Contents

1. Introduction

Biological control is an effective and necessary way to increase the amount of food for the increasing population of the world and at the same time reduces the environmental pollution, since biological control decreases yield loss (DeBach and Rosen, 1991a). DeBach (1964) defined biological control as "the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence", and this definition is still used, although sometimes in a slightly different way (DeBach and Rosen, 1991a; van Lenteren en Manzaroli, 1999).

Biological control is very often applied in greenhouses for the control of the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). The parasitic wasp *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is an effective biological control agent of *T. vaporariorum* (van Lenteren and Woets, 1998). Since the number of large greenhouses is much bigger in Europe than in the United States, the number of *E. formosa* sold is much higher in Europe (van Lenteren *et al*., 1997).

To avoid failures with natural enemies in biological control (O'Neil *et al*., 1998; Rettke, 2002) and to check whether the quality of the laboratory population has been maintained, quality control is necessary (Kim *et al*., 2001; van Lenteren *et al*., 2003). In this thesis I will focus on the development of a short-range flight test for the quality control of *E. formosa*. In order to clarify the thesis, the following subjects will be treated in the introduction: Parasitoid *E. formosa*, mass rearing and quality control, current and future research, research aims and the composition of this thesis.

1.1 Parasitoid *E. formosa*

The parasitoid *E. formosa* preferentially attacks late larval instars of the whitefly (Noldus and van Lenteren, 1990), but also feeds on the younger larvae of the whitefly, which then die (DeBach and Rosen, 1991b). After about ten days the parasitized larvae turn black (DeBach and Rosen, 1991b; Noldus and van Lenteren, 1990) and in this way the percentage of successful parasitism can be determined (Noldus and van Lenteren, 1990). *E. formosa* cannot distinguish whitefly infected leaves from uninfected leaves. *E. formosa* finds infected leaves through random flights. Romeis and Zebitz (1997) proved in their experiment that *E. formosa* is not attracted or arrested by odors over even short distances, but that colors play a role in long distance attraction of *E. formosa*. In their study, green was more attractive for *E. formosa* than yellow.

1.2 Mass rearing and quality control

E. formosa is mass reared in laboratories. Mass rearing programs are often developed by small companies with little knowledge about the conditions required for optimal development of the parasitoids (van Lenteren, 2000). Since the parasitoids are often reared in an artificial way this may cause problems if the parasitoids are released in situations like the greenhouse, which differ from the situation in the laboratory (van Lenteren, 1993). There are many conflicting requirements concerning the performance of natural enemies in a mass rearing and under greenhouse conditions (Table 1.1).

Table 1.1 Conflicting requirements concerning performance of natural enemies in a mass rearing and under greenhouse conditions (van Lenteren, 1991; van Lenteren, 1993).

	Appreciated in mass rearing	Important for greenhouse 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 behavior unnecessary and unwanted, ability to disperse minimal	Migration behavior essential
5.	Associative learning not appreciated	Associative learning appreciated

To avoid failures with natural enemies in biological control (O'Neil *et al*., 1998; Rettke, 2002) and to check whether the quality of the laboratory population has been maintained, quality control is necessary (Kim *et al*., 2001; van Lenteren *et al*., 2003). In the laboratory populations are removed from natural selection and exposed to standard conditions unlike in the field (van Lenteren and Manzaroli, 1999). There are a number of factors, which influence changes in field populations when introduced into the laboratory for mass rearing (Table 1.2).

Table 1.2 Factors influencing changes in field populations when introduced into the laboratory. (van Lenteren, 1991)

1. Laboratory populations are kept at constant environments with stable abiotic factors and constant biotic factors; 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.
2. There is no interspecific competition in laboratory populations with as result a possible change in genetic variability.
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.
4. Density-dependent behaviors may be affected in laboratory situations.
5. Mate-selection processes may be changed, because unmated or previously mated females will have restricted means of escape.
6. Dispersal characteristics, specifically adult flight behavior and larval dispersal, may be severely restricted by laboratory conditions.

Because of all the problems mentioned in Table 1.2, quality control guidelines have been developed for natural enemies by the International Organization for Biological Control for natural enemies (IOBC). Researchers take these guidelines into consideration while they try to develop simple test methods for evaluating the quality of natural enemies and thus to improve and maintain a good quality of commercially mass reared biological control products (Kim *et al*., 2001). Because of economical reasons we deal with an acceptable quality instead of a maximal or optimal quality (van Lenteren, 1993): farmers will not use biological control as soon as these products are more expensive then what they loose in yield without the use of biological control.

1.3 Current and future research

Until now only research has been done about characteristics that can easily be determined in the laboratory (Table 1.3; van Lenteren, 2002). This study will focus on the development of a short-range flight test for *E. formosa*. To support the development of a short-range flight test, supplementary experiments will be done.

Table 1.3 General quality control criteria for mass reared natural enemies.

Criteria already in use:	
Quantity:	number of living natural enemy organisms in container
Sex ratio:	minimum percentage females (male biased ratio may indicate poor rearing conditions)
Emergence:	emergence rate to be specified for all organisms sold as eggs or pupae
Fecundity:	number of offspring produced during a certain period (for parasitoids fecundity is also an indication of the host kill rate)
Longevity:	minimum longevity in days
Parasitism:	number of hosts parasitized during a certain period
Predation:	number of prey eaten during a certain period
Adult size:	hind tibia length of adults, sometimes pupal size (size is often a good indication for longevity, fecundity and parasitization/predation capacity)
Criteria to be added in near future:	
Flight:	short- or long-range flight capacity
Field performance:	capacity to locate and consume prey or parasitize hosts in crops under field conditions

Comments:

- Quality control is done under standardised test conditions of temperature (usually 22 ± 2^o C or 25 ± 2^o C), relative humidity (usually 75 ± 10 %) and light regime (usually 16 L : 8 D), that are specified for each test
- All numbers / ratios / sizes should be mentioned on the container or packaging material
- Fecundity, longevity and predation capacity tests can often be combined
- Expiration date for each shipment should be given on packaging material
- Guidelines should be usable for all product formulations

Posthuma-Doodeman *et al*. (1996) developed a flight test, but this flight test does not seem to work sufficiently reliable. Flight test are needed when the natural enemy is seriously manipulated during mass rearing and preparation for shipment, and when storage periods are long (van Lenteren *et al*., 2003). After the development of a short-range flight test the relation between performance in the short-range flight test and subsequent dispersal in the greenhouse has to be investigated further (van Schelt and Hora, 2002).

1.4 Research aims

The general aim of my research project is to develop a good short-range flight test. A good short-range flight test meets the requirements set by van Lenteren (1996):

1. The test container should be developed in such a way that a daily check of emerged parasitoids is easy.
2. The test should be suited to determine at which environmental conditions *E. formosa* still flies (e.g. temperature, light and humidity).
3. The materials used should be reusable and standardised (or easy to standardise) in order to make the test as simple as possible to conduct by producers in different countries.
4. The wasps caught on the trap should only be those that have flown. Wasps should be prevented from reaching the trap by walking or jumping.
5. Minimal flight distance covered should be 4 cm.

Besides the development of this short-range flight test, several other experiments have been done on:

- The influence of temperature on the percentage of flying wasps.
- The influence of storage period on percentage of flight at three different temperatures.
- Various preliminary tests before the flight test were developed.

1.5 Composition of thesis

Above, I presented a general introduction and the research aims (Chapter 1). This report will continue with presentation of the materials and methods in Chapter 2, were also the set-ups of the short-range flight test will be described. In Chapter 3 the results are presented, which are then discussed in Chapter 4. In Chapter 5 the conclusions are given.

2. Materials and methods

First the living materials used are presented in Section 2.1. In Section 2.2 the development of experimental set-up for the short-range flight test is described and in Section 2.3 the test of influence of temperature on percentage of flight is described. In Section 2.4 the set-up will be described for the experiment to test the influence of storage period on flight percentage. In Section 2.5 the set-ups of different preliminary experiments are described. These experiments were necessary to develop a good short-range flight test and to get a better impression about the materials and insects used.

2.1 Insects

The parasitoids *E. formosa* were delivered by Koppert Biological Systems as black pupae on paper cards. *E. formosa* is reared on the whitefly *T. vaporariorum* on tobacco (*Nicotiana tobacco* L.) plants.

2.2 Development of experimental set-up for a short-range flight test

This experiment was done to find a good set-up for the short-range flight test for *E. formosa*. The different set-ups of the experiment are shown in Figure 2.1 - Figure 2.6 and Table 2.1.

Two different kinds of cylinders made out of transparent plastic were used to develop a short-range flight test. Plastic that is normally used on an overhead projector ("soft plastic") and plastic that is normally used as a cover of a report ("hard plastic"; Table 2.1). Bottom and top of the plastic cylinders were covered by a Petri dish to prevent *E. formosa* escaping. The bottom of the cylinder was attached to the plastic by transparent tape. In some experimental set-ups glass cylinders were used.

In all the experiments a repellent was used to prevent *E. formosa* walking into a sticky trap (either the underside of a Petri dish at the top of the cylinder, or a sticky yellow card). Since *E. formosa* cannot jump more than 4 cm. (Section 3.4.1; Klapwijk, pers. comm.) and the minimal flight distance covered should be 4 cm. (Chapter 1), the layer of repellent was 4 cm. The repellents were applied as described in Section 2.5.4. The vertical wall and the bottom of the cylinders were covered with black plastic to stimulate *E. formosa* flying upwards. The experiment was done at 13 ± 0.5 ºC, 18 ± 0.5 ºC and 23 ± 0.5 ºC with a relative humidity varying between 60 % and 90 % and a light regime of 16 hours light and 8 hours of darkness. Due to the fact that the glue (Allpurposeglue Collall, The Netherlands) seemed to be toxic to *E. formosa* (Section 3.4.3), the set-up was changed in the course of the experiment. In section C and D (Table 2.1) the cards with pupae of *E. formosa* were no longer attached with glue to the bottom of the cylinder.

During the experiment the number of *E. formosa* caught in the trap was counted every 24 hours. After the experiment the number of death *E. formosa* on the bottom of the cylinder was counted. The percentage of flying wasps was determined by dividing the number of caught wasps by the number of wasps caught in the trap and the number of death wasps (Formula 1). The wasps found in the repellent were excluded from the experiment, since it is not known whether they were able to fly.

Chalk used in flight tests 13 and 14 was obtained by crunching blackboard chalk (SES, The Netherlands, 8710341002008) into powder.

% flying wasps = (# caught wasps in trap / (# caught wasps in trap + # death wasps))* 100 (1)

The results were statistically analyzed with the General Linear Model Univariate test.

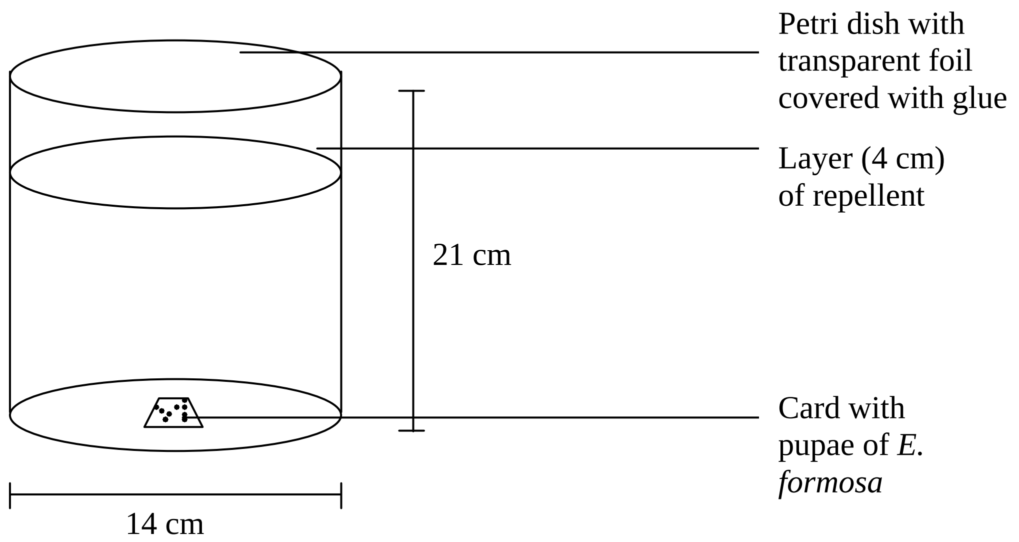

Figure 2.1 Experimental set-up 1 for the short-range flight test of *E. formosa*. The Petri dish on the top was covered with transparent foil, on which the glue was applied, making cleaning afterwards easier.

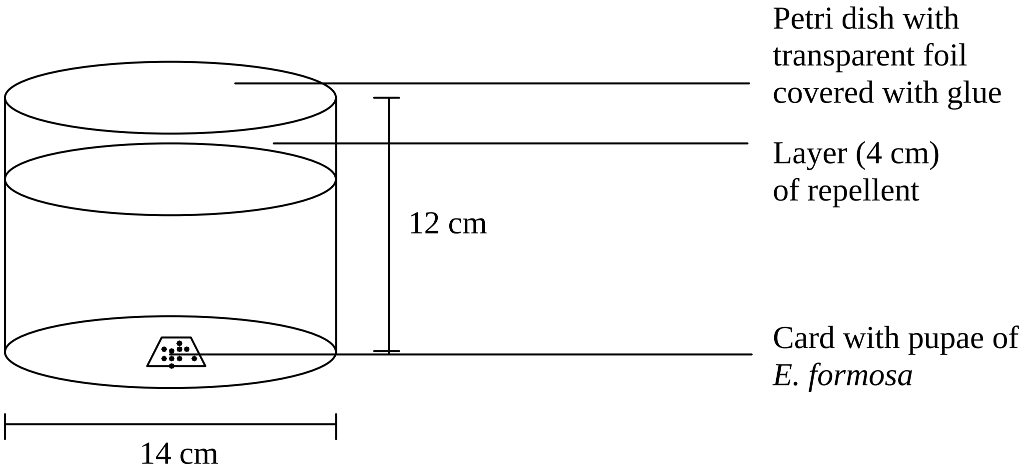

Figure 2.2 Experimental set-up 2 for the short-range flight test of *E. formosa*. The Petri dish on the top was covered with transparent foil, on which the glue was applied, making cleaning afterwards easier.

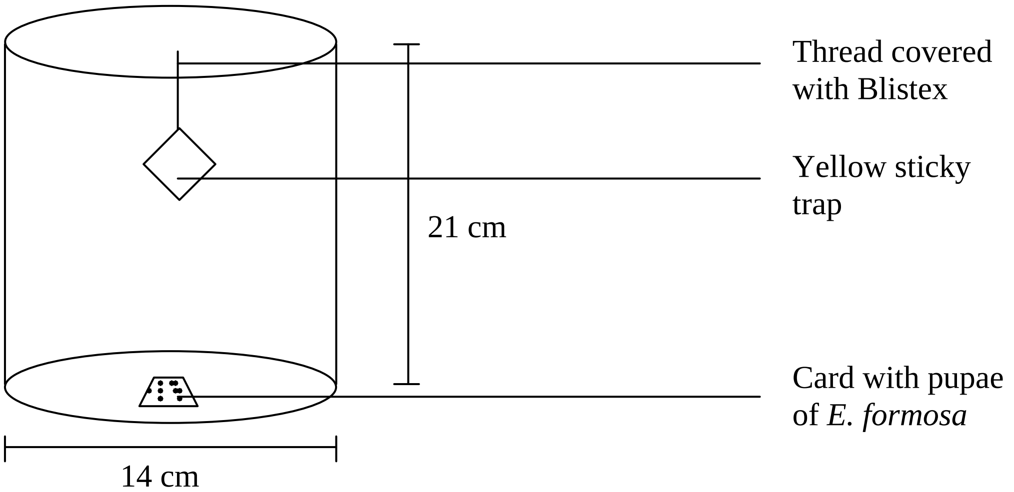

Figure 2.3 Experimental set-up 3 for the short-range flight test of *E. formosa*. The thread with the sticky trap was attached to the Petri dish on top by a drop of glue. The yellow sticky trap was attached to the thread by a staple.

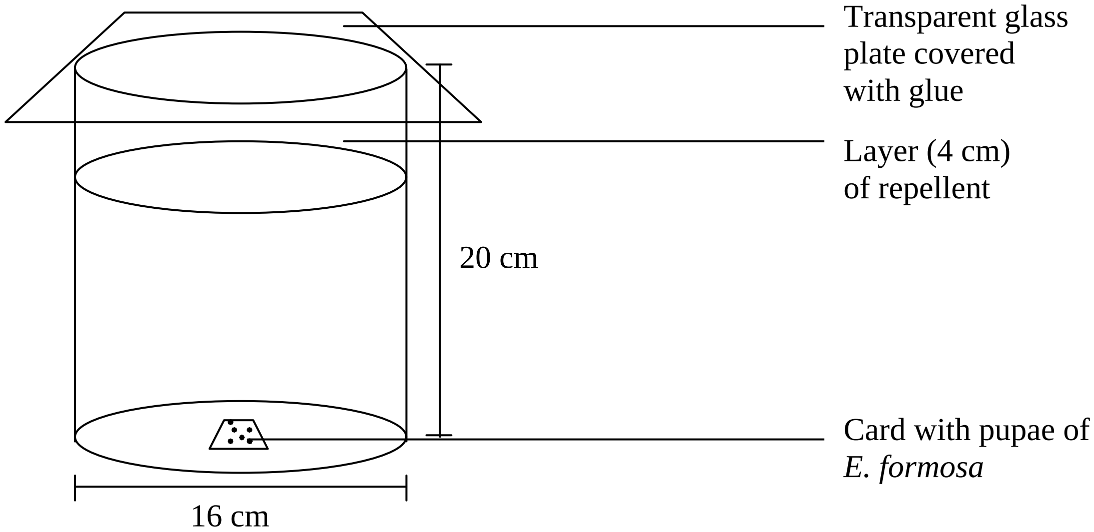

Figure 2.4 Experimental set-up 4 for the short-range flight test of *E. formosa*. The side and bottom of the cylinder were made of glass. The cylinder was covered by a transparent glass (20 x 20 cm). The glass on the top was covered with transparent foil, on which the glue was applied, making cleaning afterwards easier.

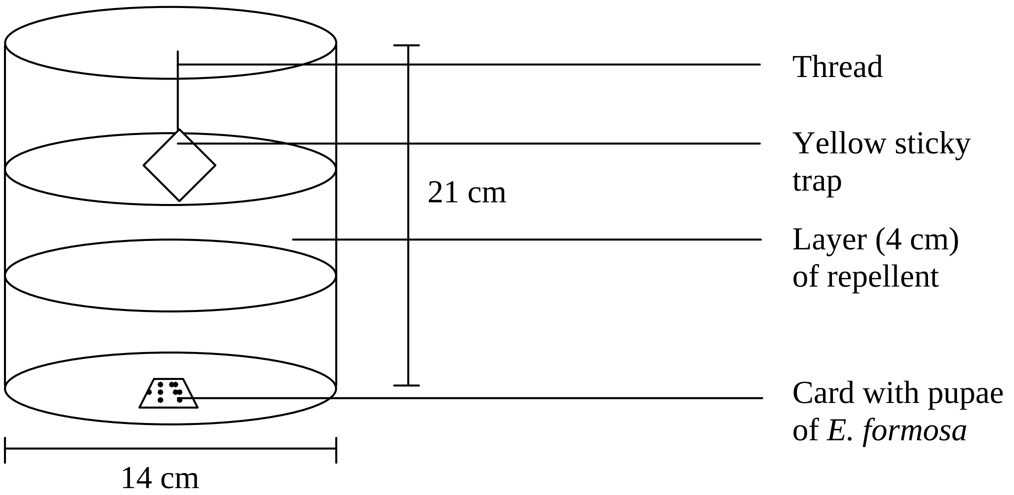

Figure 2.5 Experimental set-up 5 for the short-range flight test for *E. formosa*. The thread with the sticky trap was attached to the Petri dish on top by a drop of glue. The yellow sticky trap was attached to the thread by a staple.

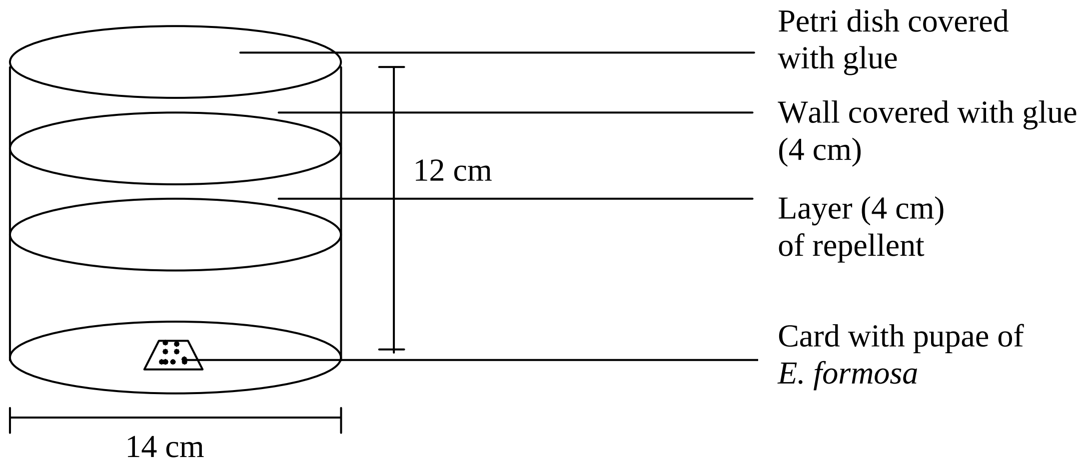

Figure 2.6 Experimental set-up 6 for the short-range flight test for *E. formosa*. Glue was directly applied to the wall and the Petri dish on top.

Table 2.1 Different flight tests and their properties. This table can also be found on the last page of the thesis.

	Flight test	T (ºC)	Duration (days)	Length of cylinder (cm)	Place of repellent	Type of repellent	Trap method	Material of cylinder	Correspond with Figure	Card glued to bottom
A	1 (n = 10)	13	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	1 (n = 10)	18	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	1 (n = 10)	23	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	2 (n = 10)	13	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	2 (n = 10)	18	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	2 (n = 10)	23	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	3 (n = 10)	13	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	3 (n = 10)	18	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	3 (n = 10)	23	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	4 (n = 8)	23	14	20	near top	Blistex	Soveurode	Glass	2.4	Yes
B	5 (n = 10)	13	18	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	5 (n = 10)	18	18	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	5 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	6 (n = 10)	13	18	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	6 (n = 10)	18	18	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	6 (n = 10)	23	14	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	7 (n = 10)	13	18	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	7 (n = 10)	18	18	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	7 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	8 (n = 5)	23	14	20	near top	Blistex	Soveurode	Glass	2.4	Yes
C	9 (n = 10)	13	19	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	9 (n = 10)	18	19	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	9 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	10 (n = 10)	13	19	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	10 (n = 10)	18	19	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	10 (n = 10)	23	14	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	11 (n = 5)	23	14	20	near top	Blistex	Tangle Trap Paste	Glass	2.4	No
D	12 (n = 5)	23	14	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	13 (n = 5)	23	14	12	middle	Chalk	Tangle Trap Paste	Strong Plastic	2.6	No
	14 (n = 5)	23	14	12	middle	Chalk with eucalyptus	Tangle Trap Paste	Strong Plastic	2.6	No

2.3 Testing the influence of temperature on percentage of flight

In this experiment the percentage of flight of wasps of *E. formosa* was determined at 13 ºC, 18 ºC and 23 ºC. The same methods as described in Section 2.2 were used for this experiment. Flight tests 4, 8, 11, 12, 13 and 14 were only done at 23 ºC; at the other flight tests all three temperatures were tested.

2.4 Testing the influence of storage period on percentage of flight

In this experiment the percentage of flight of *E. formosa* after storage was determined. The same methods as described in Section 2.2 were used for this experiment. Half of the cards with pupae of *E. formosa* was stored at 8 ºC for 11 days after the cards had arrived from Koppert Biological Systems. The other half of the cards was immediately used after arrival from Koppert Biological Systems. Both cards were received 7 days after production. Set-up 5 (Figure 2.5) was used for this experiment. The experiment was done at 13 ºC, 18 ºC and 23 ºC. Instead of 10 repetitions with one card of pupae, 5 repetitions with two cards of pupae were used.

2.5 Preliminary experiments for the flight test

These experiments were done at 24 ± 0.5 ºC and a relative humidity varying between 45 % and 50 %.

2.5.1 Distance of horizontal jumping

A one-day-old wasp was placed on a piece of white paper. The wasp was observed and every time after jumping, the place of landing was marked, so the distance of horizontal jumping could be determined. Fifty wasps were tested, 5 jumps for each wasp were determined.

2.5.2 Brand of glue

The stickiness of two different kinds of glue, Soveurode aerosol (Rhône-Poulenc, France) and Tangle Trap Paste (Tanglefoot, USA) was compared in this experiment. One glass plate (20 x 20 cm) was covered with Soveurode and another glass plate was covered with Tangle Trap Paste two days before the experiment was started. After two days wasps were allowed to jump into the glue and the place, on which the wasp landed, was marked. The time until the wasps were completely stuck in the glue, and the linear distance (Figure 2.7) they had walked in the glue were noted. Observations were stopped when the wasps could not move anymore. Twentyfive wasps were tested per kind of glue.

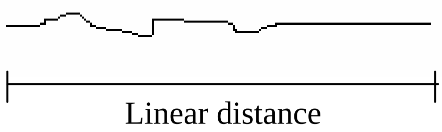

Figure 2.7 Example of linear distance measurement.

2.5.3 Toxic effect of glue

This experiment was done to investigate whether the glue (Allpurposeglue Collall, The Netherlands) used to fix the cards with pupae to the bottom of the cylinder had an effect on emergence of pupae. Ten Petri dishes were filled with one card of pupae without glue and 10 Petri dishes were filled with one card of pupae stuck to the bottom of the Petri dish with glue. After 14 days the amount of emerged wasps was counted for the two treatments.

2.5.4 Different kinds of repellent

Repellents are used in the flight test to prevent wasps walking into the glue. In this experiment different kinds of repellent were used. The different repellents (Table 2.2) were applied to the cylinder (Figure 2.8) one day before the experiment was started to let the repellent evaporate and to avoid any possible toxic effect from the repellent to the wasps. The cylinder was made of transparent hard plastic and bottom and top were covered with the bottom of a plastic Petri dish. The repellent was applied directly to the surface of the cylinder or on double-sided sticky tape. One day before the start of the experiment 10 cards with pupae of *E. formosa* were put into a Petri dish. This Petri dish with pupae was used as the bottom of the cylinder next day. From 50 wasps the time spent and the distance covered on the repellent were determined. After 14 days the experiment was repeated to ascertain whether the repellent was still working. Chalk was obtained by crunching blackboard chalk into powder.

Table 2.2 Different repellents tested.

Repellent
Blistex*
Chalk**
Chalk** on double-sided sticky tape
Mixture of 0.78 g Chalk** and 6 drops of eucalyptus oil on double-sided sticky tape
Vaseline
Mixture of 20 ml Vaseline and 6 drops of eucalyptus oil
Mixture of 20 ml Vaseline and 60 drops of eucalyptus oil
Mixture of 20 ml Vaseline and 600 drops of eucalyptus oil

* product code 8719 2018

** SES, The Netherlands, 8710341002008

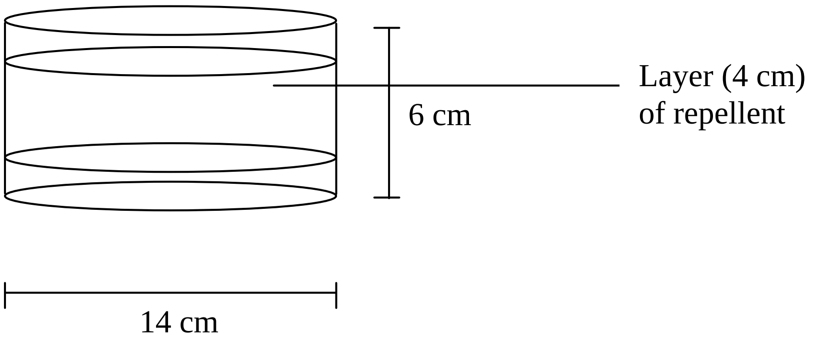

Figure 2.8 Set-up used for the test of different kinds of repellent.

2.5.5 Attraction to color

In this experiment the attraction of *E. formosa* to color was investigated. Different papers with colors were examined with a black-and-white camera. Four papers with the same grey tones were used to study the attraction from *E. formosa* to color (Appendix H). The set-up given in Figure 2.9 was used for this experiment. The glass plate was divided into four sections with papers of 4 different colors. Glue (Soveurode) was applied to the under side of the glass plate.

Twenty wasps were released from a small glass cylinder. Light was right above the cylinder. The color section, on which they landed, was determined. The experiment was stopped after 60 minutes. The experiment was repeated 18 times. Every time a different order and place of colors was used to correct for environmental influences.

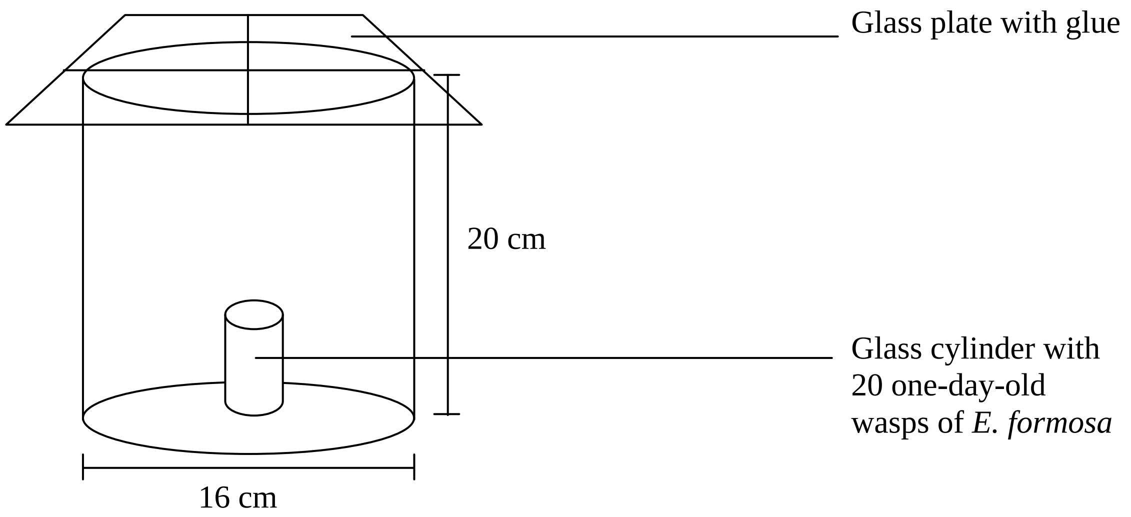

Figure 2.9 Experimental set-up for the color test.

3. Results

First, the results of the development of experimental set-up for a short-range flight test are presented in Section 3.1. In Section 3.2 the results of influence of temperature on percentage of flight and in Section 3.3 the results of the experiment to test the influence of storage period on flight percentage are presented. In Section 3.4 the results of different preliminary experiments are described.

3.1 Development of experimental set-up for a short-range flight test

This experiment was done to find a good set-up for the short-range flight test. In Figure 3.1 (Appendix A) the percentage of flying wasps for the different flight tests at 23 ºC can be seen. Flight tests 3, 6, 9, 10, 11, 12, 13 and 14 showed significantly ($p < 0.05$) the highest percentages of flight of *E. formosa*. Flight tests 1, 2, 7 and 8 showed significantly ($p < 0.05$) the lowest percentages flight of *E. formosa*.

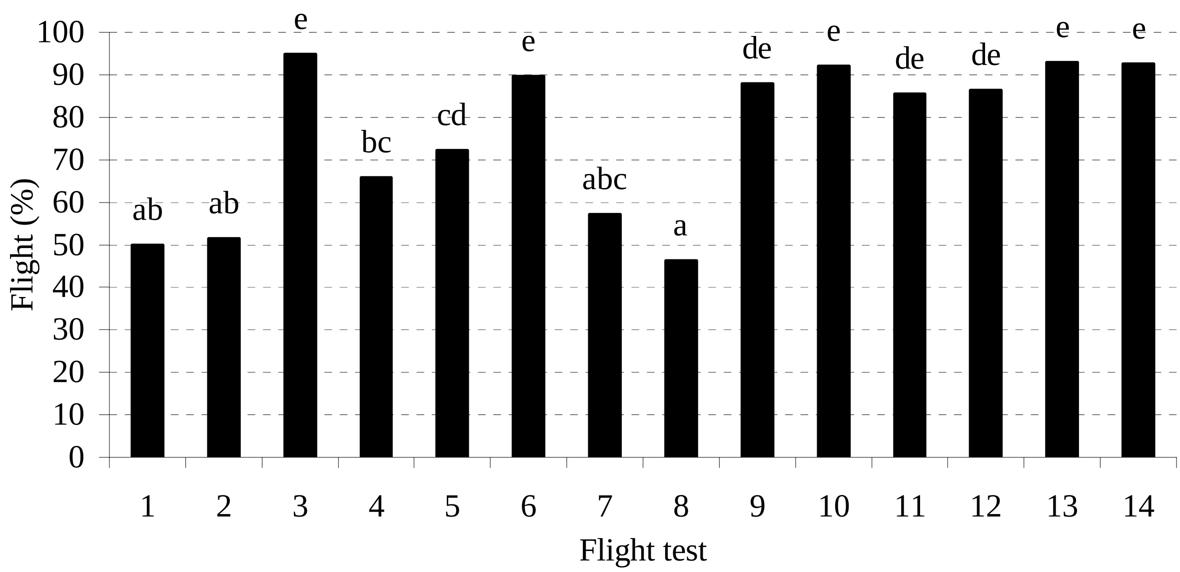

Figure 3.1 Percentage of flying wasps for the different flight tests at 23 ºC ($p < 0.05$). Flight tests correspond with the descriptions in Table 2.1.

3.2 Influence of temperature on percentage of flight

This experiment was done to investigate the influence of temperature on the percentage of flying wasps of *E. formosa*. The results of flight tests 1, 2, 3, 5, 6 and 7 were not reliable, because the glue used in these flight tests seemed to be toxic to *E. formosa* (Section 3.4.3); the results can be found in Appendix B. The percentage of flying wasps of *E. formosa* of flight tests 9 and 10 was significantly different ($p < 0.05$) at 13 ºC, 18 ºC and 23 ºC (Figure 3.2). There is a positive, linear relation between the temperature and the percentage of flight within this temperature range.

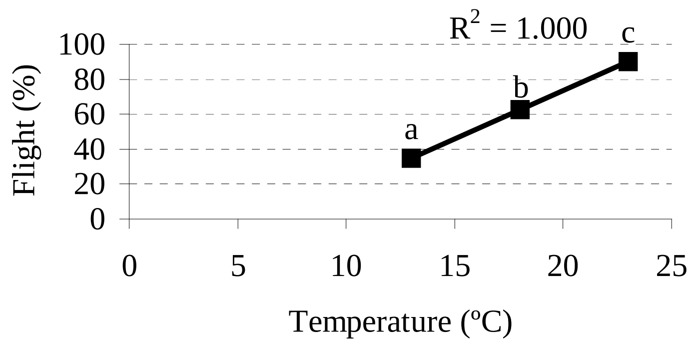

Figure 3.2 Influence of temperature (ºC) of flight tests 9 en 10 on percentage of flight of *E. formosa*.

In Figure 3.3 there is a significant difference ($p < 0.05$) between the numbers of wasps caught at each temperature. The graph shows that the maximum number of wasps caught is reached in an earlier stage at a higher temperature.

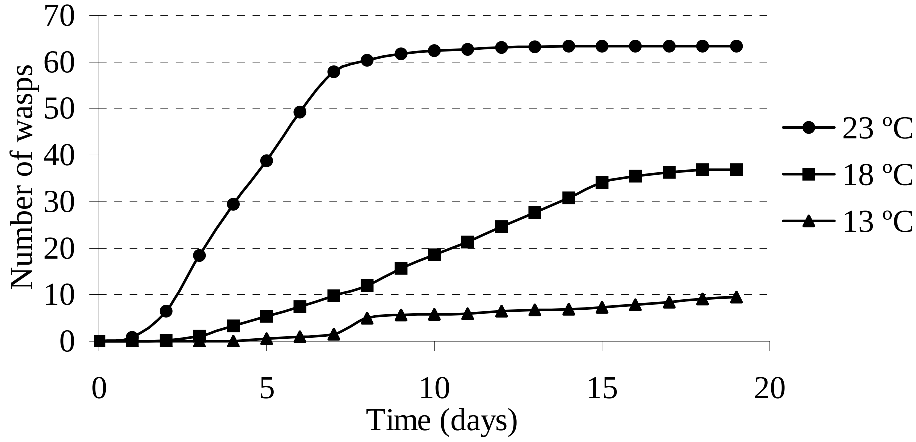

Figure 3.3 Cumulative number of wasps caught in the flight tests 9 and 10 per day at 13, 18 and 23 ºC.

In figure 3.4 the number of wasps caught per day can be seen. The higher the temperature, the shorter it took for *E. formosa* to emerge from the pupae.

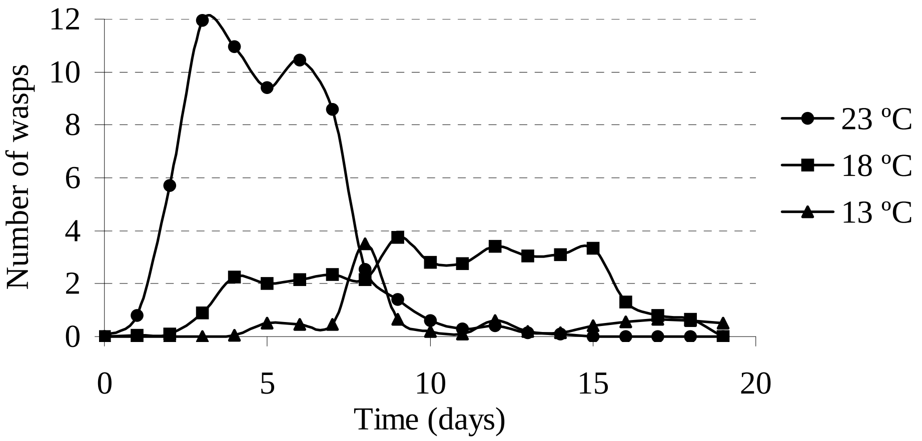

Figure 3.4 Number of wasps caught per day in the flight tests 9 and 10 at 13 ºC, 18 ºC and 23 ºC.

3.3 Influence of storage period on percentage of flight

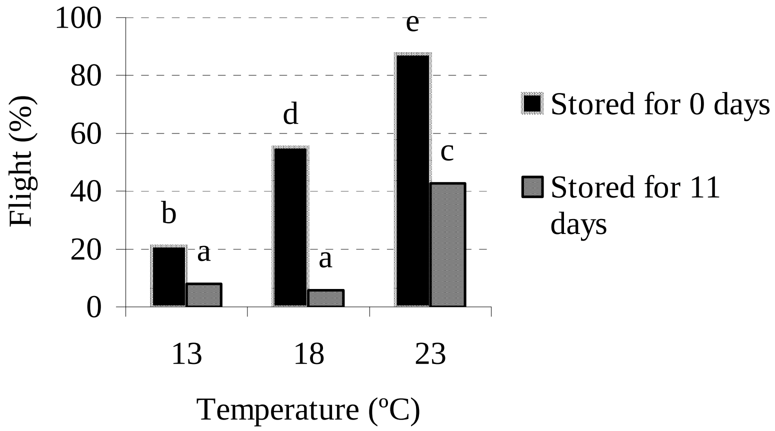

Figure 3.5 Influence of storage period on the percentage of flying wasps of *E. formosa*. Wasps were stored at 8 ºC and after that the tests were done at 13 ºC, 18 ºC and 23 ºC.

In this experiment the influence of storage period on percentage of flying wasps of *E. formosa* was determined. It has to be taken into account that the cards were received 7 days after production. The wasps that were not stored, showed a significantly ($p < 0.05$) higher percentage of flight than the wasps that were stored for 11 days (Appendix C; Figure 3.5). For the wasps that were not stored, there was a significant difference in percentage of flight ($p < 0.05$) for the three different temperatures. The higher the temperature, the higher the percentage of flight. There was no significant difference in percentage of flight at 13 ºC and 18 ºC for the wasps stored for 11 days. There was a significant difference in percentage of flight ($p < 0.05$) at 13 ºC and 23 ºC and at 18 ºC and 23 ºC for the wasps stored for 11 days.

3.4 Results of the supplementary experiments for the flight test

3.4.1 Distance of horizontal jumping

In this experiment the distance of horizontal jumping was determined. At 24 ºC the wasps jumped on average 2.1 cm. ± SEM 0.05 cm. An overview of all the results can be found in Appendix D. Only 0.8 % of the wasps were able to jump further than 4 cm.

3.4.2 Brand of glue

In this experiment the time from landing until the moment that the wasps were completely stuck in the glue, and the linear distance they could walk on the glue were significantly different (respectively $p < 0.001$ and $p < 0.007$) for Soveurode and Tangle Trap Paste (Table 3.1; Appendix E). Although it took on average 122 seconds before the wasps were completely stuck in Tangle Trap Paste, the wasps could only cover 0.04 mm. For Soveurode it took 685 seconds before the wasps were completely stuck in the glue, but they could cover a mean distance of 12.92 mm. The maximum distance the wasps could cover for Soveurode was 86 mm. and 1 mm. for Tangle Trap Paste.

Table 3.1 Time (sec.) spent on and distance (mm.) covered on the different glues by wasps of *E. formosa*.

Glue	Time (sec.)	Distance (mm.)
Soveurode	685a ± SEM 114	12.92a ± SEM 3.25
Tangle Trap Paste	122b ± SEM 15	0.04b ± SEM 0.03

Analysis of Variance: $p < 0.001$ for time and $p < 0.007$ for distance.

3.4.3 Toxic effect of glue

In this experiment it was examined whether the glue used for attaching cards of pupae to the bottom of the flight test cylinder could have any effect on emergence of pupae. In Petri dishes, in which a card of pupae was stuck to the bottom of the Petri dish with glue, 0.5 ± SEM 0.34 wasps had emerged and in Petri dishes, in which no glue was used, 33.2 ± SEM 2.20 wasps had emerged after 14 days (Table 3.2; Appendix F).

Table 3.2 Number of wasps emerged after 14 days with and without glue.

With glue	Without glue
0.5 ± SEM 0.34	33.2 ± SEM 2.20

T-Test: $p < 0.000$.

3.4.4 Different kinds of repellent

In this experiment the quality of different kind of repellents was compared. The quality was determined by the time spent and the distance covered on the repellent at one day and 14 days after application. The less time the wasps spend and the shorter distance they could cover, the better the quality. There was no significant difference in the mean time covered by wasps of *E. formosa* on the different repellents except for chalk without tape (Appendix G; Figure 3.6). Wasps did spend significantly ($p < 0.05$) more time on chalk without tape. There was no significant difference ($p < 0.05$) between the mean time covered by wasps on the different repellents at day one and at day 14.
There was significant difference ($p < 0.05$) in the distance covered by the wasps on the different repellents (Appendix G; Figure 3.7). At day 1 wasps covered a significant ($p < 0.05$) shorter distance than at day 14 for 20 ml Vaseline with 600 drops eucalyptus oil. The shortest significant ($p < 0.05$) distances on day 1 and day 14 were covered by the wasps on Blistex, chalk with tape and chalk with 6 drops eucalyptus oil.

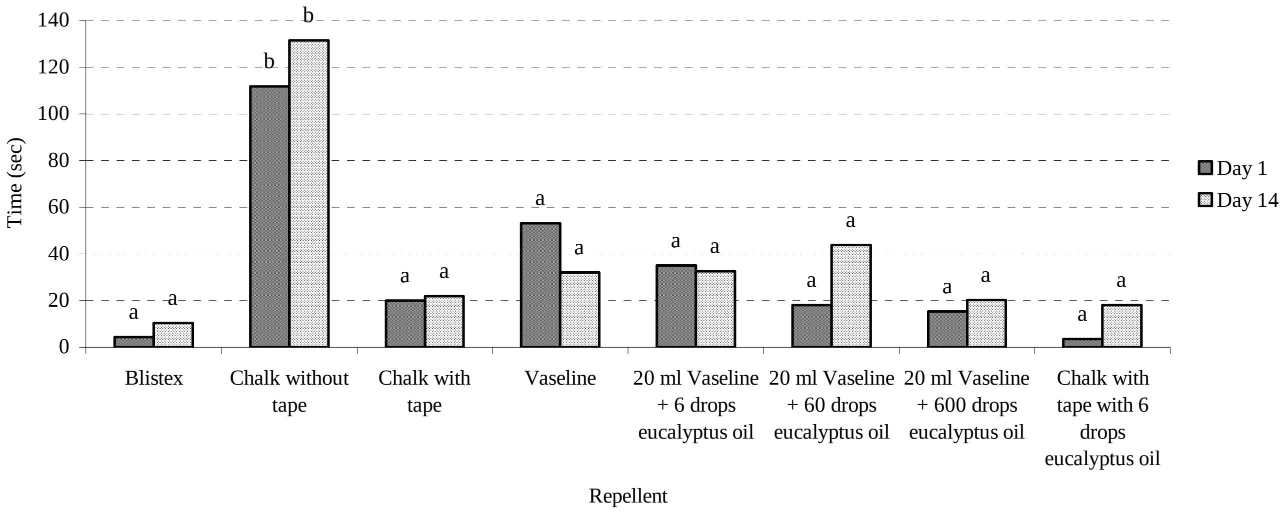

Figure 3.6 Mean time (sec.) covered by wasps of *E. formosa* on the different repellents. The experiment was done at 1 and 14 days after the repellent had been added to the wall of the cylinder at 24 ºC.

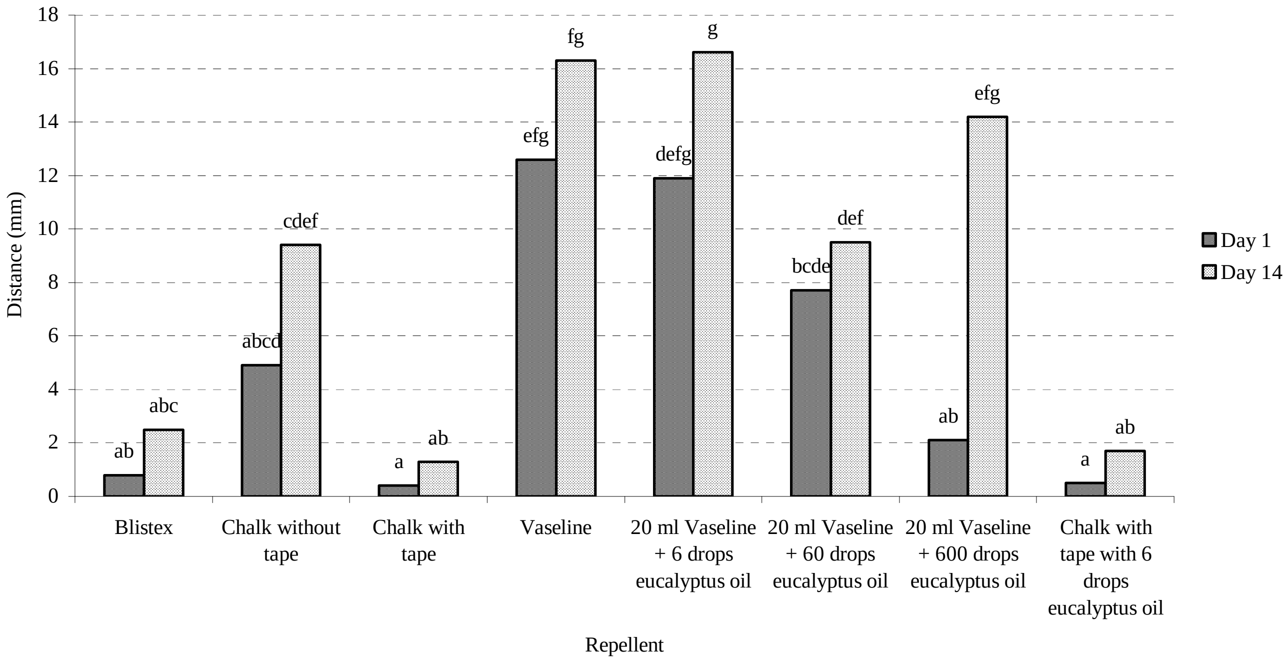

Figure 3.7 Mean distance (mm.) covered by wasps of *E. formosa* on the different repellents. The experiment was done at 1 and 14 days after the repellent had been added to the wall of the cylinder at 24 ºC.

3.4.5 Attraction to color

In this experiment the attraction from *E. formosa* to four different colors with the same grey tones was studied. Only 60 percent of the wasps were attracted by colors within 60 minutes (Appendix H). Out of this 60 percent, the wasps were significantly ($p < 0.05$) more attracted to green and to skin color than to white and yellow (Figure 3.8).

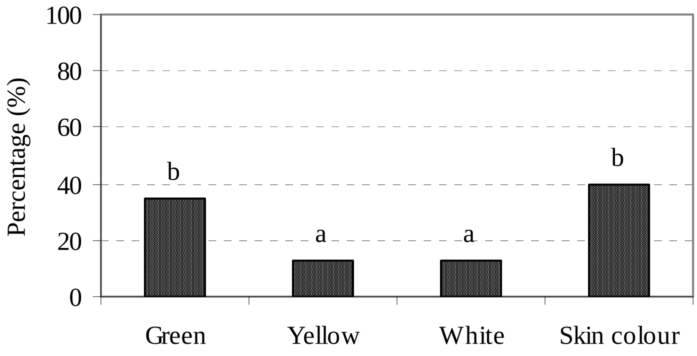

Figure 3.8 Percentage of the wasps attracted to the different colors within 60 minutes.

4. Discussion

First the development of experimental set-up for a short-range flight test is discussed in Section 4.1. In Section 4.2 the influence of temperature and in Section 4.3 the influence of light on percentage of flight are discussed. In Section 4.4 the influence of storage period on flight percentage is discussed. Finally, in Section 4.5 the attraction to color is discussed.

4.1 Development of experimental set-up for a short-range flight test

Aim of my work was to develop a good short-range flight test for the quality control of *E. formosa*. Flight tests 9, 10, 11, 12, 13 and 14 had the highest percentages of flight of *E. formosa*. In flight tests 9, 10, 11 and 12 Blistex was used as repellent, in flight test 13 chalk with tape was used, and in flight test 14 chalk with tape and eucalyptus oil was used. Flight test 13 was chosen as the best set-up for the flight test, since the repellent chalk works best and is easier to obtain than Blistex and eucalyptus oil and it is thus easier to repeat this experiment for other quality control workers. Flight test 13 is more standardized than flight tests 9, 10, 11, 12 and 14 and meets all requirements set by van Lenteren (1996) mentioned in Section 1.4. The flight test proposed for the IOBC/EC quality control test (van Lenteren *et al.*, 1996) is no longer considered a good short-range flight test for the quality control of *E. formosa*, since a flight test with Blistex, the repellent used in that set-up, is more difficult to standardize than chalk, which is used in these experiments.

Flight test 3 is not considered a good flight test, because in this set-up the wasps could have jumped from the top of the cylinder to the yellow sticky trap, since repellent was applied to the thread.

Flight tests 1 to 8 are not considered a good set-up for the short-range flight test, because in flight tests 1 to 8 glue was used to stick the cards of *E. formosa* to the bottom of the cylinders. This glue seemed to be toxic to *E. formosa* (Section 3.4.3). The glue contains aceton and ethanol. Owen (1985) showed in his research that vapour of ethanol was toxic to *Asobara persimilis* (Hymenoptera: Braconidae) at higher concentrations than 1.5 %. The results of flight tests 1 to 8 were based upon a few wasps only, since only a few wasps emerged, and were thus not reliable.

A very important aspect of the flight test is that the glue is applied just above the repellent. In this way it is easy to see whether the wasps of *E. formosa* were flying into the glue instead of walking. The glue was tested in Section 3.4.2, in which it is proved that wasps of *E. formosa* can only walk 0.04 ± SEM 0.03 mm. in Tangle Trap Paste. Since none of the wasps were found at the border between glue and repellent in flight test 13 it is a good set-up for a short-range flight test.

Although flight test 14, in which eucalyptus oil was used as repellent, had a high percentage of flight, eucalyptus oil seemed to be irritating to wasps of *E. formosa*. This was observed in Section 3.4.4, in which different kinds of repellents were tested. Wasps of *E. formosa* looked pretty disturbed and moved around like they had lost their orientation and their equilibrium. Not a lot of research has been done to the effect of eucalyptus oil, but Srivastava and Krishna (1991) found that eucalyptus oil considerably disturbed the reproductive performance of adults of *Dysdercus koenigii* (Fabr.) (Heteroptera: Pyrrhocoridae). Rezk and Gadelhak (1997) did an experiment on the control of the parasitic mite *Varroa jacobsoni* (Acari: Mesostigmata) with an oil extract from *Eucalyptus camaldulensis* and it was shown that this oil extract was effective (i.e. it killed the mite) in the treatment of *V. jacobsoni*.

It is recommended to the IOBC/EC quality control group to use flight test 13 as a standardized quality control flight test for *E. formosa*. It has to be further investigated whether this test can also be used as an indicator for the quality of other parasitoids in quality assessment. Dutton and Bigler (1995) proved that the short-range flight test for *Trichogramma* can be used as an indicator for *Trichogramma* quality in quality assessment.

4.2 Influence of temperature on percentage of flight

The percentage of flight increased with an increase in temperature within the temperature range of 13 ºC to 23 ºC. It was also shown that the maximum number of caught wasps was reached in an earlier stage at a higher temperature. The development time of insects is shorter at a higher temperature. Ekbom (1982) found that flight activity of *E. formosa* was positively correlated with temperature and could occur at temperatures as low as 13 ºC.

4.3 Influence of light on percentage of flight

At day 9 one of the light bulbs in the climate room at 13 ºC got broken. The number of wasps caught at 13 ºC dropped immediately down from an average of 3.5 per day to 0.7 per day. Light intensities in the climate room were not measured, but may have influenced the number of wasps caught. Wasps will disperse shorter distances at low light intensities (less than 500 lux) than at high light intensities (greater than 8000 lux) (van Lenteren *et al*., 1992). At low light intensities more parasites depart within the first four hours after emergence than at high light intensities. But when they fly away at a high light intensity, they span a larger distance (van Lenteren *et al*., 1992). Parr *et al*. (1976) concluded that low light intensities and short days may affect the efficacy of *E. formosa*. It is not known how low the light intensity was in the climate room at 13 ºC with one broken light. Moreover, Ekbom (1982) proved that the flight activity of *E. formosa* was positively correlated with temperature. Since 13 ºC was a relatively low temperature, it is a possibility that wasps could not be caught since they didn't span a large enough distance because of a low temperature and low light intensity.
This experiment was done in three different climate rooms. In each climate room there was another temperature. Although the same light bulbs were used, there could have been an effect of different light intensities. Moreover, the relative humidity varied between 60 % and 90 % in each room.

4.4 Influence of storage period on percentage of flight

Wasps of *E. formosa* that were not stored showed a higher percentage of flight than the wasps that were stored at 8 ºC for 11 days. Lacey *et al*. (1999) showed that although *E. formosa* pupae were killed by exposure to 2 ºC and 5 ºC for longer than 3 days, storage at 10 ºC resulted in fairly good survival for pupae stored for 13 days. Unfortunately, they did not say anything about the fitness of wasps of *E. formosa* after storage. Also the percentage of flight of *Aphidius colemani* decreased significantly after storage for 14 days at 8 ºC (van Schelt and Hora, 2002). Looking at the emergence rates, also Ganteaume *et al*. (1995a) found that parasitized pupae of *E. formosa* can be stored at 9 ºC - 12 ºC for 15 - 20 days without affecting adult emergence rates. Storage at low temperatures (9 ºC) for more than 5 days reduces adult longevity and fecundity (Ganteaume *et al*., 1995b). Storage at 8 ºC may have been too low in this research for the survival of pupae and flight activity of wasps of *E. formosa*. Moreover, it is not clear how many days pupae can be stored at 8 ºC without a decline in percentage of flight.

Before storage in this experiment, the cards with pupae were stored by the producer. It was only clear that the pupae were stored for 7 days before the experiment was started, but it is not clear at which temperature pupae were stored during these 7 days. Since the percentage of flight of pupae that were not stored was almost 90 %, the storage conditions during these 7 days seemed to be good.

Although Koppert Biological Systems advises (www.koppert.nl) to store pupae of *E. formosa* not longer than 2 days and to store them between 8 ºC and 10 ºC, more research has to be done on the effect of storage of pupae for the percentage of flight. It is very important that pupae are stored on different temperatures and on more different days of storage. These results also showed that it is important that growers do not keep pupae of *E. formosa* long before putting into the greenhouse and that they store them at the right temperatures.

4.5 Attraction to color

Wasps of *E. formosa* were more attracted to green and skin color than to yellow and white. Mellor *et al.* (1997) showed that the similarity in the shape of the efficiency curve between *E. formosa* and *T. vaporariorum* in the blue-green-yellow region could indicate a similarity in behavioral response towards yellow as both species have been found to be attracted to yellow sticky traps (*T. vaporariorum*: Berlinger, 1986; *E. formosa*: Ekbom, 1980). In the study of Oliai and King (1999) it was shown that the females of *Nasonia vitripennis* showed no innate color preference for yellow versus blue. The research of Romeis and Zebitz (1997) showed that colors play a role in long distance attraction of *E. formosa*. In their study, green was more attractive for *E. formosa* than yellow. All research mentioned here showed different results. Although four papers of the same grey tones were used, there could be a very small difference between the four papers.

5. Conclusions

- Flight test 13 (a plastic cylinder with a length of 12 cm, with Tangle Trap Paste on the underside of the top to catch the wasps and chalk on double-sided tape was used as repellent on the vertical wall of the cylinder) is the best set-up, and could serve as standard for the IOBC quality control guideline for *E. formosa*.
- The higher the temperature, the higher the percentage of flying wasps of *E. formosa*.
- The higher the temperature, the higher number of wasps was caught in the trap.
- Wasps that were not stored had a higher percentage of flight than wasps that were stored at 8 ºC for 11 days.
- At 24 ºC wasps could jump 2.1 cm. ± SEM 0.05 cm.
- Wasps got earlier stuck into Tangle Trap Paste than into Soveurode.
- Wasps could spend a larger distance (cm.) on Soveurode than on Tangle Trap Paste.
- The use of glue for attaching cards of pupae to the bottom of the flight test cylinder had a negative effect on emergence of pupae.
- Blistex, chalk with tape, and chalk with tape and 6 drops of eucalyptus oil were the best repellents, based on ease of standardization, it is advised to choose for chalk.
- Wasps were more attractive to green and skin color than to yellow and white.

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Appendix A Flight test

Table 1 Percentages of flight for the different flight tests serie A.

Flight test	1			2			3			4
Temperature (ºC)	13	18	23	13	18	23	13	18	23	23
1	33	51	53	21	56	48	78	94	85	65
2	0	100	0	50	25	59	88	92	90	75
3	50	77	60	100	26	50	67	94	96	78
4	67	75	51	60	60	60	75	98	100	60
5	50	62	52	71	50	36	86	88	96	47
6	27	47	43	58	52	29	0	96	95	49
7	59	91	58	39	50	66	76	92	98	76
8	40	62	50	40	44	47	0	93	99	76
9	57	61	71	78	74	42	93	86	99	-
10	50	63	62	61	43	76	69	97	91	-
Mean	43	69	50	58	48	51	63	93	95	66
SEM	6.1	5.3	6.1	7.0	4.7	4.5	10.9	1.2	1.6	4.6

Table 2 Percentages of flight for the different flight tests serie B.

Flight test	5			6			7			8
Temperature (ºC)	13	18	23	13	18	23	13	18	23	23
1	33	79	80	57	47	83	0	63	84	80
2	50	61	83	50	43	86	0	52	35	46
3	67	65	93	0	45	90	0	0	72	52
4	48	71	72	0	75	85	0	29	74	33
5	58	47	42	56	86	96	35	13	90	20
6	25	33	65	40	77	86	56	28	7	-
7	14	0	95	29	50	92	20	0	60	-
8	25	28	53	17	46	93	36	46	0	-
9	25	49	50	5	53	90	0	32	72	-
10	50	58	89	57	53	94	20	38	77	-
Mean	40	49	72	31	58	90	17	30	57	46
SEM	5.5	7.4	6.0	7.6	4.9	1.3	6.4	6.7	10.1	10.1

Table 3 Percentages of flight for the different flight tests serie C.

Flight test	9			10			11
Temperature (ºC)	13	18	23	13	18	23	23
1	18	52	87	80	48	88	90
2	34	59	87	48	41	89	83
3	30	81	93	15	80	96	81
4	13	56	89	46	61	91	87
5	16	47	91	50	80	94	86
6	50	71	79	27	59	89	-
7	40	56	84	47	70	90	-
8	26	31	94	43	73	99	-
9	28	72	88	43	82	91	-
10	19	75	86	22	59	93	-
Mean	27	60	88	42	65	92	85
SEM	3.7	4.8	1.4	5.7	4.4	1.1	1.5

Table 4 Percentages of flight for the different flight tests serie D.

Flight test	12	13	14
Temperature (ºC)	23	23	23
1	88	93	93
2	89	93	95
3	90	95	88
4	85	94	94
5	80	90	93
Mean	87	93	93
SEM	1.9	0.9	1.3

Appendix B Temperature

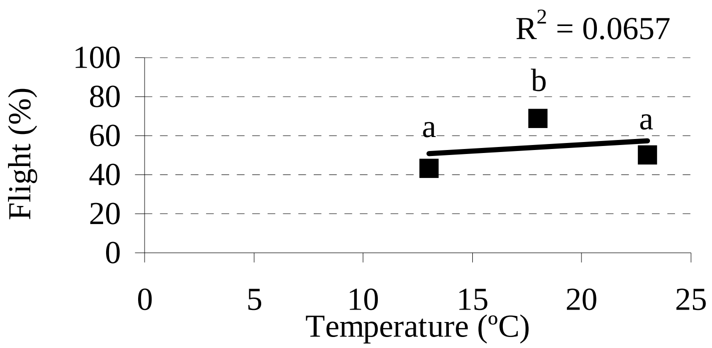

Figure 1 Influence of temperature (ºC) of flight test 1 on percentage of flight of *E. formosa*. R^2

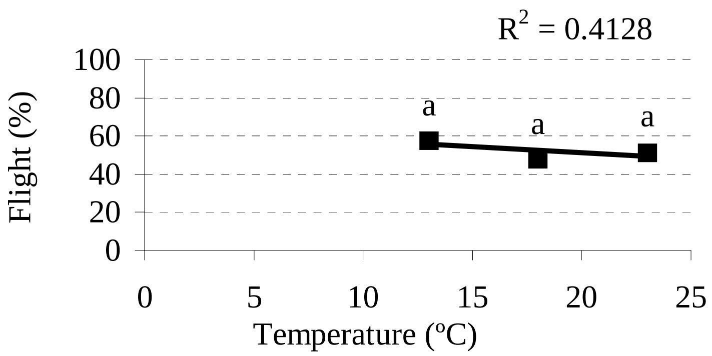

Figure 2 Influence of temperature (ºC) of flight test 2 on percentage of flight of *E. formosa*.

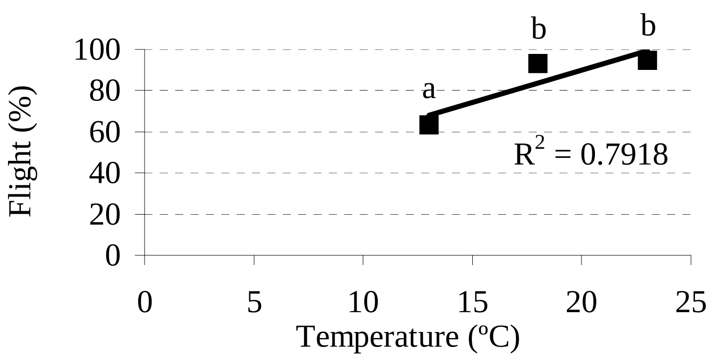

Figure 3 Influence of temperature (ºC) of flight test 3 on percentage of flight of *E. formosa*.

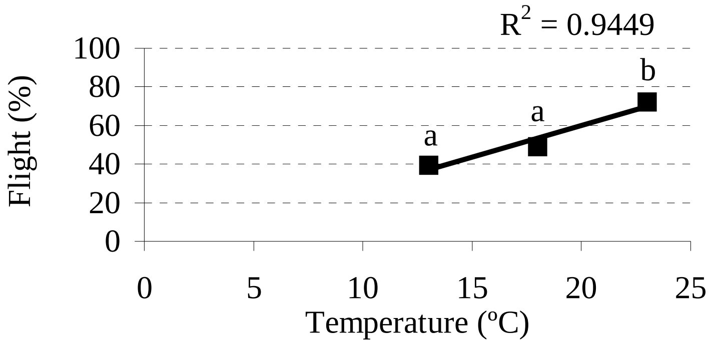

Figure 4 Influence of temperature (ºC) of flight test number 5 on percentage of flight of *E. formosa*.

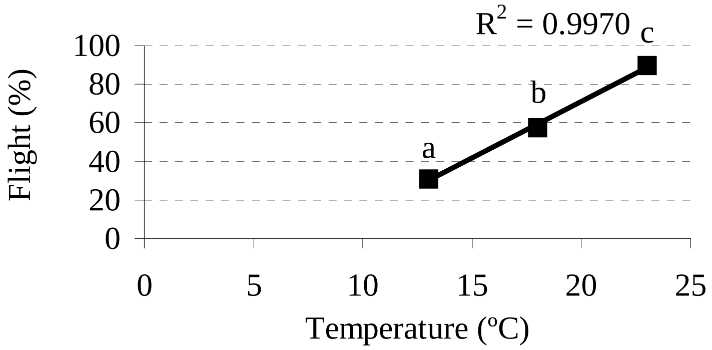

Figure 5 Influence of temperature (ºC) of flight test 6 on percentage of flight of *E. formosa*.

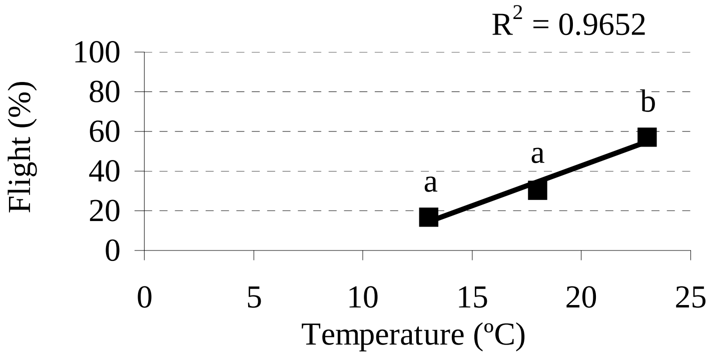

Figure 6 Influence of temperature (ºC) of flight test 7 on percentage of flight of *E. formosa*.

Table 5 Number of caught wasps of E. formosa for flight test 9, flight test 10, mean of flight tests 9 and 10 and cumulative mean of flight tests 9 and 10.

	Temperature (ºC)											
	13				18				23			
Time (days)	Flight test 9	Flight test 10	Mean	Cum	Flight test 9	Flight test 10	Mean	Cum	Flight test 9	Flight test 10	Mean	Cum
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.5	1.1	0.8	0.8
2	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	4.4	7.0	5.7	6.5
3	0.0	0.0	0.0	0.0	0.8	1.0	0.9	1.1	10.7	13.2	12.0	18.5
4	0.0	0.1	0.1	0.1	2.2	2.3	2.3	3.3	10.8	11.1	11.0	29.4
5	0.6	0.4	0.5	0.6	1.6	2.4	2.0	5.3	8.6	10.2	9.4	38.8
6	0.3	0.6	0.5	1.0	2.3	2.0	2.2	7.5	8.4	12.5	10.5	49.3
7	0.5	0.4	0.5	1.5	2.2	2.5	2.4	9.8	8.3	8.9	8.6	57.9
8	3.2	3.8	3.5	5.0	1.1	3.2	2.2	12.0	2.2	2.9	2.6	60.4
9	0.2	1.1	0.7	5.6	2.0	5.5	3.8	15.7	1.1	1.7	1.4	61.8
10	0.1	0.3	0.2	5.8	1.8	3.8	2.8	18.5	0.5	0.7	0.6	62.4
11	0.0	0.2	0.1	5.9	1.6	3.9	2.8	21.3	0.1	0.5	0.3	62.7
12	0.6	0.6	0.6	6.5	1.9	4.9	3.4	24.7	0.4	0.4	0.4	63.1
13	0.2	0.2	0.2	6.7	3.3	2.8	3.1	27.7	0.1	0.2	0.2	63.3
14	0.0	0.3	0.2	6.9	2.8	3.4	3.1	30.8	0.1	0.1	0.1	63.4
15	0.3	0.5	0.4	7.3	3.2	3.5	3.4	34.2	0.0	0.0	0.0	63.4
16	0.2	0.9	0.6	7.8	1.2	1.4	1.3	35.5	0.0	0.0	0.0	63.4
17	0.4	0.9	0.7	8.5	0.9	0.7	0.8	36.3	0.0	0.0	0.0	63.4
18	0.6	0.6	0.6	9.1	0.8	0.5	0.7	36.9	0.0	0.0	0.0	63.4
19	0.4	0.6	0.5	9.6	0.0	0.0	0.0	36.9	0.0	0.0	0.0	63.4

Appendix C Storage

Table 6 Percentages of flight for the different days of storage and temperatures.

Days of storage	0			11		
Temperature	13	18	23	13	18	23
1	16	55	78	6	10	40
2	15	61	86	3	5	47
3	25	50	90	9	6	32
4	19	57	94	8	0	47
5	29	54	89	14	8	48
Mean	21	55	87	8	6	43
SEM	2.7	1.8	2.7	1.8	1.7	3.1

Appendix D Distance of horizontal jumping

Table 7 Distances (cm.) of horizontal jumping of wasps of *E. formosa*.

	Repetition				
Wasp no.	1	2	3	4	5
1	2.4	2.4	3.1	2.6	2.7
2	2.5	1.7	2.8	2.0	1.9
3	1.8	2.6	3.7	1.8	2.7
4	2.5	2.6	3.3	3.3	1.7
5	1.7	3.2	2.1	2.8	3.0
6	3.5	2.6	3.0	2.3	2.2
7	2.2	2.5	3.7	2.5	3.6
8	4.8	3.1	1.8	2.2	2.7
9	1.2	0.7	1.2	1.8	1.9
10	1.6	3.4	0.8	2.0	1.7
11	0.7	1.8	0.7	1.3	1.1
12	3.2	2.9	1.5	1.0	2.0
13	1.7	3.0	3.6	2.7	1.2
14	2.4	1.9	1.6	1.5	2.4
15	1.7	1.9	1.3	1.4	1.5
16	2.1	2.9	2.4	2.0	2.3
17	2.5	1.8	3.2	3.6	3.4
18	1.5	1.5	1.8	1.3	2.2
19	2.5	2.2	2.2	2.3	1.8
20	1.5	3.5	2.1	2.7	1.7
21	2.2	1.9	2.5	2.2	2.3
22	1.2	1.1	1.7	1.7	1.3
23	2.3	1.3	2.9	1.9	2.0
24	1.7	1.3	0.8	2.5	2.0
25	2.9	2.2	2.3	2.0	1.5
26	2.2	4.2	2.4	2.0	2.5
27	2.7	2.0	1.3	1.9	1.7
28	1.9	2.1	2.2	2.4	3.4
29	2.3	1.7	2.7	1.7	2.8
30	2.6	2.7	1.9	1.9	3.5
31	2.0	1.3	2.2	1.9	1.5
32	3.5	3.1	2.8	3.3	3.6
33	2.9	2.1	1.8	0.8	3.3
34	3.5	2.7	2.5	3.0	2.5
35	1.4	1.7	1.7	2.2	1.7
36	1.5	2.1	1.6	2.1	1.6
37	1.5	3.5	2.7	2.6	1.7
38	1.8	2.0	1.8	2.5	2.6
39	2.0	2.3	1.7	3.0	1.4
40	2.2	1.2	2.3	1.6	1.3
41	1.2	1.1	1.1	0.8	3.7
42	1.6	1.5	1.2	2.1	1.7
43	3.6	1.9	2.0	2.0	1.7
44	3.0	2.6	2.9	2.4	2.9
45	1.2	1.8	1.7	0.9	1.6
46	1.2	1.7	1.2	1.7	1.3
47	2.1	1.8	1.5	2.5	2.1
48	1.8	2.0	1.6	1.8	2.1
49	2.7	1.8	1.8	1.9	1.9
50	2.1	1.5	1.3	2.2	2.7
				Mean	2.1
				SEM	0.05

Appendix E Brand of glue

Table 8 Time (sec.) spent on and distance (mm.) covered on the different glues by wasps of *E. formosa*.

	Glue			
	Soveurode		Tanglefoot	
	Time (sec.)	Distance (mm.)	Time (sec.)	Distance (mm.)
1	375	0	174	0
2	712	1	34	0
3	2330	52	45	0
4	1125	86	104	1
5	292	1	89	0
6	259	14	7	0
7	49	0	3	0
8	72	0	2	0
9	172	0	5	0
10	727	3	35	0
11	22	0	103	0
12	51	0	9	0
13	550	5	126	0
14	52	0	440	0
15	90	0	209	0
16	264	2	242	0
17	480	5	107	0
18	2175	14	15	0
19	75	0	176	0
20	168	1	279	0
21	2606	9	180	0
22	1007	63	256	0
23	29	0	217	0
24	1901	30	99	0
25	1549	37	101	0
Mean	685	12.92	122	0.04
SEM	114	3.25	15	0.03

Appendix F Toxic effect of glue

Table 9 Number of wasps emerged after 14 days with and without glue.

	With glue	Without glue
1	0	30
2	0	44
3	3	35
4	0	39
5	0	35
6	0	18
7	0	37
8	0	31
9	0	34
10	2	29
Mean	0.5	33.2
SEM	0.34	2.20

Appendix G Different kind of repellents

Table 10 Mean time (sec.) and mean distance (mm.) covered by wasps of *E. formosa* on the different repellents at day 1.

	Blistex		Chalk without tape		Chalk with tape		Vaseline		20 ml Vaseline + 6 drops eucalyptus oil		20 ml Vaseline + 60 drops eucalyptus oil		20 ml Vaseline + 600 drops eucalyptus oil		0.78 g Chalk + 6 drops eucalyptus oil	
	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)
1	4	0	36	2	0	0	14	0	10	12	2	0	17	0	2	2
2	4	1	21	2	358	1	7	1	30	16	9	40	3	0	3	0
3	2	1	10	0	0	0	21	40	29	31	13	22	0	0	1	0
4	2	1	49	3	0	0	38	23	24	28	12	11	0	0	2	1
5	4	0	636	38	0	0	0	0	0	0	10	13	5	0	5	1
6	2	0	26	1	0	0	36	4	0	0	24	39	2	0	4	1
7	1	0	258	6	0	0	31	40	23	6	15	40	3	0	14	4
8	5	2	498	7	0	0	2	1	6	8	48	18	2	0	4	3
9	3	5	3	1	5	1	151	4	7	5	16	4	2	0	3	0
10	3	0	33	1	30	3	0	0	4	2	2	1	1	0	1	0
11	5	1	64	3	0	0	12	6	12	4	10	10	1	0	5	2
12	9	1	21	8	19	1	10	40	194	40	7	6	4	0	3	0
13	5	1	710	6	1	0	5	2	42	7	5	1	4	1	1	0
14	5	1	98	18	0	0	174	6	15	2	29	3	10	3	1	0
15	4	0	101	10	0	0	5	7	5	1	24	4	0	0	38	1
16	4	1	2	0	180	2	12	40	64	40	4	1	3	0	1	0
17	2	1	23	3	0	0	264	7	12	5	13	9	0	0	1	0
18	4	3	4	0	0	0	272	40	32	31	7	0	31	17	2	1
19	1	0	76	4	3	0	17	14	9	2	35	37	7	1	1	0
20	3	1	66	5	0	0	73	10	125	22	4	1	2	0	1	0
21	4	0	33	2	0	0	4	4	18	1	2	0	1	0	4	1
22	4	0	4	0	0	0	61	36	29	19	0	0	6	5	2	0
23	2	1	10	2	0	0	264	17	8	4	12	11	2	1	2	0
24	2	0	730	40	0	0	9	1	14	3	3	2	7	1	1	0
25	15	1	6	3	0	0	5	6	3	1	13	4	1	0	1	0
26	1	0	5	0	0	0	14	10	67	23	33	0	156	21	1	0
27	3	1	11	1	0	0	67	3	15	14	8	0	12	4	1	0
28	3	1	90	2	94	1	2	2	8	3	16	7	5	4	1	0
29	4	3	33	2	0	0	9	6	144	40	7	3	12	5	1	0
30	3	2	227	7	0	0	197	16	7	1	4	1	1	0	1	0
31	9	2	127	3	19	2	36	12	68	6	4	0	224	11	35	3
32	2	1	78	2	0	0	21	40	35	11	5	1	0	0	1	0
33	14	2	1	0	0	0	45	0	48	32	10	1	6	1	10	3
34	5	0	79	3	0	0	30	1	21	14	31	17	2	0	2	0
35	6	1	89	5	0	0	91	31	0	0	3	1	0	0	1	0
36	5	1	61	1	0	0	109	40	33	15	68	2	21	1	1	0
37	3	0	413	7	136	1	7	1	5	4	13	4	25	0	1	0
38	4	0	1	0	0	0	17	3	39	19	3	2	4	0	1	0
39	5	0	25	1	0	0	373	34	2	1	3	2	30	2	1	0
40	7	1	61	6	0	0	3	1	11	5	11	3	1	4	1	0
41	3	0	3	0	0	0	5	2	90	13	17	4	0	6	1	0
42	5	0	27	1	0	0	9	1	28	7	19	6	1	1	1	0
43	5	1	148	2	0	0	29	15	126	23	17	5	0	3	2	1
44	6	0	20	2	145	4	10	11	11	7	102	6	0	13	1	0
45	5	0	315	17	0	0	3	4	111	40	8	2	150	0	1	0
46	7	1	90	5	0	0	26	10	33	5	6	2	0	0	1	0
47	4	0	100	7	13	2	5	2	16	6	123	24	1	0	1	0
48	2	1	40	3	0	0	26	11	36	7	5	2	2	1	2	0
49	4	0	24	1	0	0	22	13	73	6	4	0	5	1	1	0
50	6	0	9	2	0	0	13	14	6	1	60	11	0	0	1	0
Mean	4.4	0.8	111.9	4.9	20.1	0.4	53.1	12.6	35.0	11.9	18.0	7.7	15.4	2.1	3.5	0.5
SEM	0.39	0.14	25.48	1.13	8.82	0.12	11.96	1.97	5.93	1.72	3.43	1.55	6.03	0.62	1.02	0.14

Table 11 Mean time (sec.) and mean distance (mm.) covered by wasps of *E. formosa* on the different repellents at day 14.

	Blistex		Chalk without tape		Chalk with tape		Vaseline		20 ml Vaseline + 6 drops eucalyptus oil		20 ml Vaseline + 60 drops eucalyptus oil		20 ml Vaseline + 600 drops eucalyptus oil		0.78 g Chalk + 6 drops eucalyptus oil	
	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)	Time (sec)	Distance (mm)
1	2	0	72	3	1	0	3	1	1	0	11	10	12	0	1	0
2	6	5	11	1	1	0	15	10	2	1	9	3	13	17	1	0
3	5	0	15	5	2	1	25	9	27	40	6	2	8	1	1	0
4	4	0	59	5	4	0	12	2	22	40	7	8	23	0	2	0
5	93	17	27	5	1	0	7	2	30	40	5	2	18	0	1	0
6	13	3	785	40	1	0	33	40	49	25	79	15	37	40	1	0
7	12	2	321	40	1	0	31	37	14	10	5	4	28	12	1	1
8	68	4	498	7	4	0	35	40	28	21	34	22	17	40	49	3
9	13	2	5	1	11	1	4	1	9	10	11	13	12	15	15	1
10	5	2	32	1	8	0	1	0	30	11	33	8	1	0	53	7
11	10	3	68	5	1	0	20	8	16	40	1	0	7	8	1	0
12	6	2	33	9	1	0	31	15	18	2	201	18	11	0	23	2
13	6	7	51	8	1	0	23	10	29	40	23	40	44	18	2	0
14	2	0	800	20	1	0	39	40	11	1	60	5	7	0	5	0
15	9	6	5	1	74	1	15	7	1	0	88	1	3	1	2	0
16	17	10	140	4	16	1	16	11	131	40	5	0	17	13	1	0
17	3	3	36	4	114	1	2	1	211	1	2	0	16	27	11	0
18	9	2	8	2	3	2	5	0	17	8	159	40	166	12	20	12
19	12	4	80	9	155	1	1	0	1	0	13	7	3	2	29	7
20	5	0	82	6	1	1	10	0	2	1	7	4	6	0	1	0
21	10	2	3	1	10	4	7	1	20	8	4	2	5	0	48	9
22	7	5	9	2	81	2	104	35	20	25	13	5	53	22	23	0
23	8	4	10	2	62	8	155	10	4	2	10	2	10	9	1	0
24	4	0	8	5	1	0	56	38	269	35	7	3	39	28	105	2
25	5	3	9	4	24	5	3	2	9	10	23	14	27	4	1	0
26	6	0.0	924	40	22	4	22	28	34	40	9	6	7	1	21	3
27	13	2.0	11	1	130	8	30	40	9	3	3	5	22	8	3	0
28	7	0	90	2	1	0	40	33	6	2	10	14	42	18	2	0
29	2	2	33	2	2	0	55	34	14	5	2	0	9	10	12	0
30	6	1	227	40	1	0	1	0	3	1	208	6	15	40	2	0
31	5	5	127	3	1	0	44	40	1	0	243	15	15	31	63	11
32	12	3	78	2	1	0	5	1	1	0	15	3	14	10	1	0
33	7	1	1	0	1	0	6	2	1	0	247	11	18	24	15	1
34	11	2	79	40	1	0	16	40	18	1	34	18	19	20	1	0
35	9	0	89	5	1	0	19	40	49	40	70	23	3	0	4	0
36	5	2	61	1	33	1	224	16	20	32	90	40	9	11	2	0
37	9	1	413	40	81	6	5	0	8	5	3	1	8	13	1	0
38	20	2	1	0	1	0	13	4	11	19	16	2	21	40	1	0
39	24	15	28	3	124	8	74	38	23	27	30	11	31	12	1	0
40	4	1	75	9	10	2	13	19	28	33	13	1	17	23	1	0
41	10	0	5	2	1	0	13	17	103	21	139	39	33	8	2	1
42	6	1	39	6	5	0	15	22	18	8	24	2	22	40	1	0
43	1	0	233	6	1	0	15	11	65	13	28	3	3	0	246	1
44	3	0	55	6	53	5	137	21	17	9	56	5	33	40	53	6
45	4	0	486	40	7	1	21	16	3	1	55	22	1	0	1	0
46	6	2	99	8	22	1	11	21	16	11	4	2	6	3	5	0
47	6	1	156	9	1	0	140	40	34	40	5	1	17	0	1	0
48	5	0	55	6	5	0	8	10	85	40	9	1	14	40	1	0
49	2	0	30	3	1	0	5	2	38	31	5	6	6	10	45	13
50	3	0	17	5	5	0	15	2	55	36	59	11	44	40	25	7
Mean	10.4	2.5	131.6	9.4	21.8	1.3	32.0	16.3	32.6	16.6	43.9	9.5	20.2	14.2	18.2	1.7
SEM	2.18	0.49	30.37	1.83	5.51	0.32	6.38	2.18	7.13	2.23	9.06	1.55	3.47	2.00	5.55	0.49

Appendix H Color

Table 12 Percentage of wasps attracted to a color for 18 repetitions.

	Nothing	Green	Yellow	White	Skin colour
1	30	45	5	20	0
2	30	5	5	0	60
3	35	20	0	5	35
4	30	30	5	15	20
5	40	45	0	0	15
6	45	20	5	0	30
7	40	15	5	0	40
8	25	20	15	15	25
9	25	10	10	0	55
10	70	10	5	5	10
11	40	30	5	5	20
12	30	15	15	10	30
13	35	35	20	0	10
14	25	30	30	10	5
15	45	5	15	0	35
16	65	15	0	5	15
17	55	20	0	20	5
18	45	5	5	20	25
Mean	40	21	8	7	24

Table 2.1 Different flight tests and their properties.

	Flight test	T (ºC)	Duration (days)	Length of cylinder (cm)	Place of repellent	Type of repellent	Trap method	Material of cylinder	Correspond with Figure	Card glued to bottom
A	1 (n = 10)	13	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	1 (n = 10)	18	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	1 (n = 10)	23	14	21	near top	Blistex	Soveurode	Soft Plastic	2.1	Yes
	2 (n = 10)	13	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	2 (n = 10)	18	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	2 (n = 10)	23	14	12	near top	Blistex	Soveurode	Strong Plastic	2.2	Yes
	3 (n = 10)	13	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	3 (n = 10)	18	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	3 (n = 10)	23	14	21	on thread	Blistex	Yellow sticky trap	Strong Plastic	2.3	Yes
	4 (n = 8)	23	14	20	near top	Blistex	Soveurode	Glass	2.4	Yes
B	5 (n = 10)	13	18	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	5 (n = 10)	18	18	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	5 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	Yes
	6 (n = 10)	13	18	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	6 (n = 10)	18	18	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	6 (n = 10)	23	14	12	middle	Blistex	Soveurode	Strong Plastic	2.6	Yes
	7 (n = 10)	13	18	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	7 (n = 10)	18	18	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	7 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Soft Plastic	2.5	Yes
	8 (n = 5)	23	14	20	near top	Blistex	Soveurode	Glass	2.4	Yes
C	9 (n = 10)	13	19	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	9 (n = 10)	18	19	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	9 (n = 10)	23	14	21	middle	Blistex	Yellow sticky trap	Strong Plastic	2.5	No
	10 (n = 10)	13	19	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	10 (n = 10)	18	19	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	10 (n = 10)	23	14	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	11 (n = 5)	23	14	20	near top	Blistex	Tangle Trap Paste	Glass	2.4	No
D	12 (n = 5)	23	14	12	middle	Blistex	Tangle Trap Paste	Strong Plastic	2.6	No
	13 (n = 5)	23	14	12	middle	Chalk	Tangle Trap Paste	Strong Plastic	2.6	No
	14 (n = 5)	23	14	12	middle	Chalk with eucalyptus	Tangle Trap Paste	Strong Plastic	2.6	No