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LABORATORY OF ENTOMOLOGY

An internship at the JIRCAS (Tsukuba, Japan): Research on Tachinidae parasitoids

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Preface

In order to complete my Msc Biology (specialisation Entomology) at the University of Wageningen, the last thing for me to do was an internship. As I had already experienced before, doing research abroad can be very interesting and increases your intercultural experience a lot. Therefore, about one year ago I started searching for a place abroad to do my internship. It was very coincidentally that I came into contact with Dr. Y. Kainoh from the University of Tsukuba (Japan). Both the research on parasitoids and its cultural setting, Japan, grabbed my interest. Since it was financially not possible to do research at the University of Tsukuba (paying tuition fee would make the costs too high), Dr. Y. Kainoh put me into contact with Dr. S. Nakamura of the Japan International Research Centre for Agricultural Sciences (JIRCAS) in Tsukuba. Dr. S. Nakamura spends his time partly (it is his 'hobby' as he has mentioned once) on the research of several tachinid species and the tritrophic systems in which they are involved. He welcomed me in all possible ways and therefore I have spent four months in Tsukuba, Japan, to help with the research on Tachinidae. Fortunately, Dr. S. Nakamura initially believed I was an acquaintance of Dr. Y. Kainoh, otherwise he would never have accepted me as a student in his lab. And for this miscommunication I am very thankful because I have had a great time at the JIRCAS. Although my work at JIRCAS did not always go as planned and I have produced hardly any results, it was a very nice first experience with tachinid research and Japan.

First of all I would like to thank Dr. S. Nakamura for everything he has done for me. Dr. Y. Kainoh I am very thankful to for helping me to find a place for my internship. My colleagues Ryoko-san (Dr. R. Ichiko) and Giang-san (Dr. G. Ho Thi Thu) I would like to thank for their very pleasant company and teaching me a lot about tachinid research. And at last I would like to thank Yokokura-san, the technical assistant of the lab, for helping me in many ways as well.

Monique

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Summary

The Japan International Research Center for Agricultural Sciences (JIRCAS) is located in Tsukuba, Japan. At the 'Crop protection and environment division' of this institute, ecological studies on parasitoids for biological control are being carried out. In the lab a rear of seven different tachinid (Diptera: Tachinidae) species has been established and attempts are made to set up a rear of the pest insect *Brontispa longissima* (Coleoptera: Chrysomelidae), the coconut (hispid) leaf beetle which is an important pest of the coconut palm.

In order to complete my Msc Biology at the University of Wageningen, The Netherlands, I have spent four months (November, 2006 – February, 2007) at the institute for my internship. My work involved research on several species. I have looked at the mating behaviour and mating ratio of the tachinid fly *Tachina nupta* and egg survival at different temperatures and relative air humidities for *Pales pavidus* eggs. Also a small experiment was carried out in which was looked at the egg survival and development time of eggs of *B. longissima* at different relative humidities. Due to several small problems not much data have been collected but the results do give some useful suggestions for further research.

Chapter 1 / JIRCAS

1.1 Foundation

The Japan International Research Center for Agricultural Sciences (JIRCAS) is located in the 'Science City of Japan', Tsukuba. The JIRCAS was founded in 1993 by reorganization of the Tropical Agriculture Research Center (TARC, which was established in 1970), in order to include overseas forestry and fishery research. Reorganization again took place in 2001. JIRCAS now became an Incorporated Administrative Agency (IAA) under the Ministry of Agriculture, Forestry and Fisheries (MAFF). These IAA's were introduced to the Japanese government system to "enhance effectiveness, quality and transparency of public services" (brochure 1; website 1).

1.2 Research aim

Development of sustainable technologies which are applicable in the international food business, agriculture, forestry, fishery, or farming systems is the main aim of the JIRCAS. In the brochure this is literally described as: "JIRCAS is the sole national institute that undertakes comprehensive research on agriculture, forestry and fisheries technology in developing areas of tropical and subtropical regions, as well as domestic research on agriculture, forestry and fisheries, aimed at providing solutions to international food supply and environmental problems through technology development; and collects, analyzes and publishes information to grasp trends relevant to international agriculture, forestry and fisheries as well as farming systems, through international collaboration and cooperation" (brochure 1).

The JIRCAS institute is therefore very internationally orientated. It cooperates with several other agricultural institutes and also Universities in the world, especially in developing countries in Africa and Asia. JIRCAS also initiated a program, the 'JIRCAS visiting research fellowship program', by which 20 researchers of developing countries are invited yearly to the institute for doing research (paragraph 1.5). Besides these 20 researchers, yearly another 60 of several research organisations (especially from China) are invited, in order to maintain collaborative research. Further, the JIRCAS promotes young, promising Japanese researchers by the JIRCAS Post-Doctoral Fellowship Program (brochure 1).

1.3 Structure

The JIRCAS represents ten different divisions. The *Administration division* and *Research planning and coordination division* take care of administrative matters and examine what research should be carried out. The seven research divisions in Tsukuba are *Development research division*, *Biological resources division*, *Crop production and environment division*, *Animal production and grassland division*, *Post-harvest science and technology division*, *Forestry division* and *Fisheries division*. The tenth division is the Tropical agriculture research front, which is not located in Tsukuba, but at the Ishigaki Island. A total number of 163 employees work at JIRCAS of which four are executives, 117 researchers, 32 administrators and ten are involved in field management and transportation (brochure 1).

1.4 Research fields

As described in brochure 1, the research fields of JIRCAS can be summarized in five main fields:

- "Genetic research geared towards maintaining crop productivity despite adverse environments of drought, salinity and freezing". For example, research is done on the development of crops such as rice, wheat and soybeans, which are tolerant to stressful environmental conditions, by incorporating stress tolerant genes.
- "Research on biomass utilization technology which converts plant or agricultural residues into useful energy resources alternative to fossil oil". An example is a research project in Asia in which is looked at the use of cassava residues for ethanol production.
- "Research on sustainable agricultural, forestry and fisheries technology suitable for restricted space on island environments threatened by population pressure in the tropics and the subtropics". That is, for example, by development and introduction of lysimeters (which promote efficient water use) to Philippine rural farmers.
- "Research aimed at contributing to the development of stable farming villages in Asia, where 50% of the global population lives". In China, for example, research is done on the stabilization of farm

prices and income by looking at, among others, the possibility of adjustment of policies for domestic and international markets to increase rural stability.

- “Research on environment-friendly agricultural farming systems for food security in Africa, which lags behind in global development”.

1.5 The JIRCAS Visiting Research Fellowship Program

Since 1992 JIRCAS offers research contracts for one year to researchers from developing countries. These places are either in the JIRCAS locations in Okinawa or in Tsukuba. The aim is to “promote collaborative research to address various problems confronting the countries in developing regions on a global scale, including the critical situation on food production, the progression of desertification and the gradual disappearance of genetic resources” (website 1). Nowadays, yearly 20 researchers of either institutes or universities in developing countries are invited. Application for this program occurs via sending in an application form and copies of the three most important publications of the researcher. Once invited, JIRCAS offers one year of paid research in one of the JIRCAS departments, accommodation, a two-way ticket and insurance (website 1).

One of the subjects within this program is ‘Ecological studies on parasitoids for biological control’, which is researched at the laboratory I was working at during my internship. Dr. G. Ho Thi Thu, a Vietnamese lecturer of the Hanoi Agricultural University working in this lab, was one of the guest researchers within this ‘JIRCAS Visiting Research Fellowship Program’. She was studying, among others, ‘the life history parameters’ of the parasitoid fly, *Zenillia dolosa* (Diptera: Tachinidae).

1.6 The internship

I was a guest at the laboratory of Dr. S. Nakamura, Dr. R. Ichiki and guest researcher Dr. G. Ho Thi Thu. This laboratory is part of the ‘Crop protection and environment division’ of JIRCAS. We shared one office with the four of us. The lab has several incubators all with different temperatures ($T = 15, 17.5, 20, 25$ or $30.0 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16: D8 photoperiod, but can be subject to changes if necessary), and a rear of seven Tachinidae species (which are discussed in chapter three). My work involved (preliminary) research on biological aspects of the parasitic fly *Tachina nupta* (chapter four) and *Pales pavidus* (chapter five), and the pest insect *Brontispa longissima* (chapter six). Besides carrying out my own research I was sometimes involved in other lab activities, such as taking care of the rear of the tachinid *Exorista sorbillans* and inoculation of hosts by other fly species. Every week a meeting was held in the office to discuss each others activities and newly gained data.

Dr. S. Nakamura and Dr. R. Ichiki have also introduced me to some of their colleagues of other laboratories in Tsukuba, who showed me around in their labs and explained about their research. Dr. Y. Kainoh of the University of Tsukuba showed me around in his lab where research on parasitic wasps and some tachinids is carried out. Dr. A. Mochizuki of the National Institute of Agro-environmental Sciences introduced me to the research on green lacewings (Neuroptera: Chrysopidae) and explained about the mass rearing techniques for these insects. At the National Institute of Agrobiological Sciences Dr. T. Okuda introduced me to the research on the African Chironomid *Polypedilum vanderplanki*. The larvae of this insect undergo anhydrobiosis of which the exact mechanisms are being researched.

Chapter 2 / Introduction

2.1 Introduction

The use of natural enemies in the battle against insects which threaten crop harvest is nowadays receiving increased attention. As the disadvantages of the application of chemical pesticides become more visible (insect resistance, environmental problems and health issues), people seek for biological ways to control pests. Parasitoids, insects whose larvae develop by feeding on the bodies of arthropods (Godfray, 1994), are a very good alternative. As the parasitic wasps (Hymenoptera: Ichneumonidae) are the most thoroughly investigated ones, tachinid (Diptera: Tachinidae) research is on its rise as well. Many members of Ichneumonidae and Tachinidae are good possible control agents because of the ability to rear them in captivity, a crucial factor for successful use of parasitoids (Greathead, 1986).

For Tachinidae, one of the largest families within the Diptera order (superfamily Oestroidea), approximately 10.000 species have already been described worldwide. Their phylogenetic affinities are not yet well understood. Relatively little is known about the evolutionary history, ecology, diversity, abundance and behaviour of tachinids but recently they are attracting increasing attention by many scientists (Stireman *et al.*, 2006). The most important subfamily within the Tachinidae is the Exoristinae, because it is both the most numerous in genera and an important possible factor in pest control (Clausen, 1940).

Among the tachinids there is a large diversity in several aspects such as size (ranging from approximately two to 20 mm), colour and patterning for some are even mimicking Hymenoptera species in almost all morphological aspects (Stireman *et al.*, 2006). Most tachinid species are diurnal, feed on nectar or honeydew in the adult stage and parasitize a great range of hosts (Clausen, 1940). However, several variations on these characteristics are possible within the diverse group of Tachinidae, often correlated to (host) habitat and/or morphological adaptations.

2.2 Tachinidae in biological control programs

Tachinid species are closely involved in tritrophic systems and often have the tendency to attack phytophagous insects. For this reason they have already been used in many biological control programs of several pests. Though these programs often succeeded, there also have been some examples in which introduced tachinids had a negative effect on (useful) non-target organisms (Belshaw, 1994; Stireman *et al.*, 2006) and therefore partly failed. Besides, failure of the use of tachinids in pest control can be the result of other causes such as low reproductive capacity, a low host death percentage and problems with population establishment. One of the most important factors determining the choice of parasitoid is the ease of handling and whether it is possible to mass rear the species (Greathead, 1986). For the latter reason much research is done in order to optimize tachinid rearing techniques, both for further experimental work and field releases as biological control agents.

2.3 Biology and ecology

All known tachinids are parasitoids of Arthropoda, most of them attacking herbivorous insects such as species of Lepidoptera, Heteroptera, Coleoptera and Orthoptera. However, also some members of the non-insect Arthropoda, especially centipedes (class: Chilopoda) and scorpions (order: Scorpiones), belong to their host list (Stireman *et al.*, 2006).

The largest group of Tachinidae can be found in the neo-tropical region and new species are still to be discovered in all parts of the world. The reason why tachinids are very diverse in the neo-tropics, compared to other parasitoid taxa such as Ichneumonidae (parasitic Hymenoptera), is its (often) large host range and reduced susceptibility to host chemical defence. Tachinidae are found in nearly all terrestrial environments throughout the world and their habitat varies from leaves, tree trunks, flowers, and rocks to ground surface (Stireman *et al.*, 2006).

Most tachinids attack the larval stage of their host (a small five to ten percent attacks the adult stage). From the species which are known at this time, all of them emerge from the pupal stage of Arthropoda (Stireman *et al.*, 2006). Mating of adult flies often occurs very soon after emergence from the pupal stage, and takes for most species place during the morning hours. Repeated mating of both males and females has been observed in several species (Clausen, 1940). Tachinids do often not have an ovipositor (except for some which do inject the eggs) and therefore have to deposit eggs externally on

the host (direct oviposition) or near the host (indirect oviposition). Different forms of oviposition can be described, both for direct and indirect systems: ovipary - the eggs are not yet incubated- and ovilarvipary -the eggs are incubated and therefore contain fully developed larvae-.

When eggs are laid near the host, often at leaf material which is being eaten by its host, the microtype eggs (ovipary) or hatched larvae (ovilarvipary) are ingested by which they enter the host via the intestines (Stireman *et al.*, 2006). For some species the larvae are deposited in the host habitat. These larvae wait for an encounter with a host after which they penetrate the host integument (Clausen, 1940). Ovilarvipary oviposition has the advantage that tachinid first-instar larvae are able to attack concealed hosts which would otherwise be inaccessible to the tachinid adult (Belshaw, 1994). The tachinids which use direct oviposition either lay eggs at the skin of their host after which hatched larvae penetrate the skin, or inject eggs directly into the haemocoel (Stireman *et al.*, 2006). Injection of eggs or larvae precedes penetration of the skin of the host by using a piercing organ (Clausen, 1940).

Total fecundity is higher for indirect ovipositors compared to tachinids which oviposit on the host itself. Larvae almost always (except for a few species) form a respiratory funnel at some point in their development, with which they keep contact with the outer atmosphere (Belshaw, 1994; Stireman *et al.*, 2006). For tachinids which lack the ovipositor the host is not being inhibited in its development or immobilized, simply because substances which might alter development are not injected. The number of hosts that is attacked differs to a great extent among the tachinid species from one up to 200 (Stireman *et al.*, 2006).

For locating their hosts, tachinids rely on chemical cues derived from the host plants of their phytophagous insect host or from interactions between hosts and host plants. Volatile chemicals released by the host plant often attract tachinids to a specific habitat, after which certain close-range cues are used to detect the host itself. These close-range cues are either odour associated directly with the host, host secretions or excretions, or indiscriminant visual detection of the host, mainly by movement. Sometimes sexual cues (e.g. pheromones or sexual calls) of the host are used for host location by tachinids as well. Learning behaviour might also play an important role in host detection, but has not yet been demonstrated often (Stireman *et al.*, 2006). It seems that tachinids do not distinguish between hosts which have or have not been attacked by other tachinids of the same or another species, or by other Hymenoptera (Belshaw, 1994).

Chapter 3 / Rearing Tachinidae

3.1 Rearing Tachinidae

3.1.1 Introduction

Mass rearing of tachinid species is usually experienced as difficult because of several different reasons; mating rate in the lab is often low, genetic deterioration might occur and achieving high numbers often takes time. For successful rearing of tachinids the right environmental conditions (temperature, air humidity and photoperiod), hosts, inoculation methods and mating procedures have to be selected for each species separately.

3.1.2 Tachinidae rear at JIRCAS

At the JIRCAS seven species of Tachinidae are subject of research and therefore reared in the lab. All seven tachinid species require different rearing techniques but some things are common. The conditions at which the flies are kept are generally $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod. However, temperature may be kept at lower levels (15 or $20 \pm 1^\circ\text{C}$) for populations or individuals whose reproduction/development speed is decreased temporarily. Tachinid diet is similar for all species; they are fed on sugar cubes and distilled water in the adult stage and on the host species *Mythimna separata* in the larval stage. Preferably two to five day old males are used to mate with newly emerged females (Nakamura, 1994).

Factors which do differ between the rearing methods for different tachinid species are among others the number of eggs/larvae per host for inoculation which might be critical in determining reproductive success (Nakamura, 1994), the age of the 6th instar hosts, and whether a maize plant or host is provided for oviposition.

Rearing tachinids can be very difficult. Many factors have to be kept in mind and each species has to be treated differently. In order to show how rearing methods differ for different tachinid species and to give an overview of some of the rearing possibilities, the seven species which are reared at the JIRCAS lab are discussed separately in this chapter. First, the method on host rearing is described. Then, a short introduction is given for each tachinid species and the rearing methods (inoculation, mating and oviposition) are described briefly. For a description of the different cage types see figure A-1 in the appendices (chapter eight). Of course, rearing techniques for these tachinid species might be different in other laboratories.

3.2 The host (*Mythimna separata*)

3.2.1 Introduction

The host species at JIRCAS for all parasitoid flies is *Mythimna separata* (Walker) (Lepidoptera: Noctuidae). This species, which is known to be relatively easy to rear in the lab, is often used as a host in parasitoid research. Each instar (one to six) of this species takes different time periods, the last one approximately four to six days before the pre-pupation period starts.

3.2.2 Rearing method

Adult butterflies are put together in a cage (23 cm x 29 cm x 39 cm, width x length x height) to mate and oviposit. In the adult stage they are fed with a 10% sugar solution. Folded A-5 sized papers are put in the cage for female *M. separata* to oviposit on. The eggs are collected daily and sterilized for two minutes in 70% ethanol. Then they are put in Erlenmeyer flasks, 200 eggs per flask, with artificial diet until the larvae reach the 6th instar. When reaching the 6th instar they are moved to boxes (cage type D) with filter paper and diet in which they stay up to pupation. They are kept at $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod.

3.2.3. Artificial diet

The artificial diet of *M. separata* consists of 75.0 g RC4, 75.0 g wheat germ, 15.0 g dried yeast, 4.0 g ascorbic acid, 2.0 g methyl p-hydroxybenzoate, 2.0 g sorbic acid, 9.0 g agar, 1.5 ml propionic acid and 500 ml distilled water. After mixing the ingredients the mixture is put in Erlenmeyer flasks and heated in an autoclave for 16 minutes at 121°C for it to become solid.

3.3 *Tachina nupta*

3.3.1 Introduction

The fly *Tachina nupta* (Rondani) is a parasitoid of several noctuid species, of which *Mamestra brassicae*, *M. separata* and *Spodoptera litura* are yet documented (Shima, 2006). *Tachina nupta* is ovolarviparous and lays well developed eggs in the direct environment of their host, for example at stems of plants at which the hosts forage. The larvae hatch soon after oviposition and stay inactive until a host passes. Probably triggered either by movement, touch or warmth, the first instar larvae then attach to the host body after which they penetrate the integument. The larvae develop within their host where they consume the organs until the host's death. The adult flies emerge directly from the host pupae (Dr. S. Nakamura, personal communication; Shima, 2006).

3.3.2 Inoculation

Inoculation of *M. separata* with five to six *T. nupta* larvae will result in the highest reproductive success of the parasitoid (Dr. S. Nakamura, unpublished data). Inoculation occurs by hand; a host larva (6th instar, day three or preferably day four) is put close to a *T. nupta* larva until the parasitoid reaches up its body and attaches to the skin of the caterpillar. Inoculated hosts are kept in boxes (cage type D) where they will soon pupate. Approximately five to six weeks after inoculation adult flies will emerge from the host pupae (Dr. S. Nakamura, personal communication).

3.3.3 Mating and oviposition

For mating approximately ten male and ten female *T. nupta* flies are put together in a big cage (cage type A) with white filter paper at the ground surface. The paper is glued to the ground surface to prevent that the flies crawl underneath it. Oviposition occurs without the presence of plant material or host larvae on the filter paper or the plastic sides of the cages, seven to ten days after mating. It can, however, be stimulated by putting a small (maize) plant in the cage. Males are not removed from the cages since multiple mating might be needed for *T. nupta* couples (Dr. S. Nakamura, personal communication).

3.4 *Exorista* species

3.4.1 Introduction

Exorista japonica (Townsend) and *Exorista sorbillans* (Wiedemann), the latter also known as *Exorista bombycis*, are parasitoids of a great list of Lepidopteran species among which some are important pest species. They occur in a large part of Asia. The flies lay unincubated eggs (on average 475 macrotype eggs per female *E. japonica* and 460 for *E. sorbillans*) directly on the host integument. Right after hatching the larvae penetrate the host skin to enter the body (Nakamura, 1994; Puttaraju & Prakash, 2005; Shima, 2006).

3.4.2 Inoculation

The most optimal number of eggs per host is two or three (Nakamura, 1994). *Mythimna separata* larvae of day zero or day one (6th instar) are used for host inoculation by *Exorista*. Since *Exorista* larvae hatch up to approximately four days after oviposition, the host larvae should not be too old at times of inoculation since otherwise these might pupate before the larvae are able to enter the host body.

A small basket with (approximately) five *M. separata* caterpillars and some host diet should be put in a cage (cage type C) with several mated *E. sorbillans* females. The hosts are removed from the cage when having two or three eggs on them and kept in separate containers (cage type D) with filter paper and artificial food at 25°C. Within the host body the parasitoids further develop and leave the host after approximately four to seven days; they pupariate outside the host for about 11 days (Devaiah *et al.*, 1993; Nakamura, 1994).

3.4.3 Mating & oviposition

Approximately ten males are put in big cages (cage type C) just after emergence. After two days, one newly emerged female is added to the cage. If she is mating the couple is removed and a new female is added to the cage. The couple is kept in a glass vial (3 cm x 4.5 cm, diameter x height) until they separate. At this point mated females are put together (with five to ten females) in big cages (cage type C) for oviposition. Host larvae (in a small basket with diet) are presented to them for oviposition

(which takes place approximately two to five days after mating). However, it is also possible to put a number of male and female flies together and not remove mated females but just add hosts to this cage.

3.5 *Compsilura concinnata*

3.5.1 Introduction

Compsilura concinnata (Meigen) is a polyphagous parasitoid also attacking a large number of insects up to 200 different species (as reviewed in Stireman *et al.*, 2006). The fly occurs in Palaearctic and oriental regions and was introduced in North America where it established well (Shima, 2006). Most of its hosts are Lepidoptera, though some sawflies and beetles are attacked by this species as well. The eggs, approximately up to 32 per female (Bourchier, 1991), are injected in the host skin after which the larvae directly hatch and move towards the space between the gut and peritrophic membrane for further development (Bourchier, 1991; Ichiki & Shima, 2003).

3.5.2 Inoculation

Approximately 20 or 30 host larvae (6th instar, day five) are put in a cage (cage type C) with 20 or 30 mated female *C. concinnata*. The hosts are either provided in baskets or can just walk around in the cage. After one hour the hosts are removed from the cage and kept in boxes (cage type D) at 25°C until the egression of fly puparia.

It is also possible to use 5th or young 6th instar hosts for inoculation by this species since similar reproduction rates are achieved. However, by using day five (6th instar) hosts, the time needed to feed the hosts separately from the rearing population is reduced.

3.5.3 Mating and oviposition

Petri dishes (diameter: 9 cm) with two weeks old *C. concinnata* puparia are put in a big cage (cage type C) for emergence. Mating occurs soon after and it is best to remove mated females from the cages and to transfer them to separate cages for oviposition. It is, however, also possible to leave males and females in the same cage and provide hosts for oviposition in this cage.

3.6 *Pales pavid*

3.6.1 Introduction

Pales pavid (Meigen) lives in the Palaearctic region. It is also a polyphagous insect and has already been extracted from at least 46 different host species in Japan (Shima, 2006). The female flies lay microtype eggs at leaf material of several different plants which are subsequently being eaten by for example caterpillars. The parasitoid larvae soon hatch from the ingested eggs and then move from the gut to the silk glands where they further develop. Total fecundity is on average 1560 with a wide range of variety from zero to 6000 eggs (Riviere, 1975; Huang, 1981).

3.6.2 Inoculation

Leaf material with several hundreds of *P. pavid* eggs is put in a box (cage type D) with 30 ~ 50 hosts (6th instar, day one to four). After the leaf material has all been eaten, the caterpillars are fed with artificial diet. Approximately two weeks after inoculation the larvae leave the host body and pupariate; about another two weeks later the adult flies will emerge (Dr. R. Ichiki, personal communication; Huang, 1981).

3.6.3 Mating and oviposition

Pales pavid puparia are collected from the containers with the parasitized hosts and put in a Petri dish (diameter: 9 cm). The Petri dish is then put in a big cage (cage type C). The adult flies start mating soon after emergence. One week after mating cut maize leaves or maize seedlings (in a vial with tissue paper and water) are put in the cage for oviposition. Again, it is best to remove mated females and to put them in a separate cage for oviposition, but it is not necessary.

3.7 *Zenillia dolosa*

3.7.1 Introduction

Zenillia dolosa (Meigen) is also a parasitoid of several Lepidopteran (pest) species and occurs in Asia and Europe (Shima, 2006). *Zenillia dolosa* lays microtype eggs (approximately on average 1370 per female) on leaves of plants which are eaten by hosts. Soon after being eaten by host larvae, the *Z. dolosa* larvae hatch within the gut and enter the host body (Dr. G. Ho Thi Thu, personal communication and unpublished data).

3.7.2 Inoculation

Thirty to 40 caterpillars (6th instar, preferably day zero but up to the age of day three is also ok) are put together in a box (cage type D). They are provided with small strips of maize leaf (for example 3 mm x 20 mm) with *Z. dolosa* eggs; approximately 100 to 150 eggs per box. Hosts can not survive when they ingest too many *Z. dolosa* eggs; it is therefore recommended to try to feed them at most on average three eggs per caterpillar. After the leaf material has all been eaten the caterpillars are fed with artificial diet. After 16 days the *Z. dolosa* larvae will leave the host body (pupae) for pupariation (Dr. G. Ho Thi Thu, personal communication and unpublished data).

3.7.3 Mating and oviposition

Approximately ten males are put in big cages (cage type C) just after emergence. After two days, one newly emerged female is added to the cage. If she is mating the couple is removed and a new female is added to the cage. The couple is kept in a glass vial (3 cm x 4.5 cm, diameter x height) until they separate. At this point mated females are put together (with five to ten females) in big cages (cage type B) for oviposition. Every day a new maize leaf is provided for oviposition. The maize leaves with eggs are later cut in small strips and used for inoculation of the hosts.

3.8 *Drino inconspicuoidea*

3.8.1 Introduction

Drino inconspicuoidea (Baranov) is a polyphagous parasitoid attacking Lepidopteran larvae and sawflies, the former of which many are pest insects. It occurs in Asia and the whole oriental region (Shima, 2006). Female flies start oviposition within three to nine days after mating. They lay incubated macrotype eggs (approximately 164 per female) directly on the host, after which the larvae hatch within two to three seconds and enter the host body (Kaleybi & Nakamura, 2006).

3.8.2 Inoculation

Approximately 15 host larvae (6th instar, day five) are put in a cage (cage type C) with 20 ~ 30 mated female *D. inconspicuoidea*. When the hosts have two or three eggs on their skin they are removed from the cage and kept in boxes (cage type D) until the egression of puparia.

Another way to inoculate *M. separata* with this species is to put approximately five hosts underneath a plastic basket which is turned upside down together with several mated *D. inconspicuoidea* female flies. Again, as soon as the caterpillars have at least two or three eggs on their body they are removed (Dr. R. Ichiki, personal communication).

3.8.3 Mating and oviposition

Petri dishes (diameter: 9 cm) with ten day old *D. inconspicuoidea* puparia are put in a big cage (cage type C) for emergence. Mating occurs soon after and it is best to remove mated females from the cages and to transfer them to separate cages for oviposition. It is, however, also possible to leave the males and females in the same cage and provide hosts for oviposition in this cage.

Chapter 4 / *Tachina nupta*

4.1 Introduction

Rearing *T. nupta* in the lab appears to be very difficult, especially because mating rate is low. Yet there is little knowledge on its fundamental biology and in order to optimize rearing this fly for further research and practical application, knowledge on *T. nupta* biology should be increased.

Aim: “To increase fundamental knowledge on the biology of the tachinid *Tachina nupta*.”

These were the initial research questions:

- What is the mating ratio (the percentage of couples which produce offspring) of *T. nupta*?
- What is the average mating duration for *T. nupta* couples?
- How often do *T. nupta* couples mate in order to reproduce?
- What is the average fecundity for female *T. nupta*?

4.2 Methods

4.2.1 Insects

Tachina nupta was reared on *M. separata* host larvae at $20 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod (for rearing methods of both species see chapter three). The host larvae were inoculated with *T. nupta* larvae by hand. *Mythimna separata* caterpillars were inoculated with one or two *T. nupta* larvae when few hosts were available, and five or six when sufficient hosts were available to increase reproductive success.

4.2.2 Materials

- Cage type E: Cylinder shaped plastic container. Size: 9 cm height, 11 cm diameter ground surface and 10 cm diameter top surface. A lid closes the ground surface opening. At the top surface a small piece of gauze closes a square of 3.5 x 3.5 cm for air ventilation (fig. A-1 – appendices).
- Cage type F: Cylinder shaped plastic container. Size: 23 cm height and 15 cm diameter ground surface. A lid closes the top surface opening. Both the top surface and the ground surface are made of gauze and two circle shaped openings at the sides are covered with gauze as well for air ventilation (fig. A-1 – appendices).
- Cage type G: This cage is composed by combining two cages of type E. Both cages are put together with the ground surfaces attached; the lids left away (fig. A-1 – appendices).

For the experiments were also used: A weighing scale, small glass vials (diameter: 3 cm), a camera (Sony DCR-PC350), maize plants, tissue papers, filter paper, plastic foil and rubber bands.

4.2.3 Methods

Approximately seven days before emergence the fly puparia were removed from the host pupae and weighed. Newly emerged female *T. nupta* were put in cages (cage type E) together with a preferably two day old male fly to make couples. The flies were provided with water and a sugar cube as diet (at the conditions: $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod).

Nine couples were observed for possible mating behaviour by recording them using a camera, for ten subsequent days. In this set up, cages (cage type E) were put up side down and covered at the top (where usually the lid was located) with plastic foil and rubber bands to hold the foil at place. The cages were filmed from above (experimental set up: fig. A-2 – appendices). The camera made a picture every five minutes. It was observed before that mating of *T. nupta* takes about 15 minutes; a five-minute time lapse would then cover all the mating events. The results were looked back later. Recording took only place during a certain time of the light period (from 9.00 to 17.00 o'clock). During the rest of the light period and during the dark period (eight hours) the male and female were separated and therefore put in different cages (cage type E). Mating behaviour was described and the number of times mating was recorded. When the male of the couple died within these ten days and no mating had taken place yet, the male was replaced.

Other couples were kept together for whole days until the female started oviposition or one of the two flies died. This was done for 31 couples in cages of type E and one couple in cage type F. From the

number of couples which resulted in offspring, the mating ratio was determined. Only couples for which the female lived for at least eight days and the male for at least two days were included in this calculation.

Only two females started laying eggs and these were therefore transferred and kept individually in different cages (cage type G). The females were provided with water and a sugar cube as diet (at the conditions: $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod) and a small maize seedling (ten up to 20 cm) in a glass vial. Every day at 13.30 o'clock the number of larvae per female was counted until the fly's death. The maize plant was replaced every day.

4.3 Results

4.3.1 Mating behaviour

Of nine couples in cage type E the mating behaviour was observed with a camera. Of four couples the male died early and for three of these couples the male was replaced since no mating had taken place yet. For six of these nine couples the female died before the time that they had spend ten days together during recording time. Therefore, eventually only two couples were recorded for ten whole days.

Only one time mating was observed with the camera which took about 25-30 minutes, at the second day after which the male and female were put together. The next day the male fly of this couple died, so mating had only taken place one time. The female started oviposition 11 days after mating. Another time, outside the recording session, mating of a *T. nupta* couple in cage type E was observed. This mating event took about 16 minutes. The female died before starting oviposition; dissection did not take place (she was involved in a tragic accident) and thus it is not known whether she contained eggs.

4.3.2 Mating ratio and oviposition

There were 28 couples (of which eight were used in the recording experiment) of which the male lived at least *two* days and the female at least *eight* days or more and thus could have resulted in oviposition (female *T. nupta* start laying eggs earliest seven days after mating, Dr. S. Nakamura, unpublished data). Of these couples two females produced offspring. The mating ratio of *T. nupta* couples which are placed in cage type E is therefore 7.1%. One of the egg-laying females, however, laid only four eggs at the first day and ceased oviposition after that. She died nine days later. Her body did not contain eggs. The other female laid eggs for four days (in total 473) after which she died. It was observed that many flies had a swollen abdomen containing a transparent fluid, just before dying.

4.4 Discussion

4.4.1 Mating behaviour

The mating events observed in this experiment lasted 16 and 25 ~ 30 minutes, which seems to be much shorter than for many other tachinid species such as *Z. dolosa* and *D. inconspicuoidea* for which mating takes approximately 80 minutes (Dr. G. Ho Thi Thu, unpublished data; Kaleybi & Nakamura, 2006) and *E. japonica* for which mating takes on average 5.6 hours (Nakamura, 1994). The camera only took a picture every five minutes, so it could occur that mating which took less than five minutes was not recorded. This is, however, not likely for several reasons: Short mating events of only a few minutes were not observed before, the other mating events in this experiment took much longer and the couples for which mating was not observed never produced offspring. Since only two mating events have been observed, nothing can be said about the average mating duration of *T. nupta* though it seems that mating takes relatively short compared to other tachinids. There is one record in this experiment of a couple which mated only once and did produce offspring 11 days after mating. One single time mating can thus be enough for reproduction of *T. nupta*, which is not the case for other tachinids such as *Linnaemya longirostris* (Dr. S. Nakamura, personal communication), but it is not sure whether this always would be sufficient.

It was not possible to observe more than nine couples since there were not enough adult *T. nupta* flies. It is therefore recommended to repeat the experiment with a higher number of couples and possibly with bigger cages.

4.4.2 Reproductive success

In a preliminary experiment by Dr. S. Nakamura, it appeared that four out of ten *T. nupta* couples in cages of type F produced offspring. This is a much higher mating ratio (40%, though determined with a low sample size) than found in this experiment for couples caged in smaller cages (type E) for which mating ratio was only 7.1%. This might propose the importance of space for mating of *T. nupta*, which was already suggested by Kuhlmann (1995) for the tachinid *Triarthria setipennis* and by several authors for other Diptera species (e.g. for *Anopheles sinensis* by Sheu *et al.*, 1996). Because mating ratio was too low in this experiment and also *T. nupta* flies which were put together in a big cage for rearing did eventually not reproduce, it was not possible to continue the experiments as planned before. If there would have been enough offspring the experiment would have been repeated with bigger cages (cage type F) to compare reproductive success between couples caged in cages of different sizes. Now only one couple was caged in cage type F, which did not reproduce either. Besides, mating ratio might be higher when flies are offered not just one partner, but for example three or five, which might be valuable to consider during next experiments.

Another remarkable observation within this experiment was the swelling of the abdomen of many *T. nupta* flies. As soon as swelling had started it took only a few days until the fly died. After dissection the bodies appeared to be full of a transparent fluid. This phenomenon has not yet been observed for *T. nupta* flies which were caged in relatively big cages (cage type F or bigger) and might therefore be explained by excessive uptake of sugar and water in combination with little movement, causing the body to swell (Dr. S. Nakamura, personal communication).

Eventually only two couples resulted in offspring, of which one female laid only four eggs. The other female laid a higher number of eggs, but died just four days after oviposition started. Therefore, nothing can be said about fecundity of *T. nupta*.

It is suggested to repeat the experiment with a bigger type of cage to obtain more information on the fundamental biology of *T. nupta*.

Chapter 5 / *Pales pavida*

5.1 Introduction

In order to optimize rearing techniques for this fly for further research basic knowledge on *P. pavida* biology should be increased.

Aim: “To get an indication about the influence of different relative humidities at the longevity of *Pales pavida* eggs.”

Besides, this experiment might give a clue about the value of the method using salt solutions to establish different relative humidities in closed containers (RH containers). It was noticed during the experiment that the gasses which escaped from the saturated salt solutions are highly concentrated within the RH containers. It is not known what influence these gasses might have on living organisms for this experimental set up.

5.2 Methods

5.2.1 Insects

Pales pavida was reared on *M. separata* host larvae, at $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D 8 photoperiod (for rearing methods of both species see chapter three). The male *P. pavida* used for mating were one to ten days old, the female flies all mated at the day of emergence.

5.2.2 Materials

- Cage type I: Cylinder shaped plastic container. Size: 14 cm height and 8 cm in diameter. The top surface has small holes for air ventilation. The bottom is made up of a Petri dish (diameter: 9cm) (fig. A-1 – appendices).

For the experiment were also used: Relative humidity containers (paragraph 5.2.3), Petri dishes (diameter: 3.5 cm and diameter: 9 cm), filter paper, a weighing scale, maize plants, a binocular, Hobo dataloggers and temperature incubators.

5.2.3 Relative humidity containers

The containers with different relative air humidities which were used in this experiment consist of containers (cage type D) with different saturated salt solutions (NaCl, $\text{Mg}(\text{NO}_3)_6 \cdot 6 \text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ solution). The relative humidities in these containers depend partly on temperature (table 5.1). The protocol for preparation of the salt solutions is described in appendix four (chapter eight).

Table 5.1 / The relative humidity ranges (%) for different temperatures (15, 20 and 25 °C) achieved by using three different saturated salt solutions (NaCl, $\text{Mg}(\text{NO}_3)_6 \cdot 6 \text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ solution).

Temperature (°C)	Salt	RH range (%)
15	NaCl	74.0 – 80.0
15	$\text{Mg}(\text{NO}_3)_6 \cdot 6 \text{H}_2\text{O}$	53.0 – 55.5
15	$\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$	32.0 – 34.0
20	NaCl	74.0 – 79.0
20	$\text{Mg}(\text{NO}_3)_6 \cdot 6 \text{H}_2\text{O}$	53.0 – 57.5
20	$\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$	29.0 – 32.0
25	NaCl	75.0 – 81.0
25	$\text{Mg}(\text{NO}_3)_6 \cdot 6 \text{H}_2\text{O}$	50.0 – 53.0
25	$\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$	29.0 – 32.0

5.2.4 *Pales pavida* egg longevity

Several mated *P. pavida* females (weight of puparium: 0.024 g or more) were kept in a cage (cage type I) with a small maize leaf for oviposition and provided with water and a sugar cube as diet (at the conditions: $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L16:D8 photoperiod). The flies were placed at the sugar

cubes once every day to make sure they fed on it; previous experiences showed that female *P. pavida* sometimes can not find the sugar cubes themselves (Dr. S. Nakamura, personal communication).

The nine different treatments were a combination of temperature (15, 20 or 25°C) and relative air humidity (see table 5.1 for the exact relative humidity ranges for each temperature). Leaves with one to 15 hour old eggs, which were ovipositioned at the 1st to 6th day after oviposition started, were cut into pieces and put in Petri dishes (diameter: 3.5 cm). A total of 300 eggs of five different female *P. pavida* (60 eggs per female) were kept at the specific conditions of each treatment.

The eggs were removed from the incubators and RH containers after 14 or 15 days, put on artificial diet and fed to day zero (6th instar) host larvae of *M. separata* (N= +/- 50, six eggs per host). After two hours it was confirmed whether or not the larvae finished eating the artificial diet with *P. pavida* eggs. Host larvae which did not finish the food were discarded from the experiment. The hosts were kept in Petri dishes (diameter: 9 cm) with filter paper at the conditions 25 ± 1°C, 60 ± 10% R.H., and L16:D8 photoperiod. They were fed with artificial diet until the pre-pupation period. The number of *P. pavida* puparia emerging from each host and host mortality were recorded daily. Weight of the parasitoid puparia was determined seven days after egression.

A control was established by feeding artificial food with six eggs (which were ovipositioned one to 15 hours before, by five different *P. pavida* females) to 50 hosts.

5.3 Results

5.3.1 Preliminary results

Table 5.2 ('*Pales pavida* egg survival after two weeks at different temperatures (°C) and relative humidity ranges (%) determined by host mortality and number of *P. pavida* pupae emerging per host') will be included later when all results are collected. The data will be analysed with a Chi-square test (level of confidence 0.05%) using the data from the control treatment as the expected values.

Preliminary results do however suggest that survival of the eggs is much higher at the high relative humidity ranges for all temperatures, compared to the two lower relative humidity ranges. Of the puparia which egressed before I left JIRCAS, 80-100% came from hosts inoculated with eggs of the higher relative humidity ranges (+/- 74.0 – 81.0%: in total 36 puparia), and only very few puparia from the medium (+/- 50.0 – 57.5%: in total five puparia) and low (+/- 29.0 – 34.0%: in total one puparium) relative humidity ranges. Also host mortality seemed to be much higher for the high relative humidity range, compared to the two lower ranges. Exact data on this was, however, not yet collected before I left JIRCAS. Also the data for the control treatment were not yet collected and the differences between different temperatures not yet examined.

5.4 Discussion

5.4.1 Egg survival after two weeks

Since we did not want to waste the eggs which were put in the RH containers for carrying out the initially planned experiment (see paragraph 5.4.2), the eggs were all used for this preliminary experiment. In this experiment egg survival (in number of puparia emerging from the hosts and host mortality) per treatment after a time period of two weeks, was determined. Since it was not possible to completely finish the experiment within the time period of the internship, Dr. G. Ho Thi Thu has taken over observation. For this reason the results are not complete yet.

Eggs of *P. pavida* can live up to six weeks at 23 °C -RH unknown- (Huang, 1981), and more than three months at 8 °C at a relative humidity of 60% (Biliotti & Desmier de Chenon, 1971). However, Huang (1981) does already state that mortality among adult *P. pavida* is significantly higher (25%) at 50% relative humidity compared to mortality for *P. pavida* flies kept at 60% relative humidity. These results indicate a significant effect of differences in relative humidity on survival of *P. pavida*. The current experiment should point out whether relative humidity also affects egg survival to such a great extent. Up to now, before all data have been collected, it does indeed appear that survival of the *P. pavida* eggs after a time period of two weeks is much higher at a high relative humidity compared to lower relative humidities, with a difference of possibly up to 100%. Further data analyses should, however, be carried out to determine the exact differences in egg survival for the different treatments.

5.4.2 Methodology egg survival

Initially it was planned to put seven sets of 50 eggs of *P. pavida* at each treatment (nine combinations of different temperatures and relative humidity ranges). One set of 50 eggs would then be checked weekly (for the 20 and 25 °C treatments), or every two weeks (for the 15 °C treatments), to determine

egg survival at different temperatures and different relative humidities in time. The method which would be used to check egg survival involved dissolving the brown shell of the eggs with a sodium hypochlorite solution (1:1) after which could be checked with a microscope whether or not the larvae still moved and thus were still alive. However, this appeared to be very difficult, especially for eggs which were a few days to weeks old because the larvae within were relatively weak. They did not show much movement and thus it was very difficult to distinguish living from death larvae.

For this reason a different method was chosen; inoculation of 50 6th instar (day zero) host larvae with six eggs per host (based on results of an experiment on the effect of clutch size and host age on host mortality; it appeared that inoculation of 6th instar day zero hosts with six eggs results in the highest host mortality, Dr. S. Nakamura, unpublished data). The number of *P. pavidus* larvae emerging and host mortality will be determined to get a clue about egg survival. This inoculation will occur with eggs which are two weeks old (as done in this experiment), four weeks old and six weeks old. If necessary, possibly for the 15 °C treatments since at this temperature development rate is much lower, also longer time periods will be checked, but this will be determined later based on the results of the current experiment.

Since my stay at JIRCAS did not allow me to check egg survival at different temperatures and relative humidity ranges for time periods longer than two weeks, Dr. S. Nakamura will take over the experiment.

5.4.3 Methodology RH containers

Since the data were not complete before I left JIRCAS, it can not be discussed yet whether or not the method on using saturated salt solutions to establish certain relative air humidities in closed containers is valuable. Before, it was already noted that the gasses escaping from the saturated salt solutions were highly concentrated in the closed containers. These gasses might influence the survival of the organisms in the containers. The only way to establish a control treatment to test this influence is to adjust an incubator to the right relative humidity and keep eggs in this incubator for the desired time period. Since all incubators at JIRCAS were used for other experiments this was not possible, but can possibly be done later. Preliminary data do however show a linearly decrease in egg survival with a decreasing relative humidity, which might indicate a, if present, linearly related influence of the gasses on the survival of the eggs.

Chapter 6 / *Brontispa longissima*

6.1 Introduction

Brontispa longissima (Gestro) (Coleoptera: Chrysomelidae), better known as ‘the coconut (hispid) leaf beetle’ or in some cases ‘the palm leaf beetle’, occurs in many Asian countries, but also in Australia and the Pacific region. It is a severe pest of the coconut palm (*Cocos nucifera*) which is one of the economically most important tropical crops in many areas. Control by the use of natural enemies, such as parasitic wasps, fungi, ants and many others was already suggested a few decades ago (Fenner, 1984; Hollingsworth *et al.*, 1988; Voegelé, 1989).

In order to better understand this species and to investigate the possibilities of using parasitic wasps (Hymenoptera: Ichneumonidae) in order to control this pest, lab research has to be carried out for which rearing a colony would be necessary. Therefore, fundamental biology has to be extended in order to develop the most optimal rearing techniques for this species.

The initial aim of the experiment was:

“To determine egg survival and development time of the eggs of *Brontispa longissima* at different relative air humidity ranges.”

6.2 Methods

6.2.1 Insects

Adults of *B. longissima* were collected in Thailand in October 2006 and were therefore at least approximately up to four months old at times of the experiment. They were kept at $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L12:D12 photoperiod. The adults were fed on leaf material of *Typha angustifolia* Lesser Bulrush, which was replaced every two days.

6.2.2 Materials

For the experiment were used: Relative humidity containers (paragraph 6.2.3), Petri dishes (diameter: 3.5 cm), filter paper, gauze, rubbers band and Hobo dataloggers.

6.2.3 Relative humidity containers

The containers with different relative air humidities which were used in this experiment consist of containers (cage type D) with different saturated salt solutions (NaCl, $\text{Mg}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ solution). The low humidity range was 29.0 – 32.0%, the medium humidity range 50.0 – 53.0% and the high relative humidity range 75.0 – 81.0% (at 25°C). The protocol for preparation of the salt solutions is described in appendix four (chapter eight).

6.2.4 Egg survival and development time

Eggs of *B. longissima* were removed from the leaf material at which they were ovipositioned and put in Petri dishes (diameter: 3.5 cm) with filter paper. This was done twice a day; around 7.30 in the morning and 19.30 in the evening. The Petri dishes were sealed with gauze which was kept at place by a rubber band to prevent the hatched larvae from escaping. The Petri dishes were put in containers with different air humidity ranges (low, medium and high relative humidity) and kept at 25°C at L16:D8 photoperiod. Every day it was checked twice (at 7.30 in the morning and 19.30 in the evening) whether the larvae had hatched to determine egg survival and development time of the eggs. The eggs which did not hatch within ten days were dissected to check whether or not a developed larva was inside. A control was established with 43 eggs, which were put in an empty container at $25 \pm 1^\circ\text{C}$, $60 \pm 10\%$ R.H., and L12:D12 photoperiod, to check whether the salt solutions itself might have influenced egg hatchability.

6.3 Results

6.3.1 Egg survival and development time

A total of 48 eggs were kept at high relative humidity (75.0 – 81.0%), 46 eggs at medium relative humidity (50.0 – 53.0%) and 45 eggs at low relative humidity (29.0 – 32.0%) at 25°C for ten

subsequent days. Two eggs in the high relative humidity treatment hatched. Hatching rate was therefore 4.2% for the high relative humidity treatment and 0.0% for eggs in the medium and low relative humidity treatments. One of the eggs hatched after approximately 6.5 days, the other one after 7 days. Of the 43 eggs which were put in an empty container without any salt solution –the control-, two larvae hatched which is 4.7% of the total (for these larvae developmental time was not recorded).

In total 84 eggs were laid at night (during 19.30-7.30 o'clock) and 55 at day time (during 7.30-19.30 o'clock) in a period of 14 days and 15 nights (eggs of the control not included). The average number of eggs laid per night was 5.6 and 3.9 during daytime, which did not significantly differ (Mann-Whitney test, $P=0.4$).

Twenty-four out of the 48 eggs (50.0%) in the high relative humidity treatment, eight out of 46 eggs (17.4%) in the medium relative humidity treatment and zero of the 45 eggs (0.0%) in the low relative humidity treatment contained a fully developed larva. However, for many eggs, especially for the eggs in the low relative humidity treatment, the content was a dry yellow mass for which it was difficult to determine whether or not this was or used to be a larva.

6.4 Discussion

Egg survival

Since hatching rate was very low (only 4.2% for the high relative humidity treatment, 0.0% for the other two treatments and 4.7% for the control) nothing can be concluded about egg survival of *B. longissima* at different relative humidities. Besides the fact that only one third of the eggs hatch in general (as reviewed in Voegele, 1989), other reasons might explain low egg hatchability for the *B. longissima* eggs in this experiment. First, the age of the ovipositioning adults was relatively high. The beetles have been collected in October 2006 and they were therefore at the age of at least four months or older at times of the experiment. In general they live only up till three (Fenner, 1984) to seven (Voegele, 1989) months. The high age of the adult beetles might have decreased egg quality and therefore hatchability. Second, the food material at which the adults were feeding might not have been nutritious enough to produce viable eggs or achieve good egg hatchability since it is not their natural preferred food plant (experiments on this subject are yet taking place). Third, the gasses in the RH containers might have influenced egg survival. However, also in the control treatment egg hatchability was low (4.7%) and therefore this is not likely. Sample size was small for all treatments since the beetles laid only few eggs and at the end no egg-laying beetles were left at all.

The eggs were removed from the RH containers after ten days and dissected. It appeared that 50.0% of the eggs in the high relative humidity treatment contained fully developed larvae, which was lower for the treatments with medium (17.4%) and low (0.0%) relative humidity ranges. This might suggest that development of the eggs in low relative humidities is not as good as at 75.0 – 81.0% relative humidity. However, as mentioned before it was sometimes very difficult to determine whether egg content was or used to be a larva or not, especially for eggs of the low relative humidity treatments. It was not possible to check the egg content of fresh eggs (whether or not developed larvae are present in the eggs at times of oviposition), since the beetles did not lay eggs anymore when it was planned to carry out this check.

Initially it was intended to repeat the experiment, this time with younger adults of *B. longissima* hoping that these would produce more viable eggs. However, due to logistic problems the new beetles did not arrive in time for me to carry out the experiment before leaving JIRCAS. It is therefore recommended to repeat the experiment later with younger adults and also with much bigger sample sizes since natural egg hatchability is low as well (Voegele, 1989).

Egg deposition

It appeared that egg deposition of *B. longissima* is slightly higher during the dark period (average: 5.6 eggs/night) compared to the light period (average: 3.9 eggs/day), though the difference is not significant. Sample size (the number of days and nights checked) is, however, very small and variation therefore large and the difference might be significant when checked for a larger number of days and nights. It was also observed that egg deposition decreased with increasing dryness of the leaf material. Eggs were not often deposited at very dry leaf material most likely because *B. longissima* prefers healthy leaves for oviposition in order to prevent the eggs from getting lost (for example when the leaf material is decaying).

Chapter 7 / References

7.1 Literature

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7.2 Website

- Website 1: <http://www.jircas.affrc.go.jp/> (Japan International Research Center for Agricultural Sciences, 7 November, 2006)

7.3 Personal communication

- Dr. S. Nakamura, JIRCAS, senior researcher at the laboratory of 'Crop protection and environment division', personal communication between 3 November, 2006 and 27 February, 2007
- Dr. R. Ichiki, JIRCAS, JSPS research fellow (PD) at the laboratory of 'Crop protection and environment division', personal communication between 3 November, 2006 and 27 February, 2007
- Dr. G. Ho Thi Thu, JIRCAS, JIRCAS fellow at the laboratory of 'Crop protection and environment division', personal communication between 3 November, 2006 and 27 February, 2007

Chapter 8 / Appendices

Appendix 1: Cages for rearing and doing experiments

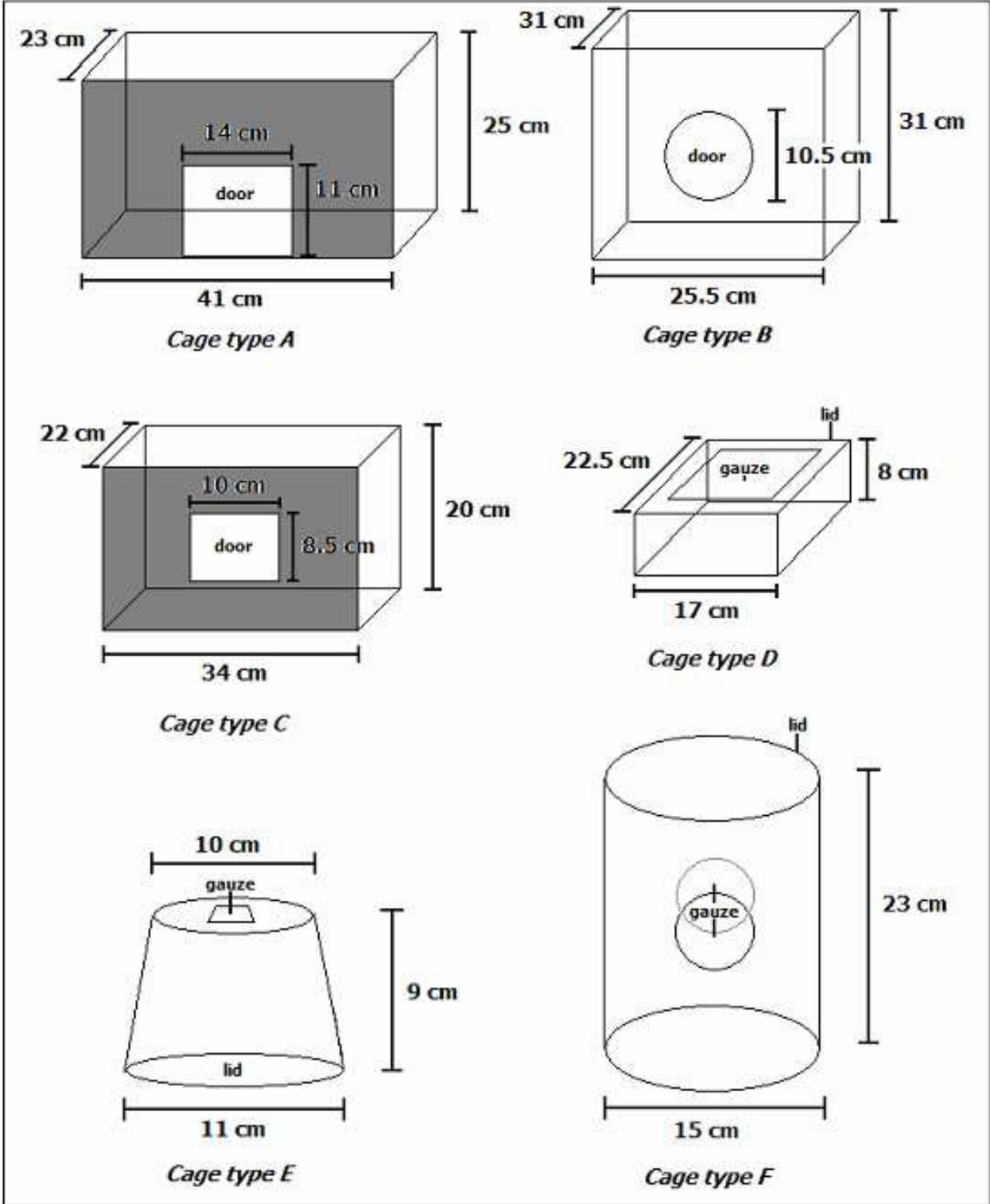


Fig. A-1-a/ The different types of cages used at JIRCAS for rearing tachinids and doing experiments

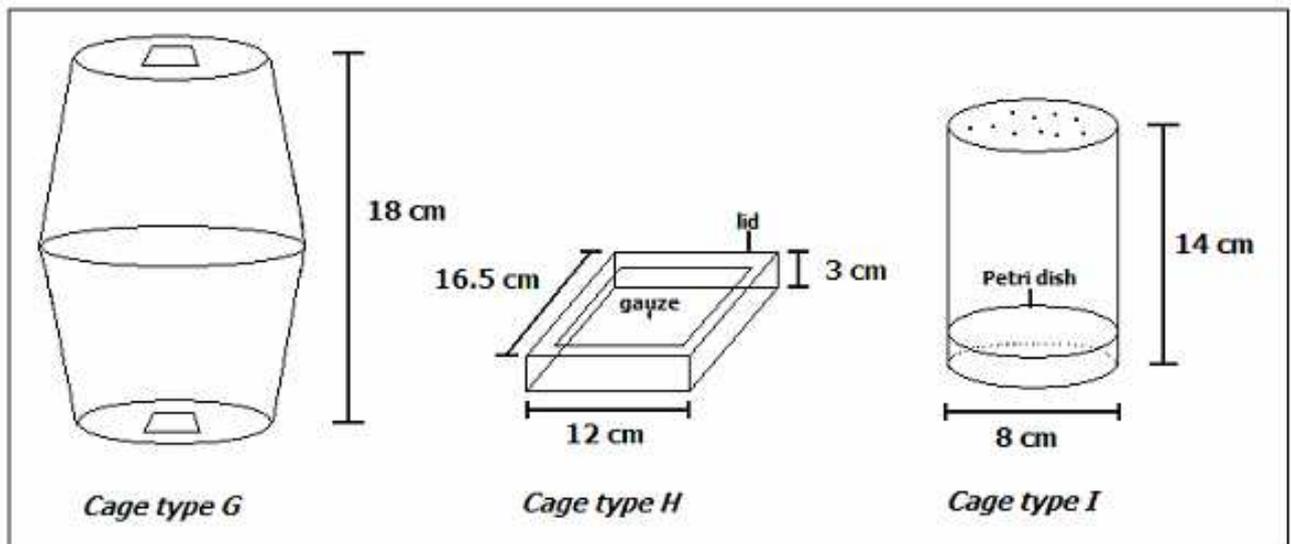


Fig. A-1-b/ The different types of cages used at JIRCAS for rearing tachinids and doing experiments

Appendix 2: Distinguishing gender

It is often relatively easy to distinguish male and female tachinid flies from each other, but the methods how to differ between the different species.

- *Tachina nupta*: To distinguish male and female flies, the tip of the abdomen should be looked at; at this location male flies have a round bulb extension (the mating organ) which is absent at the female abdomen.
- *Exorista* species: To distinguish male and female *Exorista* flies, the front side of the head should be looked at. Males have a more yellowish head, less hairs (and only one vertical line of hairs) and the width between the eyes is smaller than that of female flies. Female heads are white coloured and have more hairs (in two vertical lines next to each other on each side of the head). Besides, male flies often have bigger tarsal claws (especially the pulvillus) than females.
- *Compsilura concinnata*: The males and females can be distinguished by the amount of hair on the head; females have two lines of hair, males just one. Besides, female flies have a sickle-shaped ovipositor which can be observed by using a binocular.
- *Pales pavida* & *Drino inconspicuides*: The males and females can be distinguished by the amount of hair on the head; females have two lines of hair, males just one. Besides, the tarsal claws (especially the pulvillus) of male flies are often much bigger than that of females.
- *Zenillia dolosa*: The males and females are also distinguished by the amount of hair on the head; females have more hairs (and more vertical hair lines) than male flies.

Appendix 3: Experimental set up camera

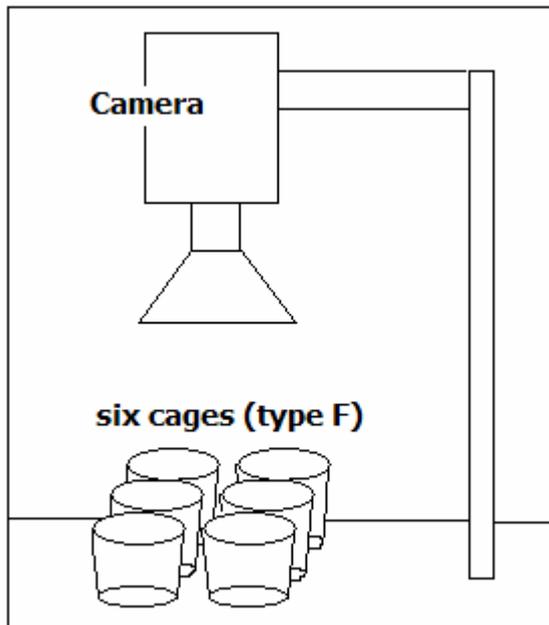


Fig. A-2 / Experimental set up to record mating behaviour of *T. nupta* (six couples at a time)

Appendix 4: Protocol for preparation of RH containers

The method to control relative air humidity in closed containers is described by Winston & Bates (1960). It appeared that there is little information in the literature on the exact method on how to prepare salt solutions in order to generate different relative humidities in closed spaces. Therefore, a brief description of the method will be given and the problems which might occur during preparation are mentioned.

For the experiments three different relative humidity ranges were tested using three different salt solutions. The absolute relative humidities will slightly differ for different temperatures (table A-1). The salt solutions were prepared by heating up demi water and adding a certain amount of salt (table A-1) to achieve complete saturation. Then, approximately 100 ml of the salt solution was put in a box (cage type H: volume approximately 595 cm³), in which one large (diameter: 9 cm) or several small (diameter 3.5 cm) Petri dishes with organisms can be put.

Table A-1 / Salts used for establishment of different relative humidities (RH in %) in closed containers, with the relative air humidity values found in the literature (lit.; Winston & Bates, 1960) and measured during the experiment (obs.).

Salt	Amount (g/l)	15 ° C (lit.)	15 ° C (obs.)	20 ° C (lit.)	20 ° C (obs.)	25 ° C (lit.)	25 ° C (obs.)
NaCl	360	75.0%	74.0-80.0%	75.0%	74.0-79.0%	75.0%	75.0-81.0%
Mg(NO ₃).6H ₂ O	6667	56.0%	53.0-55.5%	55.0%	53.0-57.5%	53.0%	50.0-53.0%
MgCl ₂ .6H ₂ O	5000	34.0%	32.0-34.0%	33.0%	29.0-32.0%	32.5%	29.0-32.0%

Table A-1 shows the amounts of salts for establishing the saturated salt solutions used in the experiments with *P. pavidus* (chapter five) and *B. longissima* (chapter six). However, some further tests have showed that slightly smaller amounts (2750 g/l for MgCl₂.6H₂O and 2500 g/l for Mg(NO₃).6 H₂O) and salt only (without being dissolved) also results in the desired relative humidities for MgCl₂.6H₂O and Mg(NO₃).6H₂O.

It is important to keep in mind during preparation of salt solutions that complete saturation has to be achieved in order to generate certain relative air humidities. If the salt solution is not completely saturated the air humidity will be different from what is aimed at. In case bigger boxes (> 1 litre) are

used it might be necessary to use an air circulation system (Watson & Bates, 1960). For all three air humidity ranges air humidity was stable again within one to two hours after opening the box and did not change a lot by opening it.

Appendix 5: Additional notes *T. nupta* experiment

Methods

Rearing numbers were recorded (number of inoculated hosts, number of fly puparia and number of flies emerging). *Tachina nupta* puparia which did not emerge were opened to check whether a developed fly was inside or not. Adult longevity of the flies in cage type E was checked every day at around 17.00 o'clock. *Tachina nupta* puparia were weighed seven days after pupariation, to determine the approximate weight of the flies.

Results

Rearing numbers suggested a clear decrease in parasitization rate and the percentage of puparia hatching with an increasing age of the ovipositioning females.

Of the 90 puparia which did not emerge, 18 were not developed at all, nine were partly developed and 63 were almost or completely developed into flies. The average age of male *T. nupta* staying in cage type E was 17.1 ± 1.0 days (average \pm SE, N=48) and that of female *T. nupta* 15.8 ± 1.5 days (N=35), which are not significantly different (Independent samples t-test, $P > 0.05$). The mean weight of male *T. nupta* is 133.2 ± 4.1 mg (N=54) and for female flies 128.2 ± 5.0 mg (N=52) which are not significantly different either (Independent samples t-test, $P > 0.05$).

Discussion

It is not clear why such a high percentage of puparia did not emerge, but it might have something to do with the age of the ovipositioning female *T. nupta* since the percentage of puparia emerging decreased with increasing age of the flies. Besides, some puparia might have been damaged during removal from the host pupae since tachinid puparia are very vulnerable. Longevity of *T. nupta* might be lower when kept in small cages (cage type E) as a result of lack of space to move, compared to flies caged in bigger spaces. However, data on adult longevity of flies kept in bigger cages should be collected and compared to these results.