups for the next generation. The experiment was continued for five generations.

Table 1. Differences in progeny allocation between geographic races or strains of *T. dendrolimi*, and between hybrids parasitizing 24-h-old eggs of *M. brassicae* (means ± Standard Deviation).

Replicate	Strain	No. wasps	Total no. eggs parasitized	% Hosts which produced one emergent	No. emergents/ host parasitized*
I	Czech.	23	241	17.8	1.9 ± 0.5^{a}
	Rom.	22	241	7.5	2.1 ± 0.5^{b}
	Czech. ♀ x Rom. ♂	25	374	9.6	2.1 ± 0.5^{b}
	Rom. ♀ x Czech. ♂	25	369	6.8	$2.0 \pm 0.4^{a,b}$
II	Czech.	25	200	15.5	1.9 ± 0.4^{a}
	Rom.	25	177	2.3	2.1 ± 0.4^{b}
	Czech. ♀ x Rom. ♂	22	166	4.8	$2.1 \pm 0.4^{b,c}$
	Rom. ♀ x Czech. ♂	25	229	7.4	2.0 ± 0.4^{c}
III	Czech.	25	303	10.9	2.0 ± 0.5^{a}
	Rom.	25	356	5.3	2.2 ± 0.5^{b}
	Czech. ♀ x Rom. ♂	25	367	5.7	2.1 ± 0.5^{c}
	Rom. ♀ x Czech. ♂	25	372	9.4	2.0 ± 0.5^{a}
IV	Czech.	23	180	28.8	1.7 ± 0.5^{a}
	Rom.	21	300	6.3	2.1 ± 0.5^{b}

*Significant differences between the means within replicates are indicated with different superscript letters (Kruskall-Wallis, analysis by rank, $p < 0.05$).

RESULTS

Experiment I

In all replications, the mean clutch size of the Romanian strain of *T. dendrolimi* was significantly greater than that of the Czechoslovakian strain (Table 1). This effect is largely the result of the higher percentage of *M. brassicae* hosts allocated a single egg by females of the Czechoslovakian strain. Hybrids of the two strains were fertile, and produced clutches of intermediate size. However, the distribution of clutch sizes allocated by the hybrids resembled that of the Romanian strain, with few hosts allocated only a single egg (Table 1).

Experiment II

Both mothers and daughters parasitized similar numbers of hosts, and mean clutch sizes did not differ significantly between the two groups (Table 2). There was a significant positive, linear correlation between maternal clutch size and that of the next generation ($r=0.86$, $p<0.05$) (Table 2).

Table 2. Comparison of Parental and F_1 generations: Parasitization of *M. sexta* hosts.

Generation	No. wasps	No. hosts parasitized/wasp	No. emergents/host
Parental	18	2.3 ± 1.3^{a}	10.8 ± 2.8^{b}
F_1	18	3.0 ± 1.8^{a}	10.0 ± 3.4^{b}

No significant differences between means with same superscript ($p>0.05$)

Linear regression:	correlation coefficient	0.74	
	regression slope	0.92	significant $p<0.05$

Experiment III

Significantly fewer progeny are allocated to partially embedded hosts ($p<0.001$). Fully exposed hosts were allocated an average of 12.6 ± 4.7 ($n=24$), while partially embedded hosts were allocated a mean of 7.9 ± 2.0 ($n=18$). The distribution of clutch sizes also differs for the two groups (Fig. 1). The range of clutch sizes allocated to fully exposed hosts completely overlaps the range for partially embedded hosts. The range of clutch sizes for partially embedded hosts is more restricted (Fig. 1).

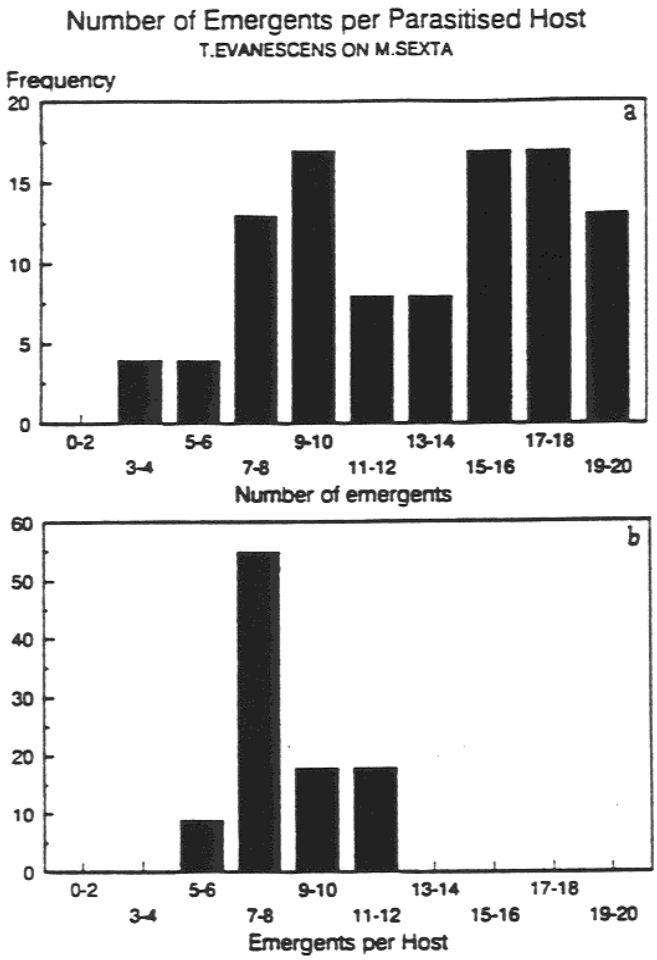

Fig. 1. Experiment III. *Trichogramma evanescens* parasitizing eggs of *Manduca sexta*. Number of progeny allocated per host.

a) Fully exposed hosts mounted on the surface.
b) Hosts partially embedded in the substrate.

Experiment IV

The results demonstrate that selection will maintain a difference in progeny allocation behaviour in *T. dendrolimi* (Table 3). In the strain selected for minimal differences, the foundress allocated an average of 2.0 ± 0.7 eggs to partially embedded hosts and 1.8 ± 0.5 eggs to fully exposed hosts. After five generations her descendants allocated an average of 2.3 ± 0.5 eggs to partially embedded hosts and 2.3 ± 0.5 eggs to fully exposed hosts. In this line non-discrimination between the two host types was maintained.

In the strain selected for maximal differences, the foundress allocated a mean of 1.5 ± 0.7 progeny to partially embedded hosts, and 2.2 ± 0.6 progeny to fully exposed hosts. This ability to discriminate was maintained consistently through five generations. The fifth-generation descendants allocated 2.1 ± 0.5 progeny to partially embedded hosts and 2.5 ± 0.5 progeny to fully exposed hosts.

Table 3. Comparison of progeny allocation for two selected strains of *T. dendrolimi* reared on *M. brassicae*. Strains were selected for minimal or maximal discrimination between partially embedded and fully exposed hosts.

Strain	Selected for Minimal Difference					Selected for Maximal Difference				
Generation	1	2	3	4	5	1	2	3	4	5
No. of wasps parasitizing	1	4	2	4	15	1	4	2	4	15
No. of partially embedded hosts parasitized	5	23	15	39	104	2	18	16	40	112
Emergents/partially embedded host	2.0±0.7	2.4±0.7	2.3±0.5	2.0±0.7	2.3±0.5	1.5±0.7	2.3±0.5	2.3±0.7	1.6±0.5	2.1±0.5
No. of fully exposed hosts parasitized	4	22	16	55	117	10	22	18	51	104
Emergents/fully exposed host	1.8±0.5	2.2±0.7	2.2±0.4	2.0±0.5	2.3±0.6	2.2±0.6	2.8±0.5	2.6±0.5	2.2±0.6	2.5±0.5
Ratio of emergents from both host types (partially embedded/fully exposed)	1.1	1.1	1.1	1.0	1.0	0.7	0.8	0.9	0.7	0.8

The pattern of distribution of eggs between the two host types differs for these two strains (Fig. 2). An important factor appears to be the increased allocation of three eggs to fully exposed hosts by the maximal difference group.

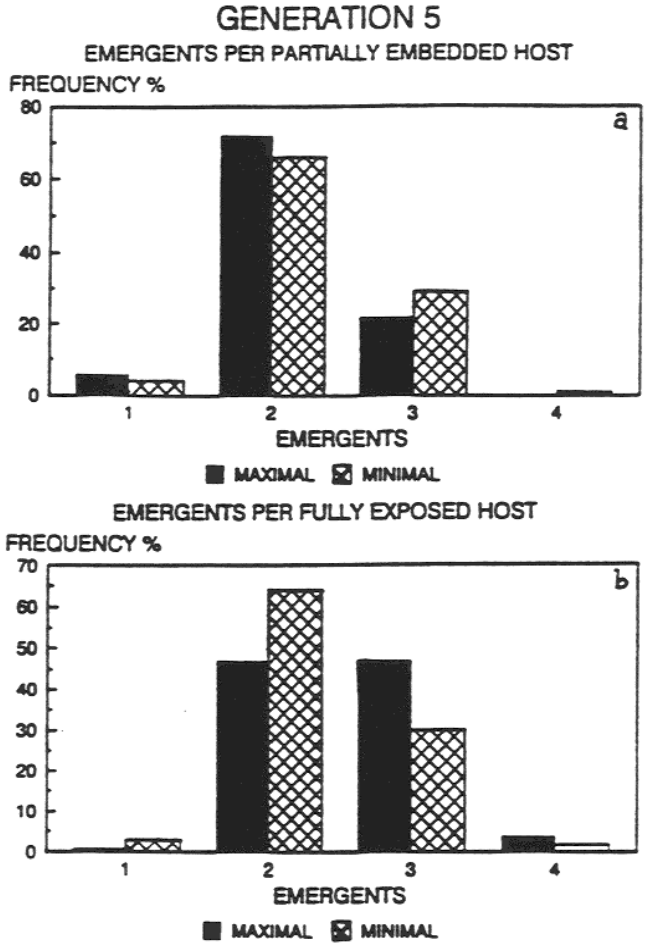

Fig. 2. Experiment IV. *Trichogramma dendrolimi* parasitizing eggs of *Mamestra brassicae*. Fifth generation of selection for minimal and maximal discrimination between differences in exposed host volume.

a) Emergents per partially embedded host.
b) Emergents per surface mounted, fully exposed host.

DISCUSSION

The results of these various experiments indicate that both clutch size and discrimination between hosts differing in exposed volume are heritable characteristics in *Trichogramma* spp. In Experiment I, two geographic strains showed consistent differences in the pattern of progeny allocation, particularly in the production of clutches containing a single egg. Hybrids of these strains produced mean clutch sizes intermediate between those of the original strains. Replications using different batches of host eggs showed that this difference was consistent despite possible differences in composition between the host eggs.

However, the setting of clutch size may not directly reflect any change in the perception of host volume itself, but could depend on differences in assessing the nutritional quality of the host contents.

In Experiment II, the heritability of clutch size was shown for variations within a population. Daughters allocated numbers of progeny to the hosts which corresponded to the clutch sizes of their mothers. Neither differences in host quality nor superparasitism (which was prevented) account for the observed correlations. Rates of parasitization and mean clutch size were similar for both mothers and daughters, indicating that the quality of hosts presented to both generations were probably comparable.

In Experiment III, the range of clutch sizes allocated to fully exposed *M. sexta* hosts was highly variable. In contrast, the range of clutches allocated to partially embedded hosts is more narrow. Importantly, the range for fully exposed hosts overlaps that for partially embedded hosts, suggesting that some wasps may not distinguish between the two host presentations. Since clutch size is partially inherited, as shown in Experiment II, the lack of discrimination of host volume may also be inherited.

To test for the inheritance of discriminatory ability, the selective breeding of discriminating and non-discriminating strains of *T. dendrolimi* was conducted. The success of this program in Experiment IV shows the potential for establishing these behavioural traits within a population of *Trichogramma*. The shift in progeny allocation that occurs among the discriminating wasps appears to be a significant reduction in the number of hosts receiving only two eggs, and an equivalent increase in the number allocated three eggs. Since the hosts and the manner of presentation were comparable for both groups, differences in response to exposed volume must account for the results.

Since both absolute clutch size, and the ability or inability to discriminate between host sizes are heritable and can be subject to selection in *Trichogramma* spp. populations, these parameters should be considered in the design of mass rearing systems. It is noteworthy that in the laboratory population from which the *T. dendrolimi* females used in Experiment IV were drawn, only 8 of 25 (32%) females screened discriminated between hosts differing in exposed volume. This suggests that the ability to discriminate was not favoured under these mass rearing conditions. This may well be the case in similar rearing systems.

ACKNOWLEDGEMENTS

Many thanks to J. Verburg, who assisted greatly in the collection of data for *T. dendrolimi*, and to K. Konze who helped collect data on *T. evanescens*. Thanks also to Dr. G. Pak and the Department of Entomology of Wageningen for the use of the insect cultures and facilities. This work was partly supported by a NATO Postdoctoral Fellowship and by the Natural Sciences and Engineering Research Council of Canada.

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GENETIC PROBLEMS CAUSED BY SEX DETERMINATION IN THE REARING OF PARASITOIDS

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Abstract

Diploid males have recently been found in several Braconid and Ichneumonid species. Such diploid males are the result of the sex-determination mechanism, in which the sex of diploid individuals is determined by the alleles on a sex-locus. Homozygous individuals become males, whereas heterozygous individuals become females. The proportion of fertilized eggs that become male depends on the number of different sex-alleles and the mating stucture of the population. When wasps from these families are cultured for several generations in insectaries a large loss of sex alleles can occur. Such reduced allelic variance at the sex locus results in a high percentage of diploid individuals becoming males, skewing the sex ratio of these cultures towards males and impairing the potential rate of increase of the population. A reduced potential rate of increase can lead to a lower rate both of establishment and of succesful biological control. Computer simulations were done which show that the loss of sex alleles in the laboratory can be minimized by maintaining either very large populations or by subdividing the original collection in many separate populations.

Introduction

Populations of natural enemies used for biological control often go through bottlenecks when only a limited number of individuals is imported for colonization. The effect of such bottlenecks is a reduced genetic variability, which is considered to be detrimental for the biological control potential of that population. In Hymenoptera

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

the reduced genetic variability causes an additional problem; the production of diploid male offspring from fertilized eggs. Fertilized eggs normally give rise to female offspring, the replacement of females by diploid males causes a reduced population growth rate. Additionally diploid males generally produce sperm that is incapable of fertilizing eggs, leaving females they mate with functionally virgins further impairing the growth rate of the wasp population.

The occurence of diploid males is a consequence of the sex determining mechanisms in certain Hymenoptera, i.e. the Whiting scheme named after A.R. & P.W. Whiting (reviewed in Whiting 1961). The Whitings studied the sex determination in Habrobracon hebetor and found that haploid individuals are always males, while diploid individuals are generally females. However under inbreeding conditions some diploid individuals become males. The occurence of diploid males has long only been known from Habrobracon hebetor, but recently diploid males have been reported from many social Hymenoptera, two sawflies and several Ichneumonoidea (reviewed in Luck et al 1991).

The purpose of this paper is to call attention to the implications of this sex determining mechanism for biological control.

Whiting scheme of sex determination.

The sex of an individual is determined by the alleles at a sex locus. Several alleles are found in a population. Haploid individuals have only one allele at the sex locus and are always males. A diploid individual has two alleles at the sex locus and can either be homozygous (have two identical copies of an allele for instance AA or BB) or heterozygous (have two different alleles for instance AB or AC). Homozygous individuals are males and heterozygous individuals females. Under inbreeding conditions, when for instance a female (AB) mates with her own son (either A or B), 50% of the fertilized eggs should give rise to diploid males, i.e. those that are either AA or BB. This is the Whiting scheme in its basic form. However in experiments where Habrobracon hebetor females are mated with their sons much less than 50% of the diploid offspring is male (Torvik 1931). Diploid males appear to suffer a higher mortality than diploid females (Petters & Mettus 1980). About 90% of the diploid males that do survive to adulthood are sterile in North American Habrobracon

hebetor (Whiting 1925, Torvik 1931). Diploid sperm of Diadromus pulchellus is able to penetrate the egg, but such fertilized eggs have a high mortality (Chauvin et al 1988).

Population effects of Whiting sex determination system.

The occurrence of diploid males in a population causes the potential rate of increase of that population to be lower because some fertilized eggs become diploid male eggs that either die during their development or become diploid male adults. In either case some eggs that in a normal population become females now fail to do so. Secondly the diploid males that do emerge as adults can inseminate females, but their insemination results in only haploid male offspring or in triploid sterile daughters. This also has a negative effect on the potential rate of increase of the population. How severe these negative effects are on a population depends on two factors i.e. the number of alleles at the sex locus and the mating system.

The number of different alleles that has been detected in Hymenoptera ranges from 9 in Habrobracon hebetor (Whiting 1943, 1961) to 19 in the honeybee Apis mellifera (Adams et al 1977). The relatedness of individuals participating in crosses also influences the proportion of diploid males produced. If there is random mating in a population with n sex alleles there will be selection for each allele to occur with a frequency of 1/n (Yokoyama & Nei 1979). In such a population the chance that a female mates with a male that carries one of her sex alleles equals 2/n. In situations where matings are not random but where strict sibmating occurs, this fraction increases dramatically, and the percentage of males among the diploids should approach 1/2.

Distibution of male diploidy in parasitic Hymenoptera

In the parasitic Hymenoptera diploid males in response to inbreeding seem to be restricted to some of the Ichneumonoidea (Habrobracon hebetor Whiting 1943, Inaba 1939, H. serinopae, Clark et al 1963, Bathyplectes curculionis, Unruh et al 1984 and Diadromus pulchellus, Hedderwick et al 1985). The diploid males found in Nasonia vitripennis are caused by a mutation and therefore are not an example

resulting from the Whiting scheme of sex determination (Whiting 1960). Parasitic Hymenoptera, other than the Ichneumononidea and Braconidae probably have a sex determining mechanism that differs from the Whiting scheme.

The Whiting scheme and biological control

When a population is imported for biological control purposes and is maintained in the lab for several generations, sex alleles are likely to be lost from the population. Initially there is a chance that the field sample contains only a subset of the sex alleles from the population. Secondly through genetic drift some sex alleles may be lost during the lab rearing, particularly if the lab rearing experiences periods reduced population sizes. The loss of many sex alleles results in two negative effects for the use of these wasps in biological control: 1) a more male biased sex ratio and 2) a reduced growth rate of the wasp population.

The sex ratio of the wasp population is important for economic reasons when large numbers have to be reared. Because only female wasps are the effective biological control agents, the sex ratio in the mass rearing directly influences the cost of a biological control progam. The intrinsic rate of increase of a population is important in bringing a pest population under control. The higher the rate of increase the faster the pest may be brought under control, but also the better the chance that the wasps become established. After release small differences in intrinsic rate of increase can make large differences over a few generations. For instance the production of 70 v.s 50 female offspring per female results in a more than a five fold difference in total number of females in the fifth generation.

The relationships between the sex ratio (SR = percentage female offspring) in a very large randomly mating population containing n alleles where each female produces R daughters, are given below. When an infinite number of sex alleles is present in the population, SR equals the fertilization rate of eggs (F), while R equals the maximal number of daughters per females (RM). When diploid males survive but do not participate in mating the relationships are: $SR(n) = F * (1-1/n)$ and $R(n) = RM * (1-1/n)$. When the all diploid males die before reaching adulthood the relationships are: $SR(n) = (n-1) * F / \{n - F\}$

and $R(n) = RM * (1-1/n)$. When diploid males survive, participate in mating, and the females they mate with produce all-male (haploid) offspring the relationships become: $SR(n) = [2n + (n-1)(1-F) + SQRT\{(1-F)^2(1-n^2) + 4n\}]/2n$ and $R(n) = RM * SR(n) / F$. Figure 1 shows these relationships plotted for the case where the fertilization rate is 50%. In all cases there is a depression of the sex ratio and of the rate of increase, which is quite substantial when the number of sex alleles left in the population is low. The expectation is therefore that populations that are relatively impoverished in sex alleles show a low sex ratio (percentage females) during rearing and a low rate of establishment in the field.

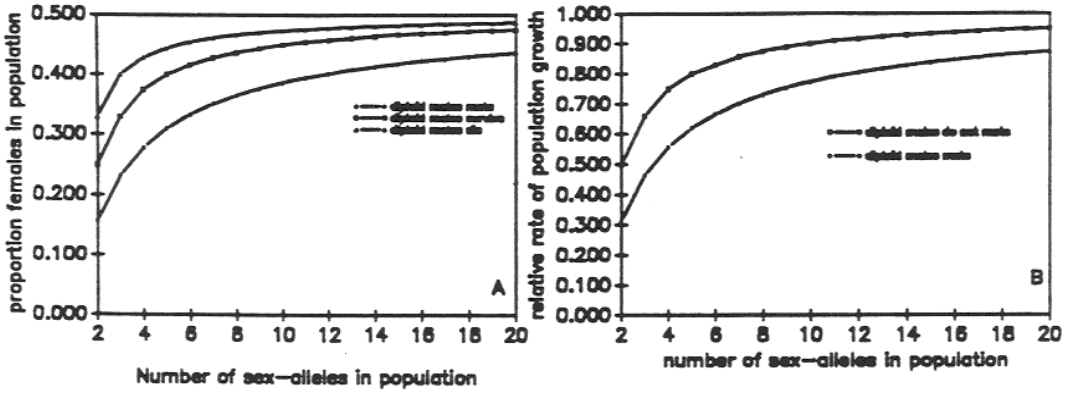

Figure 1. Relationship between (A) sex-ratio and (B) relative rate of population growth (=relative number of daugthers born per mother) with the number of sex-alleles present in a random mating population.

Effects of importation and rearing on the loss of sex alleles

To investigate the effects that normal importation and rearing practices may have on the number of sex alleles in a population several computer simulations were executed.

The first question is: How does the size of the initial collection affect the number of sex alleles in the sample? It is not known from any species how many different alleles exist, however the number has been estimated to be between 15 and 20. For the simulations the assumption was made that the population contained either 10 or 20 different alleles that each were maintained at frequencies of 1/10 and 1/20 respectively. Within the population the mating between

individuals was assumed to be random. From this population mated females were randomly chosen. The number of alleles present in a sample of n such females is shown in figure 2. With relatively low sample sizes all alleles can be obtained. However the mated females in our sample represent an idealized situation, when wasps are collected from the field they are often not collected as mated females but as parasitized hosts. For gregarious wasps all the offspring from one parasitized host only represent one mated female in our simulation sample.

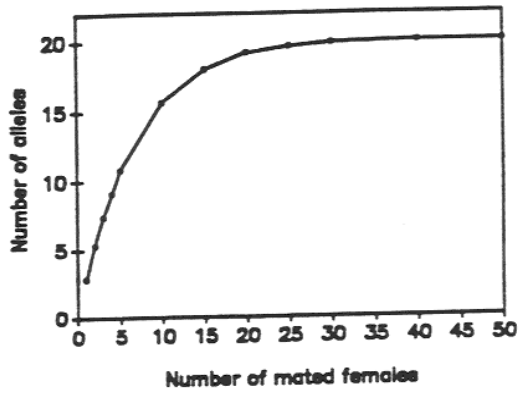

Figure 2. The number of sex alleles present in a sample of n mated females taken a field population that contains 20 different sex-alleles

Once the wasps have been collected and taken to the laboratory for propagation further loss of sex alleles can occur. A number of different factors can cause the loss of alleles. The simulations have been set up as follows. A sample of mated females from the field is taken to the laboratory where they are maintained at population sizes equal to the field sample. Each generation the pairs are randomly chosen from the offspring of the previous generation, each mated female produced the same number of offspring. If diploid males are formed they are not allowed to participate in the matings. Figure 3 shows the results of these simulations which indicate that the larger the laboratory population the slower the allele loss takes place, and also shows that the shorter a population has been maintained in the lab the smaller the loss of alleles is.

Generally not all females produce the same number of offspring

each generation. If variance is added to the number of offspring produced per female the loss of alleles occurs more rapidly. Results of simulations with increasing variance in offspring number is shown in figure 4. In laboratory populations some males mate with more than one female while other males do not mate at all. Variance in the number of matings per male also increases the loss of alleles in that population, as is illustrated in figure 4.

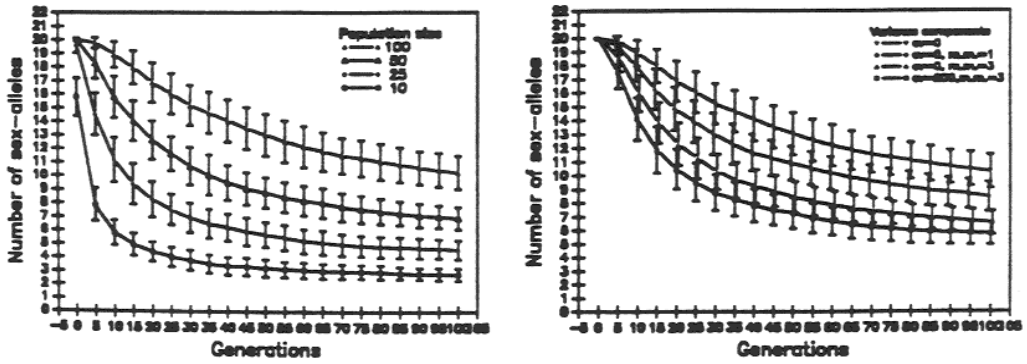

Figure 3. Relationship between the number of sex alleles in a populations (consisting of 100, 50, 25 or 10 pairs of wasps) maintained over time.

Figure 4. Relationship of the number of sex alleles maintained in a population consisting of 100 pairs each generation. The mating structure is varied by adding variance to the number of offspring produced per female (CV=200) or by allowing certain males to participate in more matings (m.m=1 or m.m=3)

Other factors that have not been simulated can also contribute to the loss of alleles in such laboratory populations, these include participation of diploid males in the matings, variance in offspring sex ratio and non random matings. From these simulations it becomes clear that there is a substantial chance that allele loss takes place during laboratory rearing.

Discussion

Based on theoretical considerations the Whiting scheme of sex determination should have an important influence on the sex ratio and

the intrinsic rate of increase of wasps populations. Both these factors are important attributes of the biological control potential of natural enemies. Although only scattered data on the distribution of this sex determining mechanism in the Hymenoptera is available, the few data suggest that in the parasitic Hymenoptera the Whiting scheme may be limited to some Braconidae and Ichneumonidae, Chalcidoidea seem to have some other sex determining mechanism.

The problems caused by this sex determining mechanism can be alleviated to a large extend. From the simulations it became clear that the initial sample used to start the laboratory culture does not need to be very large in order to include all the alleles present in a randomly mating population as long as all the individuals used to start the culture are unrelated. However larger samples are always better.

The next problem is how to maintain the different sex alleles during laboratory propagation and mass rearing. Two approaches can be taken, either the wasps can be maintained in one large population or they can be kept in a large number of small separate populations. In the first approach the loss of alleles is slow (fig 3), resulting in few specific alleles being lost, whereas in the latter approach within each subculture many alleles are lost, but at least two are retained and all the different alleles will be present in the conglomerate of small cultures.

When large cultures are maintained the following culture practices reduce the loss of alleles. 1) Build up the population as fast as possible and keep the culture population as large as feasable. 2) It is always better to have as many pairs as possible contributing offspring to the next generation, even if it is only a few. So in case of host material shortages let each female only parasitize a few hosts instead of a few females parasitizing many hosts. 3) If possible let each female contribute the same number of male and female offspring to the next generation. 4) If females mate more than once allow multiple matings to take place.

When retaining the alleles by keeping many separate cultures the number of such cultures should be kept as large as possible. One of the disadvantages of this technique is that a large proportion of hosts are wasted on the production of diploid males. This technique will have less advantages when diploid males participate in the mating

because of the very male biased sex ratios this can generate (fig 1), thus increasing the chance that some of the small populations go extinct. When these wasps are mass produced for field release individuals of all populations should be used to form one large population thus minimizing the production of diploid males.

It is clear that the Whiting scheme of sex determination can have a severely negative impact on the biological control potential of wasp populations when the appropriate precautions are not taken. It is therefore imperative that we learn more about the sex determing mechanism in wasp species used for biological control, and that sex ratio problems of laboratory cultures are reported and studied.

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GENETIC VARIATION IN THE WALKING BEHAVIOUR OF THE EGG PARASITE *TRICHOGRAMMA*

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ABSTRACT

Wasps of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) are used worldwide for inundative biological control programs of lepidopteran pests. Searching efficiency is an important criterion for pre-introductory selection of a suitable strain of the parasite. *Trichogramma* predominantly search for hosts by walking. This study therefore is concerned with genetical variability in the walking behaviour of wasps within strains. The isofemale lines method applied to populations of *T. evanescens* and *T. dendrolimi* suggests that the walking behaviour is genetically determined. This was apparent for various parameters of the walking behaviour: walking speed, turning angle, time not walked and number of flying attempts. These results indicate that artificial selection within strains for any of these parameters may be carried out. Also, a strain established in the field may undergo genetic changes according to certain environmental selection pressures.

INTRODUCTION

Wasps of the genus *Trichogramma* (Hymenoptera, Trichogrammatidae) parasitize the eggs of various insect species, especially Lepidoptera. They are used worldwide for inundative biological control programs of lepidopteran pests in various crops (Pak, 1988; Stinner, 1977). Inundative releases of mass produced natural enemies aim at immediate control of the pest. For lasting control, releases are repeated throughout the season.

Selection of a candidate natural enemy is pivotal in the development of a biocontrol program. Criteria for pre-introductory evaluation studies of potential candidates differ for

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the three methods of biological control: inoculative, seasonal inoculative and inundative (Van Lenteren, 1986). For inundative biocontrol evaluation criteria are mainly based on characteristics of the parasitization and searching behaviour, beside the premise of economic mass production (Pak, 1988). A suitable candidate natural enemy has a highly efficient rate of host or prey finding and can effectively kill the ones that are found.
For biocontrol programs employing *Trichogramma* releases, pre-introduction selection involves the study of inter- and intraspecific variability for traits related to the searching and parasitization behaviour (Pak, 1988). Variation in biological traits among *Trichogramma* strains (populations) can be environmental or genetic. Inter- and intraspecific variability has been found for many different traits (Pak, 1988), but the genetic background has hardly been investigated. Recently, Wajnberg & Pizzol (1989) found that superparasitism is genetically determined.

In this study we investigate whether the walking behaviour of *Trichogramma* is genetically determined. If a trait is variable and not genetically fixed in a population, it will be adaptive and may change according to changing environmental conditions in the field or in mass production. This may affect the natural enemy's effectiveness in biocontrol. On the other hand, effectiveness may be improved by artificial selection of the trait towards a better performance in the field.

MATERIALS AND METHODS

Experiments were conducted with females of two species, *T. dendrolimi* and *T. evanescens*. Populations were established from strains 22 and 57, respectively, of the collection of geograpical strains maintained in our laboratory on eggs of Ephestia kuehniella (Pak & Van Heiningen, 1985).

The isofemale lines method (Parsons, 1980) was used to determine genetic variabilty in the walking behaviour. The experiments were conducted with females of the first generation of multiplication. Two series of 4 mated females, from each strain, were taken from the laboratory rearing to found isofemale lines. After emergence, 10 females (less than 24 hours old) of each line were kept in gelatine capsules for about 60 minutes. The wasps were transferred to a glass petri dish (8.5 cm diameter), without any stimulating factors such as host eggs or kairomones. Then they were observed for 6 minutes by recording their walking path on video. Observations were carried out in a bioclimatic room at 22-23 °C and ca. 60% r.h., between 9.30 and 12.00 AM and between 2.00 and 4.30 PM.

Walking paths were analyzed by replaying the video-recording and drawing the track on a sheet of transparent plastic affixed over the monitor screen of the monitor. The position of the wasp on the path was marked every 5 seconds. The walking path of each

wasp was digitized for computer analysis by means of an XY-tablet with pixel distance of 0.1 mm. XY coordinates and time markers were input for a computer program, developed at our department, to compute walking distance (mm), time walked and not walked (seconds), walking speed (mm/sec) and turning angle. The measure applied in this study for the turning angle or tortuosity of the walking path is the K-value (Batschelet, 1981). K is the so-called concentration parameter of the Von Mises-distrubution, the circular equivalent of the (linear) normal distribution. The K-value increases with the straightness of the path, and decreases with a decreasing turning angle. The walking path statistics of different isofemale lines were compared by analysis of variance.

RESULTS

Data for the different walking path parameters, and for the number of flying attempts, are presented in Figure 1 (A-F). Large differences occur between the isofemale lines for the factors turning angle, walking speed, time not walked and number of flying attempts, either in the first or in the second series. Analysis of variance shows that these isofemale line effects can be highly significant. Significance levels of less than 1% are common. We may therefore conclude that these parameters of the walking behaviour are family characteristics and appear to be genetically determined in the present *Trichogramma* populations.

Are different parameters of the walking behaviour controlled by the same genetical mechanism? To answer this question, correlation tests were carried out. Figure 2 shows that there is no correlation between turning angle and walking speed. This suggests that these traits are controlled by different genetical mechanisms. Figure 3 shows a slightly negative correlation between walking speed and time not walked. Fast wasps do not interrupt their walk as often as slow wasps. On the other hand there is a slightly positive correlation between walking speed and number of flying attempts (Figure 4). Fast wasps tend to fly more often than slow wasps. Thus as far as the walking speed is concerned, there appears to be a gradation in the behaviour of wasps ranging from not walking, then slow walking and fast walking, to flying.

DISCUSSION

This study shows that there are considerable variations in the walking behaviour among *Trichogramma* wasps. Various components of the walking path investigated here seem to be partly under genetic control. No correlation could be found, however, between turning angle and walking speed, suggesting that the two components are not being controlled by the same genetical mechanism. Wasps with a specific walking speed show the whole spectrum of turning angle values. And among wasps with a specific turning

Fig. 1. 95 percent intervals for means of serveral components of the walking behaviour. Strain 22 and 57.

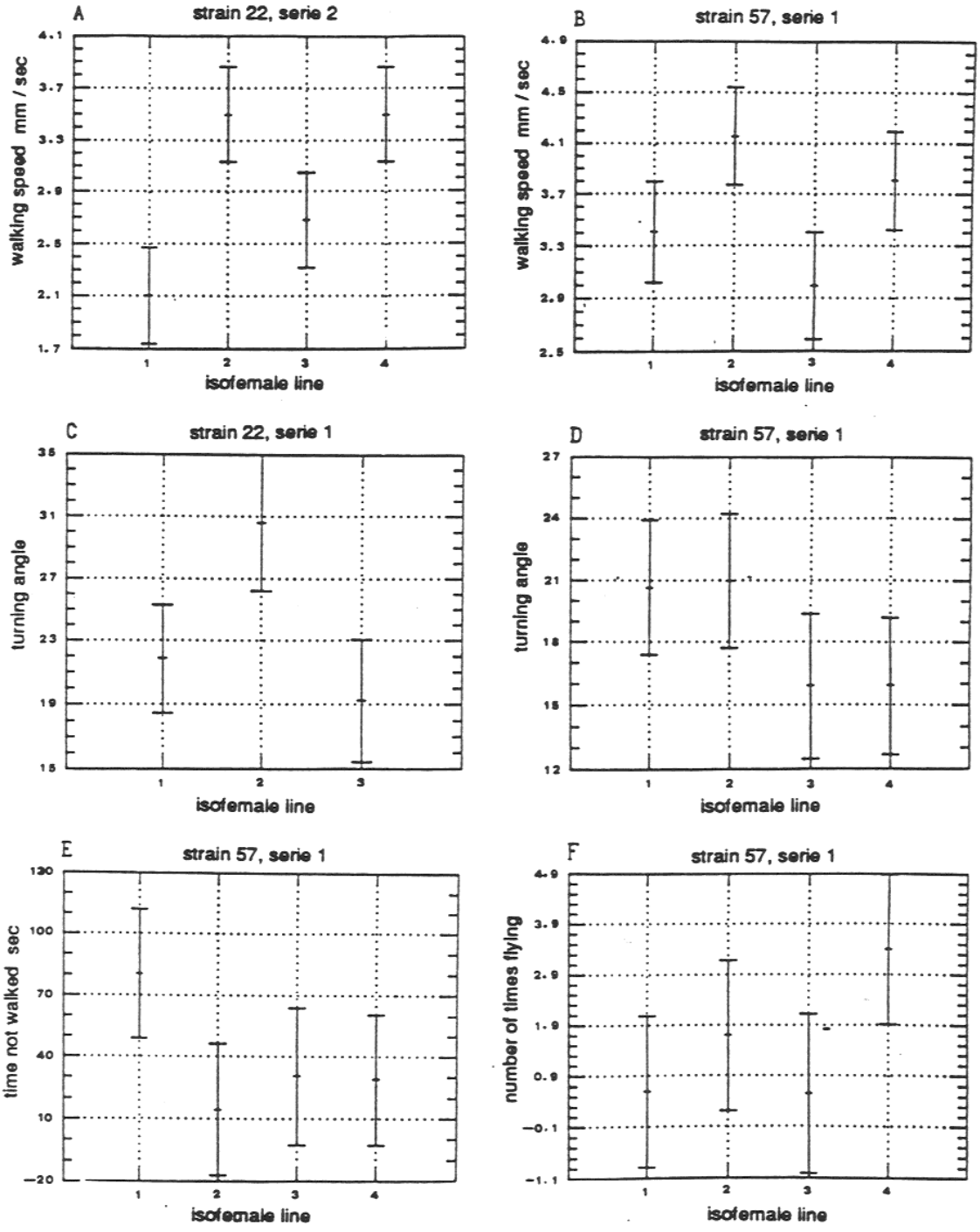


Fig. 2. Relationship between the turning angle and the walking speed. Each point represents a single Trichogramma wasp. (Strain 22 and 57, serie 1 and 2).

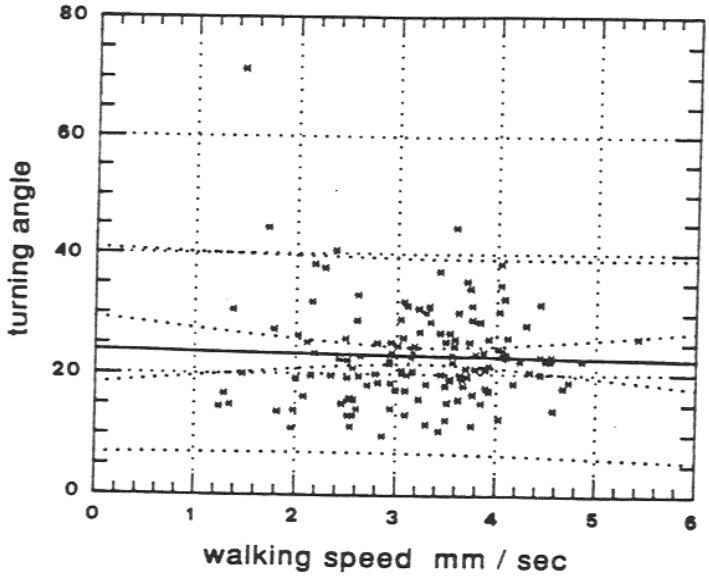


Fig. 3. Relationship between the walking speed and the time not walked. (Strain 57, serie 1 and 2).

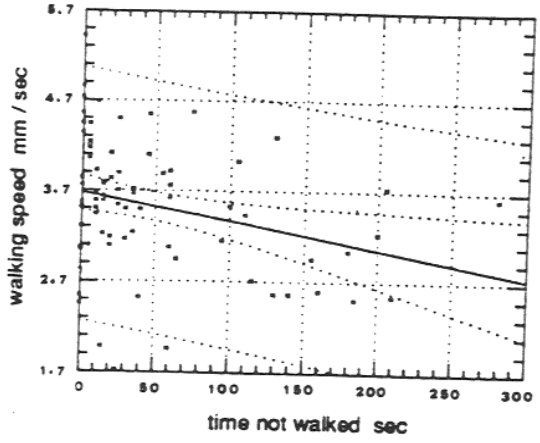


Fig. 4. Relationship between the walking speed and the number of times flying. (Strain 57, serie 1 and 2).

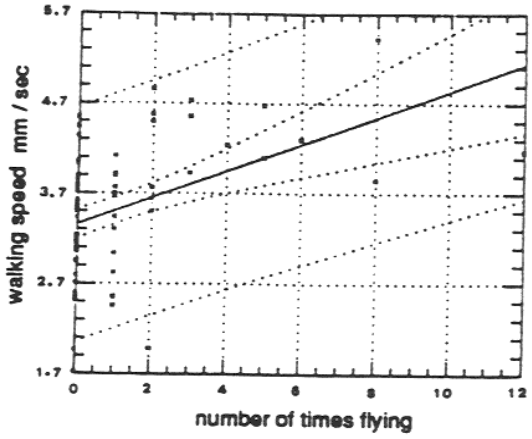


Fig. 1. 95 percent intervals for means of serveral components of the walking behaviour. Strain 22 and 57.

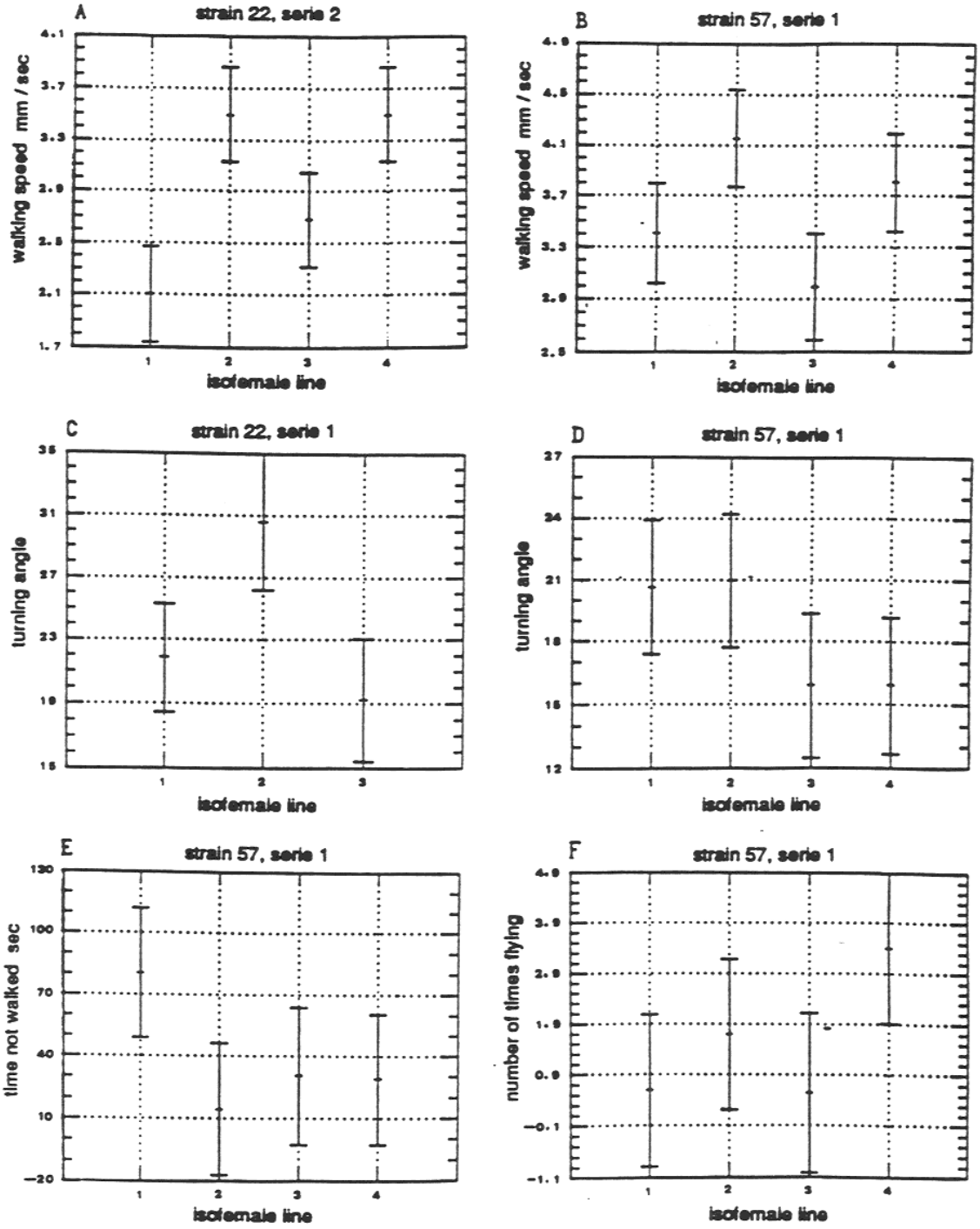


Fig. 2. Relationship between the turning angle and the walking speed. Each point represents a single Trichogramma wasp. (Strain 22 and 57, serie 1 and 2).

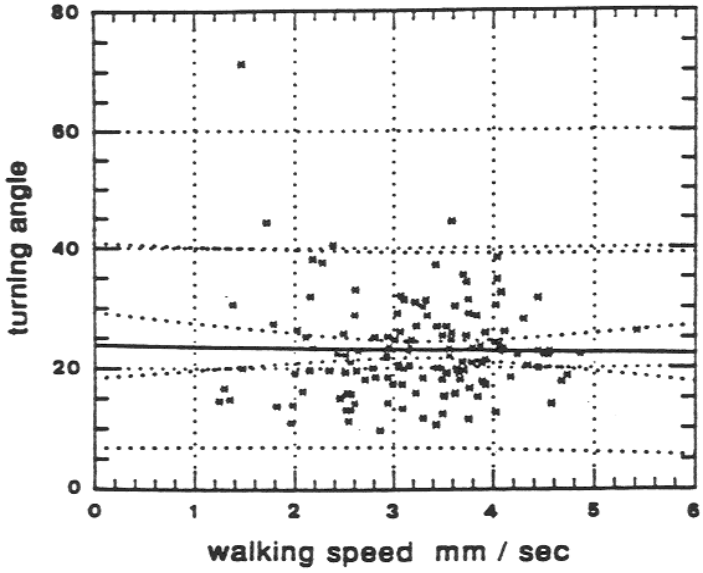


Fig. 3. Relationship between the walking speed and the time not walked. (Strain 57, serie 1 and 2).

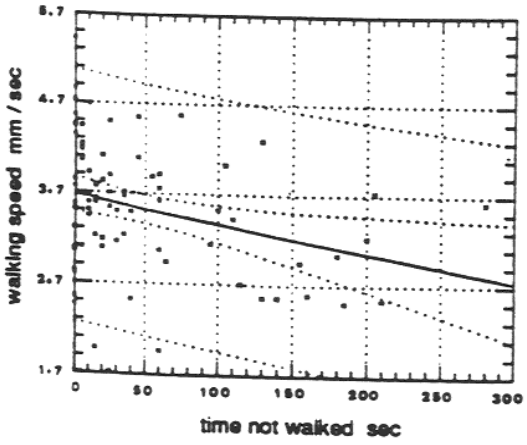
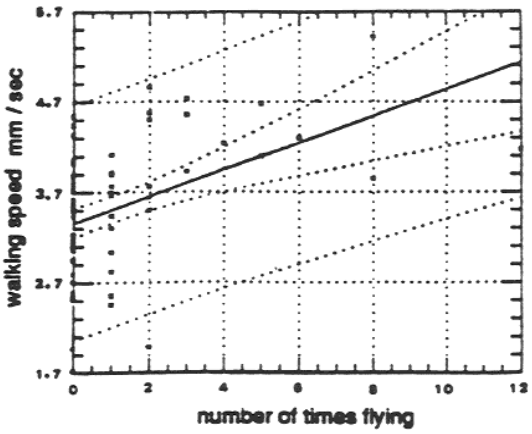


Fig. 4. Relationship between the walking speed and the number of times flying. (Strain 57, serie 1 and 2).



angle there are slow walkers as well as fast ones. This suggests that different non-linked genes or gene-sets are involved in the controlling mechanism.

The present results indicate that it is possible to develop, by means of artificial selection within a strain, a population that is best suited for release in the field. It seems feasible to select for serveral components of the walking behaviour, such as walking speed and turning angle, separately. Bigler et al. (1988) compared the locomotion of different *T. maidis* strains in the laboratory with their performance in the field. They found that the walking speed of the females of different strains is positively related to the potential for parasitism in the field. Besides a high walking speed, selection should aim at a low turning rate to optimize the searching efficiency. The straighter the walking path, the fewer will be the number of crossings (i.e. revisits to a certain location).

Parameters of the walking behaviour might be considered in relation to environmental factors. Temperature and light intensity influence walking speed and turning rate (G.A. Pak, unpublished data). Pak & Van Heiningen (1985) found that strains vary in activity at low temperature. This variability was found to translate into differences in field performance between these strains (Pak et al., 1989). In order to improve the parasitization rate in selected strains at extreme environmental conditions, selection procedures should include the effect of abiotic factors, e.g. by comparing the walking speed of selected lines at different temperatures.

A non-fixed *Trichogramma* population can easily adapt to environmental changes. The traits of a selected population are therefore difficult to maintain in the field. Maintenance of selected traits under constant selection pressure in the laboratory is possible. However, we should then be aware of bottlenecks. A single bottleneck in population size may cause a considerable loss of genetical variation from the strain, which may never be regained. On the other hand, this study shows that even a small group of inbred wasps may have a wide spectrum of genetical variation. In that case the loss of some variation might occur without a significant impact on the performance of the population.

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RESPONSE TO SYNOMONES AS A PARAMETER IN QUALITY CONTROL OF PREDATORY MITES

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Erratum

Pages 59-60 should be at the end of this article

Read: pages 56-58, 61-65 and then 59-60

ABSTRACT

Plants emit a volatile synomone in response to infestation by spider mites. This synomone enables predatory mites to distinguish olfactorily between uninfested plants and infested plants. They are attracted to infested plants. This predator characteristic seems to be essential in the extermination of spider mites. Several factors are known to affect the response of the predators. Here we report a new phenomenon. We found that the response of several predator populations of different species fluctuates widely. At some times predators prefer the odour of infested leaves to the odour of uninfested leaves whereas at other times their preference is the opposite. The change in odour preference is reversible. Several possible causes have been investigated, but this has not resulted in a final answer yet.

INTRODUCTION

Several species of predatory mites (Acarina: Phytoseiidae) are well-known for their ability to decimate populations of herbivorous mites. As a consequence man has employed these predator species in biological control in many crops (Helle & Sabelis 1985). A predator characteristic that seems to be essential in the extermination of herbivorous mites is their ability to discriminate between odours of uninfested plants and plants infested by prey mites (Sabelis & Van der Meer 1986; Dicke et al. 1990b). The odours of prey-infested plants affect predator foraging behaviour in several ways. (1) *Long-range dispersal:* predators do not take-off when chemicals related to spider-mite damage are present. Thus, upon landing after an aerial voyage, predators will stay and search for prey when volatile prey-related odours are present and they do not initiate a new aerial voyage as long as odours of prey-infested plants are present (Sabelis & Afman 1984; Sabelis & Janssen in prep.). (2) *Residence time in prey patch:* when they reach the edge of a prey patch [a group of leaves infested by prey, cf. Sabelis (1981)] predators that walk out of the odour plume emitted by the damaged leaves return towards the patch (Sabelis et al., 1984). As a result the predators stay in the prey patch as long as prey-related chemicals are present. (3) *Prey preference:* predatory mites distinguish between odours related to damage inflicted by different prey species and

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen. March 1991.

prefer some of these odours to others. This odour preference is correlated to prey-species preference (Dicke et al. 1988). Thus, odours of prey-infested plants affect several stages of predator foraging behaviour.

Because the focus of the phenomenon of predator attraction to prey-infested plants has been largely on the response of the predatory mites, the plant's role has not received the attention it deserves. Recent research has shown that the volatiles that attract the predators are produced by the plant upon infestation by spider mites (Dicke et al. 1990ab): they are herbivore-induced synomones (Vet & Dicke 1992). In addition, the effect of spider-mite damage even appears to be systemic. Uninfested leaves of infested plants produce predator attractants (Dicke et al. 1990b). Moreover, even uninfested plants that neighbour infested plants attract more predators than uninfested plants that have uninfested neighbours (Bruin et al. 1991; Dicke et al. 1990b). Thus, plants themselves employ biological control: they recruit the ravenous predatory mites after herbivorous mites have inflicted damage to the plants or their neighbours. This warrants a closer investigation of the response of predatory mites to these herbivore-induced synomones and the variation that may exist. This variation may cause variable results of biological control.

The response of predatory mites to the herbivore-induced synomones is variable. Three major factors that affect the response are known. (1) *Dietary history*. The response of predators is affected by their hunger level. Starvation generally makes the response to herbivore-induced synomones more pronounced (Sabelis & Van de Baan 1983). Deprivation of predatory mites of essential nutrients may affect the response to less-preferred prey species. For instance, the predator *Amblyseius potentillae* does not respond to volatiles emitted by leaves infested by *Tetranychus urticae*, unless the predators are reared on a diet deficient in carotenoids (Dicke et al. 1986). The spider mite *T. urticae* is an inferior prey species for *A. potentillae* because of the dense webbing this spider mite produces (Sabelis 1981). The response to leaves infested by the preferred spider mite *Panonychus ulmi* is not affected by availability of dietary carotenoids: both carotenoid-deficient and carotenoid-containing predators are attracted (Dicke et al. 1986). (2) *Learning*. Experience with certain prey-plant combinations may affect the response to odours of the experienced combinations. After 7 days of feeding on *T.urticae* on bean leaves *Phytoseiulus persimilis* prefers the odour of *Tetranychus urticae*-infested bean leaves to that of *T. urticae*-infested cucumber leaves and after 7 days of feeding on *T. urticae* on cucumber the predators show the reverse preference (Dicke et al. 1990b,c). (3) *Variation in source*. The response of predatory mites depends on the herbivore species, the plant species and even the plant cultivar (Dicke et al. 1990b). In this paper we report on a new source of variation in the response of predatory mites to allelochemicals emitted by spider-mite infested plants.

We present data for large fluctuations in the response of two species of predatory mites, *A. potentillae* and *Typhlodromus pyri*, with regard to induced synomones related to several herbivorous mites on different plant species. *Amblyseius potentillae* and *T. pyri* are polyphagous predators that can feed on e.g. spider mites, rust mites and pollen (Overmeer 1985a). They prefer the European red spider mite *Panonychus ulmi* to the spider mite *T. urticae* and the rust mite *Aculus schlechtendali* (Dicke et al. 1988). In contrast, the fluctuations observed for these polyphagous predators have not been recorded for the predatory mite *P. persimilis* which mainly feeds on spider mites in the genus *Tetranychus*.

MATERIALS AND METHODS

Predatory mites. *Amblyseius potentillae* was collected in the Netherlands in 1974 [see Dicke et al. (1989) for details on origin, rearing history] and has been reared on either broad bean (*Vicia faba*) pollen (since 1980) or on *T. urticae* on plastic plates [see

Overmeer (1985b) for rearing procedures]. In addition, another population of *A. potentillae* was obtained in July 1987 from a vineyard in Cugnasco, Switzerland (10 females) (courtesy M. Baillod). The *A. potentillae* populations will be referred to as *A. potentillae* (Vf), *A.potentillae* (Tu) and *A. potentillae* (Cugnasco) respectively. *Typhlodromus pyri* was collected in the Netherlands in 1978 [see Dicke et al. (1989) for details on origin and rearing history] and has been reared on broad bean pollen. *Phytoseiulus persimilis* was reared on *T. urticae* on Lima bean leaves [see Dicke et al. (1990a) for details on origin and rearing].

Spider mites. The two-spotted spider mite, *T. urticae*, was reared on Lima bean plants at 20-30 °C (Dicke 1986). The European red spider mite, *P. ulmi*, and the apple rust mite, *Aculus schlechtendali*, were collected on apple leaves in an unsprayed orchard in the Netherlands.

Olfactometer. A Y-tube olfactometer (Sabelis & Van de Baan 1983) was used to investigate the response of predatory mites to herbivore-induced synomones emitted by plants that are infested with herbivorous mites. For a description of the olfactometer set-up as used here, see Dicke et al. (1990c).

RESULTS AND DISCUSSION

During the past 7 years we have studied the response of *A. potentillae* (Vf) in the olfactometer towards Lima bean leaves infested with *T. urticae* vs. uninfested bean leaves (Fig. 1a). In October 1983 we discovered that 24h starved *A. potentillae* (Vf) respond to a volatile herbivore-induced synomone emitted by Lima bean plants infested by *T. urticae*: in the olfactometer 75% of the predators chose for the arm with odour of infested bean leaves and 25% chose for the arm with odour of uninfested bean leaves. The same result was obtained in April 1984. However, from the summer of 1986 through the fall of 1987 this result could not be confirmed. In one instance (summer 1986) the predator's choice was even the reverse: predators preferred the arm with uninfested leaves to the arm with infested leaves. However, when we continued this research in October 1990, the results were similar to those in 1983 and 1984 again: 77.5% of the predators preferred the arm with odour from *T. urticae*-infested leaves. But soon afterwards, in November and December of 1990 the response was the reverse again (Fig. 1a).

Similar results were obtained for satiated *A. potentillae* (Vf) females: in the experiments in 1985 and in October 1990 the predators preferred the odour of infested leaves to the odour of uninfested leaves. But in November/December 1990 75% of the predators preferred the odour of uninfested leaves to infested leaves (Fig 1b).

When the predators were reared on *T. urticae* and starved for 24 h they did not distinguish between uninfested and infested leaves in 1983 (Dicke et al. 1986). The 1983 difference between predators reared on *V. faba* pollen or *T. urticae* was the result of the pollen-reared predators being deficient in carotenoids that are needed for diapause induction: addition of crystalline ß-carotene to *V. faba* pollen resulted in a 50:50 distribution of the predators in the olfactometer. This was similar to the response of *A. potentillae* (Tu). It was concluded that *A. potentillae* (Vf) have a specific hunger for carotenoids, which makes them respond to the otherwise neglected synomone related to the inferior prey *T. urticae* which can solve the carotenoid deficiency (Dicke et al. 1986).

In 1985 we observed that more severe starvation (48 h) of *A. potentillae* (Tu) resulted in the predators preferring the arm with *T. urticae*-infested leaves. In contrast, the response of *A. potentillae* (Vf) appeared to be independent of starvation: satiated predators (but still having a specific hunger for carotenoids) preferred the odour of infested leaves to the odour of uninfested leaves (data of 1985 and October 1990). However, in November/December 1990 48h starved *A. potentillae* (Tu) and satiated *A.*

al. (1989) reported on a reduction in predation rate by *A. potentillae* (Vf). Predators reared on pollen used to have a predation rate on spider mites and rust mites that was similar to the predation rate by conspecifics reared on *T. urticae*. However, since 1985 the predation rate of *A. potentillae* (Vf) dropped to 25% of that of *A.potentillae* (Tu) (Alers 1986; Dicke et al. 1989). Its cause was found to be a lower rate of removal of ingested pollen from the gut (without reduction in rate of reproduction) which resulted in a slower prey uptake rate. After several days of feeding on spider mites, the gut was cleared from pollen and the predation rate was on its original level again. Several analogies with the phenomenon reported in this paper are apparent: (1) they relate to the same *A. potentillae* populations; (2) the reduction in predation rate appeared within the period in which the altered odour preference appeared; (3) the reduction in predation rate appeared very rapidly, i.e. within at most two days in July 1985 (Alers 1986); (4) the predation rate of *A. potentillae* (Vf) increased again to normal levels during November 1987; (5) the reduced predation rate was not caused by a genetic change.

Because, this non-genetic change in a predator characteristic appeared in exactly the same time as the change in odour preference: between July 1985 and November 1987, one wonders whether the two changes have the same cause. It is interesting to determine the predation rate of *A. potentillae* (Vf) at this moment while the predators prefer the odour of uninfested leaves to the odour of *T. urticae*-infested leaves.

Response to herbivore-induced synomones and quality control

Because the response of predatory mites to spider-mite induced synomones seems to be essential for the extermination of spider-mite populations, it is essential to understand the variation in this response. Understanding this variation may be used to manipulate predator-prey interactions (Vet & Dicke 1992) or to avoid detrimental effects such as the one described here.

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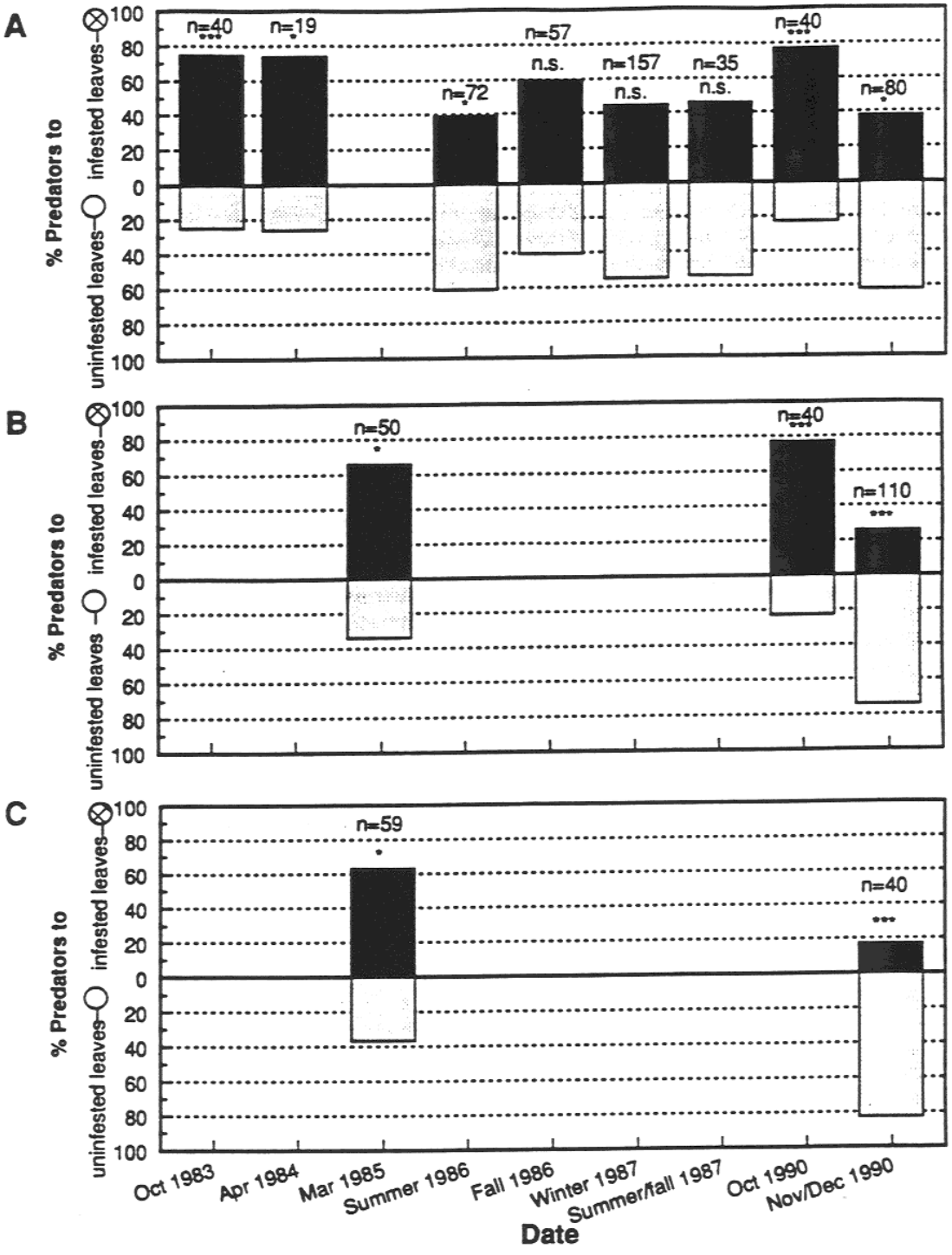


Figure 1 : Response of A. potentillae females in Y-tube olfactometer to odour of 9 Lima bean leaves infested with T. urticae vs. 9 uninfested Lima bean leaves
A- A. potentillae (Vf) 24 h starvation; B- A.potentillae (Vf) 0 h starvation;
C- A. potentillae (Tu) 48 h starvation
***: P<0.001 ; *: P<0.05; n.s.: not significant; two-sided binomial test

potentillae (Vf) preferred uninfested leaves to infested leaves (Fig. 1b and 1c).

We investigated whether the observed fluctuation in the behavioural response also occurred with respect to odours related to damage inflicted by other prey species such as *A. schlechtendali* and the most preferred prey species *P. ulmi*. *Amblyseius potentillae* (Vf) that were starved for 24 h have been reported to prefer odours of apple leaves infested by either *P. ulmi* or *A. schlechtendali* to uninfested apple leaves (Dicke & Groeneveld 1986; Dicke et al. 1986) and 24h starved *A. potentillae* (Tu) preferred the odour of apple leaves infested by *P. ulmi* to uninfested apple leaves (Sabelis & Van de Baan 1983). However, when these experiments were repeated in the summer and fall of 1987 the previous results could not be confirmed: predators distributed evenly over the two olfactometer arms (Fig 2). Also an increase in the starvation period to 48 h had no effect (Fig. 2). Thus, the fluctuation in the response to herbivore-induced synomones seems to be related to the predators rather than to the prey (Fig. 1 and 2).

Is the fluctuation caused by an altered response to infested leaves, to uninfested leaves or to both? In the period of November 1990 through February 1991 we carried out several experiments to investigate what made the predators prefer the odour of uninfested leaves over the odour of *T. urticae*-infested leaves. Is the effect caused by an increased attraction of uninfested leaves or by a decreased attraction or even repellent effect of infested leaves? Satiated *A. potentillae* (Vf) preferred the odour of uninfested bean leaves to the odour of infested leaves. They also preferred clean air to the odour of infested leaves, but there was a 50:50 distribution when uninfested leaves were offered against clean air (Fig. 3). This indicates that the preference for uninfested leaves over infested leaves is caused by a repellent effect of infested leaves. In other words, the predators still distinguish between volatiles from infested and uninfested leaves, but in contrast to their preference in 1983, 1984 and October 1990, the predators preferred uninfested leaves to infested leaves in November/December 1990.

Is the change in behavioural response restricted to* A. potentillae*? We investigated the response of *A. potentillae* (Cugnasco), *T. pyri* and *P. persimilis* in the period of November 1990 through February 1991. The responses of *A. potentillae* (Cugnasco) and *T. pyri* were similar to the response of *A. potentillae* (Tu) and (Vf), but *P. persimilis* preferred the odour of bean leaves infested with *T. urticae* to the odour of uninfested leaves (Fig. 4) . This response of *P. persimilis* has been reported by several authors (Bruin et al. 1991; Dong & Chant 1986; Sabelis & Van de Baan 1983; Dicke & Sabelis 1988) and has always been very reliable in our laboratory. These data indicate that the repellent effect of *T. urticae*-infested leaves is not restricted to one predator species, but on the other hand not all predator species are affected.

It is remarkable that a preference for odours of uninfested leaves over prey-infested leaves in a Y-tube olfactometer has also been reported for *Amblyseius andersoni* (a synonym for *A. potentillae*; Chant & Yoshida-Shaul 1990) by Dong & Chant (1986). These authors report that satiated and 8h starved *A. andersoni* females that are reared on *Tetranychus pacificus* (on cut bean leaves resting on moist cotton wool in a petri dish; supplying prey was done by shaking tetranychids from bean plants onto the cultures; D.A. Chant, pers. comm.) prefer odours of uninfested Lima bean leaves to odours of Lima bean leaves infested by *T. pacificus*. A similar preference is reported for satiated *Amblyseius degenerans* females, but these predators prefer infested leaves when starved for 8 h. In contrast, satiated and 8 h starved *P. persimilis* females preferred the odour of infested leaves. These experiments were carried out in the spring (D.A. Chant, pers. comm.). The data are very similar to our data obtained from November 1990 through February 1991.

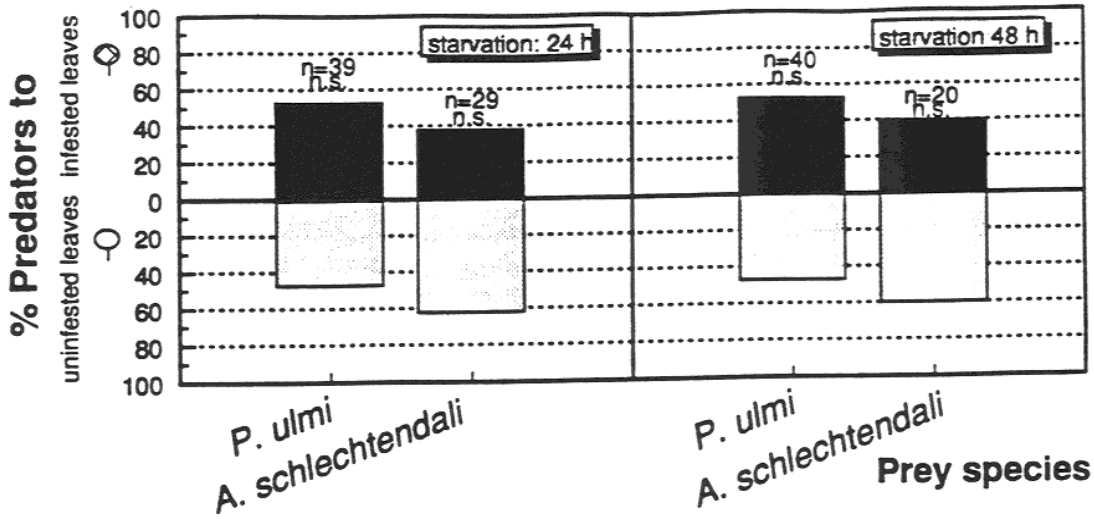
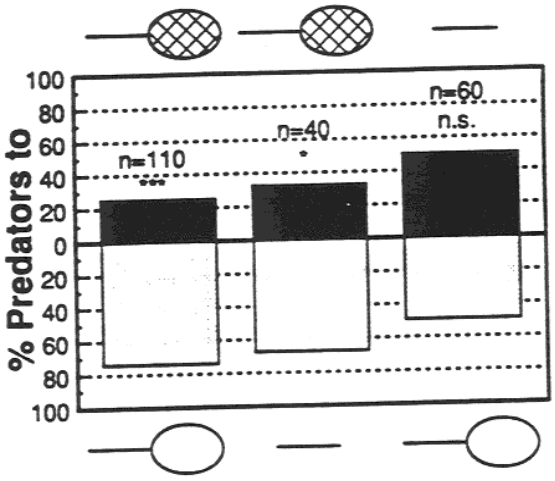


Figure 2 : Response of A. potentillae (Vf) females in Y-tube olfactometer to odour of apple leaves infested by either P. ulmi (300-1000 individuals) or A. schlechtendali (50,000 - 90,000 indiv.) vs. odour of uninfested apple leaves; n.s.: see Fig. 1; data summer/fall 1987.

Figure 3 : Response of satiated females of A. potentillae (Vf) in olfactometer to odour of 9 Lima bean leaves infested by T. urticae and/or 9 uninfested Lima bean leaves, offered in different combinations; data Oct/Nov 1990
asterisks: see Fig.1
-⊘: infested leaves
-○: uninfested leaves
—: no leaves at all



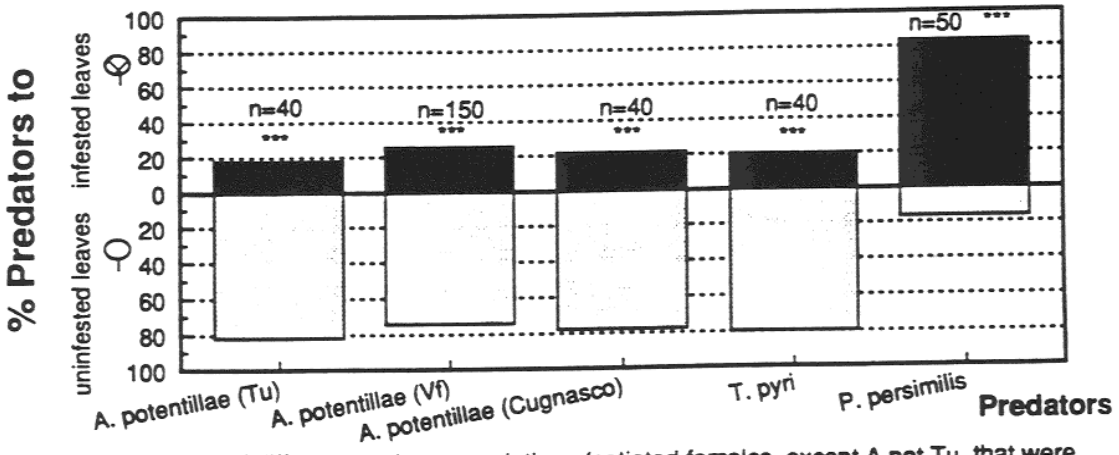


Figure 4 : Response of different predator populations (satiated females, except A.pot Tu that were starved for 48h) to odour of 9 uninfested Lima bean leaves vs. 9 T.urticae infested leaves.
(Data Nov 1990 through Feb 1991)

Possible causes for the observed fluctuations in the responses to herbivore-induced synomones.

(A) Genetic change? Three observations exclude a genetic change as the cause of the altered odour preference. (1) The fluctuations in the response occur synchronously for several isolated laboratory populations of one predator species that are reared on different food sources [*A. potentillae* (Vf), (Cugnasco) and (Tu)] and a population of another predator species (*T. pyri*). (2) The reversal in preference within the *A. potentillae* (Vf) population was observed from one day to the other in 1990, i.e. within 24 hours! (3) The reversal of odour preference is not of a permanent nature: the preference as observed in 1983 was also observed in October 1990 while it has not been observed in 1986 and 1987.

(B) Fluctuations in the odour source? The observed fluctuations do not seem to be caused by fluctuations in the odour source. In 1986 and 1987 *A. potentillae* did not respond to herbivore-induced synomones of several plant-herbivore combinations.

(C) Environmental fluctuations? The observations show that some reversible change has occurred in two predator species but not in a third species. A possible cause may be an environmental effect that differently affect different predator species. However, no correlation is apparent between time of the year and odour preference. Infested leaves have been preferred by *A. potentillae* in March, April and October, whereas uninfested leaves have been preferred during summer, fall and winter. Moreover, the reversal of preference has been observed within 24 h. Thus, a seasonal factor does not seem a likely cause. Also, suddenly occurring changes in environmental factors such as e.g. barometric pressure (Lanier & Burns 1978) do not seem to cause the observed change in odour preference, because of the prolonged period during which the change is manifest.

(D) Non-genetic changes in the predators? If non-genetic changes induced the altered odour preference in several predator species and populations, they could be related to e.g. a change in rearing techniques. However, no changes in rearing techniques have been purposefully made in November 1990 and we are not aware of unplanned changes in rearing procedures. Moreover the change in behavioural response was also observed in the fall and winter of 1986 for *A. potentillae* (Vf) reared in Amsterdam (University of Amsterdam, Department of Experimental and Applied Entomology; courtesy Y.M. van Houten) and tested in Wageningen after 24h of starvation. These predators responded in a way similar to the Wageningen strain of *A.potentillae* (Vf) (Fig. 1a): they had a 50:50 distribution in the olfactometer in which we offered *T. urticae*-infested Lima bean leaves vs. uninfested Lima bean leaves.

Some differences exist between general rearing procedures for *A.potentillae* and *T. pyri* vs. those for *P. persimilis*. The altered odour preference occurred in predator species that are polyphagous. These predators were reared on a single food source on an artificial arena consisting of a plastic plate surrounded by wet tissue. This tissue gets contaminated with microorganisms and that is the main reason for the timing of renewal of the rearing plates for predators reared on pollen. Whether or not the predators feed on these microorganisms is not known. The predator species for which a change has not been recorded (*P. persimilis*) is an oligophagous species feeding on spider mites in the genus *Tetranychus*. *Phytoseiulus persimilis* is reared on *T. urticae*-infested bean leaves on top of clay flower pots that stand upside down in a water basin, which is a water source for predators after drying of the infested leaves and depletion of all spider mites. As mentioned above, Dong & Chant (1986) reported a similar difference in odour preference between these two predator species. The rearing technique employed by these authors is different from our way of rearing *A. potentillae* (see above). Thus, the observed changes in odour preference by *A. potentillae* is not restricted to our technique of rearing this predator species.

Is the fluctuation caused by an infestation of predators with a microorganism? Microbes commonly contaminate mass-reared arthropods (Goodwin 1984; Shapiro 1984) but generally pathogenic phenomena are described. Our cultures of *A. potentillae* and *T. pyri* flourish as usual and there is no evidence for contamination with pathogens that affect development and reproduction (Dicke et al. 1989). Non-pathogenic microorganisms have been reported for e.g. *Phytoseiulus persimilis* Sutakova & Arutunyan 1990) and *Metaseiulus occidentalis* (Lighthart et al. 1988). However, non-pathogenic microbes may affect other biological characteristics of their host such as behaviour (Holmes & Bethel 1972; Smith Trail 1980). To investigate a possible effect of microorganisms on predator behaviour we added either one of two antibiotics to the pollen of *A. potentillae* (Vf): rifampicin and oxy-tetracyclin (1% w/w). This was done as crystals mixed with pollen and as a solution in honey. After one week we investigated the response of the predators when offering *T. urticae*-infested vs. uninfested Lima bean leaves. The response of these predators was similar to the response of control predators that had fed on pollen only: they preferred the odour of uninfested leaves to the odour of infested leaves (Fig. 5). Thus, we have no evidence for effects of microorganisms on the response of predatory mites. However, it remains possible that a microorganism that is not affected by the antibiotics used is responsible for the changed odour preference.

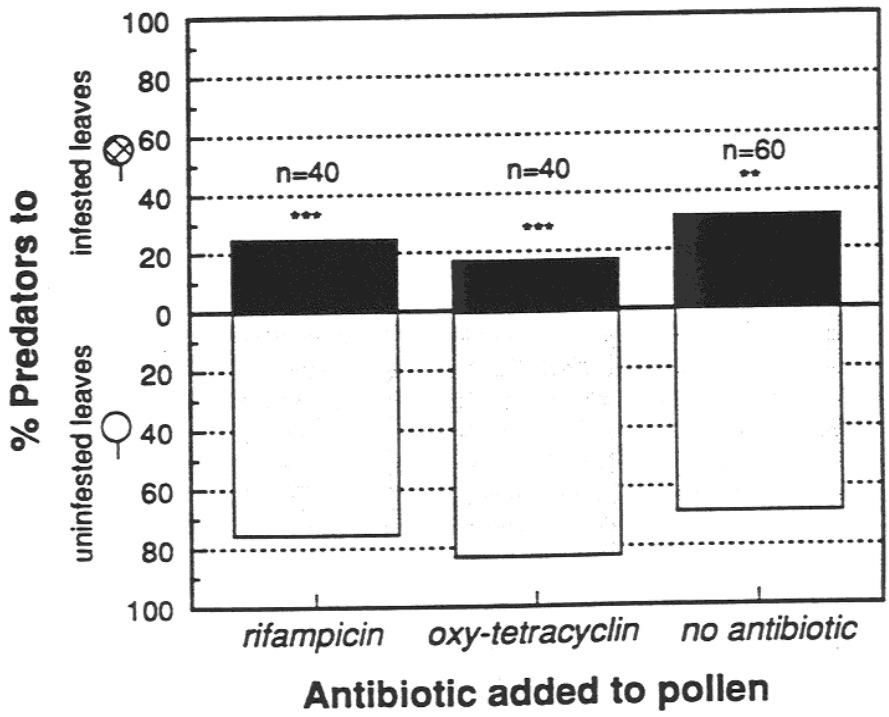


Figure 5 :Response in olfactometer of satiated A. potentillae (Vf) females after one week of feeding on V. faba pollen to which antibiotics had been added. **: P<0.01; ***: P<0.001. The responses of the three predator populations did not differ significantly (P>0.05; G-test). Data February 1991.

Other changes in predator behaviour in mass rearings. Few reports have been made on quality control in (laboratory) mass rearings of predatory mites; see e.g. Ramakers et al. (1989) who report on the contamination of mass rearings of *Amblyseius cucumeris* and *A. barkeri* with pathogens (*Nosema*). Changes in observed biological characteristics are usually not reported unless their cause was elucidated (Dicke et al. 1989). Dicke et

DIAGNOSIS OF BEHAVIOUR AS A TOOL FOR QUALITY CONTROL OF MASS REARED ARTHROPODS

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ABSTRACT

Behavioural diagnosis seems to be very useful in the process of quality control. We are capable now to gain detailed knowledge and understanding of behaviour, but ethological analysis remains still too laborious as a routine quality control procedure. However, it is possible to define and correlate automatically measurable attributes of behaviour with very complex behavioural patterns. It also seems feasible to correlate such attributes with expected efficiency in the field. This offers possibilities to develop automated equipment for behavioural diagnosis which combines sophistication with simplicity required for quality control tests. A four step procedure is discussed in detail in this paper.

INTRODUCTION

The concept of quality control has been developed in industry and is well established now. Mass reared beneficial arthropods, considered as a reliable mean for pest control, should also be tested at least before leaving "the factory" and furnished with some efficiency certificate. Efficiency of conventional pest control agents usually is related to the presence of an "active ingredient" in the field. Similarly, in mass reared beneficial arthropods, attention is paid mostly to factors which determine their presence in the field. Among such factors, survival during handling and transport, emergence, longevity and fecundity are the most frequently considered. Although more technically minded people call these mass reared arthropods a "product", we have to keep in mind that the real thing we are in fact dealing with are living, individual creatures. This simple fact, though so obvious to biologists, seems to be sometimes overlooked by producers and users. Effectiveness of mass reared beneficial arthropods

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

depends not only on their presence in the field, but also on behaviour of individuals.

Nowadays it becomes clear, that many failures in releases of mass reared arthropods should be attributed to gaps in our knowledge about their behaviour. Understanding behavioural properties of beneficial arthropods will support our efforts in efficient use. Among many aspects of behaviour, the most decisive quality factors seem to be: intensity and persistency of exploration and host searching, host acceptance and adaptability to diverse and changeable environmental conditions. Proper assessment of such behavioural characters requires simple, but quantitative methods.

Unfortunatly, many conventional behavioural bioassays were found to be inadequate and biased by a too mechanistic approach to the animals tested. Therefore it would be advantageous to use modern ethological methods for behavioural bioassays. The author's opinion concerning general usefulness and applicability of ethological research in agricultural practice was also presented earlier (Lux 1989a, Lux 1990a,b, Lux 1991). However, not long ago, ethology was considered as a pure science with little applicability to practice, mostly because its methods were very laborious and time consuming. Recently, developed computerized event recorders and programs for analysis of behavioural data made ethological methods more accessible (Lux 1989b, Noldus 1989a). To combine sophistication of ethological analysis with simplicity, required for quality control methods, the following four step procedure is proposed:

1. gaining detailed knowledge about complexity of beneficials behaviour through ethological research
2. selecting "computer digestible" attributes of behaviour, which could be easily monitored by automated devices
3. correlating selected elements of behaviour with expected performance of beneficials in the field
4. designing automatic methods and equipment for behavioural diagnosis for quality control.

EXAMPLE OF METHODS FOR ETHOLOGICAL RESEARCH

A system of programs developed in the Department of Applied Entomology, Warsaw Agriculture University (Lux 1989b) is presented hereinafter as an example of methods for ethological analysis.

The first part of the system are programs converting programmable calculators; Sharp IQ 7100M or Psion LZ-64 into very handy, portable event recorders. Data collected can be easily transmitted into a Macintosh computer.

The main part is composed of programs for quantitative ethological analysis. Each activity is characterized by means, standard deviations and variability coefficients for: number of observed events, duration of a single event and time spent for the activity during observation. Moreover, probabilities of transitions between activities (input and output chances) are calculated (Tab. 1).

Tab.1 Example of repport from ethological analysis

Trogoderma granarium Everts
The influence of age on male behaviour.
32 C, 60% RH

	data without transformation			
total time (T, SD, Σ(T^2), C.var.)	1136.87	347.29	121525110.13	0.31

standardized insects — **precopulatory phase**

resting

/48 events in 86 observ. (range; min=0.66 MAX=825)

	mean	stand. dev	sum of squar.	variab. coef.[SD/x̄]
numb. of observ. events	1.72	1.47	440.00	0.85
duration of a single activ	120.09	174.50	6640855.51	1.45
total time spent for activ./1 obs.	206.66	247.26	8930971.33	1.20

joint with	INPUT chances		OUTPUT chances	
	№ of events	probabil. [%]	№ of events	probabil. [%]
start-end	15	10.14%	24	16.22%
resting				
exploration	74	50.00%	102	68.92%
meeting with fem.	49	33.11%	20	13.51%
examination of fem.	10	6.76%	2	1.35%
attempt. to copul.				
copulation				
examin. of pherom.				

Results of such analysis provide very detailed descriptions which can be used to set quantitative standards of behaviour. Even minute deviations

from the standard can be easily detected by the next part of the program for statistical comparisons (Tab. 2). Diagrams of behaviour (ethograms) make it

Tab.2 Example of statistical comparison of repports from ethological analysis

Trogoderma granarium Everts
The influence of age on male behaviour.
32 C, 60% RH

data without transformation

comparison: 1-day males, 3-day females-(before copulation)
resting 1-da. males, 1-da. females-(before copulation)

	t-test for mean	F-test for var		df for 't'(df for 'F')
numb. of observ. events	2.51*	5.67***		60(24,36)
duration of a single activ	0.04	1.01		69(38,31)
total time spent for activ./l obs.	1.01	4.58***		60(24,36)
joint with	INPUT chances	D=3.24	OUTPUT chances	D=4.17
	partial X^2		partial X^2	
start-end	297			
resting				
exploration	0.28		1.63	
meeting with fem.			2.54	
examination of fem.				
attempt. to copul.				
copulation				
examin. of pherom.				

possible to show deviations from the standard along with the standard itself in more compact and comprehensive form (Fig. 1), revealing even subtle changes in the structure and organization of behaviour.

The successive part of the program displays changes in behaviour upon time (time budget), in other words, percentage of time spent for each activity during successive phases of observation (Fig. 2 and 3). It allows to monitor not only short term processes, but also whole life behaviour. Any significant alterations can be analyzed by the program and compared to the standard (Fig. 4). Such time budgets are very helpful in detecting distinct phases in behaviour during the lifetime or to keep track of adaptability or persistency in reaction to kairomones etc.

However, even with the aid of computer analysis, ethological research requires still considerable time for direct observation of behaviour. Thus, observations usually are performed on a limited number of replications. Together with individual variability of behaviour it leads to substantial variability of results. So, computer simulation is proposed to verify

Fig. 1 Organization of the Khapra beetle male mating behaviour.

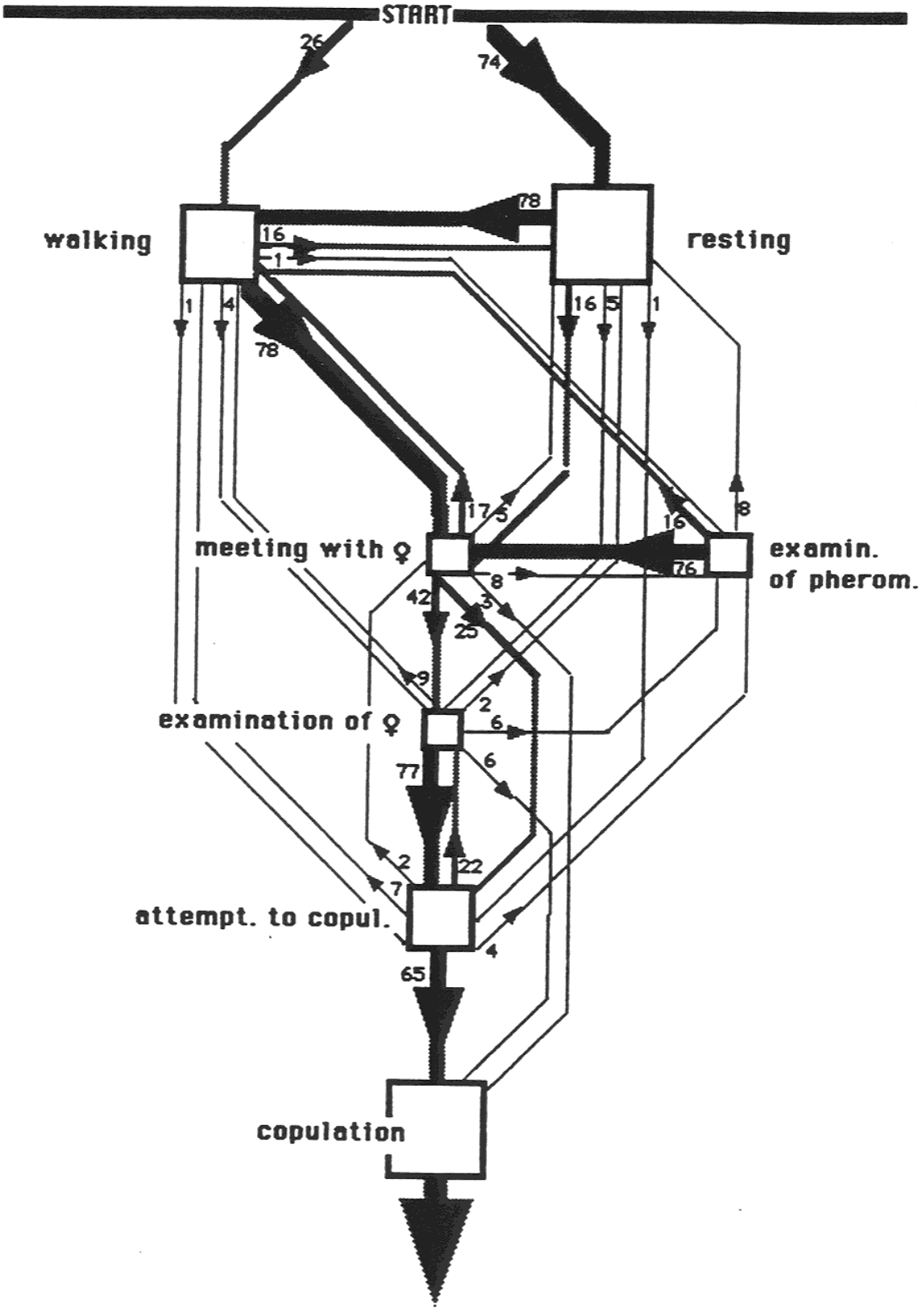


Fig. 2. Average time budget - EXPERIMENTAL DATA

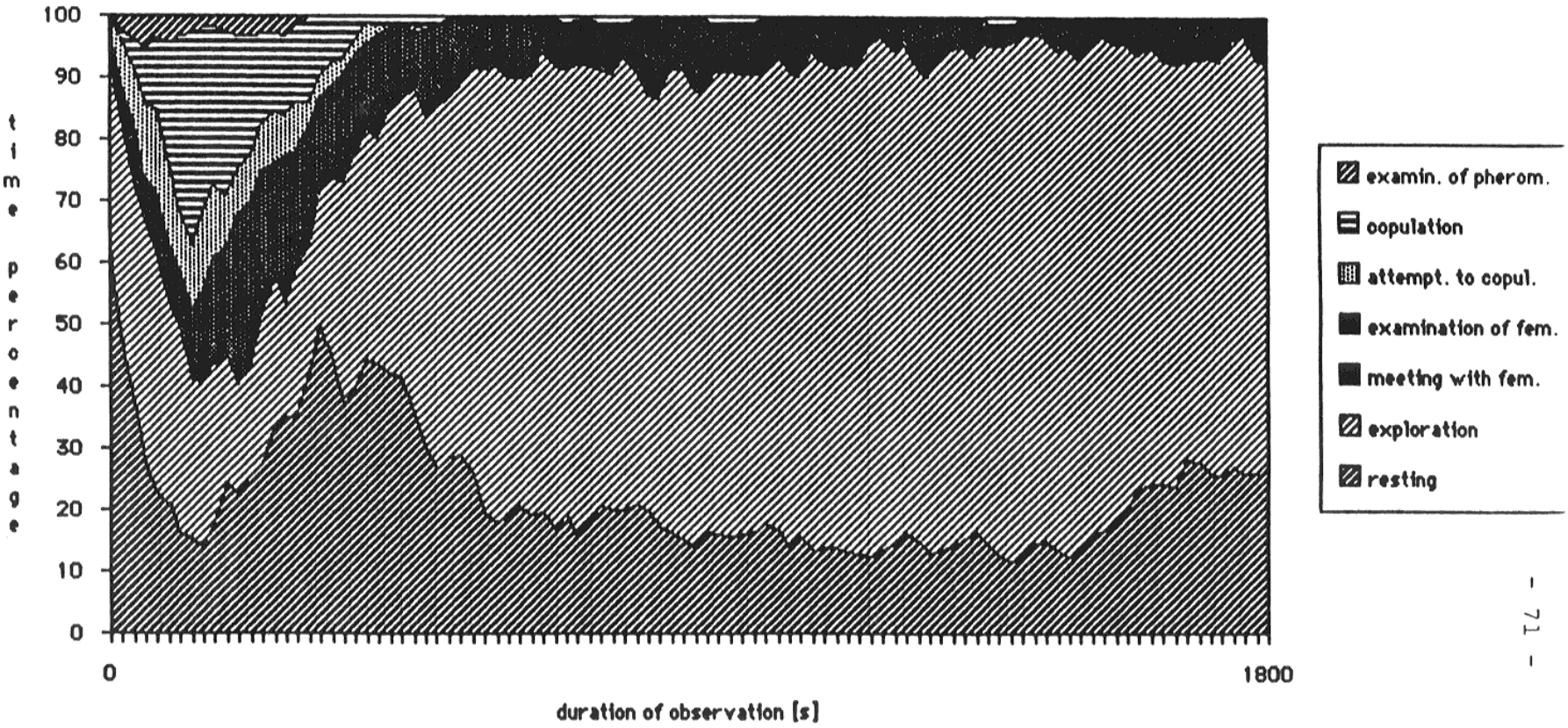
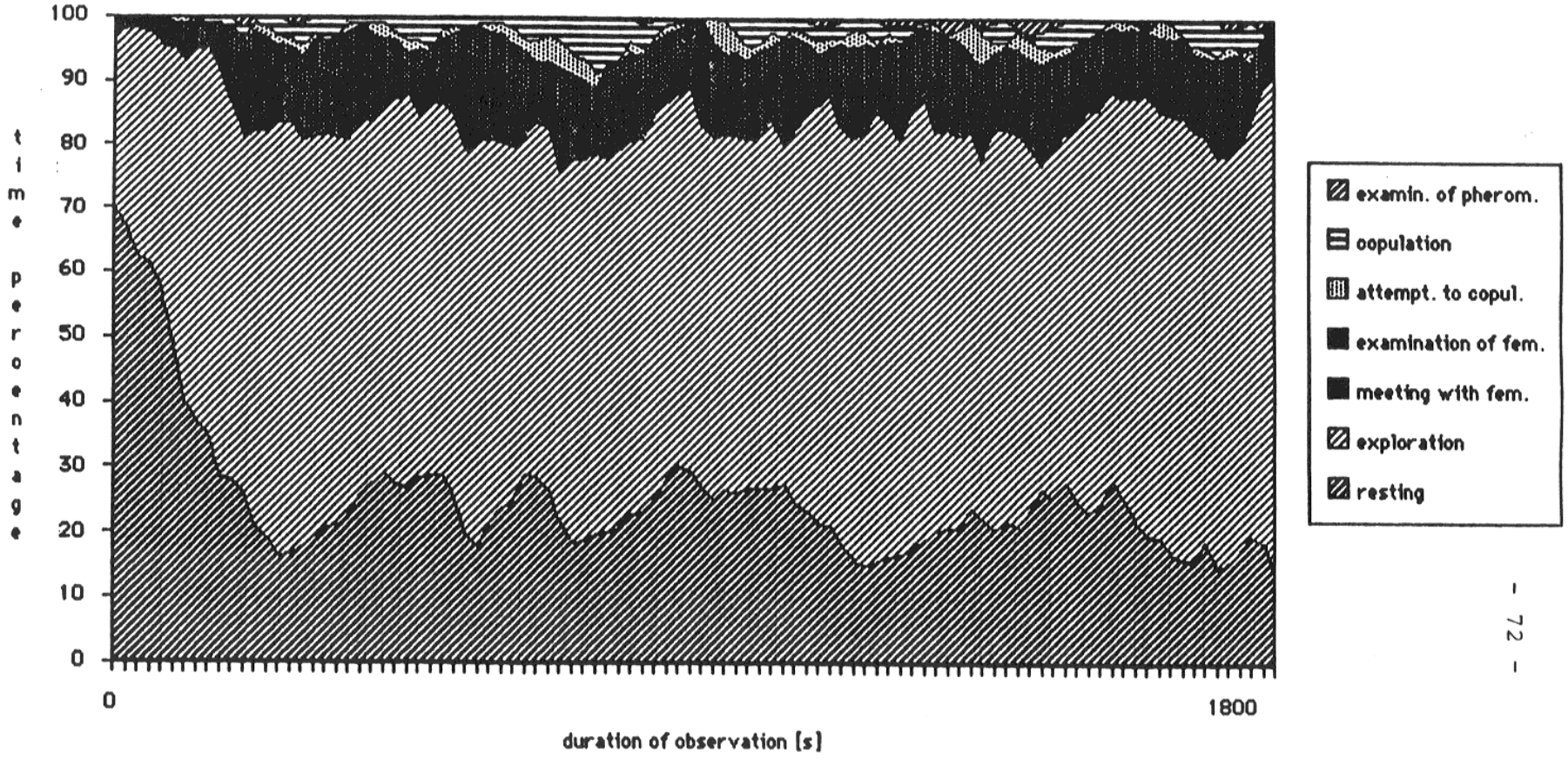


Fig. 3. Average time budget - MODEL I



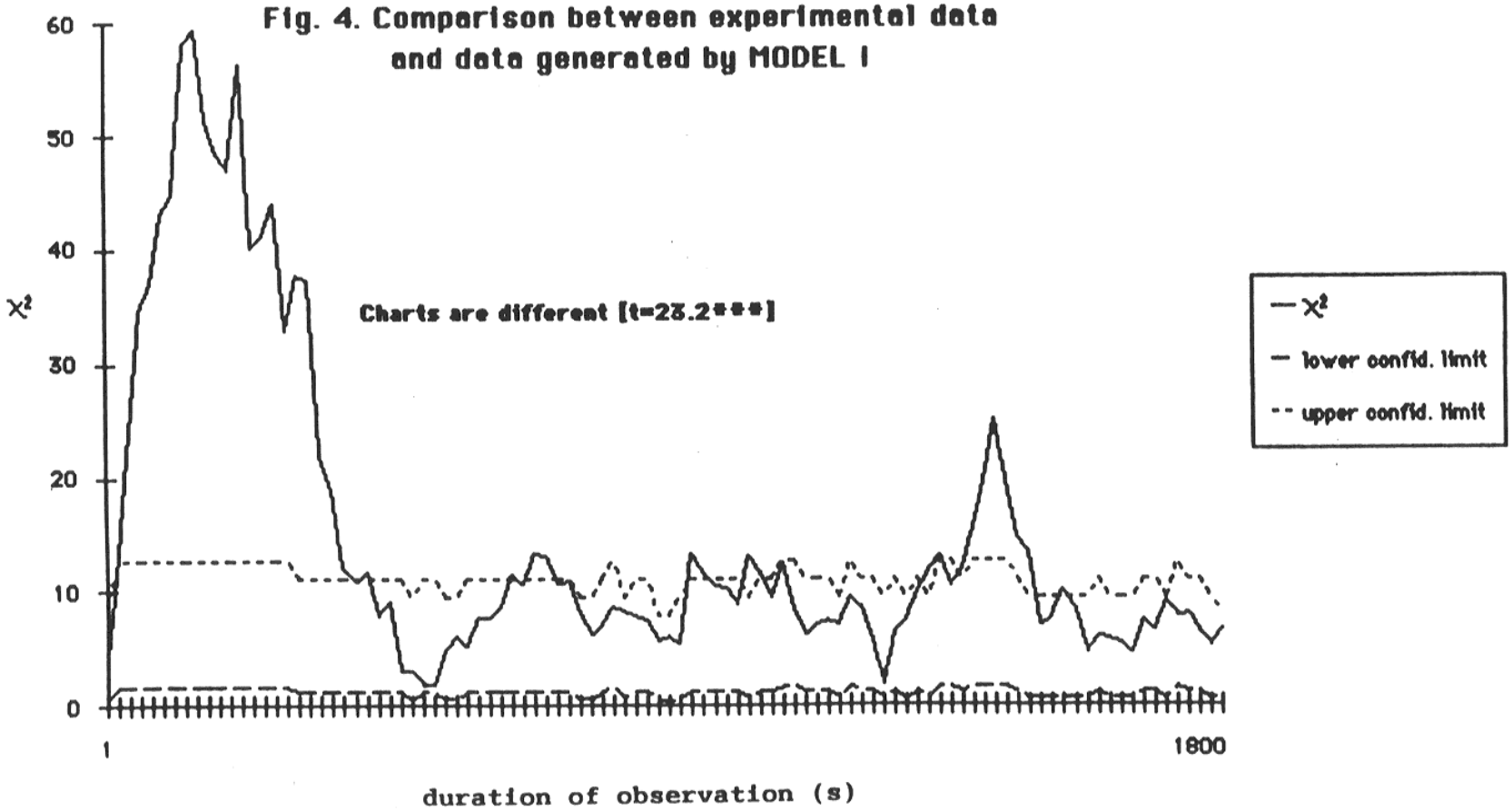


Fig. 4. Comparison between experimental data
and data generated by MODEL I
60
50
40
30
20
10
0
χ²
Charts are different [t=23.2***]
— χ²
— lower confid. limit
-- upper confid. limit
1
1800
duration of observation (s)

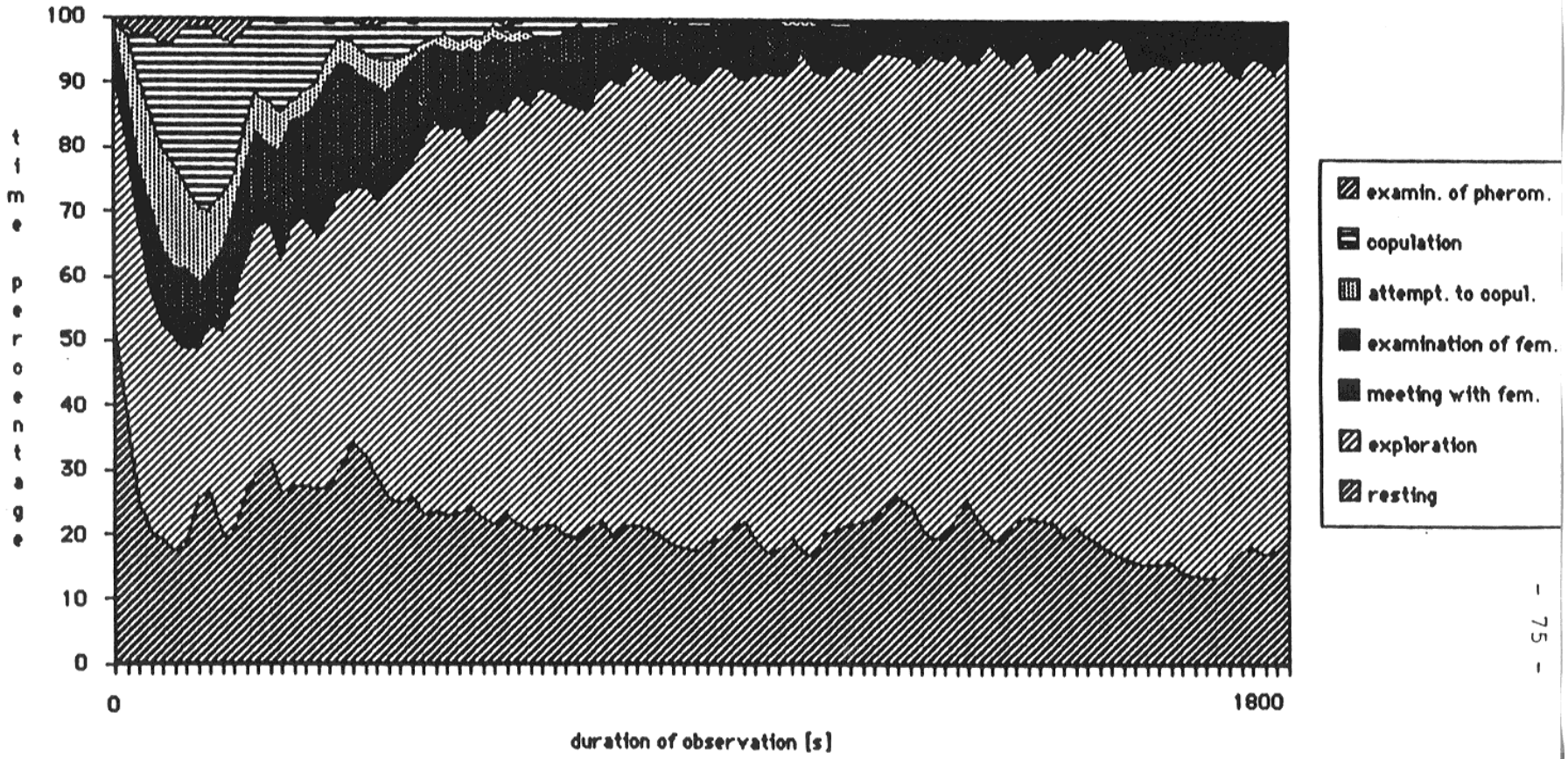
conclusions drown from ethological observation (Lux 1989b). The simulation models are based on the assumption, that behaviour may be divided into a number of periods approximated by stochastic processes (stabile, markovian, ergodic). The models are built in computer programs using parameters taken from ethological analysis. In each run, the model generates data in the same format as those from observations of an animal. So, the generated data can be analyzed the same way as those from observations with quantitative estimation of each activity in time together with statistical comparisons. The models were found to be useful in verifying hypotheses about number of distinguishable phases and the influence of different factors on behaviour etc. Data obtained during studies on *Trogoderma granarium* behaviour are presented as a brief illustration. Three models were constructed, assuming single-phase random behaviour, two- and threephase behaviour. Only results generated by the first and the third model are presented in Fig. 3-6.

APPLICATION OF ETHOLOGICAL DATA TO QUALITY CONTROL

Quantitative analysis of behaviour complemented and verified by computer simulation is a very powerful tool. Ethological observations are indispensable in gaining knowledge and understanding of beneficial's behaviour. However, writing computer algorithms for automatic recognition of complex behavioural patterns seems rather unlikely in the nearest future. But quite frequently, complex behaviours have well correlated simple attributes, which can be recognized and traced by modern image analysis techniques. For example, complex sexual behaviour may be correlated with aggregations within zones saturated with a pheromone. A sophisticated process of searching for prey may be correlated with persistency of enhanced mobility in zones with low concentration of kairomone, while host acceptance may be attributed to preference of zones with high concentration of kairomone.

Recently, considerable efforts have been made to correlate behavioural traits to performance of beneficials in the field. It was found, that efficiency could be estimated by measuring mobility of *Trichogramma spp.* which is believed to reflect its capacity for host location (Bieri et al., 1990, Bigler et al., 1988). Also host searching and host acceptance, crucial quality factors for beneficials reared on factitious hosts, may be attributed to their reaction to kairomones (Bergeijk et al., 1990, Dicke, 1988a,b, Dicke et al., 1989, Jones et al., 1973, Kaiser et al., 1989, Noldus, 1989b).

Fig. 5. Average time budget - MODEL III



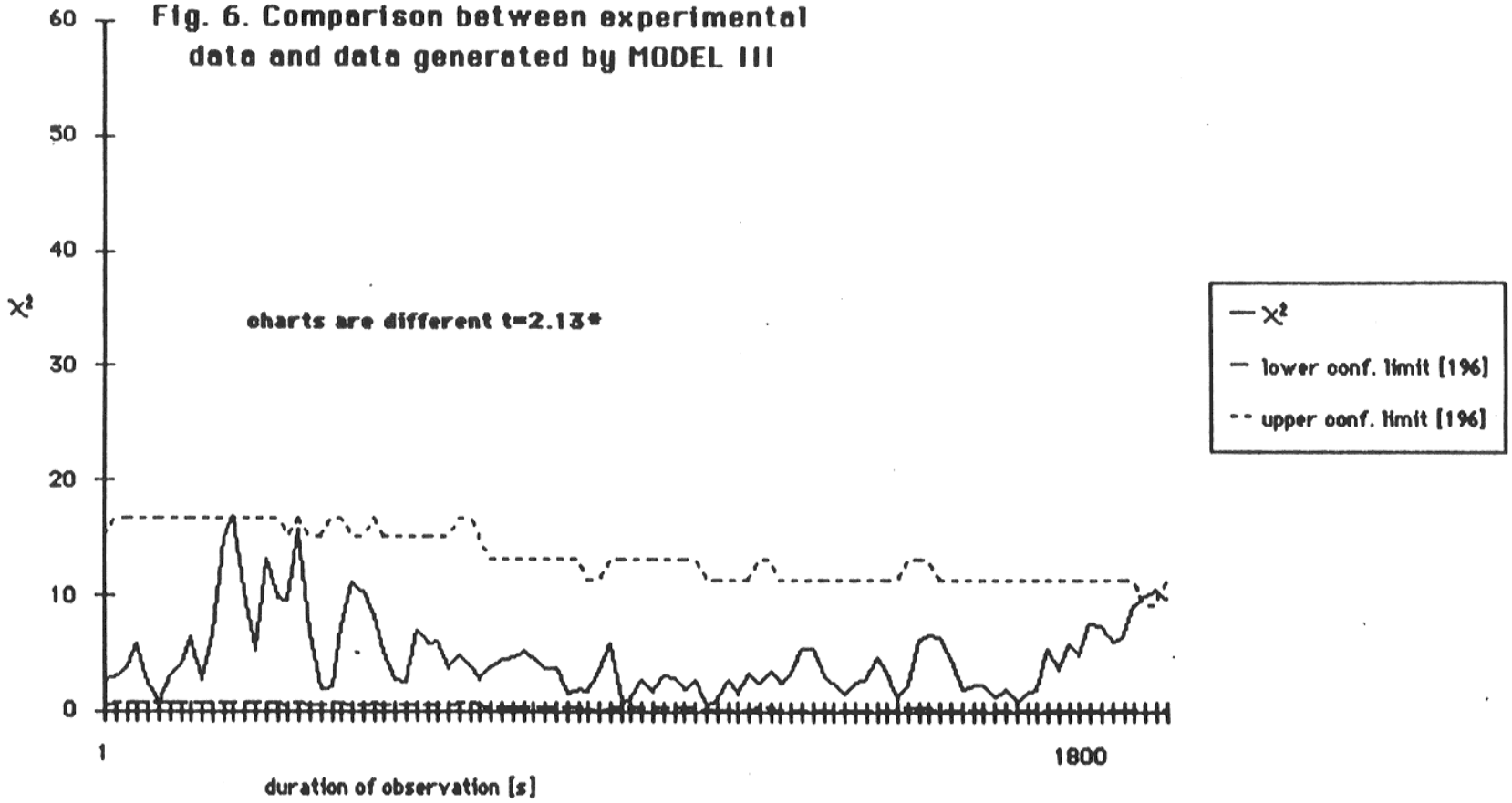


Fig. 6. Comparison between experimental data and data generated by MODEL III

Thus, measuring selected attributes of beneficial's behaviour provides information about its deviations from the standard and enables the forecast of their effectiveness in the field.

Aggregation, mobility, odour preference and other behavioural attributes can be traced by programs which recognize animals in the arena, caluclate coordinates of their position and interpret such data properly. Such programs and equipment are in use in many laboratories (Crawley et al., 1982, Dusenbery, 1985, Lenteren et al., 1979, Royce-Malgren and Watson, 1987) and some of them are available commercially. However, most of them analyse movements of only individual or few animals. Usually such programs calculate many parameters of movement, but the results are often difficult to interpret. In the case of animals exhibiting considerable individual variability of behaviour the usefulness of such programs is limited. There is a need to develop methods and equipment for automated analysis of insect behaviour in the presence of odours. Methods proposed by Bakchine et al. (1990) seem to be very promising. Also a multizone olfactometer developed by Lux and Stopinski (1991) could be useful for such purposes. The olfactometer enables long lasting observations of many insects at the same time. Such equipment with adequat programs, combined with ethological standards and requirements for particular conditions (climatic zone, kind of plants etc.) could be used as a basis for decision making systems in quality control.

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A QUALITY CONTROL TEST FOR ENCARSIA FORMOSA (HYM., APHELINIDAE) AND THE RESULTS OVER A TEN YEAR PERIOD

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Abstract

The parasite *Encarsia formosa* is used on a large area of glasshouse crops to control the greenhouse whitefly *Trialeurodes vaporariorum*. The parasite is supplied to the growers in the pupal stage adhered to paper cards. In this test parasites emerged from the cards are compared to parasites emerged from leaves. The following parameters are investigated: period of emergence and emergence rate, size and oviposition capacity.
There are no differences found between both "types" of parasites during the last ten years. The use of this test as a quality control test for *E. formosa* is discussed.

1. Introduction

The parasite *Encarsia formosa* is used worldwide to control the greenhouse whitefly *Trialeurodes vaporariorum* and is commercially reared by many producers of natural enemies (Van Lenteren & Woets, 1988). Koppert started to produce *Encarsia formosa* in 1971 and the parasites were supplied to growers in the pupal stage on leaves on which they were also produced. In the beginning cucumber was used as a host plant, later on tobacco (Ravensberg, in press) which is now a commonly used host plant for the production of this whitefly parasite.
Selling these parasites on leaf material has many disadvantages:

a) the degree of parasitism has to be checked on each leaf before harvesting and shipment;
b) the number of unparasitized whiteflies should be very low, which sometimes means that some leaves cannot be used, resulting in production inefficiency;
c) deterioration of the leaf during storage and transport;
d) tobacco leaves may release harmful vapours, that even kill *E. formosa* in closed environments, as in packages for shipment;
e) there is a risk of transferring diseases or pests with the leaf material to growers, and especially in case of export, this is unacceptable;
f) cutting the leaf into small parts and the distribution of the parts in the greenhouse is laborious.

Overall, handling the parasites in this way involves risks of loss of quality and of contamination and is very time consuming, both for the producer and the user.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

Therefore an other method was investigated resulting in removal of the black pupae of the parasite from the leaf and gluing them on paper cards. On the cards a stripe of honey (honey:water= 2:1) is provided as food for the just emerged adults. Supplying *E. formosa* on cards has clear advantages: harvesting is less critical, storage is easier, transport and introduction is more convenient and the product is free of possible pests and diseases. In 1979 the first cards with the black pupae of *E. formosa* were sold, under the commercial name EN-STRIP. Initially, these technical manipulations gave a lower percentage emergence from the cards compared to the leaves, and a poorer performance (a low searching activity and oviposition frequency) of the parasites (van Lenteren, 1986). This was caused by the technique of removing the pupae from the leaves and by the gluing. According to van Lenteren (1986) many pupae were punctured and covered by glue. During 1980 these techniques were changed and improved and at the same time we developed a quality control test to check the parasites on several quality components. The following parameters were determined in this test:

1) the position of the pupae
   The pupae are glued onto the cards in roughly three positions as a result of a random process. They are orientated in the 'natural' position, sideways and upside down. The incidence per position is determined.

2) the emergence of adults per position during time
   Emerging from 'naturally' and sideways orientated pupae occurs in the normal way, as on leaves. An adult emerging from an upside-down pupa first makes a hole in the upperside of the pupa which is now glued to the card. Then it has to turn around and gnaw an second hole in the bottomside of the pupa to crawl out. Possibly this affects the moment of emergence and the parasite's fitness in a negative way.
   Percentage emergence and the emergence during time of the differently positioned pupae are determined and compared with emergence from leaves.

3) the size of the adults
   The technical manipulations might affect the development of the parasite inside the pupa. For instance, the contact between the leaf and the bottomside of the pupa is broken, the waxy spines of the whitefly pupa are often broken off, the outer wax layer can be harmed. Does this affect the parasite inside the pupa, perhaps because of water loss by evaporation through the unprotected bottomside or the broken wax layer? Does this result in a smaller parasite with a lower fecundity? Do other technical aspects or the glue (a water-based natural glue) affect the development. In order to investigate this the body size of the wasps from cards and leaves is measured.
   The head width is taken as a reliable estimation for body size, which, once formed, does not or hardly change because of the chitin composition of the head. The abdomen length is considered as a variable parameter, which indicates the parasite's fitness. Parasites from the cards or from one of the positions on the card may be smaller in general, so both in head width and abdomen length, or they may have a smaller abdomen. The abdomen shrinks for instance when the parasite is deprived of food. A smaller abdomen may be caused by the difficult and energy consuming emergence from an upside-down positioned pupa.

4) the egg-laying capacity
   This is tested in order to compare adults emerged from leaves to adults emerged from cards from the 'natural' position and from the upside-down position. If these latter ones would suffer from the emergence this is likely to show it self the first day after emergence, rather than after a few days in which recovery after feeding can occur. So first-day oviposition has been tested; in 1990 a prolonged egg-laying test was performed to investigate the parasite's oviposition capacity.

This quality control test was performed after van Lenteren (1986) and later modified in respect to the egg-laying test. The components 1 and 2 are seen as product control parameters (Leppla & Fisher, 1989) which are measured on a regular weekly base. The components 3 and 4 have been checked on a irregular base and in case problems are suspected or expected, or if techniques are changed; they are considered to be traits of the overall quality of *E. formosa*.

2. Material and methods

To compare parasites emerged from leaves to parasites from cards, a leaf sample from the production, suited for harvesting, is taken. Two small leaf pieces, each with about 100 black pupae, are randomly taken from 2 leaves, the other leaves are processed in order to remove the pupae. These are then glued on cards, about 100 on each card. This is done following the regular procedure for EN-STRIP. This set-up was followed for the tests of 1981 to 1984. From 1985 onwards emergence is checked weekly by taking a random sample of 10 cards out of a commercial batch.

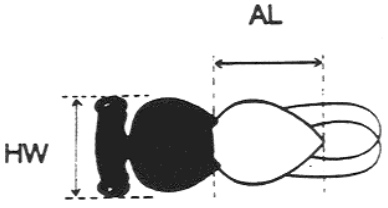
1. The position of the pupae
Their position is determined whereby 'natural' is as the position on the leaves, sideways as the pupa is glued on either of its sides, as well as sligthly hanging over to the 'natural position' or to the upside- down position whereby the emerging parasite is not hampered. Upside-down includes pupae which are positioned in such a way that the wasp cannot emerge in the normal way, but has to make two holes.

2. The emergence of adults
Each day around noon, emergence is counted, since most wasps, which generally emerge in the early morning hours, have then emerged. The position of the pupae from which the parasites have emerged is noted and the empty pupae are carefully removed. To see whether the pupal orientation affects the pupal development and the moment of emergence the mean length of the emergence period has been calculated for the parasites from the three positions. The emergence period is defined as the period starting at the day of the first emerging parasites untill the day of the last emerging parasite.

3. The size of the adults
To measure the size of parasites from leaves and from cards, freshly emerged parasites, maximally 24 hours old, are collected and anaesthetized by CO_2.
The head width is measured between the outer edges of the eyes and abdomen lenght is measured ventrally as the distance between the first abdominal segment and the abdomen tip (fig. 1) which comprises the yellow part of the female body. A magnification of 80 x is used. In the test in 1990, pupae on cards were stored for 9 days at 10 °C and measured after emergence in comparison to non-stored parasites. The effect of the availability of honey on the card on the abdomen lenght was also investigated.
The results are tested on significance by the t-test ($p<0.05$).

Fig. 1 Body size measurements, head width and abdomen length, in *E. formosa* females

HW = head width
AL = abdomen length



4. The egg-laying capacity
For the oviposition tests cards were taken from which all pupae had been removed except for the ones in the 'natural' position in order to measure their egg-laying capacity. The same was done in order to test parasites from the upside-down position.
Freshly emerged parasites, maximally 24 hours old, are collected from these cards and from leaves. The wasps from the cards were able to take up honey from the card, on the leaf honeydew was available to them. The oviposition capacity of the parasites is investigated by confining individual females in a leaf cage (ϕ 35 mm) on a leaf of a tobacco plant with an ample amount of young whitefly larvae, i.e. > 30.(c.f. Vet & van Lenteren, 1981: fig. 3 and 4). The female is allowed to parasitize for 24 hours and after two weeks the black pupae are counted.
In 1990 this test was modified since using complete plants with leaf cages askes for a lot of preparation and it is a difficult and tedious method. Some parasites escape out of the leaf cages or are lost or harmed during handling.
The test is now performed with leaf discs in a specially adapted micro test plate. This is a 6 well plate (Greiner, 657102), 82x127 mm, each well is 35 mm in ϕ and 16 mm deep. The plate is covered by a lid closing off each separate well.
In the lid 6 holes of ϕ 28 mm are made corresponding with the wells and covered with fine gauze. In the inside of the lid the notches are removed so that it closes tightly on the bottom part. As an extra ensurance small tool clips were used to press the lid on the bottom part. In each compartment 4 mm of water-agar (1 %) is poured on which a leaf disc is mounted just before the agar solidifies (at ca. 35 °C). The leaf disc should fit exactly in the well. A leaf disc contains an ample amount of whitefly larvae and in each well a parasite is put for 24 hours (23 °C, 80% RH, L:D=12:12). Each day, around noon, the wasps are anaesthetized and transferred to a newly prepared test plate. The plates with the parasitized whitefly larvae are than kept for about 20 days to count the black pupae. The leaves remain in a good condition for about 14 days.
Each female is transferred daily to a new leaf disc over a period of 14 days.

All tests were performed at a temperature of 22-24 °C, a RH of 75-90% and L:D=16:8 except for the oviposition test in 1990, which was done at L:D= 12:12.

3. Results

3.1 The position of the pupae on the cards

The pupae are mechanically glued on the cards and this results in differently orientated pupae. In table 1 the percentage of pupae in the 'natural', sideways and upside-down position is given from countings over a period of 10 years. The pupae are positioned 'naturally, sideways or upside-down mostly with a ratio of 1:2:1.

Table 1. Pupae on cards: the percentage of pupae in the 'natural', sideways and upside-down position

Date \ position	'natural'	sideways	upside-down	n	(*)
October 1981	26.4	48.6	25.0	216	(2)
November 1981	27.7	54.2	18.1	416	(4)
March 1982	23.2	61.6	15.2	232	(2)
March 1984	28.6	38.3	32.9	279	(3)
April 1990	27.5	45.6	26.9	167	(2)
Mean (%)	27.0	49.2	23.8		

n = total number of pupae
(*) = number of cards checked

3.2 Emergence

In general, the emergence time of wasps from leaves is compared to those from cards, as well as between the different positions on the cards. In the tests of 1981–1984 emergence from leaves and cards is checked during the quality control tests. Cards were then directly taken from the gluing machine and used for the test.
Since 1985 emergence is checked weekly by determining the emergence from 10 cards taken from 10 strips. The sample of strips is taken from packed material, strips in cellofane in a small box, EN-STRIP, ready for shipment.
Table 2.1 shows that emergence from cards is around 95%, about the same as from leaves. Emergence rates in the three positions are also not different. Low emergence rates from leaves in 1981 and 1982 are presumably caused by nicotine vapours from the tobacco leaves in closed jars. Hereafter leaves were kept in jars, closed with gauze.
Table 2.2 shows the mean emergence on cards per year since 1985, resulting from weekly controls of 10 cards. The year means vary between 84 and 90% with slightly larger variations within one year. The lower emergence compared to the results in 1981–1984 is apparently caused by the packaging and unpackaging handlings. The mean number of pupae per card is also shown in this table. Our standard, the minimum number of emerged adults per card, is set at 72. Over the years this standard is always amply met.

Table 2.1 Percentage emergence from leaves and cards and per position on the cards in quality control tests, 1981 to 1984 and 1990

Date		leaves	n	cards	n	'natural'	sideways	upside-down
October	1981	52.7	241	95.4	216	96.9	94.2	95.7
November	1981	88.7	220	93.5	216	87.1	97.1	87.8
March	1981	95.7	235	99.1	236	98.2	100	97.2
March	1984	95.6	203	94.5	279	94.2	94.0	93.0
April	1990	94,6	113	89,1	193	87.0	90.6	88.5

n= number of pupae

Table 2.2 Mean number of pupae per card and mean percentage emergence from cards of weekly controls of 10 cards, 1985 to 1990

Year	mean number of pupae per card		mean percentage emergence	
1985	134	(19.6)	87	(7.0)
1986	106	(18.9)	89	(5.0)
1987	112	(28.2)	85	(9.0)
1988	111	(21.5)	91	(5.8)
1989	118	(30.2)	79	(8.6)
1990	101	(20.0)	85	(7.6)

(...) = standard deviation

Table 2.3 Mean emergence period, in days, of parasites from different positions on the cards

Date \ position	'natural'	sideways	upside-down
October 1981	3.92 (54)	4.56 (97)	4.57 (35)
November 1981	4.91 (54)	5.03 (99)	4.96 (45)
March 1982	6.43 (54)	6.38 (141)	5.51 (39)
March 1984	4.13 (80)	4.74 (107)	4.18 (92)
April 1990	6.39 (71)	6.34 (163)	6.13 (57)

(...) = number of parasites

As shown in table 2.3 the emergence period is similar between the parasites from each position in the test of November 1981 and April 1990. In October 1981 this period is shorter at the 'natural' position, in March 1982 the upside-down positioned pupae have a shorter emergence period, whereas in March 1984 sideways positioned pupae show a longer emergence period. These variations do not show a clear evidence for a delayed emergence for either of the positions.

3.3. The size of the parasites

The results of the measurements of the head width and abdomen lenght are given in table 3.1. In October 1981 the head width of leaf and card parasites is not different. The abdomen length is also not significantly different. In November 1981 head width is again identical, abdomen length however is bigger in parasites from leaves compared to parasites from the cards. In March 1982 head width and abdomen length are similar in both groupes of parasites. In March 1984 the head width of the parasites is the same, but the abdomen length of the leaf parasites is significantly bigger. In 1990 head width of parasites of one leaf is smaller than of another leaf and the cards. Abdomen length is similar between the groupes.
If parasites from cards are not able to take some food this appears to affect their abdomen length immediately; in this test they are significantly smaller than the ones which are given some honey (0,294 mm versus 0,308 mm) and the ones from the leaves (0,303-0,308 mm) which are able to take some honeydew.
Pupae from cucumber, collected in a commercial greenhouse some generations after release, are bigger in head width and abdomen length. However, compared to parasites in 1984 from tobacco they are similar in size.
If pupae on cards are stored for some time, in this case 9 days at 10 °C, the body size is not affected. Comparing head width over the years of tobacco leaf parasites and card parasites, there is a variation in the means of 0,281-0,294 mm within the leaf parasites and of 0,286-0,293 mm within the card parasites. Taking into account that pupae on cards are a mixture of pupae coming from a number of leaves, it is understandable that the variation in means is less and lies within the means of the leaves, of which only small parts with a low number of pupae are used for the tests. Although not presented here, data indicate that parasites from leaves from different plants sometimes differ significantly in size, both in head width and abdomen length. The mean abdomen length in leaf parasites varies over the years between 0,294 mm and 0,323 mm, in card parasites between 0,295 mm and 0,317 mm, showing a slight tendency towards smaller abdomen lengths in card parasites. The largest difference in means found is approximately 3% (March 1984: 0,323 versus 0,314 mm).

Table 3.1 Head width and abdomen lenght (mm) of parasites from leaves and from cards

	head width		abdomen length				remark
Date	L	C	L	C	nL	nC	
October 1981	0,288 a	0,286 a	0,294 c	0,295 c	17	156	H
November 1981	0,289 a	0,291 a	0,306 c	0,299 d	113	114	H
March 1982	0,291 a	0,292 a	0,315 c	0,317 c	218	227	H
March 1984	0,294 a	0,293 a	0,323 c	0,314 d	130	181	H
April 1990	0,281 a	0,290 b	0,303 c	0,308 c	116	107	H
	0,291 b	0,288 b	0,308 c	0,294 d	132	97	–
		0,296 e		0,316 d	200		C
		0,290 a		0,294 c	132		H
		0,289 a		0,293 c	219		H,ST

L= parasites from leaves
C= parasites from cards
n= number of parasites measured
H= honey stripe available on cards
– = no honey available on cards
ST= cards stored for 9 days at 10 °C
C = parasites from a cucumber leaf

Data per row (or per set, 1990) and per size followed by the same letter are not significantly different (t-test, $p<0.05$)

3.4 Oviposition

The oviposition of parasites emerged from a leaf and from the 'natural' position and the upside-down position on a card during the first day after emergence is given in table 3.2. The results obtained in 1982 and 1984 show no difference in the number of eggs laid, actually the number of progeny, with regard to the origin of the parasite. The results of the prolonged test in 1990 (table 3.3.) show a large variation in eggs laid on the first day of oviposition. During fourteen days of oviposition, however, there is no difference between the egg-laying capacity of the parasites from a leaf or from the different positions on a card.

Table 3.2 Oviposition of *E. formosa* on the first day after emergence from leaves and from the 'natural' and upside-down position on a card

	MARCH 1982			MARCH 1984		
position	eggs/♀/day	1	n	eggs/♀/day	1	n
'natural'	7.4 a	(5.1)	10	5.6 a	(5.1)	8
upside-down	5.4 a	(3.8)	9	5.1 a	(3.0)	10
normal on leaf	5.2 a	(3.4)	10	5.3 a	(4.5)	3

(...) = standard deviation.
Data followed by the same letter are not significantly different (t-test, $p<0.05$)

Table 3.3 Oviposition of *E. formosa* from leaves and from the 'natural' and upside-down position on a card, during fourteen days

position	APRIL 1990					
	eggs/♀/day 1		eggs/♀/day *	eggs/♀/14 days		n
'natural'	7.0	a (3.5)	10.5	147.6	(30.6)	9
upside-down	2.5	b (3.6)	10.4	145.8	(19.6)	8
normal on leaf	3.2	ab (4.2)	10.3	144.0	(52.2)	9

* mean number over 14 days.
n = number of females tested
(...)= standard deviation

Data followed by the same letter are not significantly different (t-test, $p<0.05$)

4. Conclusions and discussion

This quality control test was developed and performed to see whether the removal of pupae from leaves and gluing them on cards affects the parasites. Negative effects, such as a delayed emergence and smaller parasites with a lower oviposition capacity, were expected as a result of the technical manipulations and the unnatural positions of the pupae on the cards.
Only parameters which can be measured in the laboratory are used, field performance parameters are very difficult to check and are not included.

As a result of the gluing procedure one quarter of the pupae per card is orientated in the natural position, one quarter lies upside down and about half of them is positioned on either of its sides. This appears to be expected result of a random process and means that about 25% of the parasites are facing a more difficult eclosion as described in the introduction. Emergence from leaves and cards lies around 95% and is not different. Care should be taken when emergence from leaves is checked, because tobacco leaves seem to release dangerous vapours in small, closed environments. When emergence from the three positions on the cards is compared, there are no differences in the emergence rates per position (table 2.1.) During the routine controls in 1985-1990 the emergence is approximately 86% (table 2.2.) which is lower than the results of the quality control tests done in 1981-1984 and 1990. This is caused by a different set-up of the tests, in particular, more handlings are done at the routine controls, apparently resulting in physical damage. Here, packaging and unpackaging is done, which is similar to the practical circumstances in which a grower applies EN-STRIP.
With the application of *E. formosa* in practical situations we consider an emergence of about 80% as an acceptable level.
Besides the emergence rate, the moment of emergence may also indicate possible differences between the three card positions. There is no difference (table 2.3) between the emergence period of the pupae of the three positions. This means that wasps from upside-down pupae on average do not need more time to emerge than the others.
Differences between the test results per year are attributed to differences in harvesting times and seasonal fluctuations and are not relevant in comparing emergence from the three positions. The emergence tests show that there are no differences in emergence of parasites between cards and leaves and that emergence from the three positions is similar, in rate as well as in time.
It is generally assumed that smaller parasites are of a lower quality, at least that the oviposition capacity is less and longevity is shorter. This was demonstrated in *Diglyphus begini* by Heinz & Parrella (1990), and is also expected in *E. formosa*.
Therefore the size of the parasites from leaves to parasites from cards was compared The size of the adults was determined by measuring head width and abdomen length.

Head width is similar in parasites from leaves and from cards except for parasites of one piece of leaf in 1990 which have a smaller headwidth (0,281 mm, table 3.1.)
Abdomen length only differed between leaf and card parasites in November 1981. In this case, card parasites had a smaller abdomen than leaf parasites. In these tests parasites from a certain position on the card were not measured separately, data show the mean size of all parasites. In general it can be concluded that card parasites are as big as leaf parasites.
Differences in abdomen length between the years is bigger than head width. The reason for this is not clear, perhaps abdomen length is more influenced by seasonal fluctations, hostplant quality and host size than head width.
If parasites are not able to take up some food just after emergence this causes a smaller abdomen. So the availability of honey on the card is important as an artificial food source, moreover honey positively affects life-span and oviposition (van Lenteren et al., 1987).
Parasites from cucumber were bigger in head width and abdomen lenght than the card and leaf parasites in 1990. This is in agreement with measurements of van Vianen & van Lenteren (1986a), who found that whitefly pupae on cucumber are bigger than those on tobacco resulting in bigger parasites.
They also measured parasites orginating from Koppert B.V. and found a head width of 0,293 mm, which corresponds well with our data. From other measurements they did on the head width of *E. formosa* coming from different host plant species it can be concluded that the head width found in this article lies above the mean values found by them. Therefore we consider the size of our parasites as well acceptable.
A short storage period at about 10 °C does not seem to affect the body size of the wasp.
The first day oviposition after the emergence from the 'natural' and the upside-down position on the card does not differ from oviposition of leaf parasites. The variation in the results is quite large, but they certainly do not indicate that card parasites lay fewer eggs, rather, that looking at day-one oviposition is not a good method for assessing possible differences.
Oviposition over a longer period shows, that card parasites lay as many eggs as leaf parasites, and that there is no difference in oviposition rate related to pupal position. The daily rate of oviposition is about 10 eggs which is comparable with literature data (van Vianen & van Lenteren, 1986b).

The quality control tests over a 10 year period show that there are no differences found in parasites emerged from leaves compared to parasites emerged from cards and that the unnatural pupal positions on the card do not impose negative effects on the parasites when emergence rate, time of emergence, size and oviposition is measured. Field performance is not checked but successes in the biological control of whitefly in many crops and circumstances over a long time prove the effectiveness of these *E. formosa* parasites in the field.
This quality control test is therefore considered to be an effective tool in measuring the overall control of *E. formosa* whereby emergence time and rate should be measured routinely and size and oviposition on a yearly base or when techniques are changed.

Acknowledgements

The author likes to thank Dr. J.C. van Lenteren for the cooperation in developing this test and for critical remarks and also thanks Ria Baas for the performance of the measurements in 1990 during a placement of the Agricultural Highschool of Delft.

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A QUALITY CONTROL TEST FOR THE PREDATORY MIDGE APHIDOLETES APHIDIMYZA.

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Abstract

The effect of cold storage on the emergence of *Aphidoletes aphidimyza* was tested. Five days of pupal development at 24 °C before storage and a storage temperature of 8 °C was found to be optimal. In later experiments oviposition was included. Much attention was given to mating before using individual females. It was found that providing sufficient space was crucial for mating and that females have to mate soon after emergence. Female midges were tested individually on their egg-laying capacity by using petri dishes with leaves on agar. Combining cold storage with the fecundity test showed that, though the emergence rate remained high, oviposition declined from 48 eggs per female for the none-stored to 22 eggs after one week and to 0 eggs after two and three weeks of storage.

1. Introduction

The predatory midge *Aphidoletes aphidimyza* is used as a biological control agent against aphids (Kulp et al.,1989). Since 1990 the midge has been mass produced and sold by Koppert B.V. (van Schelt et al.,1990).
Initially only the numbers of pupae per bottle, the percentage emergence and the sex ratio were used as a simple method of assessing quality of the product.
An additional test was needed to measure the fecundity as a more realistic trait of the actual overall quality in the field. This parameter is not only used for testing the quality of the product, but is also of great importance in case of alterations in the mass rearing system (e.g. storage at low temperatures).
Several fecundity tests have been described for *A. aphidimyza* (El Titi, 1976; Mansour, 1976; Gilkeson, 1986,1987). They all have in common that aphid infested plants are used or detached leaves with their petiole in water. A disadvantage of this set-up is that it needs much space and that regularly plants have to be changed or given water.
Research has been done on using artificial material for egg-laying, but the best results were always obtained with fresh leaves and a surplus of living aphids. El Titi (1976) found that 79 % of the eggs were laid on leaves with aphids and honeydew, 13% on (dead) aphids without honeydew, 8% on honeydew alone and 0% on clean leaves.
An alternative to test fecundity without the use of plants is described by Kuo (1982). Single females emerge in a glass vial and after two days the number of ripe eggs in the abdomen is counted. Although this method seems very quick for screening purposes it does not give absolute numbers because more eggs can be formed during the oviposition period. With this method 25 eggs/female was the maximum, if plants with aphids were used the number increased to 95.
In our tests a new method was developed by using petri dishes with leaves on agar.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

After some failures (almost no egg-laying observed) the impression arose that something went wrong in the mating process. Midges have to mate within several hours after emergence else the abdomen of the female will start to swell by the ripening of the eggs and mating can not take place anymore (L. Gilkeson, pers. comm.). More attention was given to mating circumstances before using the females for the actual fecundity test. Eventually the relation between cold storage of pupae, percentage emergence and fecundity was investigated.

2. Methods

2.1 Determination of the storage temperature, maximal storage time and the optimal developmental stage for storage

A. aphidimyza is harvested daily in the mass rearing, so tests can be done with large amounts of synchronized material. Pupation takes place in humidified sand. After pupation it takes 11 days at 24 °C for the majority of the midges to emerge. To assess the emergence rate 50 pupae are put individually into gelatin capsules and stored at 24 °C, 75 % RH and L:D=16:8. Every morning the number of emerged midges is counted and the sexratio is determined.
At first the optimal storage temperature, maximal storage period and optimal developmental stage for storage were established. Tests were done in environmental rooms at 3 and 8 °C. Pre-storage development was 3, 4, 5 or 6 days of pupal development at 24 °C. Storage time was 2, 4 and 8 weeks at both temperatures. In total 24 groups were tested, each group consisted of 100 pupae, individually put into gelatin capsules. After the storage period pupae were put at 24 °C to check emergence. In this preliminary experiment only the emergence rate was used as a quality parameter.

2.2 Storage of *A. aphidimyza* in combination with the fecundity

The development of the fecundity test took some time. Initially the testing material emerged in a plastic one liter box. This box contained several hundreds of pupae. Because most midges emerge late in the evening, the following morning this box was crowded with newly emerged midges. Out of this box individual females and pairs are tested on their egg-laying capacity. The ovaria of female midges are at the same time examined under the microscope by squashing the abdomen.
Oviposition is tested in round plastic petri dishes (ϕ 7 cm) which can be closed tightly. In the cover a nylon mesh is made for air exchange. In the dish 4 mm of water-agar (1%) is poured and cooled until 35 °C, just before it solidifies, then a punched sweet pepper leaf disc is put on the agar. By carefully pressing, the remaining air is pushed out and a good contact between the leaf and the agar is provided. On the leaf 25 aphids (*Myzus persicae*) are put. In a cold room the test midge(s) are carefully introduced.
The petri dishes are put away upside down in an environmental room (24 °C, 70 % RH) for four days. By placing them upside down the aphids will settle better on the leaf, also the midges prefer the underside of leaves for egg-laying. After four days the number of eggs is counted on the leaf and also on the edges of the petri dish. Tests were done with single females, one female with one male and one female with three males.
After unsatisfactory results with the emergence in the plastic one liter box five pupae were put together directly in the petri dish, the morning after emergence all midges except the test female are removed.

Finally a gauze cage (60 x 60 x 60 cm) was provided for emergence and mating. In the cage a clean sweet pepper plant on rockwool (also a water source for the midges) is put for shelter. In the morning after emergence the cage is placed in a cold room (8 °C) and midges are carefully taken out. Individual females and pairs are tested in petri dishes on their egg-laying capacity.
In combination with this method a second storage experiment was carried out. Five day old pupae (24 °C) were stored at 8 °C and put at 24 °C again after one, two and three weeks of cold storage. Simultaneously emergence rate and oviposition were assessed.

3. Results

3.1 Storage temperature and maximal storage time

Non-stored pupae give an average emergence of 93%. This was also reached after a storage time of two weeks at 8 °C with pupae with a five to six days pupal development at 24 °C before storage (table 1).
This means that a pupal development of five to six days seems to be optimal for cold storage. It is clear that storage at 3 °C is giving a high mortality, storage at 8 °C is much better and acceptable for at least two weeks with respect to emergence rate.

Table 1. Percentage emergence of *A. aphidimyza* after storage at 3 and 8 °C for two, four, or eight weeks, for pupae with a different pre-storage development

	Storage at 3 °C			Storage at 8 °C		
P.S.D.	2 weeks	4 weeks	8 weeks	2 weeks	4 weeks	8 weeks
3	2	0	0	67	26	0
4	12	0	0	90	63	2
5	28	0	0	93	67	3
6	5	0	0	93	80	3

P.S.D.= Days of pre-storage pupal development at 24 °C

3.2 The storage of *A. aphidimyza* in combination with the fecundity

Midges emerged in 1 liter box.
The first results with the fecundity test were quite bad. In spite of the fact that almost all female midges were still alive after four days the percentage females laying eggs varied between 7 and 20 % in several trials. Though almost every female had a large amount of ripe eggs, as was found after examination under the microscope, they were not laid. The test with one male and one female put together in a petri dish gave 20 % egg-laying females. One female and three males resulted in 30 % egg-laying females.

Midges emerged in petri dish
The emergence of five pupae inside the petri dish and removal of the midges except one female resulted in 20 % egg-laying females.

Midges emerged in gauze cage
The use of midges after emergence and mating during the night in the gauze cage resulted in an average of 87 % egg-laying females. So this method was further used as a standard procedure for emergence and mating before bringing the females individually into the petri dishes.

Storage combined with fecundity
With unstored midges the average number of eggs is 48 per female after four days (table 2). It was remarkable that a large amount (up to 50%) of the eggs was laid quite unnaturally on the sides of the petri-dish.
After one week of storage at 8 °C the fecundity dropped to 22 eggs per female and after two and three weeks even to zero. Although the emergence remained high cold storage had a very negative effect on the egg-laying capacity. The midges which had been stored for two and three weeks appear to be less vigorous and remained in the lower part of the mating cage.

Table 2. Emergence and oviposition of *A. aphidimyza* after several periods of storage at 8 °C

Storage time	% emergence	% ♀♀	% egg laying ♀♀	egg/♀		n
none	96	55	83	48	a	30
1 wk	98	54	92	22	b	25
2 wk	88	64	16	0.2	c	25
3 wk	77	63	0	0	c	25

Five day old pupae are stored at 8 °C for one, two and three weeks. After this storage period pupae are put at 24 °C in a large cage for mating. Percentage emergence and fecundity is assessed. Figures in one column followed by a different letter are significantly different (Mann-Whitney $p<0.05$).

4. Discussion

In this newly developed oviposition test we found that females were able to lay on average 48 eggs. In literature the mean number of eggs per female can differ widely between authors. Mansour (1976) found 8, using groups of midges in wooden cages, Gilkeson (1986) came as high as 163, during total life span, using glass globes of 11 cm ϕ and introducing one female and three males upon eclosion.
Even the amount and/or quality of the honeydew is important. Kuo (1982) found 60.8 eggs/female when young aphids were used and 95.7 eggs/female with old aphids. Gilkeson (1987) reported that providing a drinking source resulted in 248 eggs per female.
Although the number of eggs in the control test is probably not the maximum number a female can produce during the first days, the test is discriminative and quick. Because the midge adjusts the number of eggs to the colony size of the aphids, for absolute figures on the fecundity more leaf space and a higher number of aphids has to be offered. Now 20 to 30 aphids are used; this is not enough food for 50 larvae. This may explain the eggs laid on the edge of the petri dish.
It was also concluded that mating had to take place during the night of emergence and did not take place in a small and crowded environment.
From the results it was concluded that storage of *Aphidoletes* has such a negative effect on the oviposition that we changed to selling only fresh material.

We consider the described set-up as a reliable, discriminative test which can be used as a standard quality control test with respect to the parameters emergence and oviposition . This test should be used if any alterations are made in the processes of mass-rearing and if problems with the quality are suspected.

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THE EFFECT OF STORAGE CONDITIONS ON ADULT LONGEVITY, FERTILITY AND SEX-RATIO OF THE LEAFMINER PARASITOID *Diglyphus isaea* (WALKER)

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ABSTRACT

The effect of storage conditions on adults of the leafminer parasitoid *Diglyphus isaea* (Walker) was studied as part of a commercial mass production program. Freshly emerged wasps were kept for a period of 30 days at three constant temperatures: 15, 20 and 26°C. Honey was provided as a sole food source. Age and sex-specific differential mortality were monitored during the storage period, by the end of which progeny production and its sex-ratio were recorded as well.

The overall adult mortality during the 30 days of storage was very low at all three temperatures. The highest rate (2.6%) was recorded at 26°C. At 15°C there were 0.5% of dead wasps while at 20°C there was no mortality at all. Mortality of males and females did not differ significantly. Progeny production by females stored at 15°C was significantly reduced as compared to wasps which had not undergone the storage procedure. Temperatures of 20 and 26°C had an intermediate effect, i.e. higher progeny production than at 15°C but lower than the control. The higher the storage temperature the more female-biased became the F_1 progeny of the stored wasps.

With respect to adult longevity and fitness (fertility and F_1 progeny sex-ratio), storage of honey fed *D. isaea* adults at 20°C seems currently the most appropriate procedure of operative storage prior marketing.

INTRODUCTION

The parasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) is a facultative gregarious ectoparasitoid of agromyzid leafminers. Its biology was reviewed by Minkenberg and Lenteren (1986). The female wasp prefers to oviposit on the late larval instars (2nd and 3rd) of its hosts. After paralyzing the larva, the female lays between one to several eggs near or on the host. Three larval instars can be distinguished. Prior to pupation, the last larval instar builds a characteristic pupal chamber within the mine. The adult wasp emerges through a round hole which it cuts in the epidermis of the leaf. The female parasitoid has also the capability of host feeding, i.e. stinging the larva many times and then feeding upon its exudate.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

D. isaea is abundant in Europe, North Africa and Japan. It has been recently introduced into the U.S., Canada and New Zealand (Minkenberg, 1989). It has become an important component of commercial biological control programs in glasshouse vegetable crops in western and southern Europe, where it is mainly used against the leafminers *Liriomyza trifolii* (Burgess) and *L. bryoniae* (Kalt.) (Lenteren and Woets, 1988). Currently, attempts are being made to use *D. isaea* to control the newly introduced *L. huidobrensis* (Blanchard) in western European glasshouses (Linden, 1990).

In Israel *D. isaea* has been reared from a variety of leafminers including *L. trifolii* (Freidberg and Gijswijt, 1983) and *Phytomyza* spp. (S. Steinberg - unpublished). For the past two years it is mass produced by Biological Control Industries and used commercially to control *L. trifolii* and *L. bryoniae* on tomatoes, melons, watermelons and Gerbera both in greenhouses and open-fields.

Storage methods and facilities are vital in any commercial mass rearing program of natural enemies as the producer is faced with problems related to production planning and the unpredictability of demand (Lenteren, 1986a, 1986b and Lenteren and Woets, 1988). The latter expressed the common idea concerning storage of beneficial arthropods stating that: "...Storage periods normally last only few days, but reduction in fitness is still the rule. Storage during the adult stage leads to even higher and faster reduction in fitness than storage of immatures...".

D. isaea is supplied and stored in the adult stage. It is therefore essential to manipulate food and other environmental conditions during the storage period in a manner which will favour adult fitness. Measures and criteria should be developed to evaluate the effect of storage conditions on longevity, fertility and progeny sex-ratio of the adult wasps. This was carried out in the study reported herein.

MATERIALS AND METHODS

Experimental set-up

Freshly emerged wasps (maximum 24h. old) were placed in groups of 40 (sex-ratio 1:1) in glass tubes, 2cm in diameter and 14.5cm long. Each tube was plugged with a rubber stopper at one end and covered by a fine mesh netting at its other end to provide ventilation. Two pieces of 0.5X2cm honey blotting-paper which were placed on the side of the tube, served as a food source for the wasps throughout the storage period.

Adults of *D. isaea* were kept for a period of 30 days at three constant temperatures: 15, 20 and 26°C in controlled incubators, under light regime of 16L:8D and RH of 60±10%. Each treatment consisted of 5 replicates (five glass tubes).

Adult longevity

By the end of the first 24h. in storage, tubes were checked for adult mortality due to handling of the wasps prior the experiment (collecting, counting, sexing etc.). Therefore, wasps which died during this period were not taken into acount. After 7 days in storage, wasps were checked daily. The sex of dead individuals was determined. Cumulative counts of dead wasps were made until the end of the storage period. Checking and recording of the dead were

carried out at 26°C in order to differentiate between live-mobile adults and dead-motionless ones.

Adult fertility and progeny sex-ratio
By the end of the storage period, wasps were exposed to *L. trifolii* larvae to determine their fertility. Trays (31X23.5X8cm), bearing about 15 bean plants (in the cotyledon stage) infested with ca. 300 host larvae, were placed singly in screened cages of 38.5X35X54cm. The stage and size of the host larvae were kept as uniform as possible both by restricting the exposure time of the plants to ovipositing leafminer females to 24 hours and also by keeping the infested plants at the same environmental conditions, i.e. temperature of 26°C. The infested plants were isolated until the host larvae reached the age of 6 days from infestation (3rd instar), and by then were exposed to the parasitoids. This procedure was conducted for each replicate in a separate cage. Fourty wasps maximum 24h. old (five replicates; sex-ratio 1:1) served as a control.

The exposure of host larvae to the parasitoids lasted 6 days at 26°C and light regime of 16L:8D. By the end of this period, cotyledons from each replicate were picked and placed in emergence cages 31.5X20X13cm. The F_1 emerging adults were driven into glass tubes where they were counted and sexed daily.

RESULTS AND DISCUSSION

Adult longevity
The survivorship of honey-fed adults of *D. isaea*, both males and females, was hardly affected during one month of storage at 15, 20 or 26°C. The highest mortality by the end of the storage period was at 26°C (2.6%; all replicates pooled for both sexes, n=195). At 15°C only 0.5% (n=199) of the wasps died during storage, while at 20°C there was no mortality what-so-ever.

These results demonstrate a trend identical to the one reported by Minkenberg (1989) for *D. isaea* attacking *L. bryoniae* on tomato plants, without sugar additives. He found that survival of females was lowest at 25°C (mean of 10 days), highest at 20°C (32 days) and intermediate at 15°C (23 days). Minkenberg (1989) concluded that adult parasitoids could be kept alive for months at low temperatures (between 5-8°C). Allen and Charlton (1981) showed that longevity of *D. begini* (Ashmead) adults was greatly increased when provided with honey, concluding that wasps could be kept for months at 5°C. Recently, Heinz and Parrella (1990a) have demonstrated contradictory evidence on survivorship of *D. begini* attacking *L. trifolii* on chrysanthemums under greenhouse conditions: the average longevity of female wasps examined in their study was substantially lower.

The fact that carbohydrate-rich food source such as honey has a positive effect on longevity of adult parasitic Hymenoptera, is well accepted. It seems to apply also to adults of *D. isaea* in storage of at least one month period.

Adult fertility
The effect of temperature, during 30 days of storage, on fertility of *D. isaea* proved to be significant (one-way ANOVA, $F=4.35$; $DF=3$; $P<0.05$). The mean number of offsprings

produced by females stored at 15°C was significantly reduced as compared with female which were not kept in storage (Fig. 1). Fertility of adult females stored at 20 and 26°C di not differ significantly, however it decreased compared to the control and increased compare to the 15°C treatment although differences were not statistically significant (Fig. 1).

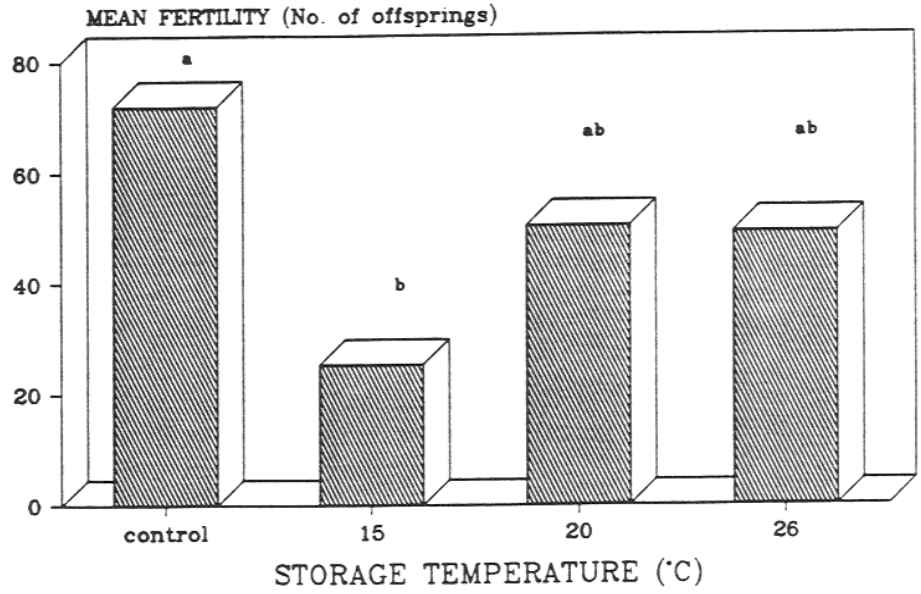

Fig. 1: The effect of storage temperature on fertility of Diglyphus isaea. Identical letterin above bars means no significant difference (Student-Newman-Keuls multiple range tes α*=0.05).*

It should be noted that the fertility criterion was not used by the present study in it classical context, i.e. number of larvae hatched. Instead, the immature stages were allowe to terminate development and eventually only the F_1 adults were considered quantitativel Mortality during the immature stages might reduce the number of emerging adults. I addition, a population of 35-40 wasps was used in each replicate to evaluate fertility (all th surviving wasps were transferred by the end of the storage period to the oviposition cages Thus reduction in oviposition rate due to mutual interference cannot be ruled out. The curre results might therefore underestimate the actual fecundity/fertility of the parasitoi Minkenberg (1989), who followed 1 day old individuals of *D. isaea* ovipositing on *bryoniae* in tomato plants under laboratory conditions, found no significant difference i fecundity at 15, 20 and 25°C, with mean number of eggs being 293, 286, 209, respectivel Nevertheless, we argue that the method applied in the present study is more appropriate quality control procedures within a mass production program, since it evaluates the fin outcome of fertility at the population level.

Reduction in fertility during storage might also be attributed to egg resorption (oosorption It has been pointed out in several parasitic Hymenoptera that lack of protein in the adult' di may lead to oosorption, the materials freed during this process can essentially be regarded nutrients, i.e. they serve as food reserves (Flanders, 1942; Engelmann, 1970). Th phenomenon might even be more prominent in a species like *D. isaea* which exploits

relatively high proportion of its hosts, through host-feeding, to maintain its protein input. The present results suggest that oosorption was highest during one month of storage at the lowest temperature (15°C), to the extent that it could not be completely reversed even by exposing the female parasitoids to favourable conditions (six days at 26°C with ample amount of hosts). In further studies it might be worthwhile to add protein ingredients to the honey in attempt to overcome the reduction in fertility during long-term storage of honey-fed wasps.

Mating is also known to influence egg maturation (Engelmann, 1970). Assuming that mating frequency during the 30 days period at 15°C was lower than at 20 or 26°C, it might contribute to the reduction in *D. isaea* fertility after storage at 15°C compared to higher temperatures. However, for *D. begini*, Heinz and Parrella (1990a) reported that mating was not necessary for oviposition because there was no difference in fecundity of females in the presence of male wasps versus females where males were absent.

Progeny sex-ratio

The higher the storage temperature of *D. isaea* adults, the more female-biased became their progeny. While after storage at 15°C it was male-biased (f/m: 0.87), there was a changeover to female-biased progeny (f/m: 1.29, 2.64) after storage at 20 and 26°C, respectively. The none-stored adults produced a female-biased progeny with an intermediate sex-ratio compared to the wasps stored at 20 and 26°C (Fig. 2).

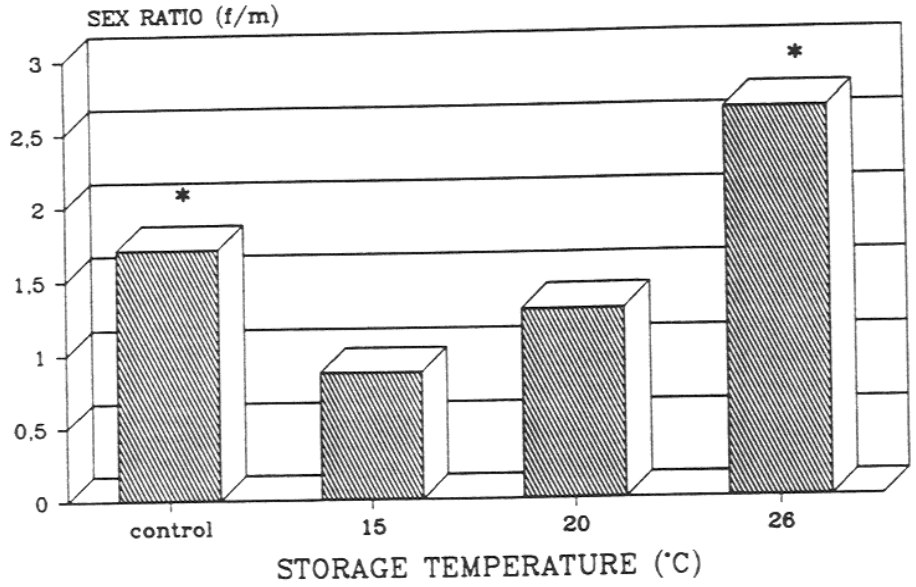

Fig 2: The effect of storage temperature on progeny sex-ratio of Diglyphus isaea. Asterisk above bar means significant deviation from 1:1 sex-ratio [G-test for goodness-of-fit, α=0.05, (Sokal and Rohlf, 1981)].

Like many other parasitic Hymenoptera, *D. isaea* is an arrhenotokous species with haplodipoloid form of sex determination. Females control the sex of their progeny by regulating egg fertilization. In addition, they are synovigenic, capable of multiple-mating during their entire life span. Possibly, the relatively low mating frequency during storage at

15°C reduced the males reproductive input in the females spermathecae, resulting in a lo proportion of females in the progeny compared to higher storage temperatures. In this respe Heinz and Parrella (1990a) showed that without the presence of male wasps, females of *begini* produced only male progeny whereas mated females were capable of producing fema progeny from the onset of oviposition.

As the number of host larvae was ample and their size kept as uniform as possib throughout the experiment (see materials and methods), it seems unlikely that the proge sex-ratio was obscured by sex-specific differential mortality caused by superparasitism developmental sex differences. Furthermore, it has presumably eliminated the effect of ho size on sex allocation by females of *D. isaea*. A detailed study by Heinz and Parrella (198 1990a, 1990b), both under laboratory and field conditions, revealed that females of *D. begi* tended to oviposit on the largest host larvae encountered and produced male progeny in hos significantly smaller than those producing female progeny.

Concluding remarks

Based upon the current study we conclude that a one month storage of honey-fed *D. isa* adults at 20°C yielded optimal results, as far as the parasitoid' vigour (survivorship, fertili and progeny sex-ratio) is concerned. We agree with Heinz and Parrella (1990a) who suggest caution when attempting to predict the effectiveness of various parasitoids to control a pe species based on laboratory analysis of life history data, because parameters might b overestimated due to the methods used. Nevertheless, we believe that the laboratory metho used in the present study to assess the quality of adult parasitoids after a storage period, ma serve as a reliable reference point to the actual performance of the wasps in the field. Th is further reinforced by the fact that mass production of *D. isaea* is carried out und greenhouse conditions, similar to those encountered by the parasitoid in the field.

In practice, we suggest that detailed quality assessment of the stored wasps (fertility an progeny sex-ratio) will be done periodically, especially off-season, while screening o survivorship will be carried out on a daily basis, prior to shipment.

For further research we suggest the following points: 1) Conducting the same type o experiment using the actual mass storage containers. The interaction between 40 adult wasp in the relatively small glass tubes used in the present study might differ from the interactio within larger populations kept in larger containers, therefore affecting adult longevity, fertilit and progeny sex-ratio in a manner other than presented here. 2) Seeking for the possibilit of storing *D. isaea* in a diapausing stage; a method recommended in general by Lentere (1986a, 1986b) and Lenteren and Woets (1988). However, data from Minkenberg an Lenteren (1986) as well as from Minkenberg (1989) did not offer any firm evidenc concerning diapause in this species nor of its diapausing stage. 3) Studying the relationshi between *D. isaea* body size and its longevity, fertility and progeny sex-ratio as major criter for quality control with or without storage. Heinz and Parrella (1990a) indicated that *D. begini*' longevity and fertility increased with wasp body size, which was in turn influence by the size of the host.

ACKNOWLEDGEMENTS

The senior author is grateful to the Department of Entomology, Wageningen Agricultural University, The Netherlands for providing the facilities to prepare this manuscript as well as the slides for the QC conference. W. Ravensberg from Koppert B.V. is thanked for critical review of the manuscript.

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A SIMPLE METHOD FOR QUALITY CONTROL OF APHIDOLETES APHIDIMYZA, APHIDIUS MATRICARIAE AND ENCARSIA FORMOSA.

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ABSTRACT

The widespread use of beneficial insects and mites has intensified in the last few years and increased the demand for biological products. Today's well educated growers demand quality products based on their fundamental knowledge of the beneficials biology and the excellent results that can be expected from biological pest control programmes. In co-operation, the Research Center for Plant Protection and Chr. Hansen's Bio Systems A/S have developed a simple quality control test, which, as well as being used by producers, can also be used by growers and distributors of beneficials. The requirements for the test were, that it was cheap, did not require special equipment, and that results could be obtained within one week. In addition to counting the number of beneficials, the test is based on indirect measurements of the quality of both predators and parasites by observing the emergence percentage from pupae. Recently the test has been extended to include observations on flight ability. Examples of test results with Aphidoletes aphidimyza, Aphidius matricariae and Encarsia formosa are presented, and other parameters which might be included in the test, are discussed.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen. March 1991.

INTRODUCTION

The use of biological control against pests on various crops has steadily expanded during the last decades, and consequently the demand for beneficial organisms has increased. The present confidence in biological control as well as the prospects for continued success and increased implementation are closely connected with production and delivery of standardized quality products. Thus, the point of view expressed by several groups of people (growers, producers, scientists working to increase use of biological control) is that availability of good quality, biological control products is essential.

Quality in relation to biological products is a comprehensive concept and elements evaluated by quality testing may consequently vary from a simple count of beneficials for a shipment to a more thorough estimation of actual performance against the pests. Cost-benefit estimates must therefore be taken into consideration when quality control tests are developed, regarding the purpose of the test, design and methodology. Furthermore, it should be noted that it would be worthwhile if the design of the test permits growers and distributors to carry out tests - or at least partial tests which are required for a simple quality evaluation. In this way, it will be possible to determine quality changes which may occur after delivery from the producer - for instance during transport or storage at growers or distributors.

Here we describe a simple quality test for three beneficials, the gallmidge Aphidoletes aphidimyza, parasitoids Aphidius matricariae and Encarsia formosa. The test has been used for more than one year at the Research Center for Plant Protection for monthly quality tests of products produced by Chr. Hansen's Bio Systems A/S. The following elements are evaluated : number of beneficials, number of other arthropods and emergence percentage of adults from the pupae. The following considerations have been made during development of the test : it should be cheap, should not require special equipment, easy to use for growers/distributors, and results should be obtainable approximately within one week.

In addition, a recent expansion of the test made in order to include information on the flight ability of adult beneficials is reported.

DESCRIPTION OF THE TEST

Samples are taken for testing from products ready for sale.

E. formosa are sold as parasitized whitefly pupae glued on pieces of cardboards. For testing, the cardboards are placed individually in small Petri dishes with the pupae facing upwards. 10 cardboards are tested simultaneously giving a total of approximately 1000 individuals. Both A. aphidimyza and A. matricariae are sold in small boxes containing beneficials (pupae and parasitized aphids, respectively) mixed with Vermiculite. For testing, these mixtures are distributed in small Petri dishes. Approximately 1000 beneficials of each species are tested individually. The Petri dishes are 5 cm in diameter and 1 cm in height. The lids of the Petri dishes are smeared with insect-trapping glue.

The dishes are inspected daily and the number of adults caught on the lid recorded. The lids are then replaced with new ones. When no further adults have emerged the total number of pupae in each dish are counted. This is, however, not done for A. aphidimyza due to difficulties in recovering the unhatched pupae. At the same time an examination is made for other arthropods and those present identified and enumerated.

The test described above has been recently expanded as follows: the beneficials are placed in 10 plastic containers, 10 cm in height and 7 cm in diameter. The lids are smeared with insect-trapping glue. To stimulate movement of beneficials towards the top of the container, the outside walls are covered with black plastic, allowing light to penetrate only through the lid. The inside walls of the container are covered with Fluon GP1 (Polytetraflourethylen) to prevent beneficials reaching the lid by crawling. Thus, the only way for insects to reach the lid and be caught in the glue is by flying. The containers are inspected daily and the number of adults caught in the lid recorded. The lids are then replaced with new ones. When no further emergence takes place, the number of emerged adults remaining on the bottom of the containers are counted. Counting of the total number of pupae as well as the procedure for dealing with other arthropods is described above. The number of beneficials tested remains the same.

EXAMPLES OF RESULTS

The results obtained from each dish are summed to express the daily and total emergence percentage. For E. formosa and A. matricariae the emergence percentage is expressed in relation to the total number of pupae, while for A. aphidimyza emergence is expressed in relation to the total number of emerged adults. Below are examples of results obtained at the Research Center for Plant Protection. The tests have been carried out in a climatic cabinet at 25°C, 60-80% R.H., and 16:8 hrs light:dark photoperiod.

Figure 1 illustrates the typical patterns of daily emergence for the three species.

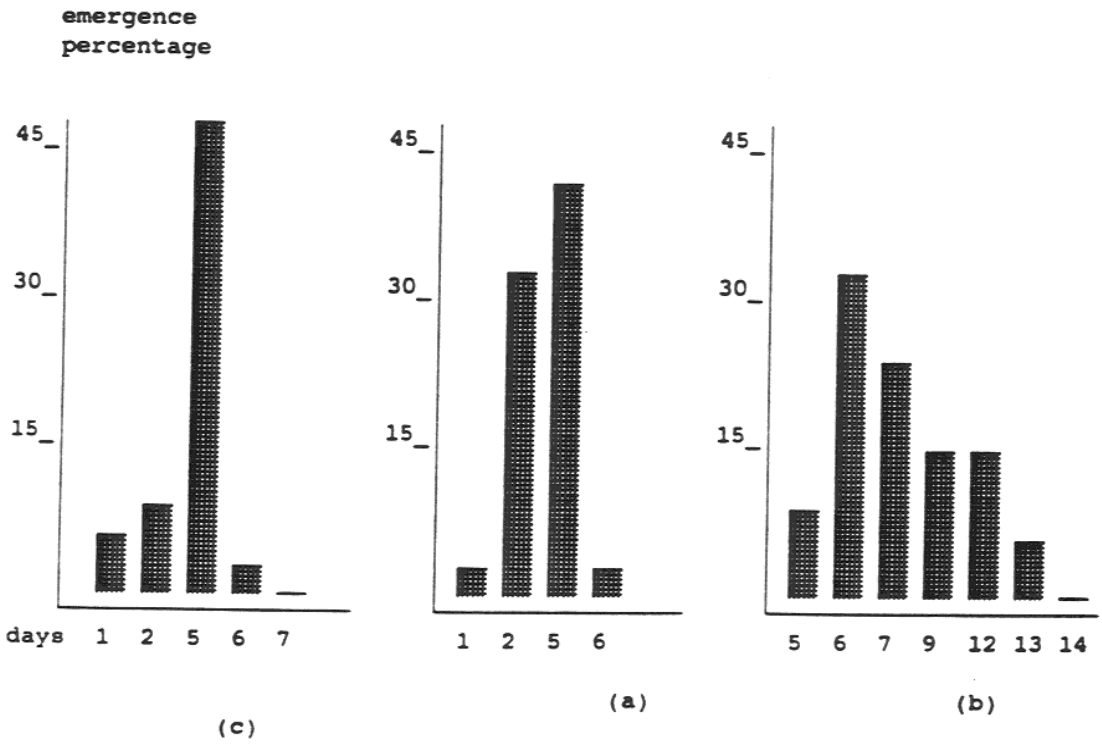

Fig. 1. Typical patterns of daily mean emergence (from the day the product has been received) of adult *E. formosa* (a), *A. matricariae* (b) and *A. aphidimyza* (c).

Adults of A. aphidimyza emerge over a relatively long period, normally lasting between 12 and 15 days with maximum emergence being reached about 6-8 days after initial eclosion. For E. formosa maximal emergence takes place after 4 to 5 days, and no further emergence occurs after 8-9 days. A. matricariae has the shortest period of emergence, only about 6 to 7 days, with a maximum reached after 2-4 days.

Adult emergence of the three species seldom varies from these typical patterns, though occasionally prolonged periods of emergence may be seen. Any correlation between the period of emergence and total percentage of adults emerging has not been found for any of the species, i.e. low emergence percentage does not correlate with extended emergence period.

Table 1 illustrates how the number of beneficials present in a sample and the total emergence percentage may vary between tests.

	E.formosa	*A.matricariae*	*A.aphidimyza*
	pr. cardboard	pr. box	pr. box
number of beneficials			
mean	158.6	1663.1	1285.1
std.error	2.4	17.3	20.8
range	87-310	1161-2083	748-2290
n	15	14	20
emergence percentage			
mean	59.5	71.9	-
std.error	3.4	3.6	-
range	46.6-83.2	51.5-95.5	-
n	15	15	-

Table 1. Variation in quality of products of the three beneficials as expressed by number of beneficials present and the emergence percentage from pupae.

The number of beneficials actually present in a sample is for all three species normally equal to or higher than specified on the product, although some variation is apparent.

The emergence percentage varies from rather low values (50% or less) to values approaching or exceeding what one might immediately regard as acceptable (80-90%).

With regard to presence of other arthropods, a few specimens of whiteflies and aphids are occasionally found in the samples of E. formosa and A. matricariae, respectively. The numbers are, however, quite low, representing at the most 1% of the number of beneficials. Organisms other than the relevant pest species found are extremely rare - the only case so far is the presence of hover flies (Syrhpidae) in a sample of A. aphidimyza.

The expanded test has been carried out only once for A. aphidimyza and A. matricariae. For both species, results from each container have been summed to express the daily and the total percentage of flying adults. This is calculated in relation to total number of adults emerged. The total emergence percentage from pupae is expressed as described above. The results in **Figure 2** and **Table 2** are presented as a mere illustration of the additional information obtained from the expanded test.

It can be seen that the percentage of emerged adults which actually fly, is quite high for both species. Still, it is apparent that the inclusion of flying as a parameter of the test has increased the level of information on the quality of beneficials. As for the patterns of daily flying, this does not differ from the patterns observed for daily emergence from pupae.

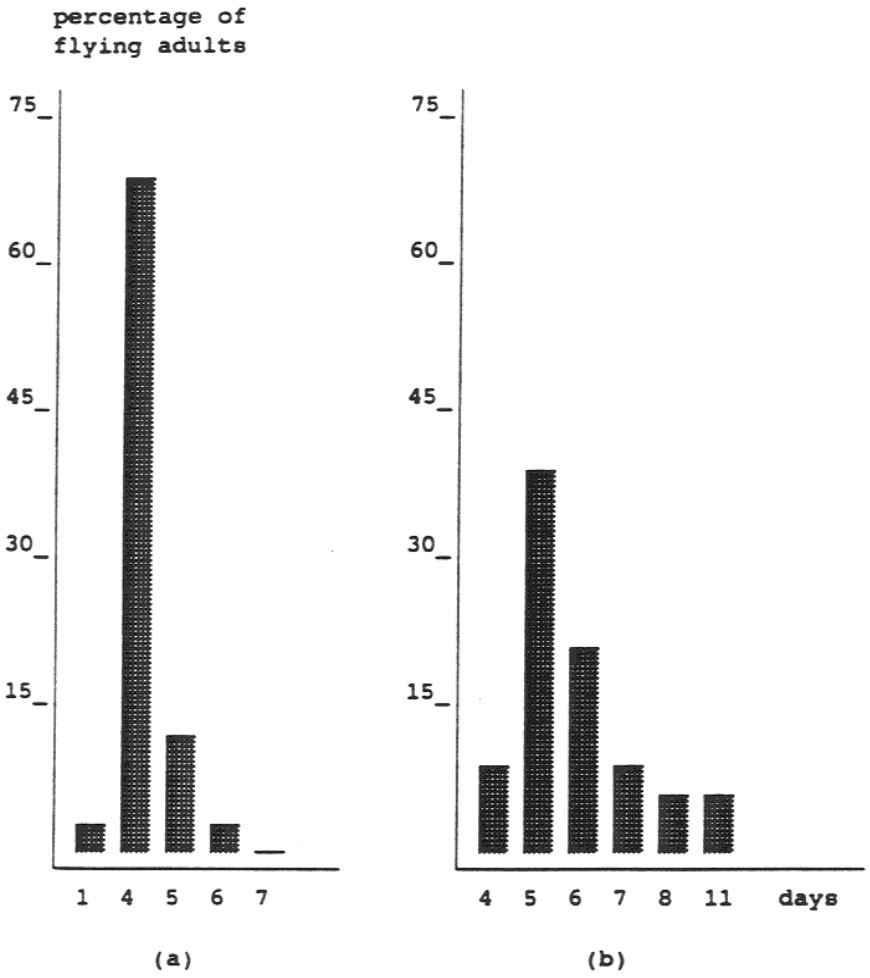

Fig. 2. Mean daily flight pattern of adult A. *matricaria* (a) and A. *aphidimyza* (b).

	A.*matricariae*	A.*aphidimyza*
percentage of flying	88.9	91.7

Table 2. Results on flight ability from a test of A. *matricariae* and A. *aphidimyza*, respectively.

DISCUSSION

The present test supplies information on various parameters relevant for judging the quality of A. aphidimyza, A. matricariae and E. formosa products.

The number of beneficials present is not a measurement of quality for the insects. Still, this parameter is important in the sense that it reveals whether the specifications made by the producer on the numbers contained in a shipment, is fulfilled or not.

The daily pattern of emergence, as determined by the present test, gives information on the duration of the period where adult beneficials are emerging, i.e. the speed with which beneficials will be released on the crop. Furthermore, it reveals how well the producer is able to synchronize both production and packaging of beneficials. Thus, although the testing results obtained from the three species have not revealed any great differences in pattern of emergence, this parameter is an important part of the test.

The total emergence percentage gives information on the actual number of beneficials that will be released on the crop, and more important on the inherent quality of the insects themselves. The tests made on the three species demonstrate how emergence percentage may vary between samples, ranging from rather low values of 50-60% emergence to increased values of around 80-90%. The cause of this variation is unknown, but may be due to conditions during insect rearing. For instance, it might be expected that emergence will be low if the parasites have been introduced to hosts at too early a stage, or if the hosts have been unhealthy, for instance underfed. Emergence percentage may also decrease due to inbreeding and other genetical changes during rearing. Finally, transport conditions and storage may influence emergence from pupae. Thus, a low emergence percentage should induce the producer to inspect the various steps of productions, packaging, transport and storage in order to discover the cause of the low quality. Increasing the number of beneficials in the shipments to compensate for low emergence should only be the last solution, one to be taken when all other improvements have failed.

A low emergence percentage may be due to the fact that adults in general are weak and thus have difficulties hatching, and emergence from pupae is not necessarily associated with the adult beneficials condition which is important for their performance against pest, e.g. parasitization/predation efficiency, searching ability and fecundity. The test was therefore expanded to include a measure of "adult performance", i.e. the ability to fly. This can be considered as minimum requirement for adult beneficials of the three species.

Although the expanded tests have been carried out only a few times, they seem to work well for both A. matricariae and A. aphidimyza. Thus, even though A. aphidimyza is nocturnal and therefore presumably not attracted to light in the same degree as the parasitoid, a high percentage of adults have been caught on the glue. The test has the shortcoming that the last emerged adults will have less time to be caught on the glue than adults emerging shortly after set-up. Therefore, at the end of the test, the adults remaining at the bottom of the containers will be a combination of adults with less ability to fly and adults just emerged, not having had the time to be caught on the glue. Keeping this in mind, however, when interpreting the percentage results of flying adults, the expanded test provides, in our opinion, useful information and will reveal changes in quality of the beneficials. Future tests will show how the ability to fly may vary and correlate with other quality estimates.

In conclusion we can state that the present expanded test represents an optimal balance between the amount of resources (materials and labour) for the test, and the type of information needed for evaluating the quality of E. formosa, A. aphidimyza and A. matricariae. Other parameters, which could be included in the test are : (1) measurements for the pupae size as the pupal size is a reflection of the size of the emerging adult and thus of the potential number of eggs produced; and (2) occurence of emerged beneficials in product on receipt as beneficials often are most efficient shortly after eclosion into adults. Thus, a product containing many emerged adults may not be as effective as one containing pupae.

ACKNOWLEDGEMENT. We wish to thank Nina Christensen and Ursula Althoff for technical assistance and useful ideas during the developing process of the tests and for carrying out the monthly quality tests; and Ingeborg Kolind for secretarial and translating assistance.

QUALITY CONTROL OF MASS-PRODUCED TRICHOGRAMMA SPECIES

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Key words: *Trichogramma*, quality control, mass-production, process control, product control

INTRODUCTION

Quality control in insect rearing has been defined as the monitoring and sophisticated control of the complex production processes for mass-rearing programs that ensures reasonably consistent product quality and ensures that the product achieves the desired performance in the field (Leppla and Ashley, 1989). However, few standard methods have been developed for quality control tests, and even fewer have been accepted and generally put into use for *Trichogramma* species, reared on a larg scale, in different areas of the world. To develop these tests, it is essential, as van Lenteren (1992) pointed out, that we separate objectives that were "nice to know" from those that we "need to know". The former are the realm of the scientist whereas the latter are of prime concern to the commercial producer of beneficial insects. There was general agreement amongst the participants (scientists and producers) that development of quality control is essential to the acceptance, by growers, of commercially produced biological control agents.

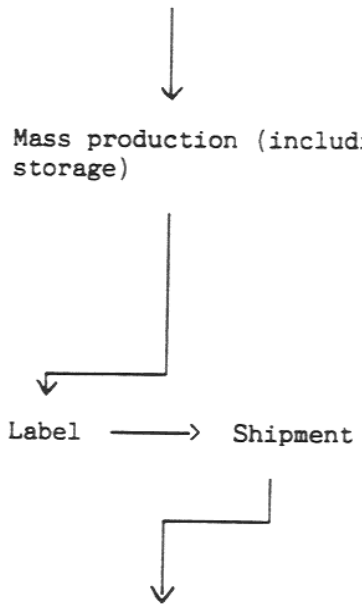
The objective of our subgroup was to describe the tests necessary for assurance of the quality of mass-produced *Trichogramma*.

PRODUCTION SCHEDULE AND QUALITY CONTROL

Although the procedures in Figure 1 are listed in the order in which they would occur during the schedule of selection, production, labelling, delivery and application, the group working with the development of quality control for *Trichogramma* found it easier to look first at product control (by the user, then the producer) and work back through the system to strain selection, while establishing the tests necessary at each stage of the process.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

Figure 1: Quality control for production and use of mass-reared *Trichogram*



Selection of the founder population

The initial use of quality or performance controls for *Trichogramma* shoul occur during the selection of a founder population of the desired species. Th "screening process" at this stage would be quite rigorous and time-consuming Many tests of quality could be applied at this stage of the process; not al tests given here would be applied to a founder population although most o them would be needed.

In an approximate order of difficulty, the tests to be used are determination of:

a. % emergence
b. sex ratio
c. fecundity
d. longevity
e. duration of emergence
f. developmental rate

g. locomotion - walking
- flight propensity

h. host acceptance (natural and factitious hosts)

i. host suitability (natural and factitious hosts)

j. temperature tolerance - thresholds

k. functional response

l. quiescence capacity

m. diapause capacity

n. semi-field or field performance.

Since many of the results from the tests listed above are temperature-dependant, it is suggested that the tests be conducted at appropriate regimes, which may depend on specific questions and field situations in which *Trichogramma* will be used. Wherever possible and significant, tests should be carried out at 23 ± 2 °C, 70 ± 10%RH and a 16L : 8D photoperiod. Tests conducted in deviating physical conditions should be specified and explained. Appropriate measures of variance, such as the standard deviation or standard error of the mean should be calculated.

Similar information to *b*, *c*, *d* and *f* above can be obtained from a calculation of the intrinsic rate of increase (r_m) of the population. However, the latter characteristic can be labour-intensive if the iterative process used in its determination is not available in a computer programme.

Tests of locomotion include walking and flight. The former could be stated as distance travelled per individual female per unit time at a given temperature. The latter is a measure of flight propensity. That is, the number of individuals of both sexes in the population that will fly at a given temperature.

Host acceptance and host suitability should be determined not only for the target host, but also the factitious host of rearing in the laboratory. Mass-rearing *Trichogramma* on factitious hosts may influence the offspring's subsequent choice of hosts. The greater the number of generations that the parasitoids are reared on a factitious host, the more pronounced may be the change in host acceptance and suitability. Thus, tests of these parameters must be run early in the selection process.

Temperature tolerance may be defined as the determination of upper and lower thresholds for such events as mating, oviposition, development, locomotion and flight. The limits of these thresholds once determined may dictate the environmnet(s) in which the parasitoids can be released.

The functional response of *Trichogramma* (number of eggs parasitized with changing host densities) should be determined at several host densities within the range found in the field.

The ability of the chosen strain to enter diapause or quiescence should be determined. If it is found that the species can enter diapause or be stored at low temperatures for a useful period, then a further hierarchy of tests should be placed in process control. At least once during the process, after parasitoids are induced to enter diapause or stored at low temperature, the tests given under mass production should be applied to those individuals obtained from storage, to ensure that their quality has not changed due to the treatment.

Semi-field or field performance tests are likely to be the most variable in methodology and will depend, to a large extent, on the target host and the host plant environment on which it is found in the field (e.g., corn vs apple vs spruce trees). One of the long-term objectives of the quality control procedures should be to relate the field efficiency of mass-produced *Trichogramma* with the tests of quality control. A simple test, easily conducted in the laboratory during both strain selection and mass production that is directly related to performance in the field, would be the ideal solution to tracking quality control in mass-reared *Trichogramma*.

Mass production

Process control

After the selection process has been completed, one or more strains or species of *Trichogramma* will be utilized in a mass-rearing program. A series of tests will be conducted that is less comprehensive and less time-consuming than those performed during strain selection. This is of necessity, because the rearing system, itself, will require a considerable proportion of the total manpower available for the program. Thus, several of the more time-consuming tests in strain selection have been omitted here. The frequency with which the following tests should be run will likely be high (once per month or more) during the start-up phase of mass-rearing, but if results do not indicate deterioration of the culture, this frequency may decline to as little as once per year.

The tests to be used during mass production are the determination of:

a. % emergence

b. sex ratio

c. fecundity
d. longevity
e. locomotion - walking
- flight propensity
f. host acceptance (natural host)
g. species strain identification

Again, because many of the results of the above tests are temperature-dependant, it is suggested that the tests be conducted at appropriate but consistently the same conditions for single test methods.

Tests of host acceptance (the natural host) become more important, the longer *Trichogramma* are reared on a factitious host. It is particularly important to check this attribute carefully just prior to diapause storage or to shipment of parasitoids for production systems without or short time storage. The only test added to mass production is "species strain identification", which is essential to the production system. Each producer must be able to verify the species or strains of *Trichogramma* that is produced. This ist particularly important in a facility where more than one species or strain of *Trichogramma* is being reared at the same time and there is a possibility that cross-contamination will occur.

If at any time during mass-production, the above tests indicate a deterioration of the quality of *Trichogramma*, the producer may wish to undertake a wider range of tests of the population including a number of tests listed under strain selection, but not included here. It is also during this phase of the project that individuals stored at low temperatures (diapause or quiescence) during the rearing process would be tested to ensure maintenance of their quality.

Production control

Production control is the regulation of consistency, reliability and timeliness of production output (Chambers and Ashley, 1984). Production of high quality parasitoids depends on the appropriate use of suitable facilities, equipment and the accurate performance of rearing operation. This includes availability of high quality host eggs, stock colony maintenance, synchronisation of emergence of the parental generations, density regulation in rearing units, optimal physical conditions and sanitation. Production control requires a permanent monitoring of the relevant factors influencing the rearing success. Operations are under control if the rearing products achieve acceptable standards.

Shipment

Product control

At this time in the production system, mass-produced *Trichogramma* are being prepared for shipment to distributors and/or users. The producer must be confident that the performance characteristics of his mass-reared *Trichogramma* will be satisfied with each batch shipped. Yet, in systems without diapause storage, it is the time when the producer is trying to maximize the numbers of parasitoids produced. Thus the time allotted to testing is necessarily restricted unless a major problem in quality detected.

The basic information to be provided by the producer is the number of healthy female *Trichogramma* per unit shipped. In order to obtain this figure, the following information is needed:

a. number of host eggs parasitized (number black eggs).
b. % emergence.
c. sex ratio.
d. % of individuals deformed (♀♀ only).

Each of the tests requires a minimum amount of time to complete so that the results will be known at the time of shipment. All of the above tests should be run on each batch to be shipped.

Handling, transfer control

Prior to shipment, the parasitoids will be conditioned within their host in order to transport and release them in an optimal stage regarding adaptation and timing of emergence. According to the production and transfer system, different handling processes may be needed. Depending on the distance and the method of shipment, the wasps may be affected negatively. Packing and transfer systems may also depend on the release system and customs of the user. All these factors have to be accounted for optimizing and control handling and transfer.

Labels

The producer should label each shipment with the following information:

a. name of parasitoid.
b. name of rearing host.
c. name(s) of intended target pest(s)
d. number of healthy female *Trichogramma*/unit or batch shipped.

Additional information on the label may be needed depending on legislation in different countries. At this point, the parasitoids could be shipped to a distributor or directly to the user.

Distributor/user

Product control

In order to determine if the wasps have been affected negatively by handling and transfer, the distributor and/or user should remove a sample from the batch (a portion of cards, capsules or several hundred parasitized eggs from a bulk shipment) and hold it as near as possible to the conditions used for this test by the producer until all parasitoids have emerged.

The percent emergence of each batch should be determined. If the *Trichogramma* do not perform well after release, this figure can be compared with that of the producer to determine if shipping has affected their quality.

CONCLUSIONS

During this workshop, the group discussed, though did not list in any detail, the obvious needs for quality controls in the rearing host, the mass-rearing production system (production control) and the requirements for commercially-produced, beneficial insects likely to be set by regulatory authorities (particularly the E.E.C. and individual Governments in Europe and North America). While these three sets of conditions will vary due to the species of host used for rearing, the species of *Trichogramma* reared and its intended use, and the often political whims of government, the participants felt that these areas should be pursued in greater detail in future workshops. The consensus of the group was that putting the quality controls into place, developing consistent methodologies for the tests, then validating the tests over the next few years, would provide the necessary data to establish protocols for use by regulatory bodies. It was noted that we should be proactive when dealing with the regulatory groups within each producing country or region.

During the next phase in the development and utilization of quality controls for *Trichogramma*, it was suggested that we develop standard methodology for the tests mentioned above and develop quality controls for host rearing, and for the production system. As we progress, further development of more sophisticated tests may occur such as tests of host-

searching behaviour, the use of kairomones in mass production, and progeny and sex allocation for individual host/species combinations. One or more of these or other tests may be incorporated into the rearing process provided that the tests are simple, generally applicable to all species of *Trichogramma* and efficient in terms of labour and time.

ACKNOWLEDGEMENTS

The procedures for determining quality control of mass-produced *Trichogramma*, described here, were derived mainly from the workshop on Quality Control of Mass-Reared Arthropods. The participants in the *Trichogramma* working group provided many of the ideas presented in this paper.

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METHODS FOR THE QUALITY EVALUATION OF *TRICHOGRAMMA EVANESCENS* WESTW. USED AGAINST THE EUROPEAN CORN BORER

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ABSTRACT

In 1991 *Trichogramma evanescens* was used on about 20'000 ha of maize in Western Europe to control the European corn borer, *Ostrinia nubilalis*. For economic reasons, the parasitoid is produced in unnatural conditions on factitious host eggs. A deterioration of the field performance of the produced *T. evanescens* was experienced formerly and a quality control system appears necessary. In Switzerland a process and product control system for *T. evanescens* were implemented during the last years. For process control, emergence rate, sex ratio, percentage of deformed females, average life span, walking activity, acceptance and suitability of *O. nubilalis* eggs and fecundity are assessed. For product control, total number of parasitized eggs per release unit, emergence rate and sex ratio of the emerged adults are determined. The article describes the methods used to assess this parameters. This control system allowed the improvement of the rearing method, the identification of critical situations and the check of commercialized products.

1. INTRODUCTION

Trichogramma evanescens WESTW. (Hymenoptera, Trichogrammatidae), formerly *T. maidis*, is used in Switzerland since 1978 to control the European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera, Pyralidae) in maize fields. In 1991 about 4'000 ha of maize were treated with *T. evanescens* in Switzerland, 11'000 ha in France and 5'000 ha in Germany (BIGLER *et al.*, 1992).

For economic reasons, the parasitoids are produced in high numbers in unnatural conditions on factitious host eggs. In Switzerland they are reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella* ZELL. (Lepidoptera, Phycitidae). Although the mother stock of the strain used is maintained continuously on natural hosts eggs in conditions close to nature, a deterioration of the field performance of the strain during

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

the mass production process must be considered (STARK, 1944; BIGLER *et al.*, 1982, 1988). Therefore, permanent quality control appears necessary.

According to BOLLER and CHAMBERS (1977) the quality of a mass reared insect has to be identified in quantifiable parameters of the major quality components. BIGLER (1989) proposes a specific choice of major quality components to be measured on *T. evanescens* used against *O. nubilalis* on maize.

According to LEPPLA and FISCHER (1989), different types of quality control have to be performed directly on the insects:

- In the so called process control, the tests are performed at different steps of the mass production and the frequency of the tests differ according to the parameters measured.
- Once the production procedure is terminated and the product is ready for sale, a few checks have to be performed on some parameters before shipment. The parameters tested in this so called product control must be quick and easy to measure because little time is left between the end of the production and the release dates.

The aim of this work is to present the methods routinely used in the process and product control of the *T. evanescens* production system.

2. PARAMETERS MEASURED

2.1 Process control

At the moment, the parameters assessed in the process control are:
emergence rate, sex ratio, percent deformed females, average life span, walking speed, acceptance and suitability of *O. nubilalis* eggs and fecundity.

2.2 Product control

For product control the following parameters are assessed:
total number of parasitized eggs per release unit, emergence rate and sex ratio of the emerged adults.

3. DESCRIPTION OF THE METHODS

3.1 Process control

3.1.1 Standardization of the females for analysis

To avoid errors related to the manipulation of the wasps, great attention has to be paid to the standardization of the rearing and handling procedures of the *Trichogramma* population which is tested. The wasps are prepared as follows: 0.1 g of parasitized eggs are placed in a small open plastic box (2 x 2 x 2 cm) which is put in a transparent plastic cylinder of 15 cm height and 10 cm diameter. The top of the cylinder is closed with a flexible plastic lid. The cylinder has 2 lateral holes of 3 cm diameter, covered with fine organdy, for ventilation. Tiny droplets of honey, about 2 per cm^2, are placed all over the lid as food. The parasitized eggs are reared at temperatures between 20 and 25 °C, a relative humidity (RH) of 80% and 16 h light (L) and 8 h dark (D). At the first day of emergence, water is provided three times a day by spraying fine droplets of water in the cylinder through the lateral holes. According to ZHANG (1984) and QIU and BIGLER (1992), the adults emerged during the first day have an higher fecundity and live longer than the ones emerged the subsequent days. For this reason only adults emerged during the first day are used. To avoid overcrowding, the small plastic box containing the parasitized eggs is removed during the first day of emergence when the population of emerged adults reaches a density of about 1000 individuals.

The next day the lid is removed and gently tapped on a white surface so that the adults get evenly scattered. They are caught individually by placing a glass tube (7.5 cm long, 1.3 cm in diameter) open end down. When the adults have walked into the vials, these are closed with a plastic plug with tiny holes for ventilation. The sex of each adult is then determined under the binocular lense. A droplet of water and a droplet of honey are then added directly to the wall of the vials containing a female.

3.1.2 Percentage of deformed females

During the sex determination at least 100 females are checked for deformed wings.

3.1.3 Walking activity

The walking activity is measured on 25 one-day old females (prepared as described in 3.1.1) with a computerized image analysis system. Each female is placed during 3 min. on a circular, flat Plexiglass arena of 10 cm diameter at a temperature of 19 °C, RH 75% and a light intensity of 3600 lux (4 lamps JUST-NORMLICHT L15W-daylight98 at 25 cm distance). Under these conditions the *Trichogramma* females show a pure walk-searching behavior without flight induction. The position of the female is recorded each 0.48 sec. by the image analysis system (TE84-B405-SYSTEM, ELTEC EL. GmbH, D-6500 Mainz; camera CCD HR 450, HENZ AG, CH-5034 Suhr) which has a resolution of 47x23.5 pixels per cm in the xy-axes. The only parameter actually used to assess the walking activity is the distance covered.

3.1.4 Average life-span

Survival is recorded daily on 25 females confined individually in glass tubes (see 3.1.1) at 25 °C, 80% RH and 16hL:8hD.

3.1.5 Acceptance and suitability of O. nubilalis eggs

25 one-day old females confined individually in glass vials (see 3.1.1), receive 2 fresh egg-masses of *O. nubilalis* with at least 20 eggs each. After 24 hours at 25 °C and 80% RH, the egg-masses are removed and incubated for 3 days at the same climatic conditions. At this moment, i.e. just before the emergence of the *O. nubilalis* larvae, the number of parasitized (black) eggs is counted.

3.1.6 Fecundity

The fecundity is assessed on eggs of *E. kuehniella*. These eggs are collected daily and UV-sterilized. They are stored for max. 4 days at 3 °C and 80-90 % RH. The experiences of VOEGELE *et al.* (1974) indicate that this kind of treatment does not affect the multiplication rate of *T. evanescens*.
PINTEREAU *et al.* (1981) established a high correlation between the first 3 days fecundity and the total fecundity of *T. maidis*. 25 pieces of cardboard (0.5 x 2 cm) with at least 250 eggs each (glued with water) are exposed individually for 3 days for parasitization to 25 one-day old females (see 3.3.1) and kept at 25 °C, 80% RH and 16hL:8hD. The pieces

of cardboard with the eggs are then removed and incubated for 4 days at 25 °C. The number of parasitized (black) eggs is then counted.

3.1.7 Emergence rate

One ml of parasitized eggs is reared at 25 °C. After the emergence of all adults, a sample of eggs is placed in a Petri dish with alcohol (70%). The proportion of hatched eggs, out of 200 parasitized eggs, is determined under a binocular lense.

3.1.8 Sex ratio

The sex ratio is assessed on 200 adults after their emergence. The wasps are placed in a Petri dish with alcohol (70%) and examined under a binocular lense.

3.2 Product control

3.2.1 Total number of parasitized eggs per release unit

The average number of parasitized eggs per release unit (i.e. cardboard strips or cardboard shells) is assessed on six units counting all parasitized eggs of each unit.

3.2.2 Emergence rate

Six release units are incubated at 25 °C and 80% RH. After the complete emergence of the adults, the rate is assessed on 200 parasitized eggs of each unit (totally: 1200 parasitized eggs).

3.2.3 Sex ratio

The sex ratio is assessed on 6 release units, incubated at 25 °C and 80% RH, till the end of the adults emergence. 100 adults per unit are checked (totally 600 adults).

4. FINAL CONSIDERATIONS

The performance of a *Trichogramma* population in the field is the product of a series of characteristics and behaviours of the released strain. Given an initial number of eggs parasitized by *Trichogramma* spp., the number of healthy females (i.e. of the organisms performing biological control) will obviously depend on the emergence rate, the sex ratio and the rate of deformed females. Other characteristics were also found to be related to field performance. GRIGORENKO (1978) noted that in fields where a *T. euproctidis* strain with an increased life-span was released, the parasitization on *Mamestra brassicae* eggs was higher than in fields with a normal strain. BIGLER *et al.* (1988) found a relation between walking activity and the percentage of *O. nubilalis* egg parasitization in the fields. VAN DIJKEN *et al.*, (1986) and VAN BERGEIJK *et al.*, (1989) observed that the capability of *Trichogramma* spp. to recognize and accept the located eggs as a host can vary greatly, depending on the strain and the rearing conditions. Other characteristics, as e.g. the reaction to chemical cues (NOLDUS, 1989) and flight propensity (BIGLER, unpubl.) can be very important, and simple tests should be developed for this characteristics, too.
A multiple data set, as the parameters measured in the quality control, does not allow the direct comparison between strains or species of *Trichogramma*. For this reason, a multivariate graphical representation or the computation of a comprehensive quality index (GRINBERG, 1992) will be helpful for decision making purposes. This comprehensive index should be verified with field data. Unfortunately, field data for establishing the relationship between the quality of strains measured under laboratory conditions and their performance in the field are still rare.

The quality control system implemented in Switzerland during the last years and presented in this paper has to be considered as a first approach. Nevertheless, it allowed the monitoring of the production process, the identification of critical situations and the check of commercialized products. Moreover, the collected data were helpful for the improvement of the rearing method. In fact it was possible to select a more appropriate strain and to develop a convenient production scheme. Our experience shows that method development, process control and product control should not be considered as completely separated spheres. Information collected in one or the other sphere can be useful to change test methods or production procedures. In our experience, the information collected in the process control allowed us to improve the production method and to simplify the product control. We realized too, that in case of changes of the production

method (e.g. production with diapause), the quality control system has to be partly redesigned and tested for the new situation.

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A SIMPLE METHOD TO CONTROL THE QUALITY OF MASS REARED EGG PARASITES OF THE GENUS TRICHOGRAMMA

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ABSTRACT

The use of replacement hosts for the mass production of *Trichgramma* such as the Angoumois grain moth *Sitotroga cerealella* (Oliv.) or the Mediterranean flour moth *Ephestia kuehniella* Zell., makes it important to monitor any loss of preference to the natural host (recognition, acceptance, suitability). In the present work, a simple laboratory method to monitor this critical aspect was developed and tested by conducting experiments with the two species: *Trichogramma evanescens* Westwood and *Trichogramma dendrolimi* Matsumura. The host preference of *Trichogramma* was tested by offering single *Trichogramma* females the choice between eggs of one target pest (the European corn borer *Ostrinia nubilalis* Hübner or the summer fruit tortrix moth *Adoxophyes orana* F.R.) together with eggs of the replacement mass rearing host *S.cerealella*.

The results showed that the host preference of one rearing line of *T. evanescens* that had no passages through the natural host *O. nubilalis* for more than 240 months before the test was significantly reduced, compared to another line that had a passage one month before the experiment. The difference between four other *T.evanescens* lines that had passages 1, 6, 8, and 13 months before the test were not significant. This seems to suggest that the increased preference to the target pest may last for about one year after a passage was carried out. Four different

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen. March 1991.

T.dendrolimi lines that had passages though *Adoxophyes* eggs 2, 7, 40 and >108 months before the experiment did not significantly differ in their preference towards this host.

The use of this test to control the quality of *Trichogramma* at intervals, especially before the start of a mass production is recommended.

INTRODUCTION

Interest in the use of egg parasites of the genus *Trichogramma* to control agricultural pests has rapidly increased in the last few years. Effective mass rearing systems to produce the necessary quantities of the natural enemy species with defined qualities for field releases are being developed. Production techniques that insure the preservation of the genetic structure of the population and that prevent any deterioration in the natural attributes of the parasite are required. Beside monitoring efficacy in the field, laboratory methods to detect any changes in the characteristics of the parasite in the rearing should be made available for the producers (Bigler 1989).

To facilitate discussion on quality control one should differentiate between (1) biological characteristics of the species such as longevity, fertility, host spectrum and habitat preference, (2) efficiency of production such as the number of *Trichogramma* produced per day, degree of parasitism, occurrence of unwanted organisms, and (3) product characteristics such as the number of parasitized eggs per unit, age structure, period of storage before delivery and purity.

In order to develop a quality control method to monitor any changes in the biological characteristics of a *Trichogramma* species in the mass rearing one should consider choosing a character (natural attribute) relevant to the purpose of the rearing. The effectiveness of *Trichogramma* in the field depends on its (1) searching ability (habitat location, host location), (2) host preference (recognition, acceptance, suitability) as well as (3) tolerance to environmental conditions. Whereas searching behaviour ought to be examined in the presence of plants, in semi-field or in field experiments, host preference and tolerance to environmental

conditions could be satisfactorily tested in simple laboratory experiments.

Because *Trichgramma* is reared on an replacement host in mass production units such as the Angoumois grain moth *Sitotroga cerealella* (Oliv.) or the Mediterranean flour moth *Ephestia kuehniella* Zell., the aspect of host preference (recognition, acceptance, suitability) becomes to be critical. Bigler et al. (1982) showed that the field performance of T. *evanescens* was inferior after the constant propagation of the parasite on *E.kuehniella* in the mass rearing. Neuffer (1987) demonstrated that host acceptance and suitability decreased with increasing number of generations reared on factitious hosts.

To study and compare the host preference of 3 *Trichogramma* species towards 4 different lepidopterous pests, Quednau (1955 & 1956) estimated the parasitization capacity by offering host eggs to single parasite females. Schieferdecker (1969) conducted host preference tests with 2 *Trichogramma* and 70 lepidopterous species by placing samples of host eggs in parasite emerging units. The cages included *Sitotroga* eggs in abundance to avoid super parasitism. A direct observation method to study host selection of *Trichogramma* was used by Dijken et al. (1986). Eggs of two cabbage lepidopterous pests were placed in a draughtboard pattern and the acceptance to contact ratio of one *Trichogramma* female was directly observed for 2 hours.

In the present work, a simple laboratory method to monitor the host preference of *Trichogramma* was developed and tested by conducting experiments with the two species: *Trichogramma evanescens* Westwood and *Trichogramma dendrolimi* Matsumura. Experiments to test the effect of a passage through eggs of the target host on the host preference quality of *Trichogramma* was carried out. In previous work, the method was used to select effective *Trichogramma* strains to control the European corn borer *Ostrinia nubilalis* by HASSAN and GUO (1991) and the codling moth *Cydia pomonella* and the summer fruit tortrix moth *Adoxophyes orana* by HASSAN (1989).

MATERIALS AND METHODS

The host preference of *Trichogramma* was tested by offering the parasite the choice between eggs of one of the two target pests: (1) the European corn borer *Ostrinia nubilalis* Hübner and (2) the summer fruit tortrix moth *Adoxophyes orana* F.R. and eggs of the replacement standard laboratory mass rearing host *S.cerealella*. A single *Trichogramma* female (12 to 24 hours old) was released in a glass tube (100 mm long and 26 mm in diameter) together with 60 eggs (2 x 30 patches) of the standard mass rearing host *S. cerealella* and eggs of one of the two target pests (2 x 30 Adoxophyes or 2 x 20 *Ostrinia*). Host eggs laid on small pieces of paper or cellophane (including the host eggs) were glued near the 4 corners of a larger piece of paper (2 x 2 cm). The number was reduced to 40 eggs (2 x 20) with *Ostrinia* because the eggs are larger. To separate single females, parasites were scattered on a smooth surface and captured by placing small tubes (50 mm long and 9 mm in diameter), open end down, to cover one parasite. *Trichogramma* walked up the tube and were easily examined using a binocular. The single females were transferred to the larger testing tubes by allowing them to walk towards a source of light. The females were allowed to parasitize the host eggs for a period of 4 hours after which they were removed from the tubes.

Monitoring was carried out by (1) checking all the tubes 8 times during the first 24 hours of the experiment and recording the location of the parasite (target pest eggs, *S.cerealella* eggs, elsewhere). A minimum of 30 minutes was left between observation; (2) counting the number of *Trichogramma* pupae within the host eggs 4 to 5 days after parasitism , in the case of *Sitotroga* where only one *Trichogramma* egg is laid, the number of black parasitized eggs was counted. In some cases, when it was not possible to see *Trichogramma* through the host egg chorion, the number of adults emerging from the host eggs was counted and used as basis for comparison.

Observing the location of the parasite in the first monitoring reflected the preference of *Trichogramma* to contact and remain on the different host eggs. Counting the number of *Trichogramma* developing in the host eggs in the second monitoring showed the preference of the parasite to lay eggs and indicated its ability to develop in these eggs. As most of *Trichogramma* eggs were laid during the first few hours, the removal of the parasite from the testing tubes 4 hours after the beginning of the experiment prevented superparasitism. To avoid odour disturbance, no food was offered in these experiments. The test was repeated at least 30 times (3 x 10 tubes) for each host insect and *Trichogramma* strain. The experiments were carried out in an environmental cabinet at 27 ± 1 ^{o}C, 16 h light, 8 h dark and 60 to 70 % relative humidity.

In additional experiments it was found that higher levels of parasitism can be obtained when the females were left in the tubes for their entire life and when they were fed with honey/agar before and during the experiment.

Effect of a passage through a natural host on the quality of the parasite:

In the present investigation the effect of rearing *Trichogramma* for one generation on eggs of a target pest (passage through a natural host) on its preference to this host was studied by applying the standard laboratory *Trichogramma* preference test. Five different lines of *T.evanescens* were reared on eggs of *O.nubilalis* for one generation then reared again on *Sitotroga* eggs for 1, 6, 8, 13 and >120 months. Similarly, four lines of *T.dendrolimi* were given passage on eggs of *A.orana* for one generation then reared on *Sitotroga* eggs for 2, 7, >40 and >108 months. The effect of the time intervals between the passage through eggs of the target pest and the beginning of the experiment on the host preference of the parasite was tested. Each of the 5 *T.evanescens* strains, and *the 4 T.dendrolimi* strains were offered the choice between the eggs of *Sitotroga/ Ostrinia*; *Sitotroga/ Adoxophyes* and *Ostrinia/ Adoxophyes*

Rearing of Trichogramma strains:

Each of the two *Trichogramma* species was kept in several glass tubes (145 mm long and 26 mm in diameter) with a cloth cover. The tubes were confined in a plastic container with one side darkened by black paper to keep the parasites away from the cover of the tubes. About 1500 parasitized host eggs were placed in each tube and the emerging adults were offered ca. 50,000 host eggs glued on stripes of paper (egg card). About one week later, when all the adults have died, new parasitized host eggs were taken by cutting about one third of the egg card and placing it in a new tube. To insure the purity of the different *Trichogramma* strains, care was taken, that *Sitotroga* eggs used for the rearing were completely free of *Trichogramma*. The parasites were kept in an environmental cabinet at 25 ± 1 ^{0}C, 16 h light, 8 h dark and 60 to 70 % relative humidity.

RESULTS AND DISCUSSION

The results of the experiments to test the effect of a passage through a target pest on the host preference quality of *Trichogramma* were given in Figure 1 for *T.evanescens* and in Figure 2 for *T.dendrolimi*. The combinations of hosts offered to each of the two species were: *Ostrinia / Sitotroga*; *Adoxophyes / Sitotroga* or *Ostrinia / Adoxophyes*. Five different lines of *T.evanescens* were given passages through *Ostrinia* eggs 1, 6, 8, 13 and >120 months before the test. With *T.dendrolimi*, passages on *Adoxophyes* eggs were carried out 2, 7, >40 and >108 months before the test (see Figures 1 and 2).

It is clearly seen in Figure 1 that *T.evanescens* had a strong preference to *Ostrinia* eggs compared to *Adoxophyes* eggs. The number of *Trichogramma* eggs laid (Figure 1 a) as well as the number of parasite contacts with the host eggs (Figure 1 b) showed that when *Ostrinia* and *Sitotroga* eggs were offered, both types of eggs were accepted. When *Adoxophyes* and *Sitotroga* were offered, the first host was nearly always rejected. The

choice between *Ostrinia* and *Adoxophyes* resulted in a clear preference for the first. This Figure also shows that a passage of *T.evanescens* on eggs of the European corn borer significantly improved its preference to this host. The difference between a passage one month and more than 240 months before the test were significant for both contacts and parasitism. The difference between four *T.evanescens* lines that had passage 1, 6, 8, and 13 months before the test were not significant. This seems to suggest that the increased preference to the target pest may last for about one year after a passage is carried out, but this has to be confirmed.

The results in Figure 2 (a and b) show that *T.dendrolimi* had a clear preference for *Adoxophyes* eggs compared to *Ostrinia*. European corn borer eggs were nearly totally rejected in the presence of *Sitotroga* or *Adoxophyes* eggs. The different *T.dendrolimi* lines that had passages though *Adoxophyes* eggs did not significantly differ in their preference towards this host (Figure 2).

These results showed that the method developed in this work can be used to monitor changes in the host preference of *Trichogramma*, a quality control aspects. The conduction of this test on continuously reared parasites at intervals especially before mass production would indicate relevant quality changes.

ZUSAMMENFASSUNG

Eine leicht durchführbare Methode zur Prüfung der Qualität von in Massen gezüchteten Eiparasiten der Gattung *Trichogramma*

Um Fehlschläge in der Praxis bei der Verwendung von Eiparasiten der Gattung *Trichogramma* zu vermeiden, dürfen Aspekte der Qualitätskontrolle und der Qualitätserhaltung der Nützlinge in der Massenzucht nicht vernachlässigt werden. Obwohl praktische Einsätze mit *Trichogramma* zur biologischen Bekämpfung von Schadlepidopteren in mehreren Ländern bereits zum festen Bestandteil integrierter Programme zur Schädlingsbekämpfung gehören, sind bisher keine Verfahren zur Prüfung der Qualität dieser Nützlinge in Massenzuchten bekannt. Da *Trichogramma* in der

Massenproduktion auf Eiern von Ersatswirten gezüchtet werden, ist darauf zu achten, daß ihre Präferenz gegenüber den Zielorganismen nicht vermindert wird.

Zur Erfassung der Wirtspräferenz von *Trichogramma evanescens* und *Trichogramma dendrolimi* wurden einzelnen *Trichogramma*- Weibchen Eier des Zielschädlings (*Ostrinia nubilalis* oder *Adoxophyes orana*) zusammen mit Eiern des Ersatzwirtes *Sitotroga cerealella* Oliv. (Getreidemotte) angeboten. Als Maßstab für die Wirtspräferenz dienten (a) die Häufigkeit der beobachteten Kontakte mit den natürlichen Wirten sowie (b) die Parasitierungsleistung der Nützlinge. Die Ergebnisse (Abbildung 1 und 2) lassen erkennen, daß die Aufzucht von *T. evanescens* auf Eiern des Ersatzwirtes *Sitotroga* für die Dauer von > 120 Monaten ohne Passagen durch den natürlichen Wirt zu deutlichen Qualitätsverlusten führte (signifikante Veränderung in ihrer Wirtspräferenz). Bei der Dauerzucht von *T. dendrolimi* über einen Zeitraum von 108 Monaten hinaus traten dagegen keine meßbaren Leistungsminderungen auf. Die intervallmäßige Anvendung dieser Methode wird empfohlen, um die Qualität von *Trichogramma* zu prüfen.

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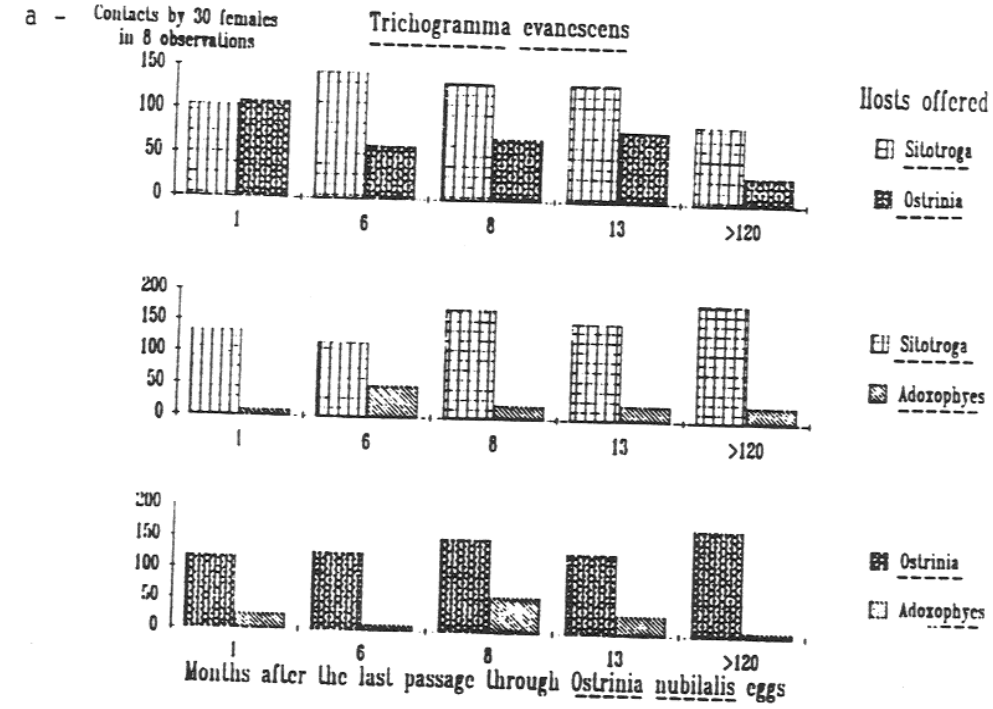


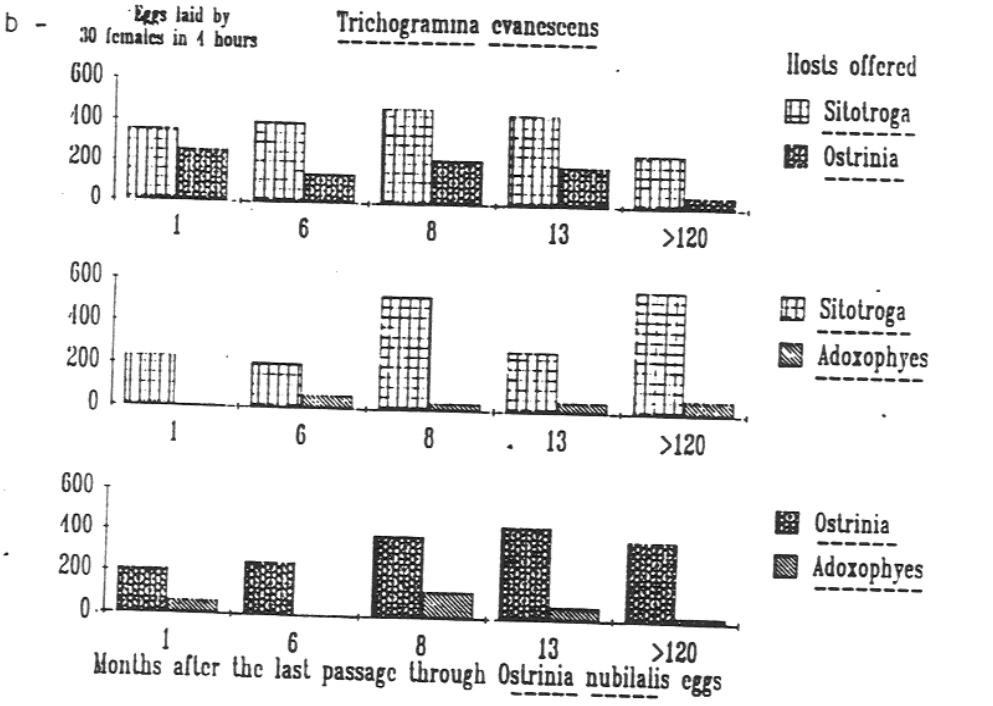
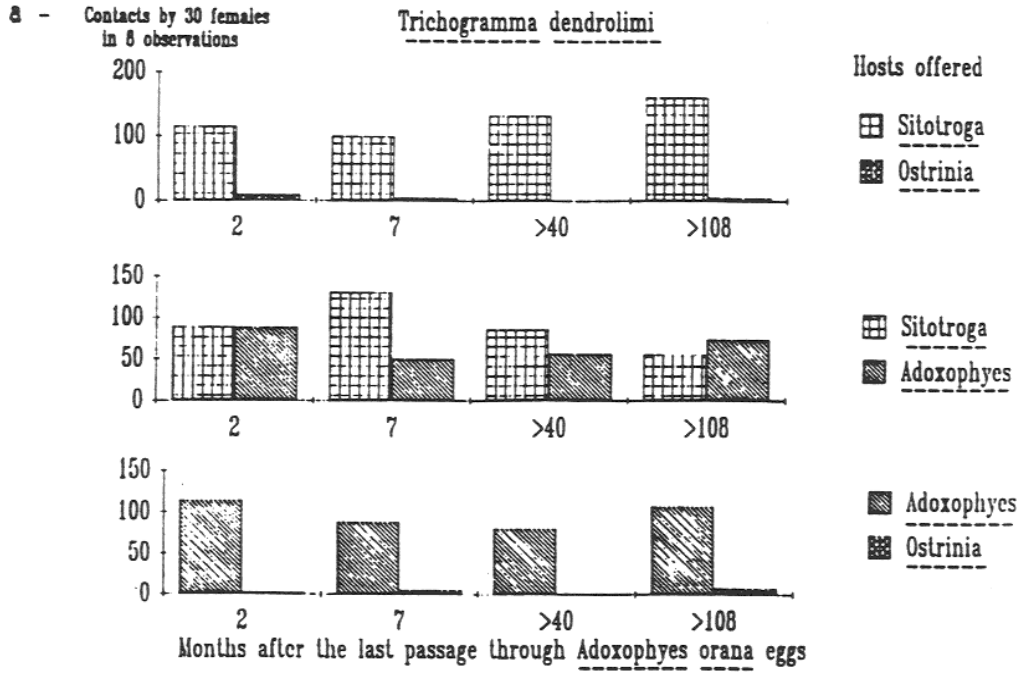


Figure 1: Host preference of Trichogramma evanescens tested at 5 different time intervals after a passage through the natural host. a - Contacts (above), b - Parasitism (below).



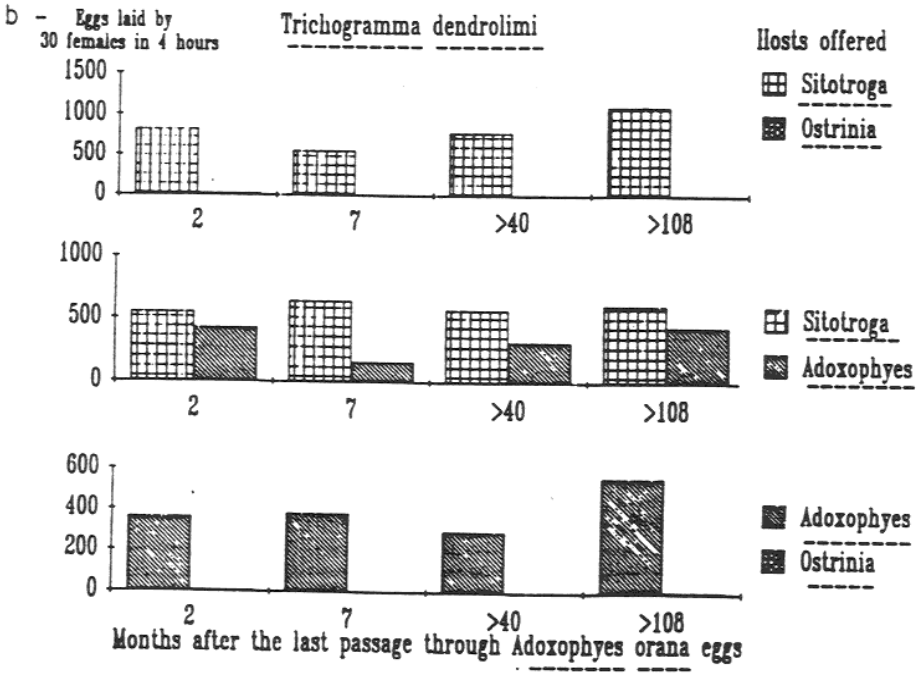


Figure 2: Host preference of Trichogramma dendrolimi tested at 4 different time intervals after a passage through the natural host. a - Contacts (above), b - Parasitism (below).

EVALUATION TECHNIQUES FOR TRICHOGRAMMA QUALITY

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ABSTRACT

The article describes methods and techniques for evaluating the quality of *Trichogramma spp.* used for biological control of pests. A method used to assess the searching ability and acceptance of natural host eggs is presented. Combining searching ability with emergence rate, sex ratio and fecundity leads to a generalized quality criterium which is related to field efficacy. Olfactometer studies are useful in quality control and allow the classification of mass-produced *Trichogramma*.

INTRODUCTION

Trichogramma is one of the main biocontrol agent used worldwide against insect pests. Successful application of *Trichogramma* considerably promotes further studies and practice of biological control in general. Moreover, *Trichogramma* is a good test object and the results can be used for the development of production and application techniques of other entomophagous insects.

Trichogramma effectiveness considerably depends on its quality. Development of objective methods to evaluate the entomphage's quality and its standardization allows not only scientifically sound research but it helps producers and users to make decisions for improving product quality. Existing methods of *Trichogramma* quality evaluation are cumbersome and need further development.

In USSR, the main investigations on parasite quality evaluation have been carried out since 1977 in the *Trichogramma* laboratory of the All-Union Institute of Biological Methods in Plant Protection.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

METHODS AND TECHNIQUES

Searching ability

To develop the technique for assessing the searching ability special boxes of acrylic plastic and model plants were used. Experiments were carried out under controlled, optimal environmental conditions specific to a certain species. *Trichogramma evanescens* Westw., *Mamestra brassicae* L. and *Ostrinia nubilalis* Hb. served as objects for the investigation. A grid consisting of 49 squares (4 x 4 cm) was drawn in the bottom of the box (30 x 30 x 5 cm). One fresh egg of *Mamestra brassicae* was placed into each square (each egg was fastened to a paper strip (5 x 10 mm) by 10% sugar solution). The boxes were placed bottoms up on an even, black surface. 25-30 young females were selected randomly and placed into the boxes (1 female/box). *Trichogramma* contacts with host eggs lasted 24 hours under the temperatures 23 ± 1 °C, relative humidity 80 ± 5% and a photoperiod of 16 hours. After 24 hours, egg cards were transferred into vials and kept under the above mentioned conditiones until they turned black. The total number of parasitized eggs in all boxes (N_m) was counted.

To determine searching ability of *Trichogramma* for eggs of *Ostrinia nubilalis*, egg clusters (on average 9-10 eggs/cluster) were placed into boxes at 5 or 10 points with an equal distance from each other and one or two females were released per box respectively. All the other procedures were the same as described for *Mamestra brassicae*.

Cards with pest eggs were hung on model plants covered by cages 1 x 1 x 1 m for cabbage and 1.5 x 1.5 x 0.5 m for maize; then Trichogramma was released (1 female/50 eggs).

Searching ability (y_2) of the parasites was determined as:

$$Y_2 = \frac{N_m}{N_o} \times 100\%$$

N_o is the initial number of host egss.

It was established that *Trichogramma's* searching ability is considered low at parasitization rates up to 10% of host eggs, satisfactory for 10-20% good for 20-30% and excellent for more than 30% (Greenberg et al. 1983).

Emergence, sex ratio and fecundity

Searching ability is one of the parameters evaluating *Trichogramma* quality. Additionally, emergence (α_1), sex ratio (α_2) and fecundity were taken into account.

400 parasitized eggs are randomly selected from an initial sample. 300 eggs are used to determine emergence and sex ratio. For these purposes, eggs are glued with 10% sugar solution onto 3 paper strips (3 x 1 cm; 100 eggs/strip). Every strip is placed into a vial and the latter are kept under optimal conditions (23 ± 1°C and RH 80 ± 5%). After complete emergence and natural mortality of the adults, the emergence index $\alpha_1 = \frac{N}{300}$ was evaluated.

Sex ratio was established by counting the number of emerged insects (N_1) in all 3 vials and by determining the number of females (N_2)

$$\alpha_2 = \frac{N_2}{N_1}$$

To determine the fecundity, groups of 10 parasitized eggs were placed into 10 vials and closed by cotton plugs. The vials were stored under the above mentioned conditions. At the beginning of *Trichogramma* emergence and repeatedly during the whole life span, cards with fresh *Sitotroga cerealella* eggs (not less than 200) were placed into the vials. After death of all adults, the fecundity (F) is assessed by counting the numer of females (ni) and the number of parasitized eggs (Ni).

$$F = \frac{\Sigma Ni}{\Sigma ni}$$

The generalized quality criterium and biological efficacy

All above mentioned parameters are summarized to the generalized quality criterium (D). Its value is determined by the equation

$$D = \sqrt[1.7]{d_1 \cdot d_2^{0.7}}$$

where $d_1 = \exp [- \exp (1.089 - 0.136\, y_1)]$

$d_2 = \exp [- \exp (2 - 9y_2)]$

$y_1 = \alpha_1 \cdot \alpha_2 \cdot F$

y_1 is considered as a statistical critierium of the quality. The biological sense of it (assuming a simple process) means the number of parasitized

eggs/1 infested *Trichogramma* host egg (e.g. 200 eggs parasitized by 10 infested ones, $y_1 = 20$).

D can be determined graphically as well (Greenberg et al., 1979).

$D = f_1 \cdot f_2$, where $f_1 = f_1(y_1)$ and $f_2 = f_2(y_2)$

Values for f_1 and f_2 are found by graphical interpolations of Figures 1 and 2.

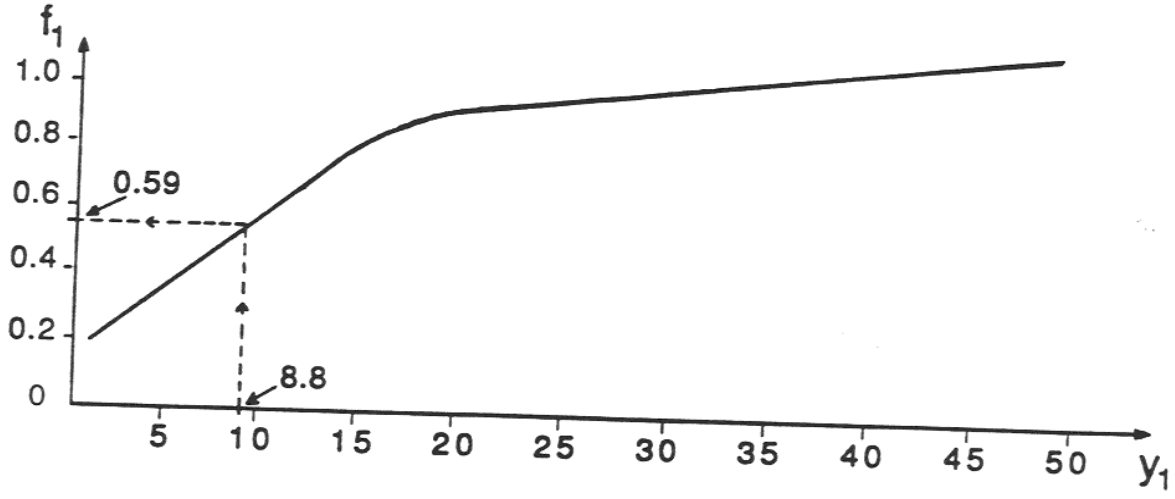

Figure 1: The number of parasitized eggs per one host egg containing a Trichogramma (y_1) and its relationship to the quality index (f_1)

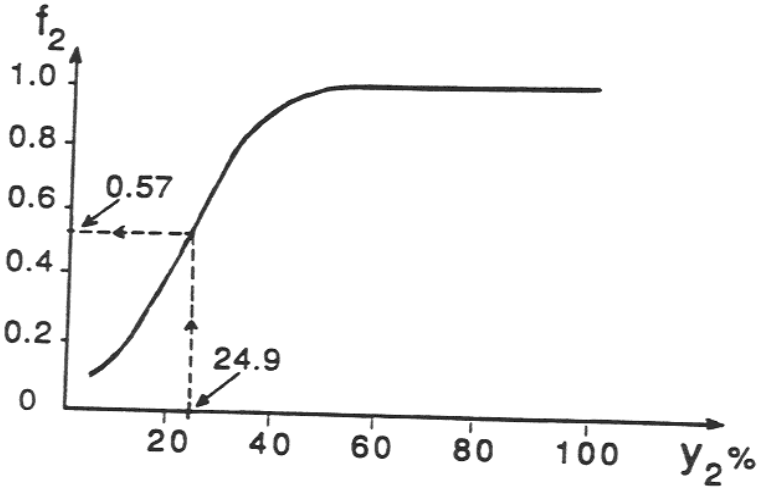

Figure 2: Percent parasitism of host eggs (y_2) as assessed in the searching ability test and its relationship to the quality index (f_2)

Division of the *Trichogramma* material into quality classes has been proposed on this base (Greenberg et al., 1980, 1986). A relationship between quality criteria and expected biological effectiveness of the entomophage (E) in the field under optimal environmental conditions (Table 1) has been established (Greenberg and Podberezskaya, 1984).

Table 1. Biological effectiveness of *Trichogramma* depending on the quality

Intervals for values of the generalized quality criterium (D)	Quality class	Forecasted biological effectiveness (E)
0.7 - 1.0	1	> 80 %
0.5 - 0.7	2	55-80 %
0.3 - 0.5	3	33-55 %
Less than 0.3	non-standard	<33 %
E = - 0.53 +113 · D		

When climatic conditions deviate from optimal ones, the generalized quality criterium is corrected during *Trichogramma* releases. It is determined in accordance with the regression equation:

$D = 0.857 - 0.6213\ T - 0.000227\ TW + 0.000049\ W^2$. The regression is calculated on the base of experimental data and is explained in detail by Medoni and Mencher (1980).

Olfactometers

Quick methods are being developed for *Trichogramma* quality evaluation using indexes as e.g. activity for searching host eggs by means of olfactometers (I_1) and movement activity (I_2) by means of a set of glass tubes. The technique described by French scientists (Ferreira et al., 1979) was used to carry out olfactometer studies. Two vials (one containing 50 adult *Trichogramma*, another one was used for their collection) were tigtly put onto two glass tubes with the length of 80-100 cm and a diameter of 3 cm. The tubes and vials containing parasites were covered with a black cloth.

The number of individuals which went during 30 minutes into the illuminated vials was counted. This exposition was determined experimentally. Relationships were determined between the variables and the generalized quality criterium. Models have been built for the relationships between host searching activity or moving activity and the generalized

quality criterium: $D_1 = 0.42 + 0.1\ (I_1 - 5.7)$, where D is an expected generalized quality criterium for *Trichogramma* and I_1 the activity for host searching. $D_2 = 0.49 + 0.01\ (I_2 - 29.93)$, where I_2 is the moving activity. The correlation coefficient (r) between estimated and actual generalized quality criterium is in the first case 0.8 - 0.9, in the second 0.55 - 0.6.

The analysis of experimental data allows to establish the following gradation of *Trichogramma* based on searching activity for host eggs:

at $I_1 \leq 4.5$ corresponds to non-standard *Trichogramma*
$I_1 = 4.6 - 6.5$ corresponds to the 3rd class
$I_1 = 6.6 - 8.5$ corresponds to the 2nd class
$I_1 > 8.5$ corresponds to the 1st class

The integral index

Another device has been designed together with the Moscow State University and Research Production Unit "Agropribor". It estimates *Trichogramma* quality using the integral index, i.e. the number of parasitized eggs of the natural host under a given environmental regime. This integral quality index depends on the infestation, emergence, sex ratio, number of deformed individuals, migration, searching abilities and fecundity of *Trichogramma* (Tschernyshev et al., 1986).

Each chamber of the device consists of two parts: one is used for *Trichogramma* release another for placing the host eggs. They are connected with a twisting tube with the total length of 3 m, corresponding to the radius of the effective activity of the entomophage in the field (Fig. 3). 0.5g of *Trichogramma* ready to emerge from host eggs is put into the first part of the device. 500 host eggs (*Heliothis, Mamestra* etc.) are placed on cards into the other part. Three such chambers are left in a climatic room for 8 hours under specific conditions. The number of adults and host eggs was determined empirically.

After 8 hours, cards with host eggs are withdrawn and placed for incubation. The average number of parasitized eggs in three chambers is considered as the integral index for the quality of *Trichogramma* (IP). When IP is < 30%, the given *Trichogramma* population is estimated as being of low quality, at IP 30% to 50% of medium quality and at IP > 50% of high quality (Torgovetsky et al., 1988).

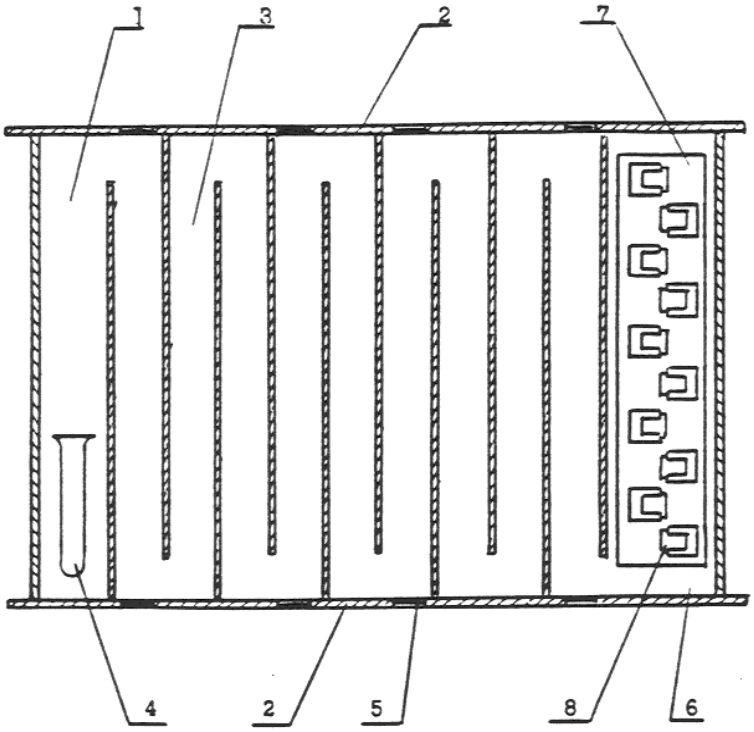

Figure 3: Quality control chamber for *Trichogramma*

1. section for Trichogramma release
2. cover
3. twisting canal
4. vial with Trichogramma
5. ventilation window
6. section for parasitism
7. tray
8. cards with host eggs

The effectivenes of *Trichogramma* depends not only on their quality, but also on the density of pest eggs and the number released. In order to release an optimal number of wasps, we developed mathematical models which take into account forecasted *Trichogramma* effectiveness under specific abiotic conditions, quality of the material used and the state of plants. The optimal number of parasites can be calculated for each specific case.

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MASS REARING OF TRICHOGRAMMA BRASSICAE USED AGAINST THE EUROPEAN CORN BORER OSTRINIA NUBILALIS

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ABSTRACT

During the mass rearing of *Trichogramma* for biological control of the European corn borer it is necessary to take some precautions in order to produce insects of high quality. For more than 6 years UNCAA has used a process which allows to produce about 5 billion parasitoids per year with a good and regular efficiency.

INTRODUCTION

In collaboration with INRA, UNCAA created a pilot plant to produce *Trichogramma brassicae* used against the European corn borer (ECB), *Ostrinia nubilalis*. The present paper gives some informations about the results of this project.

MASS REARING OF EPHESTIA KUEHNIELLA

Mass production of *Ephestia kuehniella* has been conducted for about 15 years in INRA's laboratory at Antibes (FRANCE). In the pilot plant, *E. kuehniella* has been reared for more than 6 years. The productivity has been improved substantially in the last years. The main checks in the rearing process concern abiotic and health conditions. Until now, the production potential has not shown any decrease (Figures 1 and 2).

Concerning the parasitoids, their rearing is realized with more precaution, particularly because of the great number of generations during the production cycle (about 25 generations per year) and also because these insects are intended to be commercialized. So, a control system has been set

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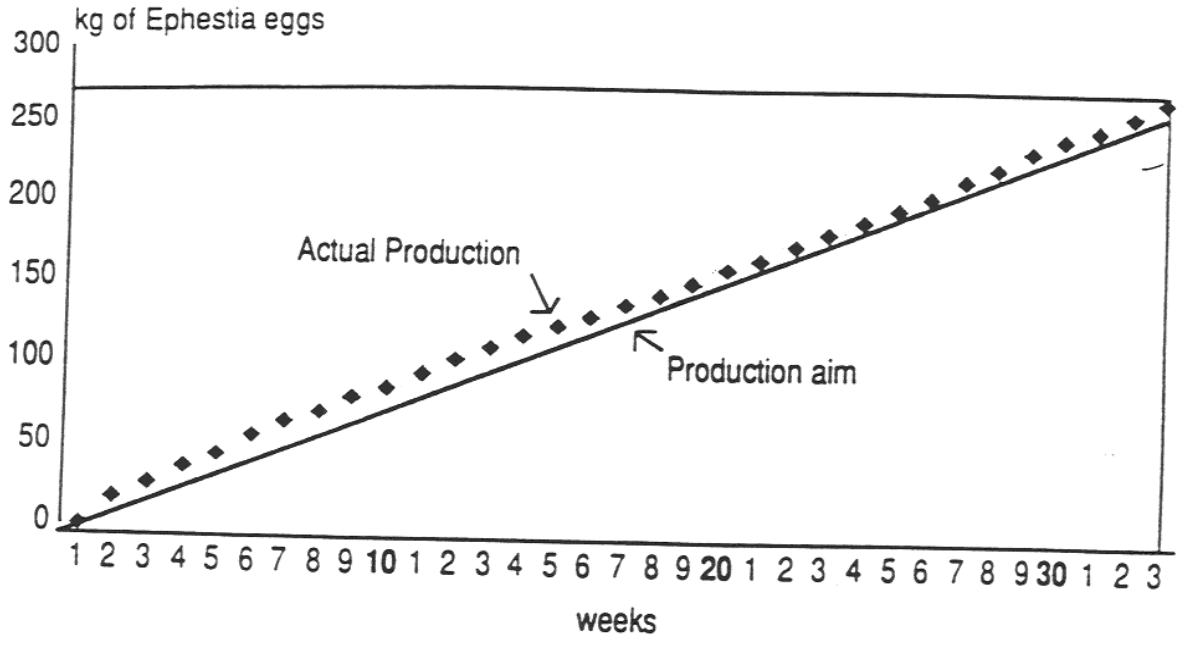


Figure 1: Production of Ephestia kuehniella eggs in 1990

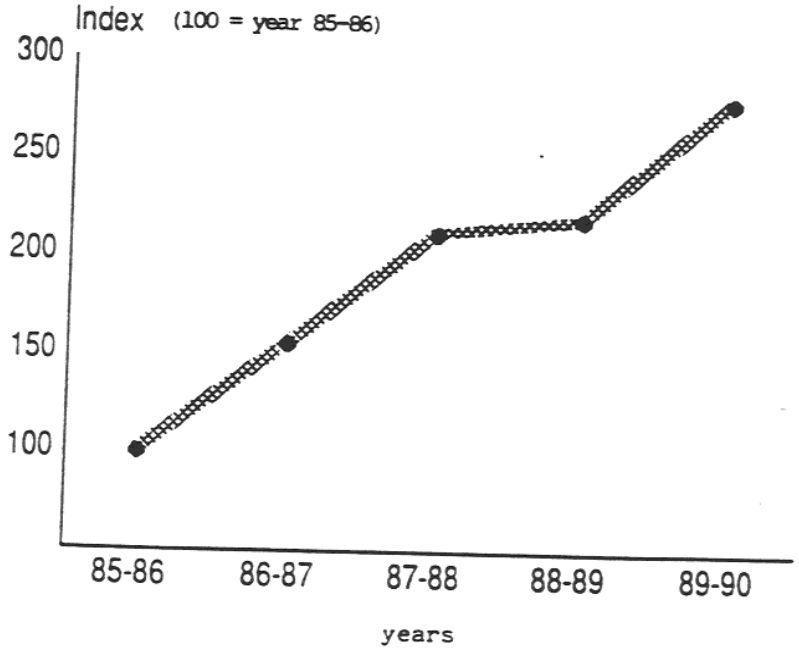


Figure 2: Improvement of the productivity of Ephestia rearing (eggs/kg of food)

up to check the quality of paraitoids and to maintain it during all the production cycle.

OBTAINING THE INITIAL POPULATION (FOUNDER POPULATION, INOCULUM)

The parasitoid population is renewed at the beginning of each production cycle (Figure 3). This population is obtained by crossing insects from different origins:

a. from the inoculum used to rear parasitoids during the current season
b. isofemale strains consisting of females collected in fields treated the previous years
c. isofemale strains of wild *Trichogramma*. These strains are controlled by electrophoretic esterases tests to check their specificity.

After multiplying the parasitoids, the strain is used for 25 generations (about 20 million insects per generation produced) in the laboratory.
With this method, a great number of Trichogramma which were able to find and parasitize ECB egg-masses in natural conditions are introduced in our population.

QUALITY CONTROL OF THE INOCULUM AND IN THE COMMERCIAL PRODUCTION

The inoculum is controlled at each generation. Several samples of the commercial product are checked after the reactivation of diapausing insects before their shipment to users. The comparison of results with standards allows to estimate the quality. Tests and standard levels are:

	Inoculum	Commercial product
Fecundity in 7 days (number of *Ephestia* eggs parasitized by 1 female)	65	55
Mortality after 7 days (%)	< 10	< 10
Sex ratio (%)	> 70	> 70
Rate of emergence (%)	> 85	> 85

The control of fecundity is realized with sterilized eggs of *E. kuehniella* using 20 isolated females fed with honey in controlled conditions (23 ± 1°C; 75% ± 5% RH).

Some other tests are also realized to evaluate the mobility and the ability to find ECB eggmasses (in olfactometer).

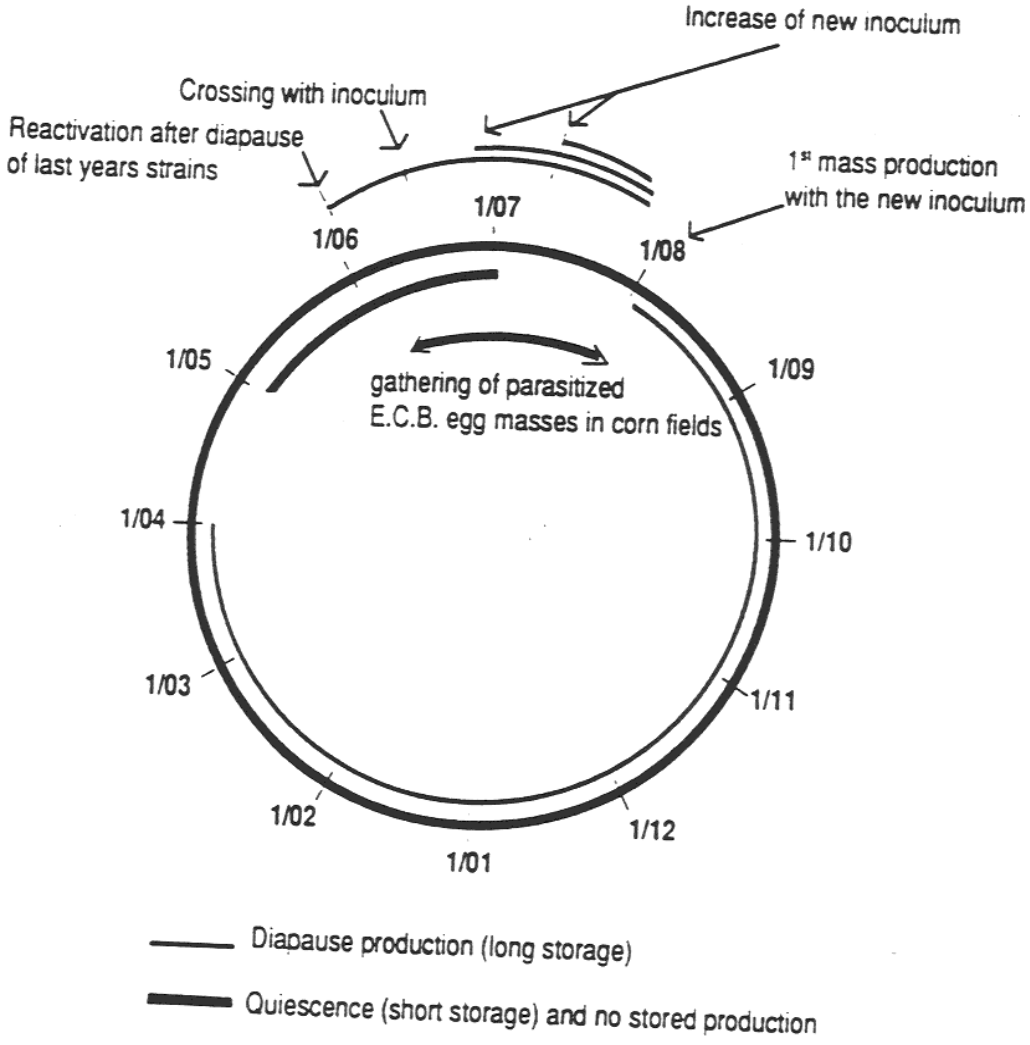


Figure 3: Scheme illustrating the preparation of the founder population (= inoculum) of *Trichogramma brassicae* and the mass rearing cycle. ——— Diapause production (long storage), —— Quiescence (short storage) and not stored production

Table 1: Efficacy of Trichogramma brassicae against the European corn borer assessed as larval reduction

Year-Locality		Number of fields treated with Trichogr.	Numer of egg-masses/100 plants	% parasitism	Larvae/plant in treated fields	Larvae/plant in untreated fields	Efficieny
1988							
Hochfelden	o	5	-	-	0,04	0,78	94%
Blois	o	5	18,5	86,2	0,11	0,43	74,4%
Beaurepaire	o	6	30,9	78	0,08	0,44	82%
Beaurepaire	•	2	32,5	67	0,55	2,8	80,3%
1989							
Beaurepaire	o	2	16,5	92	0,03	-	-
Beaurepaire	•	5	24,5	81	0,5	2,5	80%
1990							
Heyrieux	o	8	45	85	0,26	1,8	85%
Hochfelden	o	3	-	-	0,05	0,8	93,7%
St. Maximin	•	2	15	74	-	-	-

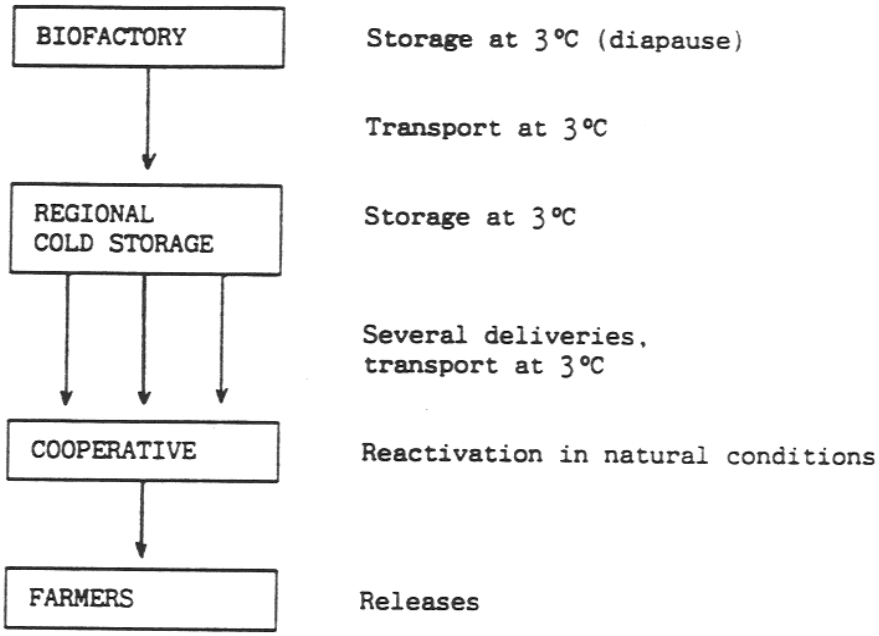
o 1st generation of European corn borer
• 2nd generation of ECB in seed corn

CONTROL OF THE PARASITIZATION EFFICIENCY

The efficiency of the produced parasitoids is checked every year in field trials. The number of ECB egg-masses and parasitization rates are recorded. The efficacy in terms of larval reduction is also assessed (Table 1). During these trials, parasitized egg-masses are gathered for next years inoculum.

Good field results depend on the quality of the parasitoids and on the distribution method of the product preserving insects' potential until they reach the farmers. This method is

presented in the following Figure:



CONCLUSION

Industrial production of *T. brassicae* used for biological control of ECB is realized in UNCAA's pilot plant with a process which allows:

- a constant production of great numbers of *Ephestia kuehniella* eggs (presently about 40 million eggs per day all year long)
- to maintain a good parasitization potential of commercialized parasitoids (about 5 billion *Trichogramma* per year)

THE USE OF ENZYME TESTS TO CONTROL THE QUALITY OF ENTOMOPHAGES UNDER MASS REARING WITH SPECIAL REFERENCE TO CHRYSOPA CARNEA STEPH.

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ABSTRACT

The multiple forms of different enzymes were studied in natural Moldavian populations and laboratory cultures of *C. carnea*. The alkaline phosphatase and acid RNase were found to be the most interpretable and informative enzymes for investigation of changes in genetic structure of natural populations and processes proceeding during the introduction into the culture. Direct evidence, confirming the importance of the period of insects collection for a stock colony, was obtained. This is due to the occurance of seasonal microevolution processes in the local population of insects.

INTRODUCTION

Insect rearing conditions in biolaboratories are known to be different from those in nature. There are differences in all aspects of insect life cycles, e.g. the regimes of feeding, abiotic conditions (temperature, moisture, photoperiod), changes in the character of development of insect populations and the nondiapausing reproduction during a number of generations. This continuous rearing under unnatural conditions may result in the reduction of viability and productivity and even in partial degeneration and mortality due to genetic shift and selection processes (Tamarina, 1987). Thus, artificial insect mass rearing demands a constant control of character traits and quality of insects, in particular, changes in genetic structure of an insect population.

Changes in the genetic structure of natural and laboratory populations are reflected in changes of enzyme and protein polymorphism. Nowadays, one of the traditional methods to control the genetic variability is monitoring the occurrence of different enzyme forms, a kind of genetic determination which has been well investigated on a variety of insect species.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

This investigation is devoted to the analysis of quality alteration processes occurring in *Chrysopa carnea* Steph. during its introduction in the laboratory and its mass rearing.

MATERIALS AND METHODS

Insects were collected in the neighbourhood of Kishinev. One of the laboratory cultures (KG6) was founded on the base of 500 ovipositing females caught in July 1986. Insects from this culture were constantly reared under long-day (16-hours) photoperiod. They had a continuous access to eggs of *Sitotroga cerealella* Oliv. (in the larval stage) and to a mixture of autolyzate of brewer's yeast with honey (1:1 v/v) and water as adults. The offspring from field collected insects reared under these conditions for more then 36 generations lost their ability of diapausing under the short-day (10-hours) photoperiod.

A second culture of *C. carnea* (PD) of which 100% were undergoing diapause under the short-day photoperiod was reared. The stock colony for this culture consisted of 5 diapausing insects, caught in early spring and reactivated in the laboratory. Oviposited eggs were collected and hold for development in short-days. Resulting adults (F1 laboratory generation) began diapausing. This culture was conserved as diapausing adults until the beginning of our experiments. Studies were made on adults of the second laboratory generation (F2).

While rearing insects under short-day conditions, adults were fed additionally 40% solution of sucrose. Diapausing insects were distinguished by the development of a waxy appearance and yellowish-green to reddish-brown body color with characteristic dorsal dark spots, and by the absence of oviposition during the experimental period (until 60 days in some cases).

Carrying out studies on the structure of natural and laboratory populations we analyzed the composition of enzyme multiple forms with Polyacrylamide Gel Electrophoresis (PAGE). We tested the following enzymes: alkaline and acid phosphatases, acid RNAse, digestive nuclease, G-6-PhDG, MDG, GPhDG, complex esterase. These enzymes were tested preliminarly in insects of all stages fed on different food in the laboratory.

RESULTS

Investigation of a natural population

Preliminary work showed the alkaline phosphatase (APase) and acid RNAse to be the enzymes with the highest information.

Depending on electrophoretic mobility, 7 different enzyme forms of APhase (Fig. 1) were discovered. These forms were found to occur with different frequencies during the field season. The most significant changes were detected in the 7th zone of enzyme activity (Rf 0.35): starting with zero in springtime (i.e. at the beginning of population development) the frequency of this enzyme form increased up to 0.20 - 0.60 (depending on the year) in midsummer (July) and then decreased to 0.02 - 0.05 in autumn, i.e. at the end of the favourable period for populaton development.

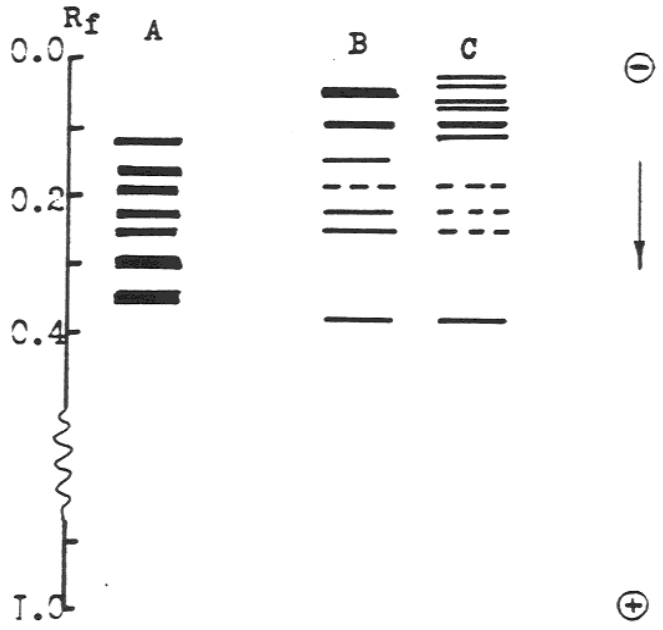

Fig 1. Zymogramm patterns of the alkaline phosphatase (A) and acid RNase (B, C) of imago *Ch. carnea*. A, B - imago fed with protein-sucrose diet; C - imago fed with 40% solution of sucrose. Electrophoresis was carried out in 7,5% polyacrylamide gel in 0.1M tris-borate buffer at pH 8.7.

When we studied the expression of different forms of this enzyme in ontogenesis and with different food supply, it was found to depend on feeding regimes: insects caught in nature and fed in the laboratory during 10 days showed a 3-fold increase of APhase7 (0.07 before and 0.296 after feeding in the laboratory). This form of enzyme may therefore be assumed to be an adaptive one connected with changing food supply during the season. This is an evidence of the natural population heterogeneity i.e. the possibility to change the level of natural polymorphism when introduced into laboratory culture. We also got additional data with acid RNase (acRNase) confirming the change during the season according to the food regime of *C. carnea*.

At the beginning we found clear differences in enzyme zone activity sets between active and diapausing adults (Fig. 1). In most insects, "diapausing" zones of activity appeared in August, i.e. in the period when visible traits of diapausing insects were absent. Insects caught in July, however, had enzyme activity zones identical to those laboratory insects getting a suitable protein-carbohydrate diet.

To clarify the reasons of this we carried out laboratory experiments with insects, feeding them either only carbohydrate solution of sucrose or protein-carbohydrate diet [water and dry mixture of brewer's yeast with sucrose - 1:1 (m/m)]. Parallel analysis of enzyme activity zones showed that pure carbohydrate feeding led to the "diapausing" type whereas in the other case (protein - carbohydrate feeding) characteristic zones of active (non-diapausing) insects were observed.

Studies on changes in laboratory cultures

For checking the possible changes of natural polymorphism during artificial rearing of *C. carnea*, we monitored aPh7 along with the introduction of insects into culture while forming the KG6 strain. The results obtained are shown in Table 1.

The analysis of these data proves the selection process during the introduction into the culture. The individuals with the possibility to express the aPh7 are selected primarily. We compared these results with those obtained in natural populations and came to the conclusion that one of the possible reasons for the selection process, occurring in insects reared in the laboratory, was connected with changes of the feeding regime. It was due to the fact that in the laboratory insects obtained food enriched

Table 1: Changes in frequency of the enzyme aPh7 during introduction of insects in the laboratory

Generation	Number of analysed adults	Frequency of enzyme aPh7
P	71	0.21
F1	86	0.27
F2	64	0.14
F3	87	0.17
F9	98	0.98
F10	50	0.96
F11	37	0.81

with protein compared to that in nature. In mid-summer, there were more opportunities for ingesting proteins and other different food constituents in nature due to the occurrence of pollen in a wide variety of blooming plants. In summer season, the ratio of insects having aPh7 is increased. On the contrary, in springtime and autumn, when food supply is poorer, the ratio decreased to about zero. However, what is the mechanism allowing a stable "zero" springtime/autumn fequency of that enzyme? And what is the way for insects to loose the ability to enter diapause when reared under short-day conditions?

The most appropriate hypothesis explaining these events is to suppose that this enzyme is involved in insect adaption to a more full-value food supply as well as in photoperiodic sensitivity. That is, we adapted the hypothesis about pleiothropic action of a respective gene.

The main factor responsible for *C. carnea* entering diapause is photoperiod. So it is likely to suppose selective death of individuals with expressed aPh7 during unfavourable periods of development. This would be the simpliest and most effective way of eliminating them from the population if such insects did not enter diapause with decreasing day length.

For experimental testing this hypothesis, the group selection for lacking photoperiodic sensitivity was made. The insects caught in July were maintained under diapause-inducing conditions (short-day) with access to the full-value protein-carbohydrate diet. Each subsequent generation was initiated with eggs only from those insects that reproduced without entering diapause. Parallel monitoring of aPh7 occurrence showed a sharply increasing frequency for this enzyme (to 0,88) in the 3rd generation. Thus, our suppositions of the relation between the insect's ability to express aPh7 and the absence of photoperiodic reactions proved to be true.

It should be mentioned that elimination of insects with expressed aPh7 during the unfavourable period of the year seems to be not the only mechanism responsible for the dynamic ballance of this allele during the development of local populations. There are some other intrinsic mechanisms leading to death in a part of the population followed by decreasing frequency of that allele in the population. For example, individuals of the 4th selected generation passed through the "bottle neck" divided in diapausing and nondiapausing insects. In the next generations (from F4 to F12), the frequency of this enzyme again sharply decreased (the enzyme was not detected in individuals from generation 12 to 20).

Analogous changes were detected while rearing insects of strain KG6 (Table 1). The frequency of this enzyme decreased until disappearance in F30-F50. An explanation of this phenomenon could be the "evolution of dominancy". This process was investigated quite well on *Drosophila*. The reason might be the parallel evolution of mutations and systems of genes-modificators providing its expression (Dubinin, 1986).

DISCUSSION

The results obtained show the following: Firstly, they prove different characters of lacewing's feeding in natural populations during the field season. There is a regular change of carbohydrate-protein feeding. Secondly, taking into account, the abundance of flowering plants in August in Moldavia, one can suppose that the autumn change of lacewing's feeding is not due to the absence of sufficient protein food. The change of feeding might be due to the decreased day-length. This change seems to be connected with insects preparing to diapause.

The last affirmation is in agreement with investigations made by Shyiko (1974) who studied the optimal feeding regimes for *C. carnea* entering diapause. The author discovered that carbohydrate feeding of insects while entering diapause increased their oviposition after reactivation. We obtained similar results (Fig. 2). Moreover, insects from the PD culture did not oviposit during a month in the long-day photoperiod fed with only carbohydrate. It seems that carbohydrate feeding provides not only successful preparing of *C. carnea* to diapause, but to a large extent it causes entering the first stage of diapause of some insects in a population. This stage is expressed by ending oviposition and changing in acRNase activity from "active" to "diapausing" type. This hypothesis is in

accordance with experimental data obtained by Tauber and Tauber (1987) when they investigated the inheritance of diapause in *C. carnea* from different populations from the West of North America. Analysis made by the authors showed that food supply is one of the factors responsible for *C. carnea* entering diapause.

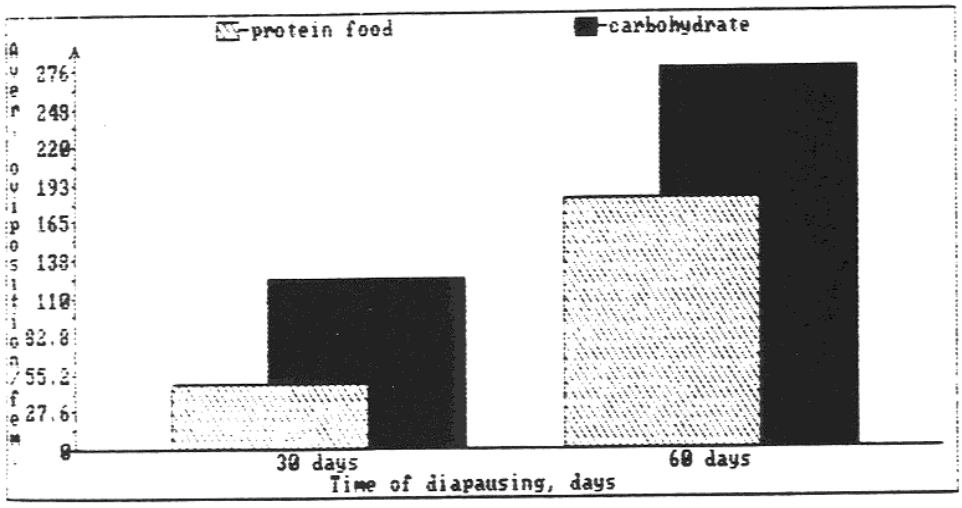

Fig 2. The influence of food and time of diapausing on oviposition of females from PD culture during 28 days after introducing them into long-day photoperiod. The influence of feeding and time of diapausing is statistically significant at 95% level.

Experiments carried out with (natural) insects caught at different periods during the field season seem to be of even greater importance. The results prove the fact that not only the location but also the period when insects were caught is of great importance for founding insect laboratory cultures. Considerable changes in genetic structure of populations were found during its development. This is in accordance with changes of the conditions.

From this point of view a logical interpretation may be found for all our experimental data concerning changes in natural Moldavian populations of *C. carnea* as well as for selection processes during establishing and rearing laboratory cultures. Insects naturally reactivated in spring have different gentoypes adapted to changing environment. Two extreme types of insects may be distinguished: on one hand, successfully diapausing ones having sharp photoperiodic reaction, and on the other hand, those without

photoperiodic reaction, dying without convenient food supply and under low temperatures. These two types of insects are likely to have different generative abilities: the first one has a middle or low productivity under optimal conditions while the second has a high one under the same conditions. Thus, these two groups of insects, provide a maximal stability and a maximal size of the population under conditions of continuously changing environment.

Another hypothesis for the occurrance of different groups of insects in a natural population is more attractive. Two relatively unconnected groups with a different speed of mutation may exist. This supposition is quit real if taking into account only statistical postulates. The number of replications occurring in ovaries differs 2-fold in two individuals, one being 2-fold more productive. If the frequency of spontaneous mistakes in reading DNA is stable, the probability of mutant offsprings from more productive individuals is double. Additionally, other specific physiological and biochemical mechanisms increasing the mutation are possible for more fertile individuals. In this case, the existance of such intrapopulation structures allows (at the level of population) two known functions of stable informative systems: conservativeness and changeability.

Results obtained in laboratory cultures of strain KG6 may be interpreted in the following way: This culture was founded from insects caught in July, i.e. the period when the natural population was enriched with individuals of "mutant" structure, having the opportunity to express aPh7. These individuals are characterized by increased ability to oviposit if protein food supply is available in long-day conditions. Upon rearing on suitable laboratory diet (the mixture of autolyzate brewer's yeast with honey (1:1 v/v) their contribution to the next laboratory generation constantly increased. This resulted in a culture without photoperiodic reaction, i.e. a "nondiapausing" strain of insects, sensitive to food supply.

Some other mechanisms leading to the formation of such insect cultures are possible. For example, spontaneous diapause entrance by some insects when reared under stable conditions. This will automatically eliminate such insects in the subsequent generations. Forming of diapausing types of insects under stable conditions is described in some other species (Tamarina, 1987). It is possible that these two mechanisms are interactive and contribute to "nondiapausing" laboratory cultures.

CONCLUSIONS

1. From the investigations made we conclude that microevolutionary processes in a natural Moldavian population of *C. carnea* occur. We evidenced the importance of season for catching insects for stock colonies.

2. Both, aPhase and acRNase are informative and promising enzymes regarding the changes in genetic structure and feeding requirements of natural and laboratory populations of *C. carnea*.

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PUPAL AND ADULT INDICES OF QUALITY FOR LEPIDOPTERA: THE CASE OF CNEPHASIA JACTATANA (WALKER) (LEPIDOPTERA: TORTRICIDAE) AND CHILO PARTELLUS SWINHOE (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The threshhold to pupation is the larval critical weight (Lcw). The highest weight attained by a larva is termed as the larval maximum weight (Lmw). In the tortricid leafroller *Cnephasia jactatana* and the spotted stem-borer *Chilo partellus* there were constant decreases in weight from Lmw to the pupal weight (Dp) and to the adult weight (Da). There was also a relationship between pupal and adult weights with the fecundity of the female insects. This information is used to describe pupal and adult indices of quality (PQI and AQI) which are useful in quality assessment of laboratory reared insects.

Key words: *Chilo partellus*, *Cnephasia jactatana*, colonisation, fecundity, indices, larval critical weight, metamorphosis, quality, reproductive performance, rearing

INTRODUCTION

Modern Pest Management programmes research and implement areas of integrated pest management that include bionomics and applied ecology, mechanisms of cultivar resistance, sterile insect release methods as well as biological control among others so as to chart out sustainable control strategies (see for examples, Knipling, 1966, 1984; Saxena, 1990). Research of this type depends to a large extent on insect supplies from cultures maintained in artificial rearing systems either on synthetic or on natural food sources. In other words, research results to be applied in the natural field conditions are obtained from experimenting with insects produced in simulated environments, insects often feeding on unfamiliar foods and consequently they may be very different from the target populations.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

The constant monitoring of the biological performance of insects reared in captivity is of prime importance (Moore et al., 1985; Leppla & Fisher, 1989; Ochieng'-Odero, 1990a). The process of rearing (whether as large or small scale) is not a cheap effort. In the final instance, the success of any such process will be only gauged by how successful the research has been useful in controlling the target population.

Insect size is directly related to its performance (see for example Gunn and Gatehouse, 1986; Wagner et al., 1987; Fitt, 1990). In many rearing facilities pupal weight is commonly monitored as a measure of quality (Leppla et al., 1980; Chambers and Ashley, 1984). Pupal weight is a convenient parameter as it is easy to assess without affecting the biological performance of the insect.

The final larval instar in many insects is crucial to its reproductive fitness. During this instar the insect must grow sufficiently large and accumulate enough energy for metabolic activities in the moult to an adult and/or during its non-feeding pupal stage. In many instances the duration of the final instar is longer and with higher food utilization efficiency than earlier instars (see review by Slansky and Scriber, 1985).

The existence of a larval critical weight (Lcw) as a threshold to pupation has been established in several insect species (Nijhout, 1975, 1979, 1981; Nijhout & Williams, 1974a, b; Woodring, 1983; Slansky and Scriber, 1985). Ochieng'-Odero (1990b and unpublished) investigated the larval critical weight (Lcw) as a stable standard in pupal and adult quality of the New Zealand native leafroller *Cnephasia jactatana* (Walker) (Lepidoptera: Tortricidae) and in the spotted stem-borer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). This paper reviews the results of those two studies.

MATERIALS AND METHODS

The study material

Larvae used for the study on *C. jactatana* were derived from a laboratory population reared on GPD artificial diet (Singh, 1983) for twelve successive generations at the Department of Scientific and Industrial Research (DSIR), Auckland, New Zealand. Progeny of wild larvae (Gw) collected by light trapping adult moths was used for comparison studies. Rearing and

experimentation was conducted at 20 ±1 °C, 75 ± 5%RH with a photoperiod of LD 18:6.

The study on *C. partellus* was conducted on insects derived from two laboratory populations originally collected from farmers fields near the ICIPE's Mbita Point Field Station (MPFS) on the shores of Lake Victoria in western Kenya. The cultures were maintained for over 50 successive generations on a semi-synthetic diet (Ochieng' et al., 1985). One population was maintained in a controlled environment (26 ±2 °C, 65 ± 5%RH, LD 12:12) at the ICIPE headquarters, Duduville, Nairobi; while the other was maintained in uncontrolled ambient conditions at MPFS where conditions may fluctuate between 24-30 °C and 30-70% RH. Progeny of wild larvae used in the comparison with the laboratory population were collected from farmers fields in the environs of MPFS.

Identification of an Lcw

In each study, final instar medium sized larvae were starved from day of moult to final instar (day 0) in plastic vials for 20 days and 6 days in *C. jactatana* and *C. partellus*, respectively. Larvae which had not pupated after that duration were transferred onto artificial diet until pupation whereupon their sex was determined. To precisely establish Lcw, 100 medium sized larvae of various weights which had fed for 1-24 hrs after moult were starved. In each case, those larvae failing to pupate after the proscribed period were transferred onto artificial diet until pupation.

The establishment of a relationship between final instar duration, pupal weight and reproductive performance

To evaluate the effect of the larval duration and pupal weight on adult reproductive performance, pupae formed from all starved larvae were weighed and incubated until eclosion and females weighed and mated in perspex tubes with average sized males (of about 30mg pupal weight) derived from the stock population. The number of eggs laid were counted and longevity was determined for each female.

To determine weight gain over consecutive days in the final instar freshly moulted final instar larvae were transferred into plastic vials containing 1.5g of artificial diet. Each larva was thereafter daily weighed individually between 1200-1300 o'clock. Progeny of Gw were similarly

measured to test for differences with the laboratory population. The experiments were carried out under controlled conditions for two successive generations for the laboratory populations and once for the progeny of Gw.

RESULTS

Table 1 provides a comparison of the results obtained from the two species studied. Figures 1 and 2 represent sequential models of wet weight changes in final instar larvae of the two species.

C. jactatana

Larvae of *C. jactatana* undergo 4 moults in about 21 days at 20°C. Only 5 instars were recorded in 12 generations of successive rearing of *C. jactatana*. It therefore appears that 5 instars is a determinate character for the species. The Lcw for *C. jactatana* was 29.0 and 36.4 mg for males and females of medium size, respectively. Lcw was stable for the three laboratory generations examined and Gw.

Decrease in weight from larval maximum weight (Lmw) to pupal weight (Pw) was not significantly different for the generations and Gw tested, remaining relatively constant at about 30% for males and 25% for females. The percentage decrease from Lmw to the adult weight in the generations and Gw tested were not significantly different, remaining at about 50% for males and 40% for females. A decrease of 15% and 10% in pupal weight was due to the pupal case in males and females, respectively. The only apparent relationship between Lcw and Lmw was that Lcw was consistently about 75% of the mean Lmw for both sexes.

C. partellus

In the laboratory, larvae of *C. partellus* underwent 5 moults in about 20 days in controlled temperature conditions of about 26°C. The laboratory populations recorded six instars under normal conditions and an extra supernumerary instar in 25% of the starved larvae. Most of the starved larve recording a seventh instar were females (83%). The Lcw for *C. partellus* was found to be 54.6 $\pm$1.0 (standard error of the mean) mg for males and females of the three populations tested.

Table 1. Summary of data from the two species studied

Parameter	C. jactatana	C. partellus
Final Instar	L_5	L_6
Supernumerary moult	Nil	1
% of L_{CW}/L_{MW}	m 75% f 75%	m 63% f 39%
L_{CW}	m 29 mg f 36.4 mg	m 54.6 ± 1 mg f 54.6 ± 1 mg
D_P	m 0.3 f 0.25	m 0.35 f 0.35
D_A	m 0.6 f 0.5	m 0.71 f 0.56
Latent feeding period (days)	m 3.4 ± 0.7 f 4.8 ± 0.5	m 4.2 ± 0.4 f 7.9 ± 0.5
Correlation: coefficient latent feeding period/pupal weight	+ 0.8	+ 0.9
Regression: fecundity on pupal weight	y = 160 + 7.83x	y = 6.32x
P_{CW} (mg)	m 27 f 20.3	m 35.5 f 35.5
A_{CW} (mg)	m 18 f 11.6	m 15.8 f 24
Lowest fecundity (eggs/female)	52	224

L_{CW}: larval critical weight
P_{CW}: pupal critical weight
L_{MW}: larval maximum weight
A_{CW}: adult critical weight
D_P : constant of weight decrease from L_{MW} to pupal weight
D_A : constant of weight decrease from L_{MW} to adult weight
m : male, f: female

Figure 1: Sequential model of wet weight change from the final insta adult *C. jactatana*

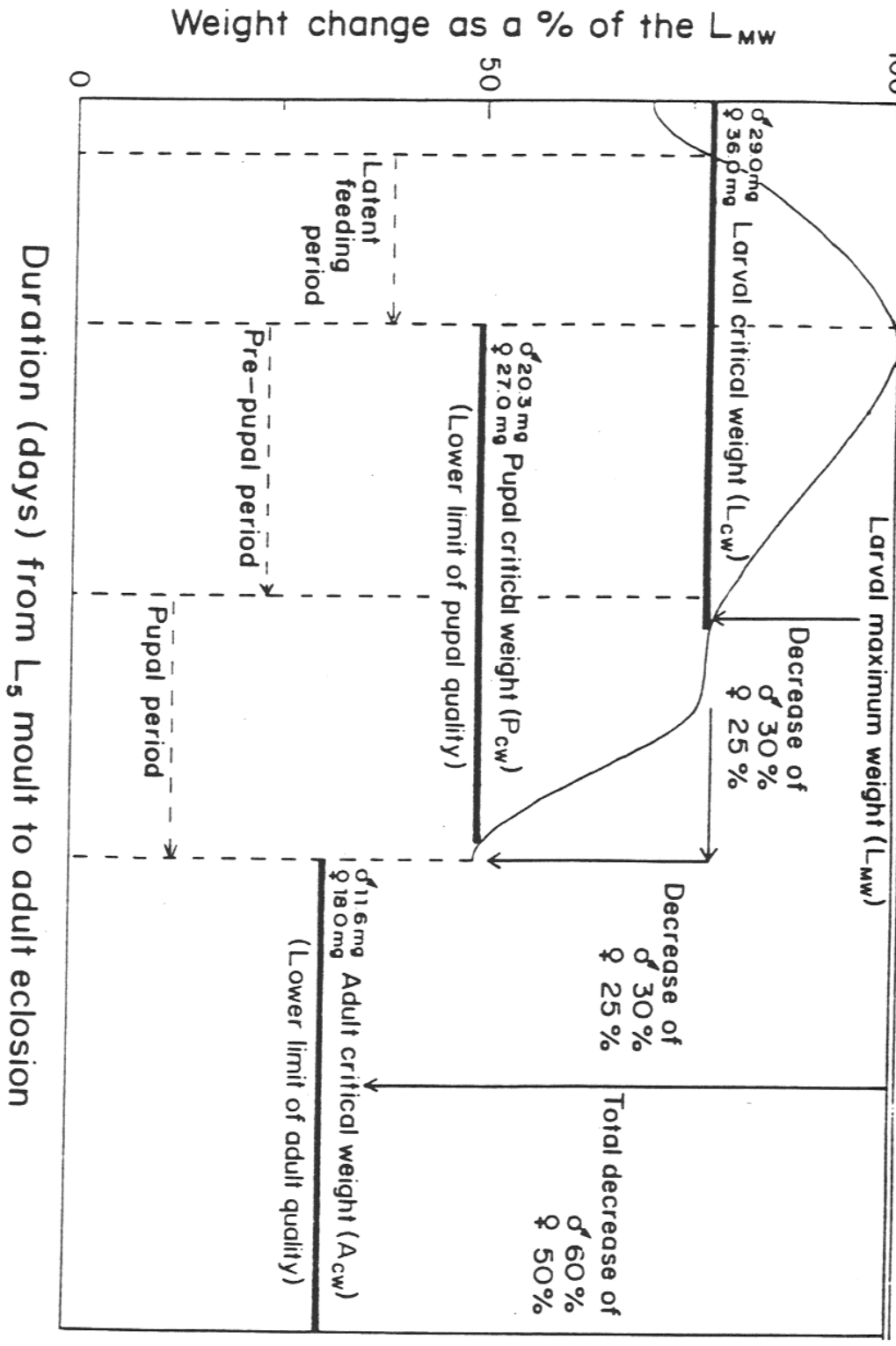
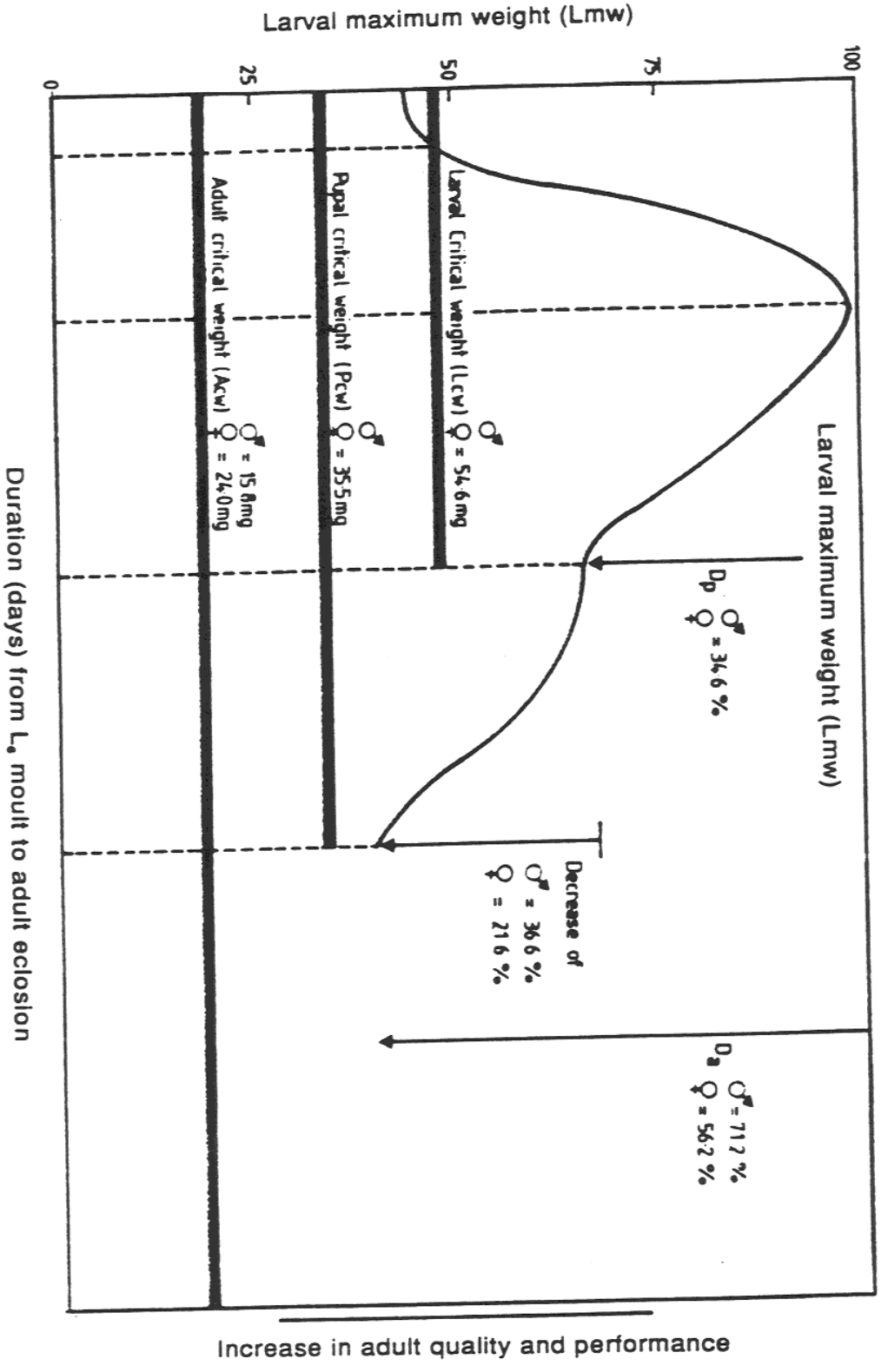


Figure 2: Sequential model of wet weight change from the final instar to adult *Chilo partellus*



The Lcw was 63% and 39% of the male and female Lmw, respectively. Unlike in *C. jactatana* (Ochieng'-Odero, 1990a), the Lcw of *C. partellus* is not different for males and females, however, its relation to the Lmw is dimorphic.

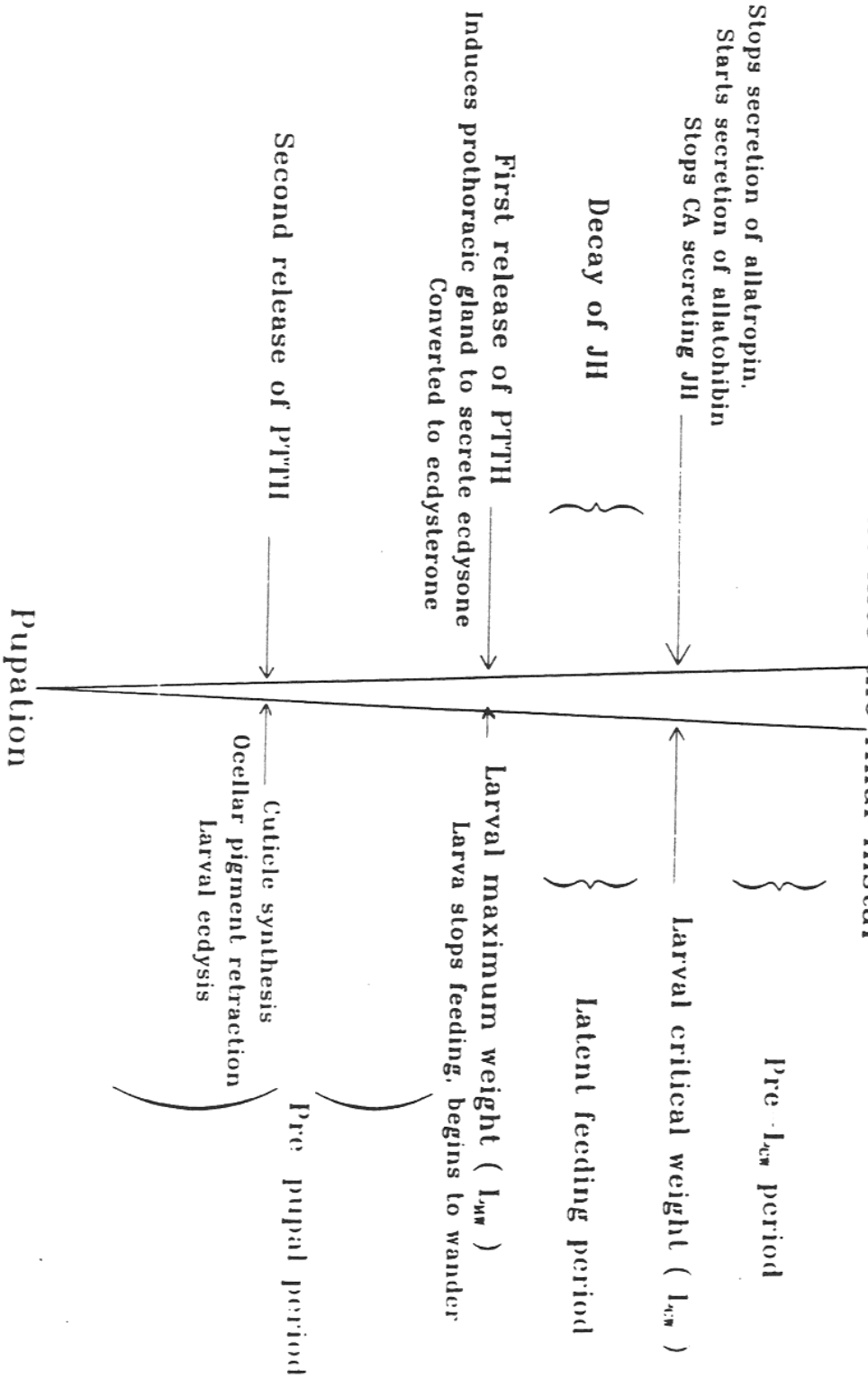
DISCUSSION

The programme of events leading to pupation in holometabolous insects may be summarised in Figure 3 as follows: Final instar larvae feed from the day of moult until they attain a larval critical weight or size (Nijhout and Williams, 1974a; Safranek and Williams, 1984a, b). The critical size in itself is not causative of moulting but triggers the release of hormonal substances that act on the *corpora allata* to stop any further secretion of juvenile hormone. It has been shown that the half life or decay of the juvenile hormone takes about 1.5 days in *Manduca sexta*. During this "latent" or "lag" period (Nijhout and Williams, 1974b) the larvae continue feeding, attaining weights above the Lcw.

Slansky and Scriber (1985) considered that the latent period between achieving Lcw and the actual cessation of feeding permits the larva to reach an "ideal" or optimum weight if food quality, food quantity and environmental factors are suitable. Lcw is therefore only a minimal weight for pupation that allows production of a "functional" adult (competent to survive and reproduce). The size or weight achieved by a larva above Lcw is the major factor that dictates the adult reproductive performance and hence, overall quality (ability to survive and colonise) of the insect.

The Lmw peak corresponds to the first release of PTTH from the brain after total decay of the juvenile hormone (Nijhout and Williams, 1974a; Williams, 1975; Jones et al., 1981). Lmw signals the onset of the pre-pupal phase. In *C. jactatana*, L_5 larvae stop feeding, wander to the tops of rearing containers, and initiate spinning. There is also some lightening of colour, the dorsal vessel and gut purge becomes progressively evident. In some *Lepidoptera* the attainment of Lmw is the onset of pre-pupal morphological markers such as pale green colouration in *Trichoplusia ni* (Jones et al., 1981) and negative phototaxis leading to digging into soil in *M. sexta* (Nijhout and Williams, 1974b).

Figure 3: Scheme of events occuring between moult into final instar and pupation in holometabolous insects



The period between day 0 and achievement of L_{CW} is described as the pre-L_{CW} period. The period between day 0 and L_{MW} (pre-L_{MW} period) is the active feeding phase. The latent feeding period is the period between attainment of L_{CW} and L_{MW}. There is a gradual decrease of weight from L_{MW} to pupa during the pre-pupal period. Weight decreases further during the pupal period to give the adult weight.

Relationship between L_{MW}, pupal and adult weight are therefore represented as:

$$\text{Pupal weight } (P_W) = L_{MW} - (L_{MW} \times D_P)$$
$$\text{Adult weight } (A_W) = L_{MW} - (L_{MW} \times D_A)$$

where D_P = a constant of weight decrease from L_{MW} to P_W
D_A = a constant of weight decrease from L_{MW} to A_W

Pupal critical weight (P_{CW}) and adult critical weight (A_{CW}) are derived as:

$$P_{CW} = L_{CW} - (L_{CW} \times D_P)$$
$$A_{CW} = L_{CW} - (L_{CW} \times D_A)$$

P_{CW} and A_{CW} are useful as descriptive indices of insect quality for both male and female insects.

$$\text{Pupal quality index (PQI)} = P_W/P_{CW}$$
$$\text{Adult quality index (AQI)} = A_W/A_{CW}$$

Thus, where the mean pupal or adult weight for the population is equal to the critical weight, the indices would be 1. As the weights increase above the critical weights, the indices would climb above 1 representing an increase in quality.

The value of using these quality indices is the fact that they are derived from stable values of L_{CW}, D_P, and D_A which have been shown not to be dependent on diet quality, temperature, photoperiod, thermophotoperiod or selection pressure (Ochieng'-Odero, 1989).

ACKNOWLEDEMENTS

I am grateful to Professor T.R. Odhiambo, Director of the ICIPE and Professor K.N. Saxena for their support in the completion of this work and for funds so that the paper may be read at the 5th Workshop on Quality Control of Massreared Arthropods, March 25-28th, 1991 in Wageningen, The Netherlands.

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DIETARY CONTROL IN INSECTS. THE CASE OF THE OLIVE FRUIT FLY, Dacus oleae (GMEL.).

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ABSTRACT

Adequate control of nutritional and of other dietary factors is essential to the reproducibility of data and for the expression of genetic potential of any organism. This presentation deals with ways to control diets and nutrition in experiments with insects. Reference is made to the main nutritional components such as amino acids, inorganic elements, vitamins, to nutritional imbalancies or toxicities, modifications of ingredients in the diet, allelochemicals, pH, contaminations and to other factors which affect growth and development of reared insects. The experimental results presented illustrate the importance and the effects of certain nutritional and other factors upon the performance of the olive fruit fly, Dacus oleae, GMEL. (Diptera: Tephritidae).

INTRODUCTION

Many scientists today use artificial diets to produce insects for entomological research and pest control. It is quite well known that diet is one of the more important variables but its importance in insect mass rearing and experimentation has not been sufficiently emphasized. Changes in enzyme systems, microbial flora, behavior and often body and product composition may be influenced by dietary factors. Many conflicting results in literature can be attributed to difference in dietary factors employed by various investigators. Deficiencies or imbalances of nutrients can affect the response of an insect to a given treatment. Uncontrolled sources of contaminants and microorganisms may give false and misleading data.

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This report gives some of the main components of control of diets. It is mostly referred to practical (oligidic) and purified (meridic) diets commonly used for insect rearing and nutritional experiments. Examples were taken from experiments with larval diets of olive fruit fly, Dacus oleae, GMEL. (Diptera:Tephritidae).

CONTROL OF NUTRITIONAL COMPOSITION

The lack of knowledge on the quantitative requirements and on the relative availability of nutrients in many insects is a serious weakness since it makes it difficult to precisely relate nutritional findings to other aspects of insect growth and behavior. The situation becomes much more problematic because not much attention is given by many investigators and insect rearing specialists to reporting the nutritional profile of the diet. No matter what level of knowledge has been achieved for the nutritional requirements of an insect a published diet should be nutritionally adequate.

For practical diets, most well established companies contain adequate amouts of the known essential nutrients. They are usually nutritionally adequate unless the diets are stored for long times or at high temperatures or contain unidentified contaminants. On the contrary, purified diets may be deficient or highly excessive in one or more nutrients and in this case metabolic disorders can be expected. Diets for insect rearing have been recently reviewed by Singh and Moore [1985].

For insects, for which nutrient requirements are published investigators should check the nutritional adequacy of the diets according to feed intake. For insects for which requirements are not known, as it is the case for most insects of economic importance, a minimum of information should be given. Table 1 and Table 2 present such information for basal diet used for rearing the larvae of the olive fruit fly.

Table 1. Basal Larval Diet for the Olive Fruit fly, Dacus oleae, GMEL[1]

Ingredients	Content
Water, ml	55.00
Brewer's yeast [Schwechät, Austria], g	7.50
Soy hydrolysate [Nutritional Bioch.Co.USA], g	3.00
Olive oil [Minerva Co., Greece], ml	2.00
Sucrose [Sugar Co., Greece], g	2.00
Tween 80 [Merck AG, Germany], ml	0.75
K-sorbate [Merck AG, Germany], g	0.05
Vitamin mixture [Supplier, address], unit	-
Mineral mixture [Supplier, address], unit	-
Amino acids [Supplier, address], unit	-
Other chemicals [Supplier, address], unit	-
HCL, 2N, [to make pH 3.8-4.1] ml	3.00
Cellulose [Schleicher-Schüll, Germany], g	26.50

[1]This is Basal diet PS reported by Manoukas, [1975], and it is based on diet P described by Tzanakakis et al [1970], from which peanuts and agar were omitted and cellulose increased.

This diet gave acceptable growth and development rates [Manoukas and Tsiropoulos, 1977] and certain quality properties of adults [Tsiropoulos and Manoukas, 1977]. It was found to give satisfactory number of pupae at relatively low cost and it was proved adequate to maintain the olive fruit fly colony at "Demokritos". Proximate analysis is the most widely used method for chemical measurement of nutritive quality. Details can be found in Methods of Analysis published by AOAC [1965].

Table 2. Proximate analysis and energy content of Brewer's yeast [Schwechat, Austria], soy hydrolysate [Nutritional Bioch. Co., USA] and of basal diet [Table 1].

Component %	Brewer's yeast [1]	Soy hyd. [2]	Basal diet [2]
Water	8.9	9.6	59.0
Lipids	2.1	3.7	2.3
Nx6.25 [3]	52.8	57.1	5.7
Fiber	3.2	0.8	7.8
Ash	7.4	8.8	0.8
NFE [4]	25.6	20.0	4.4
Kcal/g [5]	3.0	3.5	0.66

[1]Determined in this Laboratory, according to methods of AOAC [1965].
[2]Calculated on the basis of ingredient content of the diet [table 1].
[3]Total nitrogen x6.25 of which 7.6, 19.9 and 1.2% is non protein nitrogen, [mainly free amino acids], for yeast, soy and diet, respectively.

[4]Nitrogen Free Extract calculated by difference from 100.-
[5]Energy determined in Kcal/g by bomb calorimeter.
[6]Calculated on the basis that lipids, proteins, and NFE contain 9.4, 4.1 and 3.7 Kcal/g, respectively. Cellulose added in the
artificial diet [26.5 g/100 g diet] was not included in the calculations for energy content.

It should be emphasized, however, that many biological and metabolic variations were found between the artificially reared and the naturally grown insects and most of these variations were attributed to the differences in the larval diet [Manoukas, 1983]. The recognition that diet exercises selection which affects insect metabolism and behavior may be a very exciting and challenging development in years ahead. For the olive fruit fly it is advisable to compensate for any

loss of water which exceeds 10% of initial dietary water content [Manoukas, 1980]. Water and agar or cellulose give to the diets the desired texture and form in connection with the other ingredients and the preparation procedure. Further more water and cellulose may affect availability of nutrients and acts as a substrate for receiving some of products of catabolism. Ingredients such as Brewer's yeast and soy hydrolysate may be deficient, or excessive in nutrients. For example, Brewer's yeast purchased from the same company at different times [unpublished data] gave different growth on the olive fruit fly larvae. Care should be excersized when classic mineral mixtures are used to supplement diets because they may have contained excesses or deleterious imbalances for the particular insect. Furthermore, it is important to realize that some vitamins are very susceptible to destruction either by other ingredients in the diet or by exposure to heat and air. Allelochemicals, without known action on a particular insect, are not recommended and they may complicate interpretation of results. For the same reason nutrient mixtures and other additives should not be added if their effect is not documented.

Investigators often wish to make changes in the content of main nutritional components (protein, fat, carbohydrates). Such changes may cause different consumption rates of other components in the diet. In low-fat vs. high fat diets there will be a major change in the consumption of other dietary components. Many other factors may reduce feed consumption. In all these cases of reduced consumption the test insects may not be nutritionally or biochemically comparable to the control insects. To preclude effects of decreased food intake in treated groups several approaches may be used but the best way is to make diets isocaloric,if applicable.

To allow for variations in requirements in individual insects and also to compensate for possible losses of unstable nutrients, the requirements levels, if known, should be exceeded by factors that vary for different nutrients and insects. If requirements are not known much effort and money can be saved

by knowing the tolerance levels of certain ingredients or nutrients or other chemical components of diet. For the olive fruit fly, the tolerance limits of the main dietary components including water and cellulose have been established [Manoukas, 1977, Manoukas, 1986]. Food composition and tolerance levels for amino acids and minerals have been also reported [Manoukas, 1981, 1982 and Manoukas et al, 1973, 1978]. Labels may show minimum and maximum values for protein, fat, ash, fiber e.t.c. but they do not necessarily provide the exact nutrient levels or constancy of ingredient composition from batch to batch. Accurate nutritional control must include consideration of the ingredients that are used to make up the diet. Ways to assure ingredient quality, sources of ingredients and general considerations for storage and handling have been given by Brewer's and Lindig (1984). The composition in most ingredients are relatively stable but in some instances nutrient availability may be very low. Other ingredients may vary markedly in their trace element content, vitamin content and some times in protein and other compounds.

CONTROL OF OTHER ENVIRONMENTAL FACTORS

The problem of pathogens and microbial contamination in insect rearing and the role of antimicrobials in diet preservation, has been very well covered by Shapiro (1984). Additionally it should be stressed that contaminants can be introduced with either one or more ingredients in a diet or through unclear diet mixing apparatus. Copper and iron can vary due to contamination introduced by processing equipment during ingredient preparation. Potential contaminants are cadmium, selenium, mercury, lead, arsenic e.t.c. Examples of industrial contaminants are the polychlorinated biphenyls and some insecticides. Naturally occurring toxicants include aflatoxins and other mycotoxins associated with moldy ingredients. Water can be an important source of many contaminants and chemical elements. It will be of importance

to know the composition of water especially in experiments with minerals.
It should be mentioned also the well known fact that physical and other non-nutritional dietary factors should be also controlled as efficiently as possible. In diets, like that used for the olive fruit fly monitoring of pH during larval growth and development can be very important. In high larval density pH may be as high as 7 by the 3th day of larval growth with undesirable consequences (Manoukas and Tsiropoulos, 1977). Other factors to be considered in connection with the diet is the microflora larval density type and size of cups, holders, cages, e.t.c. Environmental factors such as temperature and light cycles can affect food intake and food intake patterns which in turn may affect daily rhythms of certain enzymes.

CONCLUSION

The role of nutrition as a variable in the quality of reared insects has been overlooked, too long. Even in the case in which nutritional requirements are not known, information on the dietary composition, proximate analysis and other critical dietary factors is essential to produce a standard quality of insects and to allow others to evaluate the work and to repeat it as precisely as possible.

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TWO METHODS FOR ESTIMATION OF ORIENTAL FRUIT FLY AND ASSOCIATED OPIINE PARASITOID ABUNDANCE

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ABSTRACT

Sticky spheres and canopy fogging were evaluated for estimation of oriental fruit fly, Dacus dorsalis Hendel, and opiine parasitoid (Hymenoptera: Braconidae) numbers in guava, Psidium guajava L., trees of a commercial orchard. Oriental fruit fly and four species of opiine parasitoids [Biosteres arisanus (Sonan), Psyttalia incisi (Silvestri), Biosteres vandenboschi (Fullaway), and Diachasmimorpha longicaudata (Ashmead)] were recovered on spheres and by canopy fogging. Applications of these studies are discussed with respect to monitoring both oriental fruit fly and parasitoid abundance, distribution, and dispersal in the context of areawide parasitoid release programs.

1 Introduction

Oriental fruit fly, Dacus dorsalis Hendel, infests more than 150 species of fruits and vegetables (Christenson & Foote 1960). In Hawaii oriental fruit fly is the most serious agricultural pest of tree fruit crops. Studies have indicated that 95% of the oriental fruit fly population develops in yellow common guava fruits, Psidium guajava L., and that population cycles are determined primarily by fruiting of wild guava (Newell & Haramoto 1968, Vargas et al. 1983). In studies of guava fruit infestation by tephritid fruit flies, of which oriental fruit fly was the predominant species, 88% of the fruit samples were infested during summer-fall and 39% during winter (Haramoto & Bess 1970).

Braconid wasp parasitization of oriental fruit fly reared from guava fruits often averages greater than 75% annually (Bess et al. 1961). The dominant oriental fruit fly parasitoid (>90%) reared from guava is Opius oophilus Fullaway [=Biosteres arisanus (Sonan)] (Haramoto & Bess 1970); other less common species are Opius persulcatus (Silvestri), Opius longicaudatus Ashmead [=Diachasmimorpha longicaudata (Ashmead)], and Opius incisi Silvestri [=Psyttalia incisi (Silvestri)] (Bess et al. 1950, Haramoto

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

& Bess 1970). The first of these species oviposits in eggs, the second in first-instar larvae, and the third and fourth in second- and third-instar larvae of tephritid fruit fly hosts (van den Bosch & Haramoto 1953).

Large scale augmentative releases of tephritid parasitoids offer a potentially effective method for suppressing fruit fly populations and may prove to be especially effective as an adjunct to the Sterile Insect Technique (SIT) (Knipling 1979, Wong et al. 1984). Prior to initiating parasitoid releases, baseline data on abundance and distribution of an established oriental fruit fly and parasitoid population in a control area are essential. Past field data on wild populations were obtained from fruit collections (Vargas et al. 1983, Vargas et al. 1990). However, Wong & Ramadan (1987) theorized that fruit collections may not provide an accurate picture of the number and kinds of parasitoids present in the field, particularly if the fruit is picked from the tree and not collected from the ground. Egg parasitoids may be well represented when ripe fruits are sampled, but larval parasitoids may be under represented or lacking altogether because the fruits are removed from the field before larval parasitoids have an opportunity to oviposit. Also, only the immature stages of parasitoids are sampled when fruit collection is the sole method of data gathering. Therefore, gaps exist regarding field biology and behavior of adults, and a method is needed to accurately determine their abundance and distribution. The objective of the present study was to develop methods that could be used to estimate adult oriental fruit fly and associated parasitoid abundance under field conditions.

2. Materials and Methods

Experiments were conducted from July 1988 to December 1989 in commercial guava orchards at Kilauea, Kauai, Hawaii. Highest oriental fruit fly abundance occurred between August and November and was correlated with the number of ripe guava fruit present in orchards (Stark et al. In Press).

2.1 Colored Sphere Experiments

Spheres (Prokopy 1968, Katsoyannos 1989, Agnello et al. 1990, Vargas et al. In Press) were hung randomly at ca. eye level throughout the guava tree canopy near both green and yellow fruit. After 1 wk, spheres were removed from trees, placed in boxes, and transported to the laboratory where insect counts were made. Two experiments were conducted: Experiment 1 tested the response of oriental fruit fly and parasitoids to eight colors. Eight 4-cm spheres (treatments = white, yellow, orange, red, light green, dark green, blue, and black) were hung in each of eight guava

trees (eight replicates) selected randomly.
Experiment 2 tested the response of oriental fruit fly and parasitoids to white and yellow spheres over time. A 4-cm white sphere was paired with a 4-cm yellow sphere in each of 8 guava trees (eight replicates) selected randomly during four separate study periods from Nov. to Dec. 1989.

Data Analysis. Oriental fruit fly and parasitoid capture data from experiment 1 were subjected to an analysis of variance and treatment means compared using the Waller-Duncan k-ratio t test at the $P = 0.05$ level (SAS Institute 1985). Data were transformed by $(x + 0.5)^{1/2}$ before analysis, but untransformed means are presented here. Oriental fruit fly and parasitoid capture data from experiment 2 were analyzed with the t test (SAS Institute 1985).

2.2 Canopy Fogging Experiments

The sampling area was divided into 36 blocks; each block contained 25 trees. One tree was chosen at random within each block for treatment on each sample date.

Two 3.7 x 7.3 m plastic tarps (Z. W. International, Pomona, Ca.) were placed under each tree one to either side of the trunk such that there was an overlap of ca. 2 cm. A solution of Pyrenone Crop Spray (Fairfield American Corporation, Newark, N. J.) consisting of 2 ml Pyrenone/liter water was applied to each tree at the rate of 1.5 liter/tree with a Solo Port-423 Mist Blower (Solo Inc., Sindelfingen, W. Germany). The mist blower was calibrated to deliver 1.5 liter in 2 min. This amount of spray gave complete coverage of the treated trees. Four min. after application of the spray, each tree was vigorously shaken to dislodge any remaining insects. The two tarps were removed immediately, the flies and parasitoids were placed into labeled plastic bags, and identified at a later date. Sampling was conducted from 7:30 a.m. to 2:00 p.m.

3 Results

3.1 Sphere Experiments

Response of B. arisanus to Sphere Color. Mean oriental fruit fly captures per sphere differed significantly ($F = 10.9$, $df = 7, 56$; $P < 0.001$) with color. Highest mean oriental fruit fly captures were on white and yellow spheres; lowest on dark green spheres. Only the opiine parasitoid, B. arisanus, was captured in adequate numbers to merit analysis for color preference. Mean B. arisanus captures per sphere per tree differed significantly ($F = 3.5$, $df = 7, 56$; $P < 0.01$) according to color. Highest mean B. arisanus captures were on light colored spheres; lowest mean B. arisanus captures were on dark colored spheres.

Table 1. Mean number of oriental fruit flies and B. arisanus captured on colored spheres.

Sphere color	$\bar{x}$ ± SEM flies/sphere/tree	$\bar{x}$ ± SEM wasps/sphere/tree
White	7.6 ± 1.1a	1.6 ± 0.5ab
Yellow	4.8 ± 1.1a	2.5 ± 0.6a
Blue	1.9 ± 0.8b	0.1 ± 0.1c
Orange	1.6 ± 0.6c	2.4 ± 0.8a
Black	1.6 ± 0.6c	0.4 ± 0.3b
Red	1.4 ± 0.5c	1.4 ± 0.4a
Light green	1.3 ± 0.2c	1.9 ± 0.6ab
Dark green	1.1 ± 0.3c	1.5 ± 0.4abc

Values represent means of 8 replicates. Figures in the same column followed by the same letter were not significantly different ($P > 0.05$; Waller-Duncan k-ratio t test [SAS Institute 1985]).

Table 2. Mean number of oriental fruit flies captured on white and yellow spheres suspended in guava trees for four test weeks during 1989.

Collection date	Sphere color	n	$\bar{x}$ ± SEM flies/sphere/tree
1 Nov	w	8	5.2 ± 1.6a
	y	8	4.9 ± 1.6a
17 Nov	w	8	2.4 ± 0.6a
	y	8	1.4 ± 0.4a
5 Dec	w	8	7.6 ± 1.1a
	y	8	4.8 ± 1.1a
28 Dec	w	8	8.8 ± 1.7a
	y	8	6.5 ± 1.3a
Total	w	32	6.0 ± 0.8a
Total	y	32	4.4 ± 0.7a

Values in the same column for each date followed by the same letter are not significantly different ($P > 0.05$; t test procedure [SAS Institute 1985]).

Table 3. Mean number of B. arisanus, P. incisi, B. vandenboschi, and D. longicaudata captured on yellow and white spheres suspended in guava trees for four test weeks during 1989.

Species	Sphere color	n	$\bar{x}$ ± SEM wasps/sphere/tree
B. arisanus	w	32	0.8 ± 0.2
	y	32	1.6 ± 0.3
P. incisi	w	32	0.1 ± 0.04
	y	32	0.1 ± 0.04
B. vanden-boschi	w	32	0.03 ± 0.03
	y	32	0.1 ± 0.04
D. longi-caudata	w	32	0.03 ± 0.03
	y	32	0.1 ± 0.04

Captures of Oriental Fruit Fly and Parasitoids with Time. Table 2 summarizes oriental fruit fly captures on white and yellow spheres during four study periods. Mean oriental fruit fly captures were not significantly different for yellow and white spheres for any test period ($0.21 \leq t \leq 2.6$, $df = 14$, $P \geq 0.06$). Of the total flies captured over all collection dates for white and yellow spheres 91.7 and 86.4% were females and 8.3 and 13.6% were males, respectively. Mean (± SEM) number of oriental fruit flies captured for all 4 wk collection periods was 6.0 ± 0.8 and 4.4 ± 0.7 for white and yellow spheres, respectively. Four species of opiine parasitoids were captured on white and yellow spheres: B. arisanus, P. incisi, Biosteres vandenboschi (Fullaway), and D. longicaudata. Overall mean (± SEM) numbers of parasitoids captured during the four study periods were pooled and are summarized in Table 3. B. arisanus, P. incisi, B. vandenboschi, and D. longicaudatus constituted 83.3, 10.5, 3.1, and 3.1%, for white spheres (n = 32), and 84.1, 5.3, 5.3, and 5.3%, for yellow spheres (n = 32), respectively, of the total braconid wasps captured.

3.2 Canopy Fogging Experiments

There were virtually no oriental fruit flies in the orchard at the beginning of the study in 1988 and 1989 (Fig. 1). Populations increased in number as fruits ripened and then declined as the number of ripe fruits declined. However, maximum fly populations lagged 2 wk behind maximum guava production in 1988. Populations reached a maximum of 2.6 ± 3.8 (x ± SD) flies per tree in early October 1988. Highest levels of oriental fruit fly were present 2 wk earlier in 1989 than in 1988. The mean (± SD) number of flies per tree was 0.6 ± 0.8 in 1988 and 0.3 ± 0.5 in 1989.

Four parasitoid species, B. arisanus, D. longicaudata, P. incisi, and B. vandenboshchi were recovered from guava trees during 1988 and 1989 (Fig. 2).

B. arisanus, the most abundant parasitoid species, accounted for ca. 80% of the total number of parasitoids recovered at the study site in 1988 and 88% in 1989. Mean (± SD) number of B. arisanus per tree was 9.7 ± 9.4 in 1988 and 6.5 ± 5.1 in 1989. Highest numbers of B. arisanus occurred 2 wk earlier in 1989 than in 1988. The B. arisanus population reached a maximum two weeks after maximum fruit yield in 1988 and on the same date as maximum fruit yield in 1989.

D. longicaudata accounted for ca. 10% of the total number of parasitoids found at the study site in 1988 and 9% in 1989. Mean (± SD) number of D. longicaudata per tree was 1.3 ± 1.8 in 1988 and 0.7 ± 0.6 in 1989. Population trends in 1989 were similar to those of 1988 but highest numbers occurred 2 wk earlier in 1989 than in 1988. Maximum population levels occurred 2 wk later than maximum fruit yield in 1988 and on the same day as maximum fruit yield in

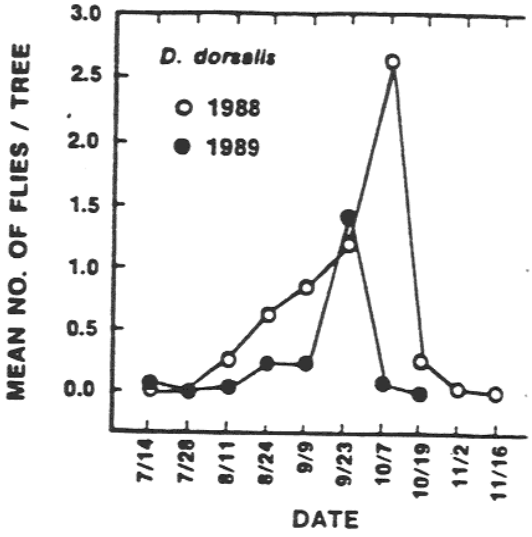
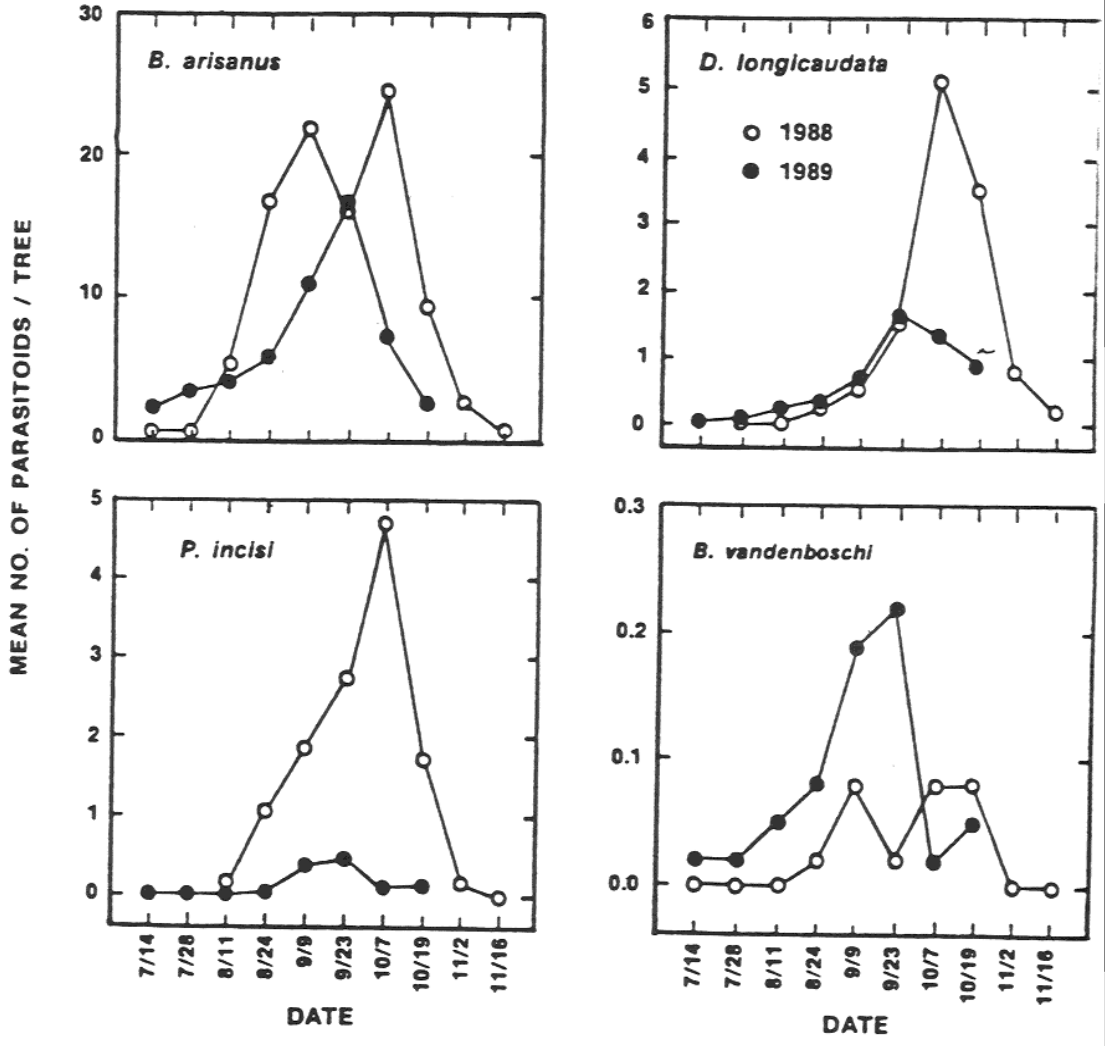


Fig. 1. Population trends of oriental fruit fly in guava trees as determined by canopy fogging.

Fig. 2. Population trends of oriental fruit fly parasitoids in guava trees as determined by canopy fogging.



1989.

P. incisi accounted for ca. 10% of the total number of parasitoids found at the study site in 1988 and 2% in 1989. Mean number of P. incisi per tree was 1.3 ± 1.6 (x ± SD) in 1988 and 0.1 ± 0.2 in 1989. Population trends in 1989 were similar to those of 1988. Highest numbers occurred 2 wk earlier in 1989 than in 1988. Populations reached a maximum 2 wk later than maximum fruit yield in 1988 and on the same date as maximum fruit yield in 1989.

The least abundant parasitoid species, B. vandenboschi, accounted for 0.25% of the total number of parasitoids found in the study site in 1988 and 1% in 1989. Mean (± SD) number of B. vandenboschi per tree was 0.03 ± 0.04 in 1988 and 0.08 ± 0.07 in 1989. Population trends in 1989 were similar to those of 1988. Two population increases were observed in 1988 and 1989. The first population increase occurred 2 wk earlier than maximum fruit yield in 1988 and occurred on the same date as maximum fruit yield in 1989.

4 Discussion

4.1 Sphere Experiments

During the past 5 yr we have been interested in development of a simple device for the study of oriental fruit fly female behavior and population dynamics in the field. Although very effective as monitoring tools, methyl eugenol traps attract predominantly males from unknown distances, while MacPhail traps, although attractive to females, are cumbersome and messy to evaluate. On the other hand, yellow or white sticky spheres, as used in the present study, would appear to be simple inexpensive tools that attract primarily females. Estimation of female abundance in the field has important applications for determination of oriental fruit fly breeding areas and monitoring oriental fruit fly distribution and abundance in pest management programs designed for orchard agroecosystems. Furthermore, we have discovered a simple device for the study of the ecology of braconid wasps associated with fruit flies. Captures of parasitoids on sticky spheres provide a simple abundance estimation procedure useful in studies of population dynamics and distribution of parasitoids. Interestingly, even the scarce opiine species, B. vandenboschi and P. incisi, were captured on yellow and white sticky spheres. Capture of both parasitoids and oriental fruit fly on the same sphere provide a method of investigating oriental fruit fly and parasitoid interactions under field conditions.

4.2 Canopy Fogging Experiments

Canopy fogging also appears to be an excellent method to monitor populations of tephritid fruit fly parasitoids in orchards. Because tephritid parasitoids are poor fliers they are unable, in most instances, to avoid pyrenone sprays. Applications of pyrenone result in very fast knockdown of parasitoids. We rarely observed parasitoids flying away from trees as they were sprayed. Because oriental fruit fly is a strong flier, we did observe some adults fleeing pyrenone sprays and an underestimation of the true adult population in the orchard may have occurred, however, a relative population estimate of oriental fruit fly should be obtainable with this method.

Canopy fogging can be used to estimate the size of naturally occurring fruit fly and parasitoid populations. Furthermore, the method can be used to determine the number of parasitoids needed, the frequency of releases, and the number of release sites necesssary for an augmentative parasitoid release program. Finally canopy fogging can be used to determine longevity, dispersal and success of laboratory-reared, field-released dye-marked parasitoids.

Acknowledgment

We greatly appreciate the coopeation of Jack Gushiken and Scott Enright of Kilauea Agronomics. We are also grateful to Ron Thalman, Bambi Torres, Alan Spencer, Chris Albrecht, Grant Uchida and Dale Kanehisa (Tropical Fruit and Vegetable Research Laboratory, USDA-ARS, Honolulu and Kapaa, Hawaii) for their help in recording field data. We also thank Ron Prokopy (Department of Entomology, University of Massachusetts), John W. Beardsley, Toshiyuki Nishida, and Mohsen Ramadan (Department of Entomology, University of Hawaii), in addition to Tim Wong (Tropical Fruit and Vegetable Research Laboratory, Honolulu, Hawaii), for critical reviews of an earlier version of this manuscript.

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C O N C L U S I O N S

A PRELIMINARY LIST OF CRITERIA FOR QUALITY CONTROL OF BENEFICIAL ARTHROPODS USED COMMERCIALLY IN GREENHOUSE CROPS

(guidelines outlined by the greenhouse group)

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INTRODUCTION

The criteria listed herein refer to **product control** procedures, not to production or process control. They were designed to be as uniform as possible so they can be used in a standardized manner by many producers. These measures should preferably be carried out by the producer **after all** handling procedures before shipment. Inspection authorities must have the ability to perform the same measures. The user (farmer), however, should only perform a simple quality test, e.g. percent emergence of parasitoids .

Some tests are to be carried out frequently by the producer, i.e. on a daily/weekly basis. Others will be done less frequently, i.e. on an annual/seasonal basis, or when the rearing procedure will be changed. This is specified for each criterion in the lists below.

For each beneficial, a coordinator was appointed to follow up the application of the quality control tests by the producers and, upon their feed-back, to reassess the technical and economic feasibility of those tests. If necessary, the coordinator will contact relevant scientists or producers in order to design and carry out further studies which are essential for the completion of the quality control tests list. A short workshop of circa 10 specialists in 1992 might stimulate the development of quality control tests, which, in a somewhat mature form can be discussed with supporting data at the next full meeting in Italy in 1993.

Our future goal will be to relate quantitatively the results of the quality control tests conducted under laboratory conditions to the actual performance of the beneficial in the field.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

***Encarsia formosa* Gahan (Hymenoptera: Aphelinidae)**

Test conditions:	Temperature: 22°C (20-25°C); 60%<RH<90%; Light regime: 16L:8D
Quantity:	>= the number specified at the label; a weekly or batch-wise test;
Emergence rate:	>= 75% within 3 weeks; n=500; a weekly or batch-wise test (question for next workshop: should percentage emergence be higher).
Sex-ratio:	98% females; n=500; a weekly or batch-wise test
Adult size:	head width >= 0.28 mm; n=20 females; an annual test (question for next workshop: if parasite pupal size is related to adult size, would measurement of pupal size suffice?).
Fecundity:	> 7 eggs/female/day; n=10; an annual test. Procedure: will be described by Ravensberg (1991, Wageningnen proceedings) for days 2, 3 and 4 of the adult life of female wasp.
Flight activity:	to be developed, see e.g. test of Enkegaard & Reitzel (1991, Wageningen proceedings).
Comments:	Due to procedures to remove pupae from the leaf, to attach them to a substrate, etc. delays in emergence and a decrease in performance may occur. When mass production methods are changed in this respect, comparative tests should be performed with parasites developing on a leaf and parasites on a substrate after processing (see e.g. van Lenteren 1986).
Coordinators:	van Lenteren and Ravensberg (for address see list of participants)

***Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae)**

Test conditions:	Temperature: 22°C; RH: 60±5%; Light regime: 16L:8D.
Quantity:	>= the number specified at the label; a weekly or batch-wise test
Adult mortality:	< 10% per package; a weekly or batch-wise test; n=500
Sex-ratio:	> 50% females; n=500; conducted once in 3-4 weeks.
Fecundity:	> 100 eggs/female within 7 days; n=10; an annual test; Procedure: daily oviposition of a single pair of wasps on brown beans (*Phasoleus vulgaris*) infested with sufficient *Liriomyza trifolii*, if insufficient hosts are offered many might be killed by host feeding
Adult size:	to be investigated (the relationship between female body size and fecundity; may be a minimum size will suffice).

Comments: No need for flight activity test as flight propensity is a trait that should be exhibited by the parasitoid during the mass rearing procedure.

Coordinators: Fleurynck C.

***Dacnusa sibirica* Telenga (Hymenoptera: Braconidae)**

Test conditions: Temperature: 22°C; RH: 60±5%; Light regime: 16L:8D.

Quantity: >= the number specified at the label; a weekly or batch-wise test

Adult mortality: < 10% per package; a weekly or batch-wise test; n=500

Sex-ratio: > 50% females; n=500; conducted once in 3-4 weeks.

Fecundity: > 50 eggs/female within 7 days; n=10; an annual test; Procedure: daily oviposition of a single pair of wasps on brown beans (*Phasoleus vulgaris*) infested with sufficient *Liriomyza trifolii*, if insufficient hosts are offered many might be killed by host feeding

Adult size: to be investigated (the relationship between female body size and fecundity; may be a minimum size will suffice).

Comments: No need for flight activity test as flight propensity is a trait that should be exhibited by the parasitoid during the mass rearing procedure.

Coordinators: Greatrex R.

***Aphidius* sp. (Hymenoptera: Braconidae)**

Test conditions: Temperature: 22°C; RH: 60-80% ; Light regime: 16L:8D.

Quantity: >= the number specified on the package; a weekly test;

Emergence rate: 70%; 50% emergence within 5 days; a weekly test; n=500

Sex-ratio: > 45% females; a weekly test; n=500

Fecundity: > 65% parasitism (indirect measure); n=25; to be conducted at least 4 times a year; Procedure: each female wasp is offered 20 *Myzus persicae* aphids in a Petri dish.

Comments: The above mentioned tests refer to the parasite delivered in a pupal stage within mummies of the host aphid. A test for flight capacity has to be developed, see e.g. test of Enkegaard & Reitzel (1991, Wageningen proceedings).

Coordinator: van Schelt J.

***Aphidoletes aphidimyza* (Diptera: Cecidomyiidae)**

Test conditions: Temperature: 22°C; RH: 60-80%; Light regime: 16L:8D.

Quantity: >= the number specified on the package; a weekly test;

Emergence rate: 70%; 50% emergence within 7 days; a weekly test; n=500

Sex-ratio: > 45% females; a weekly test; n=500

Fecundity: > 40 eggs/female within 4 days; n=25; to be conducted at least 4 times a year; Procedure: females feeding individually on *Myzus persicae* infesting sweet pepper.

Flight activity: 90% flying adults; to be conducted at least 4 times a year. A test for flight capacity has to be developed, see e.g. test of Enkegaard & Reitzel (1991, Wageningen proceedings).

Coordinator: White A.

***Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae)**

Test conditions: Temperature: 23°C (20-25°C); RH: 70±20%; Light regime: 16L:8D.

Quantity: number of live predators >= the number specified on the package; a weekly test.

Longevity: minimum 5 days, reached by at least 80% of the females in the sample; n=20; a seasonal test.

Fecundity: > 2 eggs/female/day for 5 days after egg-laying starts; n=20; a seasonal test.

Comments: The longevity and fecundity tests will be conducted simultaneously on brown beans (*Phaseolus vulgaris*) infested by ample amount and all developmental stages of the two-spotted spider mite *Tetranychus urticae*.

Coordinator: Goldman D.

Chrysoperla carnea

Test conditions: Temperature: 25°C; RH: 70-90%; Light regime: 16L:8D.

When shipped as eggs

Quantity: number of eggs >= the number specified on the package; a weekly test.

Longevity: minimum 5 days, reached by at least 80% of the females in the sample; n=20; a seasonal test.

Hatching ratio: > 65% within 5 days; n=200; eggs must be isolated to prevent cannibalism after emergence; a weekly test

Predation quality: > 65% of newly-hatched larvae has to develop to 2nd instar larvae within 4 days; to be conducted once a year or when the rearing system is changed; procedure: offer individual, freshly emerged larvae at least 50 prey larvae on a leaf on agar in a petridish (30 cm diameter); n=30; three species of aphids can be used: *Aphis gossypii* on cucumber, *Macrosiphum euphorbiae* on strawberry or potato, or *Myzus persicae* on sweet pepper.

when sold as 2nd larval stage

Quantity: number of live predators >= the number specified on the package; a weekly test.

Composition: >= 65% of larvae in package should be 2nd larval instar, <= 35% 1st larval instar; weekly test

Predation quality: > 80% of 2nd instar larvae has to develop to 3rd instar larvae within 5 days; to be conducted once a year or when the rearing system is changed; procedure: offer individual, freshly moulted 2nd larvae at least 100 prey larvae on a leaf on agar in a petridish (30 cm diameter); n=30; three species of aphids can be used: *Aphis gossypii* on cucumber, *Macrosiphum euphorbiae* on strawberry or potato, or *Myzus persicae* on sweet pepper.

Comments: A test to evaluate the searching capacity of 1st and 2nd instar larvae of *Chrysopa* should be developed.

Coordinator: Benuzzi M.

FIRST DRAFT OF CRITERIA FOR QUALITY CONTROL (PRODUCT CONTROL) OF TRICHOGRAMMA

(guidelines outlines by the Trichogramma group)

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INTRODUCTION

The objective of our subgroup was to draft the tests necessary for assurance of the quality of mass-produced *Trichogramma*. Although we were aware that the test sequence in a rearing programme includes selection, production, labelling, delivery and application, the group, working with the development of quality controls for *Trichogramma*, found it easier to look first at **product control** (by the user, then the producer) and work back through the system to strain selection, while establishing the tests necessary at each stage of the process. **Product control** evaluates the final insect stage produced and prepared for shipment to distributors and/or users. The **producer** must be confident that the performance of his mass-reared *Trichogramma* will be satisfied with each batch shipped. A batch may consist of a unit produced and further manipulated (stored, prepared for delivery, etc.) uniformly. Each of the tests requires a minimum amount of time to complete so that the results will be known at the time of shipment. Therefore the tests must be quick and simple (not expensive) but accurate and reliable enough to indicate any deviation from a standard quality of the product.

Depending on the distance shipped and the method of shipment, the parasitoids may be affected negatively. In order to determine if this has occurred, **the user** should remove a sample from the batch and hold it until all parasitoids have emerged. The emergence rate will give a minimal information on the possible negative effects during shipment.

A number of tests for product control has been proposed and discussed during the workshop. It was agreed at the end that the basic information to be provided by the producer is the number of healthy females per unit shipped. In order to obtain this, a few tests are necessary and methods for them were proposed.

Proceedings of the 5th workshop of the global IOBC working group "Quality control of mass reared organisms". Wageningen, March 1991.

It was agreed that the practical application and the economics of these methods should be assessed by *Trichogramma* producers in order to improve quality control procedures in the future. The reevaluation of the methods will be possible during a short workshop in fall 1992 where mainly those colleagues who are involved in mass rearing of beneficials for greenhouse and field crops will meet.

Product control for *Trichogramma evanescens* and *T. minutum* (Hymenoptera: Trichogrammatidae)

Rearing host: *Ephestia kuehniella* or *Sitotroga cerealella*

Test conditions: Temperature 24 +/-1 °C; RH 70+/-10%; Light regime: 16L:8D.

Number of host eggs parasitized per mg host eggs:
when loose material (not glued): number of parasitized eggs per mg (the relationship between % parasitization and number of parasitized eggs per mg must be established)
weekly or batch-wise test

Emergence rate: >= 90% assessed on 5x100 parasitized host eggs (duration of the test according to the expected emergence period)
weekly or batch-wise test

Sex ratio: =>50% females assessed on 5x100 adults (duration of the test according to the expected emergence period)
weekly or batch-wise test

% of individuals with deformed wings: 5%<= assessed on 200 females
weekly or batch-wise test

Comments: additional tests can be included for a more detailed product control:

Longevity: Average life span measured on 25 fed and mated females, isolated in vials without host eggs

Fecundity: Average number of eggs parasitized per female, measured on 25 isolated 1-day old, fed and mated females, exposed during 3 days to at least 300 eggs of the rearing host

Walking speed: in cm/sec, measured on 25 1-day old fed and mated females, measured at 19 °C, 3600 Lux daylight during 3 min.

Flight propensity: to be developed

The additional tests are recommended especially if Trichogramma had been stored at low temperatures (slow down of development or diapause). Our experience shows that storage under suboptimal conditions often reduces longevity, fecundity and walking speed.

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