The influence of foliar pubescens on searching activity of the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) on different poinsettia (*Euphorbia pulcherrima* Willd.ex Koltz.) cultivars and temperatures.

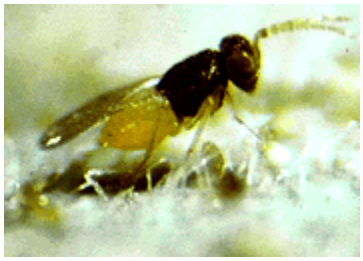
And

The influence of temperature in parasitization of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) by the whitefly parasitoid *E. formosa* on the poinsettia cultivar Lilo.

Påvirkningen av hår tetthet på søke adferden for *Encarsia fromosa* Gahan (Hymenoptera: Aphelinidae) på ulike julestjerne (*Euphorbia pulcherrima* Willd.ex Koltz.) kulturer og temperaturer.

Og

Påvirkningen av ulike temperaturer på *E. formosa* ved parasitering av *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) nymfer på julestjerne kulturen Lilo.



Cand. scient. thesis
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Preface

My interest for insects led me to Planteforsk and Nina Svae Johansen. She was to begin work on a new project in January 2002 (Biological control of *B. tabaci* in poinsettia), and she kindly invited me to participate in a part of this venture. The opportunity to take part in a larger project, seeing it through from beginning to almost the end was a hugely beneficial and enjoyable experience for me as I had never done this before. A new computer program, EthoVision, was to be used, and a considerable amount of my time was spent acquainting myself with, experimenting with and implementing this program, before I could commence with my own (behaviour) experiments. This thesis is composed of two parts. In part one I worked with EthoVision and in the second I undertook the experiments.

Unfortunately, due to a broken collar bone in winter 2003, I was unable to begin my thesis as scheduled and subsequently had to delay my post-graduate thesis for two months.

I would like to thank Nina Svae Johansen for her proposal of the problem, for her encouragement and support during this process. Toril Sagen for technical assistance, providing me with poinsettia cultivars and for maintaining the *B. tabaci* colonies. Anette Sundbye for undertaking a substantial amount of the first experiment and allowing me to use her results, and for her guidance. Eline Hågvar for reading, guidance and giving me advice for writing my thesis. Roar Moe for responding my question about poinsettia production in Norway.

I would also like to thank all my friends for encouraging me, reading my thesis and advising me. I would like to thank Nina Gullan for her language support.

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CONTENTS

Abstract

One of the factors that can cause problems in the biological control of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on poinsettia (*Euphorbia pulcherrima* Willd.ex Koltz.) by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), is the leaf trichomes, another is the temperature. Two experiments were conducted to examine these factors. The objective of the first study was to determine whether a difference in hairiness of poinsettia plants affects the foraging behaviour of the parasitoid *E. formosa* on clean leaves, at two temperatures. A second experiment was carried out on poinsettia leaves with *B. tabaci* as hosts, where parasitization and feeding by *E. formosa* was determined at two different temperatures.

Two poinsettia cultivars with different trichome density were used in the first experiment, Lilo (2029, 5 trichomes per cm^2) and Sonora (449, 0 trichomes per cm^2). The experiment was carried out at two different temperatures (18 and 20 ºC). One inexperienced, less than 24 hour old *E. formosa* female was individually video tracked for 7 minutes on a clean poinsettia leaf. The different foraging behaviour was measured using the automated video tracking program called EthoVision (walking velocity, walking activity and turn angle). Analysis of the searching behaviour of *E. formosa* showed that this parasite was not influenced by the trichome density on leaves, however a significant effect was found between the two temperatures for walking velocity at both poinsettia cultivars. A rise in temperature from 18ºC to 20 ºC leads to a higher walking velocity and it increases with 26, 5 % on Lilo, from 0, 444 mm/s to 0, 604 mm/s. For Sonora the walking velocity increased with 18, 9 %, from 0, 421 mm/s to 0, 519 mm/s. For the other parameters, the walking activity was found to be between 80 and 90 %, and the turn angle between 23 and 26 degrees for both poinsettia cultivars and temperatures. There were no significant differences within these two last parameters.

In the second experiment, the parasitization rates for *E. formosa* at *B. tabaci* on poinsettia leaves were studied. Host feeding was also examined. Only one poinsettia cultivar (Lilo) was used, although two temperatures (18 ºC and 21 ºC) were used during the experiments. A significant difference in parasitization ratio of *B. tabaci* was found between the two temperatures. Average percentage *B. tabaci* nymphs parasitized by *E. formosa* on each leaf increased from 1, 7 % to 4, 3 % at a rise in temperature from 18 ºC to 21 ºC.

The increase in walking velocity due to temperature rise may be of importance for biological control in greenhouses where temperatures are at 18 – 21 ºC for several hours most days. A higher walking velocity in *E. formosa* results in a higher parasitization of *B. tabaci.* It seems that a high temperature in greenhouses can leads to a better control of *B. tabaci* with use of *E. formosa*.

The consequences of the results of the searching behaviour of *E. formosa* on poinsettia cultivars are discussed later in the paper in regards to the possibilities of biological control on this ornamental plant.

Sammendrag

En av de faktorene som kan bli et problem i biologisk bekjempelse av *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) på julestjerner (*Euphorbia pulcherrima* Willd.ex Koltz.) med *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), er blad behåring, en annen faktor er temperaturen. To eksperimenter ble utført for å undersøke disse påstandene. Målsettingen med det første eksperimentet var å bestemme om ulikheter i hår tetthet på julestjerne planter påvirker søke adferden for parasitoiden *E. formosa* på rene julestjerne blader. Første forsøk ble gjennomført ved to temperaturer. Det andre eksperimentet ble utført på julestjerne blader med *B. tabaci* som verter. Her ble parasitering og vert spising av *E. formosa* studert ved to ulike temperaturer.

I det første eksperimentet ble det brukt to ulik julestjerne kulturer med forskjellig hår tetthet på bladene, Lilo (2029, 5 hår/cm^2) og Sonora (449, 0 hår/cm^2). Forsøkene ble utført ved to ulike temperaturer (18 og 20 ºC). En uerfaren, mindre enn 24 timer gammel *E. formosa* ble filmet 7 minutter alene på et julestjerne blad uten vertsdyr. De forskjellige søkeadferdene som: gang hastighet, gang aktivitet og retnings vinkel i gang mønstret, ble målt ved bruk av et automatisk video søke program, kalt EthoVision. Søkeadferden for *E. formosa* på Lilo og Sonora ble ikke hindret av hår tettheten på bladene, men en signifikant effekt ble funnet mellom de to temperaturene for gang hastigheten på begge julestjerne kulturene. En økning fra 18 til 20 ºC i forsøket, førte til en høyere gang hastighet. For Lilo var økningen på 26, 5 %, fra 0, 444 mm/s til 0, 519 mm/s, for Sonora var økningen på 18, 9 %, fra 0, 421 mm/s til 0, 519 mm/s. For de andre parametrene ble gang aktiviteten målt til mellom 80 og 90 %, og retnings vinkel lå på mellom 23 og 26 grader for begge julestjerne kulturene og temperaturene. Det var ingen signifikant forskjell innen de to siste parametrene.

Parasiterings prosenten for *E. formosa* på *B. tabaci* nymfer på julestjerne blader ble studert i det andre eksperimentet. Vert spising ble også undersøkt. To temperaturer (18 og 21 ºC) og en julestjerne kultur (Lilo) ble brukt i forsøket. En signifikant forskjell i parasiterings raten ble funnet mellom de to temperaturene. Gjennomsnitt i prosent *B. tabaci* nymfer parasittert av *E. formosa* på hvert blad økt fra 1, 7 % til 4, 3 % ved en økning i temperaturen fra 18 til 21 ºC.

Økningen i gang hastigheten i henhold til temperatur økningen kan være en viktig faktor i biologisk bekjempelse av *B. tabaci* i drivhus, hvor temperaturer på 18 til 21 ºC forekommer flere timer daglig. En høyere gang hastighet for *E. formosa* resulterte i en høyere parasitering av *B. tabaci* nymfer. Det ser ut til at en høy temperatur i drivhus kan føre til en bedre kontroll av *B. tabaci* ved bruk av *E. formosa*.

Konsekvensene av resultatene for søkeadferden for *E. formosa* på julestjerne kulturer blir diskutert senere i oppgaven med hensyn til en mulig biologisk bekjempelse på disse prydplantene.

1. Literature review

Poinsettia

History

Figure 1. The poinsettia cultivar Cranberry Punch, Photo: Ecke ranch (Ecke, 2003)

The poinsettia, *Euphorbia pulcherrima* Willd (figure 1), is a tropical plant indigenous to Mesoamerica and tropical Mexico, and belongs to the *Euphorbiaceae* family.

In the early 1900s, the Ecke family of southern California grew poinsettias outdoors for use as landscape plants and as cut flowers. Eventually the family grew poinsettias in greenhouses and is today recognized as the leading producer of poinsettias in the United States (31 University of Illinois). Across the world, in countries such as USA and Germany, poinsettia is one of the main pot plants (Vik, 2003). Total U.S. poinsettia production was valued to $325 million in 1997 (Ecke, 2003), and in Germany 26, 6 million plants were sold in 2000, not including imported poinsettia plants that year (Vik, 2003).

Norwegian Poinsettia production

Poinsettias were introduced in Norway towards the end of the1950's (Schulze, 2003), and the production has increased every year since (Statistisk sentralbyrå, 1999). In 1998, 4 843 000 million plants of poinsettia were produced in Norway for further culture (cuttings) and 5 774 000 million plants were ready for sale. 845 000 mini – pot plants of poinsettia were

ready for sale (Statistisk sentralbyrå, 1999). The majority of the production takes place in the east of the country (Vestfold, Østfold and Oslo and Akershus) and in the western counties of Norway (Statistisk sentralbyrå, 1999). Every year Norway imports 1 million cuttings (both rooted and not rooted), a proportion of this is to renew the assortment and to use in sales (Roar Moe, pers. comm.). Norwegian inhabitants purchase approximately six million poinsettias per anumm (Salvesen, 2003).

There are many different poinsettia cultivars with a great variety of colours e.g. red, pink, white and purple. The most significant cultures in Norway are: Millenium, Cortez, Prestige, Primero, Freedom, Cortez White and Lilo (Roar Moe, pers. comm.).

Culture practice

In order to produce a fine and strong poinsettia, there are a number of factors to be considered. Two morph types of poinsettia cultivars are grown commercially (Stimart, 1983); one is a restricted-branching morph type characterized by a strong apical dominance and few axillary's shoots and bracts. The other is a free-branching morph type characterized by a weak apical dominance and many axillary's shoots and bracts. When growing pinched and non – pinched crops of self – branching poinsettia cultivars, there are some differences due to temperature and day length. The first 2 to 4 weeks after potting, 18 hour light is needed to promote good vegetative growth, before SD (short day). High irradiation is needed to improve the quality by increasing the florescence diameter and number of bracts. In the following 10 weeks the plants need SD. SD is required to promote the flowering of poinsettia and its floral development (Vik, 2003). The temperature of the greenhouse interacts with daytime temperature and is decreased during the following weeks. Throughout bract expansion and development a normal temperature is maintained. (24-26°C during the day, 18-19°C at night for most cultivars)(Ecke, 2003). Temperature requirements differ from cultivar to cultivar, failure to adhere to these differences result in small leaves and bracts, uneven length of lateral shoots and a poor plant quality. High temperature (22 ºC) is recommended in the vegetative period, followed by a decrease in temperature, resulting in a temperature of approximately 16 ºC at the marketing stage. For the first 2 – 4 weeks a temperature of 22 ºC is required up to the start of SD (If no SD treatment the following is needed; temperature decline to 17 – 19 ºC in greenhouses where the cultures are produced by naturally SD). Then 4 weeks at 20 ºC, followed by 18 ºC for 2- 4 weeks, ending with 16 ºC for a 4 week period. Approximatly12

weeks are needed to complete a poinsettia culture, depending on the type of poinsettia. Some of the newest cultivars require a lower temperature when the plants are established, Cortez and Freedom in particular (Roar Moe, pers. comm.). The greenhouses are fitted with high pressure sodium lamps, e.g. SON – T bulbs can be used, giving a light intensity (irradiation) of approximately 10 000 lux at plant level (Strømme, 1994). A balanced fertilizer regime is important to achieve a successful plant growth and the temperature should not exceed 32 ºC, during the daytime (Ecke, 2003).

Poinsettia is attacked by various pests which need to be controlled. In poinsettia crops cultivated in Norway, the most common insect pests are whiteflies (*Aleurodidae*) e.g. *Bemisia tabcai* (Gennadius) (Homoptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), and sciarid flies (*Bradysia* spp.) (Strømme, 1994).Other pests found in USA, are *Frankliniella minuta*, melon aphid *Aphis gossypii*, green peach aphid *Myzus persicae*, citrus leaf miner *Phyllocnistis citrella* and serpentine leaf miner *Liriomyza trifolii* (Ecke, 2003). Buprofezin was previously used against the whitefly *B. tabaci* in Norway, but it is now prohibited. Ecke (2003) mentions some other insecticides that can be used against whiteflies. They act well against *B. tabaci*, which have yet to develop resistance to the active components of the insecticide. The insecticides referred to are: dimetoat, fenpropatrin, imidakloprid, lambda – cyhalotrin and pyriproxyfen (Ecke, 2003). They are all approved for use in Norwegian greenhouses (Mattilsynet, 2003a). In the past adult *B. tabaci* were easily killed with insecticides, but pesticide resistance in *B. tabaci* populations is a common problem faced by many growers today.

Diseases often found in Poinsettia are:

- grey mould *Botrytis cinerea* Pers. Ex Nocca & Balb. (the most destructive disease for the poinsettia).
- *Phytophthora*, *Pythium*.
- *Chalara elegans* Nag Raj & Kendrick/*Thielaviopsis basicola* (Berk. & Broome) Ferraris (this black root rot has been an important disease of poinsettia).
- *Rhizoctonia solani* Kühn –Black scurf, (a root and stem rot disease).

The most important virus facing the poinsettia is the poinsettia mosaic virus, and the most significant amongst the bacterial diseases is the soft –rot bacterium *Erwinia carotovora*

subsp.*Carotovora* (Jones) which is common in Norway (Strømme, 1994). There exist several pesticides in Norway for use against the afore mentioned diseases (Strømme, 1994). The use of pesticides in general is very restricted in Norway due to strict legislation. A large number of the pesticides used in other countries are prohibited in Norway and extensive use has lead to resistance in some insect pests. As a result biological control and IPM (integrated pest management) has increased.

Bemisia tabaci

Biology of *B. tabaci*

The development of *B. tabaci* consists of: an egg stage, four nymphal stages and the adult stage. Eggs are light yellow-green and later turn light brown. The first nymphal stage, called crawlers, is transparent and small (0, 25 mm in length), later the nymphs turn yellow and become larger, 0, 50 mm in length (3rd nymphal stage) (figure 2b). The last nymphal stage is often referred to as the pupa, and is rather flat and transparent or yellow in colour. The red eyes of the adult within the pupae are clearly visible. The adult female is slightly larger than 1 mm in length, the males slightly less than 1 mm. The body remains light yellow with a light dusting of wax (figure 2a.) (Malais & Ravensberg, 1992).

a)  **b)** 

Figure 2. a) *B.tabaci* adult. Photo: Scott Bauer, (2004) (Naranjo *et al.* 2004). **b)** *B. tabaci* nymphs, 3rd, 4th nymphal stages and empty nymphal shell. Photo: University of California. (Anonymous, 2003)

B. tabaci has an arrhenotokous parthenogenetic reproduction where virgin females can only lay haploid eggs that give rise to males. Mated females can produce haploid and diploid (female) eggs. *B. tabaci* adult lifespan is strongly dependent on temperature (table 1) and the

nature of the crop. Optimal development temperatures are 25-30 ºC. Females deposit their eggs on the underside of leaves. Winged adults can move freely over the whole plant (Malais & Ravenberg, 1992).

The *B. tabaci* is a highly polyphagous insect, and has more than 500 host plant species, with a preference for cotton, beans, sunflower, aubergine, potato, capsicum, tobacco, tomato, *Citrus* and various ornamental plants (Fraval, 1998). The consumption of plant sap and secretion of honeydew (which can cause growth of sooty mold) (Fraval, 1998) by *B. tabaci* results in crop damage. Symptoms of damage on plants includes the appearance of chlorotic patches, yellowing, fruit –and leaf –fall and the veins of young leaves turning yellow, giving rise to "mosaic vein" (Malais & Ravenberg, 1992). *B. tabaci* is a feared carrier of viruses, transmitting more than 25. Particularly tomato yellow leaf curl virus (TYLCV), which is a persistent virus causing serious diseases, mainly in tropical crops. Females are better vectors than males. In contrast to the smooth cultivar, highly hairy cotton cultivars support larger populations of *B. tabaci* (Heinz & Zalom 1995).

Table 1. The population growth of *B. tabaci* on poinsettia at different temperatures (Enkegaard, 1993).

	Temperature (ºC)				
	16	19	22	25	28
Development time (days)					
Egg	34,3	17,6	12,7	10,5	7,8
Egg-adult	137,2	66,8	38,7	31,9	23,2
Po-period (days)*	4,3		3,0		2,2
Lifespan ♀	50,8		21,8		16,0
Mortality (%)					
Egg	19,3	9,2	2,1	1,0	2,8
Egg-adult	95,0	60,4	60,6	39,3	6,1

* po-period = pre-oviposition period, *i.e.* period from becoming adult to first egg-laying

Encarsia formosa

The aphilinid *Encarsia formosa* Gahan (Hymenoptera: Aleyrodidae) is used worldwide for commercial control of whiteflies in greenhouse crops (Hoddle, *et al.*1998a). Its exact origin is unknown though it is thought to have originated from a tropical or subtropical region. It is assumed that *E. formosa* comes from the same areas as its host *T. vaporariorum* (tropical or subtropical America), and are now found in Europe, Australia, New Zealand, Japan, Canada

and the United States (Malais & Ravenberg, 1992). *E. formosa* was identified from specimens reared from an unknown aleyrodid on geranium (*Pelargonium* sp.) in 1924 in a greenhouse in Idaho (USA) (Gahan, 1924). Morphological description of all life stages are provided by Speyer (1927).

Biology of *E. formosa*

E. formosa are primary thelytokus parasitoids, i.e. females are produced parthenogenically on a phytopagous host insect, like *B. tabaci* and *T. vaporariorum* (van Lenteren *et al.*1996). They are also solitary (only one *E. formosa* emerge from one nymph) and endoparasitoid, (lays one egg inside a host insect) (Hoddle *et al.* 1998a). The *E. formosa* females are small (0, 6 mm long) (Sutterlin & van Lenteren, 1996; Sutterlin & van Lenteren, 1997), the head and thorax are black and the abdomen is yellow (figure 3). The males are completely black and very rare. Males develop when an *E. formosa* lays an egg hyper parasitically in an *E. formosa* larva and is readily distinguished by the dark abdomen (Hussey, *et al.* 1969). Enkegaard (1993) was assuming a sex – ratio for the progeny of *E. formosa* on *B. tabaci* of 1:0 (♀: ♂) when constructing life tables (Enkegaard, 1993).

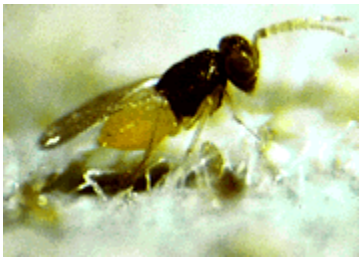


Figure 3. *E. formosa* adult female. Photo: Mark Hoddle. (Hoddle *s. a.*)

Host location and acceptance

For *E. formosa* to successfully reproduce, it must first locate potential hosts, approve host quality, and use nymphs appropriate for host – feeding or parasitism. *E. formosa* has at least 15 host species in eight aleyrodid genera which it can successfully parasitize (Hoddle, *et al.* 1998a). The wasp searches to find infested plants, infested leaves and whitefly patches via random flight, landing and walking, without visual or olfactory cues (Hoddle, *et al.* 1998a &

van Lenteren, *et al.* 1976). However a recent study from Birkett *et al.* (2003) indicate that flight searching of *E. formosa* is regulated by compounds from the plant – host complex, as olfactory cues for host location. Bean plants (*Phaseolus vulgaris*) infested with the greenhouse whitefly (*T. vaporariorum*) were used in this study (Birkett, *et al.* 2003). More experiments are considered necessary to see whether olfactory cues have an impact or not on the *E. formosa* searching behaviour on other plant – host complex. After landing, the wasps search for hosts by randomly walking, while drumming with their antennae on the leaf surface until they hit a host. van Lenteren *et al.* (1996) found that *E. formosa's* searching is random on all levels, and after a host has been found and assessed the search pattern does not alter (Lenteren, *et al.* 1976 & van Lenteren, *et al.* 1996). It appeared that *E. formosa* is not capable of locating an individual host from a distance, and it may pass *T. vaporariorum* at a range of less than one mm and yet does not show any obvious reaction to it (Lenteren, *et al.* 1976). Walking velocity on the leaves might therefore be important for host finding and effectiveness in biological control. The searching behaviour of *E. formosa* may be influenced by hair densities on the leaf surface (Hulspas-Jordaan & van Lenteren, 1978). van Lenteren *et al.* (1995) found that the parasitoid turns more on hairy leaves, which can lead to searching on previously searched places. This may cause longer host searching. Hairy leaves contains more honeydew excreted from whiteflies than smooth leaves, parasitoids then, run more often into honeydew. Contacts with droplets of honeydews, makes parasitoids spend more time preening otherwise they drown and die in the liquid. Time spent preening, can not be used for searching hosts (Lenteren, *et al.* 1995). When an *E.formosa* encounters antennal contact with the host, it will inspect the host with antenna and/or ovipositor. *E. formosa* can reject the host after antennal contact (antennal rejection) or after ovipositor contact (ovipositor rejection, may shows as black spots). A host previously parasitized and/or too small nymphal may be a cause of rejection. When the ovipositor is inserted into the host, it can be accepted for oviposition or for host feeding (Sütterlin & van Lenteren, 1999 & van Lenteren, *et al.* 1980).

Encarsia walking pattern and velocity on infested leaves is the same as on uninfected leaves and is not affected by host encounters (Hoddle, *et al.* 1998a). However when a host is encountered, Sütterlin & van Lenteren (1993) observed that *E. formosa* remain at least twice as long on infested *Gerbera* leaves than on uninfected leaves.

When *E. formosa* reproduce they prefer the 4th nymphal stage, the pupa of *B. tabaci* for oviposition followed by the 3rd and 2nd nymphal stage (Enkegaard, 1993). There are few

experiments undertaken to prove how *E. formosa* determine host size (Hoddle, *et al.* 1998a). With her ovipositor, an egg is placed inside the host nymph, and 14 -16 days thereafter (temperature dependent) the parasitized *B. tabaci* nymph becomes transparent brown (Stenseth, 1991). An unparasitized nymph will remain yellow. Oviposition lasted for four minutes for *E. formosa* on *B. tabaci* (Enkegaard, 1993). *E. formosa* females are synovigenic which means that females mature eggs during their adult life (Qiu *et al.* 2004), the maximum number of eggs that can be laid is dependent on the number of ovarioles. *Encarsia* species are also anautogenous, which means that females do not have mature eggs in their ovaries at emergence (Qiu *et al.* 2004). After sunrise and if hosts are abundantly available, the first batch of about 8 eggs is laid within one hour. The temperature during the day is important for egg laying and influences how many eggs that will mature. The period of active searching, and thus how many eggs might be laid, are determined by length of day light (van Lenteren *et al.* 1992) and number of ovariols. The searching time decreases with decreasing egg load (van Lenteren *et al.* 1996). The parasitoid usually shows no host searching behaviour for one to several hours after all mature eggs have been laid. In order for the parasitoid to produce additional matured eggs, a certain amount of time must lapse before she is able to lay eggs again (van Lenteren *et al.* 1996). In the winter, if the greenhouse temperature is kept at 18 ºC - 20 ºC and the day length is less than 8 hours, egg maturation is slow. Less than 8 eggs per 24h will be laid by *E. formosa* (van Lenteren *et al.* 1992). *E. formosa's* egg laying capacity is at a maximum at 25 ºC, and stops when the temperature falls beneath 12 ºC or rises above 40 ºC (Stenseth, 1991).

Whitefly (*T. vaporariorum*) life stage influences *E. formosa* mortality rates and developmental times. Nechols & Tauber (1977) found that parasite egg development and hatching can occur in all four whitefly nymphs of *T. vaporariorum.* When *E. formosa* parasitized 1st nymphal stages, ca. ¼ of the parasites survived to adult emergence. However, *E. formosa* development does not proceed past the 1st nymphal stage until after the host reaches 4th nymphal stage. *E. formosa* completes its life cycle and emerges as an adult from 4th whitefly nymph stage (Nechols & Tauber, 1977). Both fecundity and longevity can be affected by the host from which the wasps are reared (Hoddle *et al.* 1998a). Szabo *et al.* (1993) found a high mortality (40 – 56 %) of *E. formosa* when developed on either *B. tabaci* or *T. vaporariorum* on poinsettia. Developmental time for *E. formosa* with *B. tabaci* as a host on poinsettia decreases with increasing temperature. Egg to adult took from 2 months at a low temperature (16 ºC) whilst at a high temperature (28 ºC) it was completed within 14 days

(Enkegaard, 1993). 16 days can pass from the first nymphs were parasitized to when they turn brown in colour (Szabo *et al.* 1993). The adult longevity of *E. formosa* decreased with increasing temperature, from 1 month at 16 ºC to 9 days at 28 ºC (Enkegaard, 1993). It is important to have a good knowledge of the parasitoid development, when controlling whiteflies.

E. formosa are synovigenic (require a protein meal) and often host – feed in order to mature a full complement of eggs (Gerling *et al.* 2001). Killing a host for nutritional purposes is termed host feeding. Adult wasps obtain energy and nutrients by consuming honeydew and hemolymph from hosts. *E. formosa* will host feed on pupae and all nymphal stages of *B. tabaci*, with no apparent preference of certain stages (Enkegaard, 1993). To host feed, *E. formosa* wounds nymphs or pupae by probing with the ovipositor for up to 6 min and then feeds from the wounds (Hoddle *et al.* 1998a). This feeding kills the host. Nymphs that have been used for feeding are not used for oviposition and host feeding never occurs on a parasitized host (Nell *et al.* 1976).

Greenhouse temperatures influence the flight activity of *E. formosa*. The correlation is such that at a lower temperature fewer *E. formosa* engage in flight activity than at a higher temperature. The normal greenhouse temperature during poinsettia production commences at 22 ºC and by the end of the production time, it has declined to 16 ºC (Strømme, 1994). Despite the fact that the lowest average temperature is 16 ºC, flight activity has been registered right down to 13 ºC (Hoddle *et al.* 1998a). van Lenteren *et al.* (1995) also found that *E. formosa* was capable of foraging at that temperature.

Biological control of *B. tabaci* with *E. formosa*

Speyer (1927) was the first to attempt to develop biological control in commercial greenhouses during the 1920's. He used the parasitoid *E. formosa* as a control for the whitefly *T. vaporariorum*. In the post-war era, the development of DDT and other effective insecticides reduced the whitefly problem, and the use of parasitoids almost disappeared. No further development in biological control took place until the 1950's (Hussey *et al.* 1969). Today *E. formosa* is used worldwide for the commercial control of greenhouse whitefly *T. vaporariorum* on greenhouse grown vegetable crops (Enkegaard, 1990; de Courcy & Wright

1999; Hoddle *et al.* 1997a). It has been an exceptional success (Parrella *et al.* 1991), and *E. formosa* is the most important species commercially applied in augmentative releases against whiteflies (van Lenteren *et al.* 1996).

Use of *E. formosa* to control *B. tabaci* has shown mixed results. In Europe, parasitoids of the genera *Encarsia* and *Eretmocerus* are among the natural enemies used for the biological control of *B. tabaci.* They have received the most attention (de Courcy &Wright 1999) followed by predator bugs of the genera *Macrolophus* or *Dicyphus* and the insect pathogenic fungus *Verticillum lecanii* and the fungus genus *Aschersonia* (van Lenteren *et al.* 1996). *Dicyphus* and *Aschersonia* are not approved for use in Norway. *B. tabaci* is not the optimal host for *E. formosa*. In particular, its small size does not suit the normal development of the wasp. In a choice situation, *E. formosa* will always favour parasitizing *T. vaporariorum* before *B. tabaci* (Malais & Ravenberg1992). Work done by Hoddle & van Driesche (1999) shows that *Eretmocerus eremicus* Rose & Zolnerowich effectively can control *B. argentifolii* (*B. tabaci* strain B) on poinsettia (Bellows *et al.* 1994; Hoddel *et al.* 1998b & Johansen, 2003). Furthermore they concluded that *E. formosa* was an ineffective natural enemy for the control of *B. argentifolii* (Hoddle & van Driesche, 1999). Hoddle *et al.* (1998a), states that at present the biological control of whiteflies on ornamentals with *E. formosa* is not commercially feasible. However during a 2 year trial experiment in 1988-89, Benuzzi *et al.* (1990) proved that *E. formosa* was effective when used in the biological control of both *B. tabaci* and *T. vaporariorum* on poinsettia. Boisclair *et al.* (1990) predicted that the control of *B. tabaci* might be gained through the repeated introduction of *E. formosa* during the cropping season. Stenseth (1993) found that a dosage of 1, 3 *E. formosa* per plant in mother plants of poinsettia and repeated releases throughout the crop period, controlled the *B. tabaci* to a level that was found to be acceptable. At the end of the period, the whitefly population tended to increase, possibly because the plants were too large for the parasitoids to search for whitefly nymphs (Stenseth, 1993). A life table analyses done by Hoddle *et al.* (1997b), showed that *E. formosa* Beltsville strain released at 1 wasp per plant per week, (low release rate) and 3 wasp per plant per week (high release rate) exerted a suppressive effect on *B. argentifolii* population growth on poinsettia, when compared with control plants in the greenhouse that did not receive the parasitoid.

The release of *E. formosa* into greenhouses for whitefly control can be done using four distinct methods. Three of these ("pest in first", "dribble", and "banker plant") are inoculating

in nature and establish a reproducing parasitoid population, after which releases are discontinued (Hoddle *et al.* 1998a).

Pest in first method: Adult whiteflies are introduced into the crop at a fixed rate. *E. formosa* is later introduced when the whitefly nymphs begin to develop at regular intervals (1 to 3 times). This method has not been widely approved because of the concern of releasing pests into the crop (Hoddle *et al.* 1998a).

Dribble method: Weekly parasitoid introductions begin at planting or when whiteflies are first observed. This because one expects a development of whitefly population. The regular introduction of parasitoids continues until high levels of parasitized whitefly pupae exist in the crop (Hoddle *et al.* 1998a).

Banker – plant method: Established colonies of parasitoids reard on whitefly infested plants are introduced into the crop. Banker plants are introduced at a fixed rate. Mesh screens can be used to cage banker plants to contain whiteflies, allowing the smaller adults of *E. formosa* to disperse into the crop to feed and parasitize the host (van Lenteren *et al.* 1997 & Hoddle *et al.* 1998a).

After an inoculated introduction of *E. formosa,* whiteflies are usually not eliminated, however fluctuations will occur in the host and parasite stock (Stenseth, 1991). This is mostly used in the growth of vegetables.

The fourth method, inundative programs require regular releases of high numbers *E. formosa*. Establishment and reproduction of the parasitoid in the crop are not expected. This method occurs most frequently in ornamental crops (Hoddle *et al.* 1998a), and is more likely to be used against cotton whitefly than greenhouse whitefly (Stenseth, 1991).

E. formosa is readily available in Norway as black parasitized pupae glued on cards (En – strips). This is imported from Koppert, Holland and distributed through LOG. Other importing firms are Vekstmiljø and Fruktlager Handel AS (Mattilsynet 2003b).

2. Introduction.

The subtropical and tropical pest, the cotton whitefly, *Bemisia tabaci*, has recently beome a serious pest affecting the greenhouse crops of the temperate regions in Europe and USA. It is especially attracted to ornamental crops (Enkegaard, 1993) such as poinsettia (*Euphorbia pulcherrima*), hibiscus and gerbera (*Gerbera jamesonii*) (Enkegaard, 1994). *B. tabaci,* was introduced to Norway in 1987, and is a well-known threat to poinsettia in the greenhouse environment (Johansen, 1997 & 2004). Whiteflies are the major arthropod pest of poinsettia (Heinz & Parrella, 1994a) and they can damage much of commercial poinsettia production. Adult whiteflies prefer young poinsettia leaves for feeding and oviposition (Noldus *et al.*1986). Preliminary damage is a reduction in the aesthetic quality of the crop. Whiteflies harm the host plant by sucking plant sap on the leaves and secreting honeydew, which in turn gives rise to the growth of sooty mould. All this reduces its market value (Enkegaard, 1990 & Heinz & Parrella, 1994b). Whiteflies can also serve as vector carriers for several viruses which can cause serious diseases on plants. In greenhouses' insecticides have been the primary agent used against *B. tabaci*, but this whitefly has recently developed resistance to many of them, and is therefore hard to control. Pesticide use may also lead to decimation of natural enemies, and may create other pest problems (van Lenteren & Noldus, 1990). Alternatives to conventional chemical control are therefore necessary (Heinz & Parrella, 1994b). Pesticide use has begun to decrease, and biological products are more widely used. Further basic research is therefore essential, to improve biological control or to develop other control methods (van Lenteren & Noldus, 1990). It is important to increase the use of biological control agents that can effectively control the pest, and that are safe for the environment whilst being acceptable to farmers and greenhouse growers (Heinz, 1995). Biological control agents are imperative in the management and controlling of the whitefly due to the development of resistance to them.

Encarsia formosa has for a long time been applied as an agent against *Trialeurodes vaporariorum. E. formosa* has been used in several greenhouse vegetable crops, e.g. tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and eggplant (*Solanum melongena* var. *esculenta*). It has also been tested in several ornamental plant species like gerbera, poinsettia, marigolds (*Tagetes erecta*) and strawberry (*Fragaria x ananassa*) (Hoddle *et al.* 1998a). The different plants have different qualities as host plants, such as dissimilar trichome

density, this leads to differences in whitefly population growth (Hulspas-Jordaan van Lenteren 1978).

E. formosa show different degrees of control in different crops. The searching behaviour of *E. formosa* may be influenced by length, thickness and density of the trichome (van Lenteren *et al.* 1976 & 1995), leaf venation (van Lenteren *et al.* 1976), contact with honeydew (Gerling, 1990 & van Lenteren *et al.* 1995), nymphs suitable for host feeding or parasitism (van Lenteren *et al.* 1976) and temperature (van Roermund & van Lenteren, 1995). Microclimate in the hair layer may also influence the walking velocity. In cucumber crops, the control has been moderately successful, but in tomato plants, it has given good results (Hulspas-Jordaan & van Lenteren 1978). Hua *et al.* (1987) found that *E. formosa*, controlling *T. vaporariorum* on cucumber, will be able to find more whitefly nymphs on a less hairy cucumber leaf than on a hairy leaf in the same amount of time (Hua *et al.* 1987). Sütterlin & van Lenteren (2000) found that the difference in leaf hairiness of *G. jamesonii* did not influence parasitism at a low host density, but at a high host density. There was a higher rate of parasitization on less hairy leaves. van Lenteren *et al.* (1995) stated that hair may drastically slow down the walking velocity of *E. formosa* on cucumber leaves.

Both Boisclair *et al.* (1990) and Szabo *et al.* (1993) found that parasitoids reared from *B. tabaci* have lower fecundity and longevity compared to wasps reared from the larger host *T. vaporariorum*. They also found that the number of ovipositions and host feedings are similar for parasitoids developed on both host species (Boisclair, 1990 & Szabo, 1993). They had an opinion that *E. formosa* could not be used exclusively to control *B. tabaci*, because it is not adequately sufficient. On the other hand, an inundative release of *E. formosa* during cropping season has the ability to obtain control of *B. tabaci* (Boisclair *et al.* 1990 & Szabo *et al.* 1993). Henter *et al.* (1993) stated that the parasitization rate for *E. formosa* on *B. tabaci* is dependent on the wasp species from which it is reared upon from the start. Henter *et al.* (1993) found a lower parasitization ratio for *E. formosa* when first reared on *T. vaporariorum* before being tested on *B. tabaci*, compared to when it was first reared on *B. tabaci*. Enkegaard (1993) found that the possibility of using *E. formosa* to control infestations of *B. tabaci* on poinsettia seemed to be very good when the temperature was within the range of 16 ºC - 28 ºC. This is the normal temperature in greenhouses growing poinsettia in the temperate regions. Even at low temperatures when the development of the parasitoid is typically slow, the biological control of *B. tabaci* with *E. formosa* was good (Enkegaard, 1993).

When using *E. formosa* in the biological control of poinsettia, it is necessary to understand the degree to which the wasp can move upon the (hairy) leaf. If it is exceedingly difficult to move upon the leaf (either too hairy or smooth), it can become a problem when host searching, and lower the chances of parasitization. The availability of hosts and the temperature also have an effect on *E. formosa's* behaviour on plant leaves. All of these factors may present serious problems for pest management and the gardener's cultivation of plants.

The aim of this experiment was to study how the influence of the hairiness of poinsettia cultivars and different temperatures affected the walking velocity, walking activity and turn angle of the parasitoid *E. formosa*.

In the first study, clean poinsettia leaves were used. Six poinsettia cultivars were first tested for differences in trichome density and length. The two poinsettia cultivars that differed most in hair density upon the leaves, (Lilo and Sonora) were compared to see whether foraging behaviour of the parasitoids differed. The parameters used in the analysis were; walking velocity, walking activity, and turn angle. In a non-experimental environment the greenhouse temperature would be expected to vary between 15 ºC and 25 ºC when growing poinsettia (Strømme, 1994). For this study only the effect of temperatures at 18 ºC and 20 ºC were tested on the foraging behaviour of *E. formosa* on clean poinsettia leaves. This was done in laboratory conditions. To determine whether an effect of temperature on searching behaviour would affect the effectiveness of the parasitoid, I also measured the rate of parasitization and feeding at *B. tabaci* at two different temperatures.

3. Material and methods

The first experiment took place in May – June 2002 at The Norwegian Plant Research Institute (Planteforsk) undertaken by Anette Sundbye and myself. The second was carried out during the autumn of 2003 and spring 2004 at SKP, Centre for Plant Research in Controlled Climate (Senter for Klimaregulert Plantelaboratorie) a modern centre with climate regulated laboratory compartments.The third took place in June and July 2004 at Kirkejordet south, also SKP. The second experiment was carried out in a non climate regulated room, although an air conditioner & dehumidifier machine were used. All experiments where undertaken in Ås, Norway.

Material

1. Plant material

Six cultivars of poinsettia were used for measuring trichome density and length; Sonora, Cortez, Millennium, Primera, Snowcap and Lilo. The plants were grown in greenhouse compartments at a temperature of 24 ºC, L18:D6 and 70% RH (relative humidity), the light intensity was measured at 10 000 lux. Each plant cultivar had a minimum of seven full-grown leaves and was one month old after potting, when used in the experiments.

2. Whiteflies

A laboratory culture of *B. tabaci* was maintained on poinsettia cultivars Lilo and Sonora in a glass cage inside a greenhouse compartment at 24 ºC, L16:D8 and 70 % RH. The *B. tabaci* culture used to infest the poinsettia plants originated from a culture at Ljones gartneri, and has been reared on the poinsettia plant at Planteforsk since 1998.

To produce infested poinsettia plants for use in parasitizing experiments, 10 *B. tabaci* female adults per poinsettia plant were put into cages with clean poinsettia, and kept there for three days. After approximately three weeks, the nymphs were of stage L3 – L4, the stages

preferred by *E. formosa* for oviposition. Leaves (between 6. and 10. leaf) were regularly removed, for use in this experiment.

3. Parasitoids

Whitefly pupae that were parasitized with *E. formosa* (En Strip) were obtained weekly on cards from "Koppert Biological Systems". The *E. formosa* was reared on *T. vaporariorum* feeding on tobacco, *Nicotiana tabacum* (Sütterlin, 2000). One strip was then placed in each of the glass vials, 10 cm by 2, 5 cm, figure 4. The parasitoids that emerged were given a droplet of honey that was placed on top of the glass, and a moist piece of cotton wool was placed at the bottom. *E. formosa* females, 0 – 24 hours old, were used in the behavioural and parasitizing experiments. The *E. formosa* culture was kept at a temperature of 25 ºC, and at L16:D8. *E. formosa* was used directly from the vials, and first walked on a poinsettia leaf during the behaviour and parasitizing experiments.

Figure 4. Cards (enstrip) with black parasitized whitefly pupae with *E. formosa* on vial. Photo: Benedicte Milton, 2004.

EthoVision

EthoVision is an automated video tracking, motion analysis and behaviour recognition system. The Ethovision program offers a wide range of video tracking options, extensive analysis of locomotory tracks, and automatic behaviour recognition (Noldus Information Technology 2002). The video camera used was a Panasonic super Dynamic II, WV-GP460 and the monitor was a Panasonic WV-CM1480. The video camera converts the arena (scene) into an image, and is then passed to the frame grabber in the computer. One video image from the camera is called a frame. EthoVision transforms the analogue video signal into digital video signal and each frame is converted to a bitmap composed of a grid of pixels. The brightness of each image is represented as a grey value between 0 (black) and 255 (white), (grey scaling). EthoVision has to distinguish a group of adjacent pixels from the background, to identify the object. The method described above converts each video frame of the moving parasitoid into a series of numbers, x, y coordinates and the parasitoids size. This raw data is used in the Analysis module of EthoVision to produce a series of parameters describing the behaviour of the *E. formosa*. It is possible that some pixels will be identified as being objects that are just system noise, or reflections. These can be excluded by defining an arena (pixels outside the arena are ignored) or by making various settings to exclude noise. More information under item 4 in the results chapter and the EthoVision Reference manual version 2.3 (Noldus Information Technology 2002).

This system is used throughout all of the experiments.

Experimental set-ups

Experiment 1: Leaf hair density and length

Ten plants from each of the six cultivars were randomly chosen. The seventh leaf, counted from the youngest unfolded leaf of the rosette, was taken from each of the poinsettia plants, i.e. 60 leaves in total. Three leaf discs with a diameter of 4 mm where taken from each poinsettia leaf (from the top, centre and base of the leaf, figure 5) (at each plant and

analyzed). A total of 180 leaf discs were analyzed. The hair density was determined by counting the number of trichomes on the underside of the leaf discs using a light microscope (Leitz DM RB), at a magnitude of x80; aided by the data program Leica Q 500 MC.

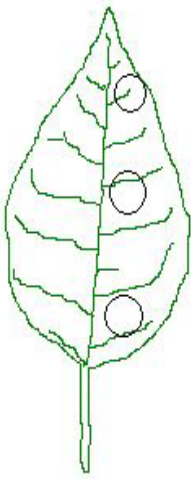


Figure 5. A poinsettia leaf, the black circles indicate where the three leaf discs were taken from.

The same leaf discs used for the registration of leaf hair density were also used for the registration of hair length. The length of ten random hairs from each leaf disc was measured with the use of the same light microscope and data program as for the hair density registration.

Differences in trichome length and density between cultivars were tested with an ANOVA, Fishers pair wise comparison test $p<0,05$.

Experiment 2: *E. formosa's* behaviour without hosts

At two temperatures, two cultivars representing opposite trichome density were selected to find out whether a deviation in these would lead to differences in parasitoid behaviour. Based on the length and density of the leaf trichomes (from experiment 1.), Lilo and Sonora were chosen: Lilo (with a high hair density of 2029,5 trichomes per cm^2 and long hair (18,0 µm)) and Sonora (with a low hair density of 449,0 trichomes per cm^2 and medium long hairs (11,8 µm)). Leaves from Lilo and Sonora where regularly harvested from the poinsettia culture for use in the experiment. The experiments were carried out at 20 ºC (± 0,5 ºC) and 18 ºC (± 0,5 ºC). When observations were carried out at 20 ºC, the vials with *E. formosa* were placed at 20 ºC for 30 minutes to acclimatize the parasitoids, the procedure was repeated at 18 ºC. The

seventh leaf, counted from the youngest unfolded leaf of the rosette, was cut in half, (along the vein), and placed adaxial on a Perspex plate. Another plate, with a rectangle cut out in the middle, the arena (2.5 x 2.0 cm), and with a Petri dish over the arena, tagged with tape, was placed on top of the leaf together with an *E. formosa* (figure 6). Every recording was done with a fresh leaf without hosts and one new individual, naïve (inexperienced), less than 24-hour-old parasitoid.

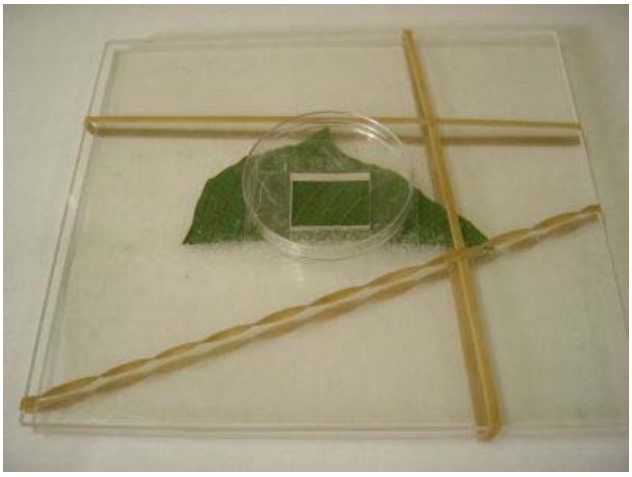


Figure 6. The Perspex with a leaf, the Petri dish is laying over the square in the middle, which is the arena. Photo: Benedicte Milton, 2004.

The Perspex was mounted with the leaf upside down on a burette holder, (figure 7a and figure 8). The whole setup was hidden from the rest of the room by a box with curtains, (figure 7b). To measure light intensity a Quentum/Radiometer/Photometer: model LI – 185B was used. The Perspex plate was standing 20 cm below the light source, and about 4000 ± 213 (n=10) lux was measured above the leaf. Light intensity 0,50 cm below the leaf measured 274 ± 53 (n=10) lux.

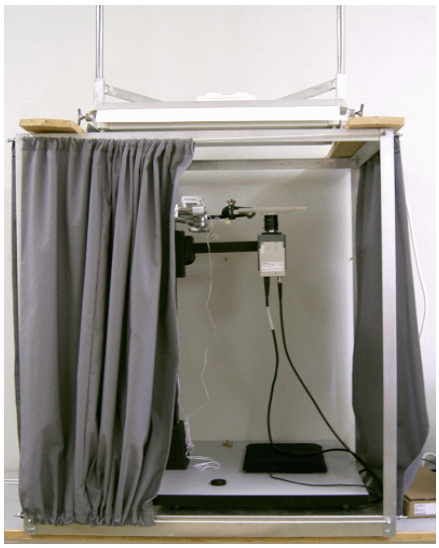
a)  **b)** 

Figure 7.**a)** Camera with the Perspex and **b)** Camera set up. Photo: Benedicte Milton, 2004.

The behaviour of *E. formosa* was videotaped through the Petri dish lid, while the parasitoid was walking on the leaf. The tracking and the camera signal was transferred to the computer that controls the EthoVision software. The walking behaviour was recorded for a maximum of seven minutes per parasitoid. Recording was started simultaneously with the start of each test. If the *E. formosa* stood still for more than one minute at the beginning of the test, walked on the Perspex plate or the Petri dish for longer than two minutes, after the recording had started, the recording was stopped and the procedure was repeated. For both cultivars and temperature, 40 or more searching tracks of *E. formosa* were analyzed from the behavioural observation in EthoVision.

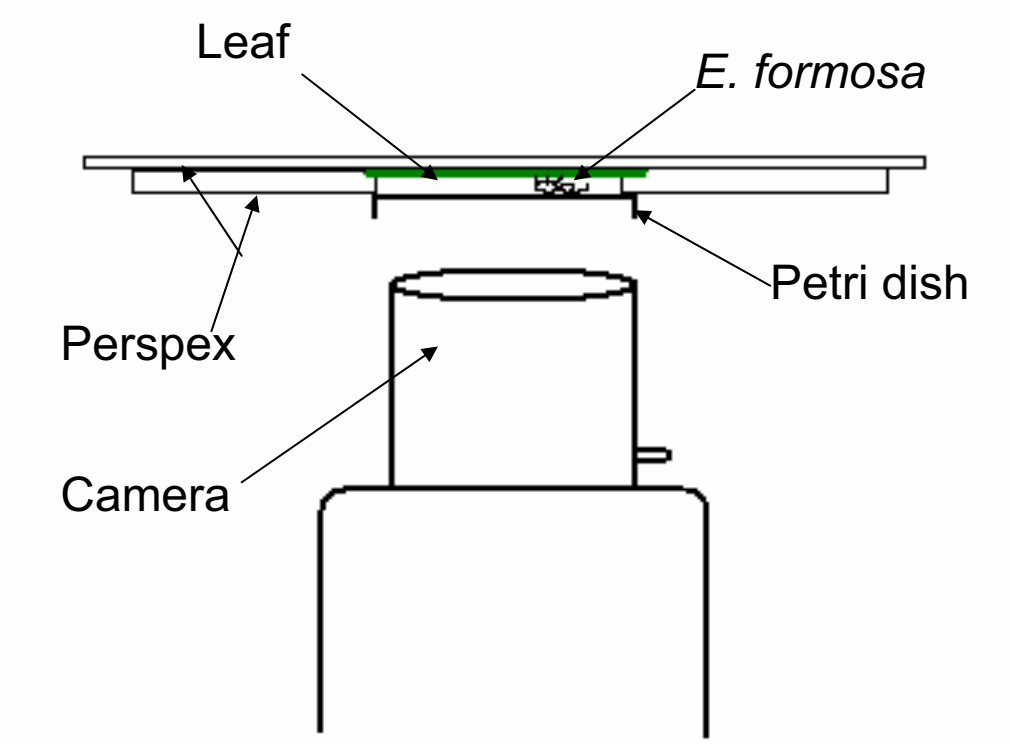


Figure 8. A drawing of the camera with the Perspex, the drawing does not indicate the actual proportions.

The parameters analysed were: walking velocity, turn angle, distance moved and walking activity. A definition of the mentioned terms is as follows:
Walking velocity: the distance moved per unit time while the parasitoid regularly touches the leaf with the antennae.
Turn angle: defined as change in the direction of an object's movement between two following samples.
Walking activity: defined as percentage time spent walking while drumming on the leaf surface with the antennae, compared with the total time spent on the leaf.

When the setup was ready, as in figure 7a, the definition of arena and zone had to be programmed into the EthoVision program. A fixed arena was used; it was on average 4,66 cm^2. Inhibited by the Perspex plate, the zone was somewhat smaller inside the arena. A zone is used to avoid the edge effect when the *E. formosa* is walking along the Perspex plate.

Before starting each trial, some acquisition settings had to be set. These settings remained constant except for "Update detection variables" which were changed at the onset of each individual trial. This incorporated updating the grey scale thresholds, setting low and high limits, between 0 - 130 were used. For object detecting, grey scaling was used. It distinguishes

between the background and the object. Sample rate, was set to 2,27 samples/second. The sample rate is the maximum image capture rate. With noise removal, it is possible to prevent objects that are the wrong size from being detected; this was not incorporated in all of the trails. Two image filtering settings, erosion and dilation were used to improve the differences between objects and non-objects. Erosion was set to 1 (a layer of pixels is removed) and Dilation was set to 3 (a layer of pixels is added). For these experiments I have used the settings erode and then dilate. This filter is used to avoid miscalculation of the *E. formosas'* position and to eliminate the detection of thin objects, such as antenna. Once the acquisition settings are complete, the experiment can commence.

To measure the walking velocity, turn angle, and walking activity parameters, on the hairy leaves, it was necessary to decide how long the steps of the parasitoids' were. The minimum distance moved was set to 0,03 cm, to prevent minor movements being recorded as authentic displacement. 0,03 cm was half the length of an *E. formosa* female and is supposed to be its average step length (Hua, 1987). Therefore, EthoVision did not start recording before the *E formosa* had moved 0,03 cm. In that way one avoided recording when the parasitoid stood still or was grooming. This input filter was used for all the three parameters. For walking activity there were two options, one that included start and stop velocity, and the last was averaging interval. Averaging interval was used to reduce the sensitivity of the parameter to short changes in velocity. The data can be smoothed by taking the running average of the last "n" sample. This number is referred to as the averaging interval and was set to 1 sample. This means that the average velocities are calculated from the 1st sample onwards. On the background of the visual examination of five tracks, start and stop velocity was set to 0,30 mm/s and 0,10 mm/s respectively. The EthoVision would not measure any data until these options were fulfilled. Differences in velocity, activity and turn angle between the cultivars and temperatures were tested with the Mann – Whitney test (p=0.05).

Experiment 3: Parasitization of *B. tabaci*

This experiment compares the ability of the *E. formosa* to parasitize whiteflies on Lilo at different temperatures. Two other elements of behavior were observed and recorded. One leaf (between 6.-10. leaves counted from the youngest unfolded leaf of the rosette) was taken from 30 Lilo plants. Each leaf was examined and the total number of living nymphs and eggs were

counted. At least ten whitefly nymphs in 3rd and 4th nymph stadium on each leaf were required for the experiment. One leaf was then placed inside a transparent box (5 x 8 x 12 cm) with the petiole in a vial with water, (figure 9). A single naïve *E. formosa* female, not older than 24 hours, was positioned on the leaf. 15 boxes were then placed at 18 ºC, and the rest at 21 ºC with L10:D14 and 70% RH. The wasp was removed after 48 hours, and the boxes remained at the two temperatures for possible *E. formosa* development inside whitefly nymphs. After 14 to 16 days the leaf was removed, and with the use of a stero microscope at a magnitude of x40, the following occurrences were recorded manually. 1) Parasitization – the *B. tabaci* pupae have turned transparent brown, 2) Host feeding/dead nymphs – the nymphs are hollow, some are loose from the leaf and transparent. This category contains all the samples in which natural mortality or host – feeding was found. 3) Black spot – the pupae were alive and had one or several black spots on the back. Black spots depict puncture marks. These show where the *E. formosa* have tried to oviposit, but not laid an egg. Only when a wasp has exhibited one or more of the aforementioned occurrences was it recorded.

Figure 9. Poinsettia leaf with *B. tabaci* nymphs with one *E. formosa*, inside a tight box, allowing air flow. Photo: Benedicte Milton, 2004.

Differences in parasitization of *B. tabaci* nymphs, host feeding and registration of black spots were measured using the Mann – Whitney test, in Minitab.

4. Results

Experiment 1: Leaf hair density and length

1. Trichome density and length

Leaf hair density and length for the different poinsettia cultivars are listed in table 2. A large variation in trichome density was seen among the tested cultivars. The average density of leaf hairs on Lilo was approximately 4,5 times higher than on Sonora. The trichome length on all poinsettia cultivars exceeds 0,6 mm, which is the length of *E. formosa*. Length of leaf hairs varied from 1,09 to 1,80 mm when outstretched. The hair length on the different poinsettia cultivars did not always correlate with hair density. Snowcap that had a high density of hairs had very short hair due to Lilo which had the same hair density, but longer hair. On the basis of trichome density, Lilo and Sonora were chosen for further experiments.

Table 2. Hair density and length (mm) on leaves from six different poinsettia cultivars. Standard deviation is given in brackets*.

Poinsettia cultivar	Number of hairs/cm^2 ± (StDev)	Hair length, mm ± (StDev)
Sonora	449,0 a (60,0)	1.18 ac (0,1)
Cortez	609,9 ac (257,1)	1,09 a (0,9)
Millenium	886,1 ac (237,3)	1,12 ac (1,2)
Primera	1251,6 c (250,7)	1,74 b (0,7)
Snowcap	2027,9 b (760,5)	1,28 c (0,7)
Lilo	2029,5 b (289,4)	1.80 b (0,2)

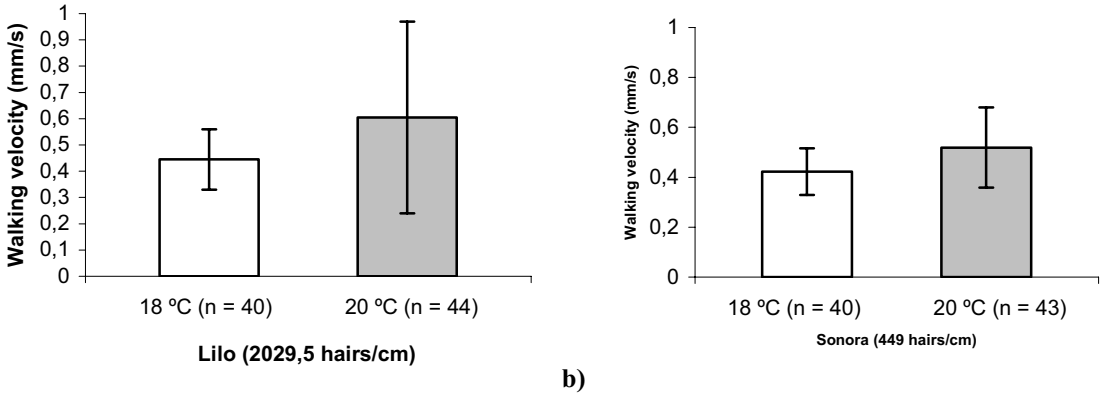
*ANOVA(Fishers pairwise comparisons p< 0,05, Minitab). Different letters indicate significant differences in hair density and hair length between cultivars.

Experiment 2: E. formosa's *behaviour*

1. *E. formosa's* walking velocity

The walking velocity measurements of *E. formosa* on the two poinsettia cultivars had a wide range. On Lilo at 18 ºC, *E. formosa's* walking velocity varied between 0,227 and 0,775 mm/s

(indicates mean velocity per female), at 20 ºC it varied between 0,256 to 2,091 mm/s.On Lilo this averaged out at 0,444 ± 0,115 mm/s (mean ± StDev) when tested at 18 ºC and 0,604 ± 0,365 when tested at 20 ºC respectively, for all the tested females (figure 10a). The variation in walking velocity for *E. formosa* on Sonora when tested at 18 ºC was between 0,220 and 0,732 mm/s and at 20 ºC the velocity was between 0,267 to 0,973 mm/s. This gave an average at 0,421 ± 0,094 mm/s at 18 ºC and 0,519 ± 0,160 mm/s at 20 ºC respectively on Sonora (figure 10b). Under these experiments the walking velocity of the insects on Lilo and Sonora; at 18 ºC was significantly lower than at 20 ºC ($p<0,05$). There was no significant difference in the walking velocity of *E. formosa* when tested on either Lilo or Sonora at 18 ºC or 20 ºC.



a) **b)**

Figure 10. The average walking velocity of *E. formosa* adult females on leaf discs on the poinsettia cultivars without hosts at 18 ºC (± 0,5) and 20 ºC (± 0,5). n are given between brackets. (Vertical bars: standard deviations). **a)** Lilo and **b)** Sonora

Figure 11 to 14 illustrates the breakdown of the frequency distribution (%) of *E. formosa* when searching at different velocities. The findings show that most females were found in the walking velocity group 0,30 – 0,40 mm/s for Lilo and Sonora when tested at 18 ºC. At 20 ºC there is no apparent highest frequency group, however higher velocities were more apparent.

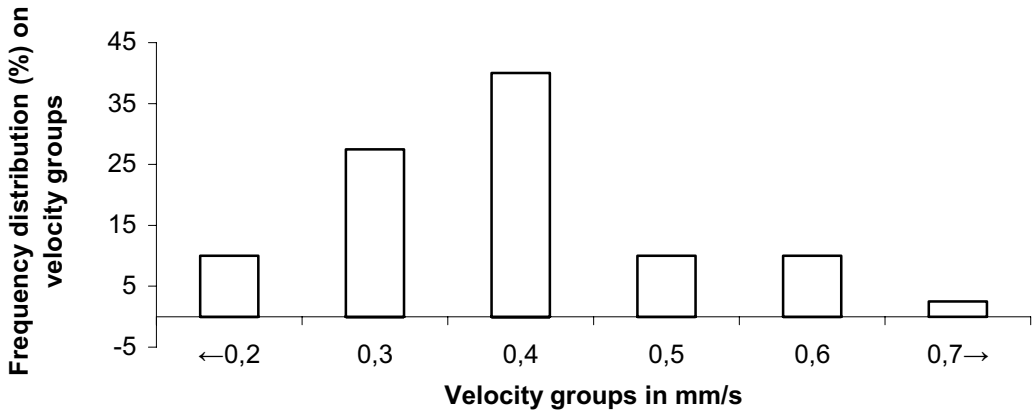


Figure 11. Lilo 18 ºC, frequency distribution (%) of walking trials falling into a certain velocity group. Group ← 0,2 and 0,7 → contain smaller and larger velocity measurements.

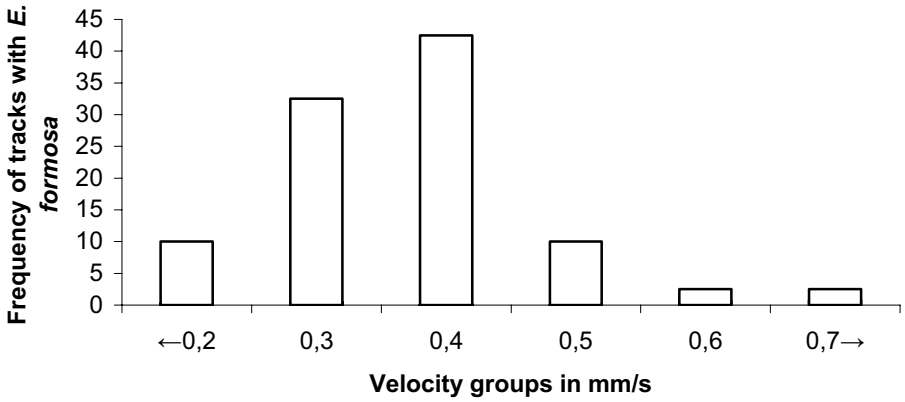


Figure 12. Sonora 18 ºC, frequency distribution (%) of walking trials falling into a certain velocity group. Group ← 0,2 and 0,7 → contain smaller and larger velocity measurements.

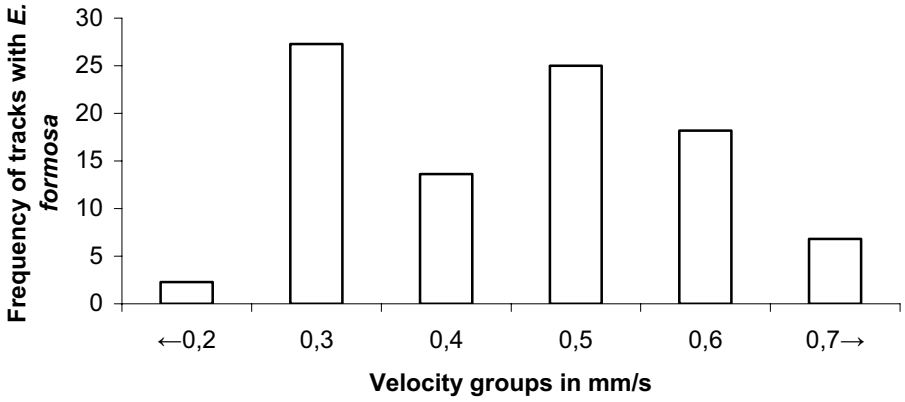


Figure 13. Lilo 20 ºC, frequency distribution (%) of walking trials falling into a certain velocity group. Group ← 0,2 and 0,7 → contain smaller and larger velocity measurements.

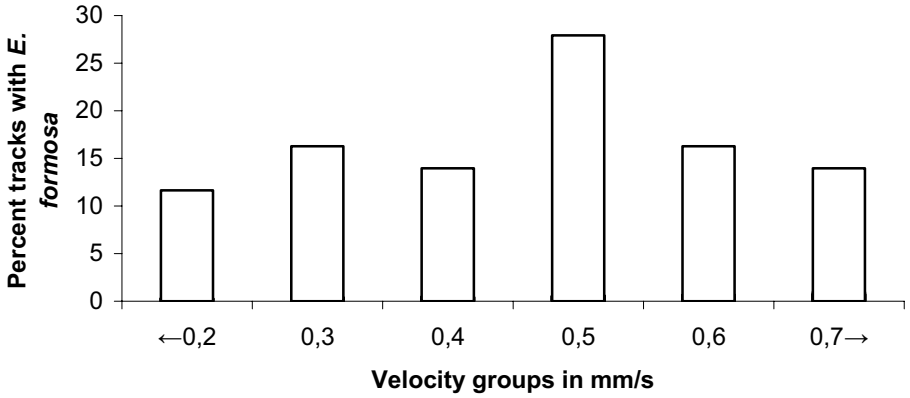
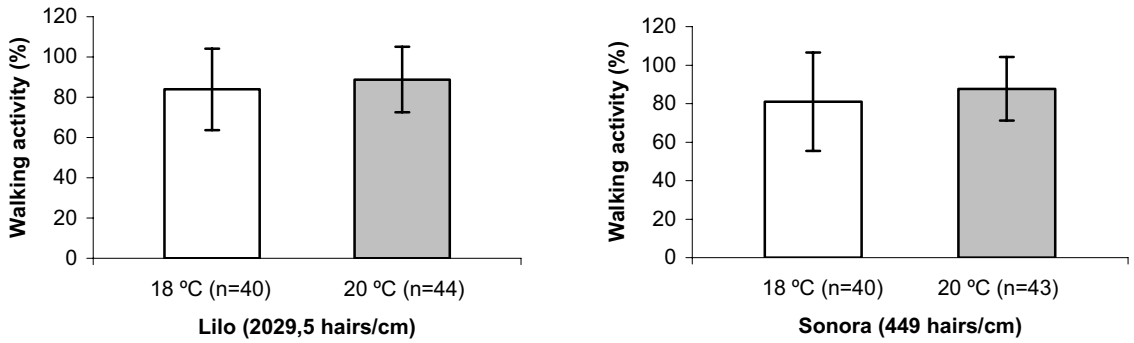


Figure 14. Sonora 20 ºC, frequency distribution (%) of walking trials falling into a certain velocity group. Group ← 0,2 and 0,7 → contain smaller and larger velocity measurements.

2. *E. formosa's* walking activity

Walking activity is expressed as the time walking while drumming on the leaf surface, i.e. searching time as a percentage of the total time spent on the leaf. Walking activity of the parasitoid on the two poinsettia cultivars had a huge variation, and ranged from very low, 2,73 % to almost 100 %. This was not dependant on the temperature or cultivars. Figure 15a and 15b show the average walking activity for all the measurements for Sonora and Lilo at 18 ºC and 20 ºC, which ranged between 80 and 90 %. The StDev ranged between 16 and 25 %. There were no significant differences in the walking activity of *E. formosa* between the two cultivars or the temperatures (Mann – Whitney test, p>0,05).



a) **b)**

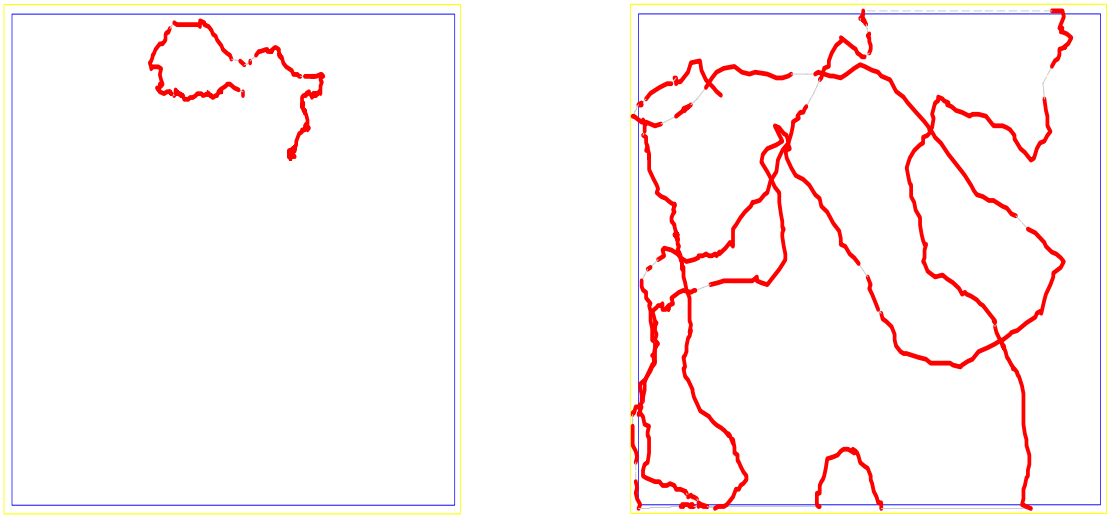
Figure 15. Average walking activity of *E. formosa* adult females on leaf discs on the poinsettia cultivars without host at 18 ºC (± 0,5) and 20 ºC (± 0,5). n is given in brackets. (Vertical bars: standard: deviations) **a)** Lilo **b)** Sonora

3. *E. formosa's* turn angle

Turn angle is defined as the changes in the direction of the movement of *E. formosa* between two samples. The turn angle for the parasitoid was pinnacle on Sonora at both temperatures; however there were no significant differences between Sonora and Lilo when tested at the same temperatures (Mann – Whitney test, $p>0,05$). The maximum turn angle for Sonora was 57,01 (20 ºC) and 53,62 (18 ºC). The maximum turn angle for Lilo was at 39,30 (20 ºC) and 36,36 (18 ºC) respectively. The average turn angle for both cultivars and temperatures lay between 23 and 26 degrees. A low turning rate indicates that the *E. formosa* walks mostly straight, whilst a high turning rate describes a direction of movement that turns more.

4. Analyzed data from EthoVision

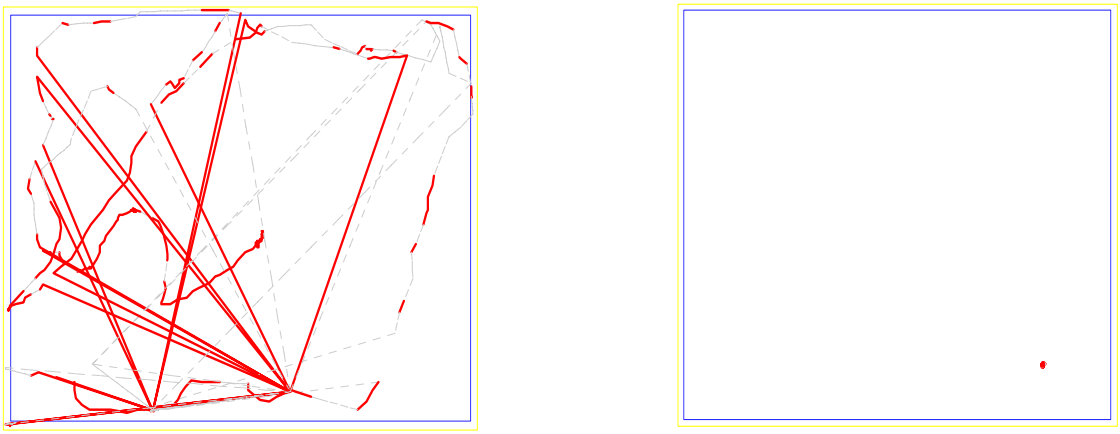
An example of a typical walking pattern on a poinsettia leaf is shown below, (figure 16). The yellow square stipulates the arena, and the blue square indicates the zone border. Figure 16a shows the walking trail for a wasp with low walking activity, figure 16b demonstrates a much higher walking activity. All the measurements were estimated inside the arena, walking velocity, walking activity, and turn angle. Average walking velocity was estimated on the whole track, not separate intervals within one track. The missing samples were not included in the measurements.



a) **b)**

Figure 16. Walking pattern of *E. formosa* on Lilo at 18 ºC without any host. The red solid line shows were EthoVision has registered the wasp walking, the black dotted line is missing samples. a) shows a *E. formosa* that have moved very little, track 4 in table 3, b) shows a very active parasitoid, track 11in table 3.

After running a set trails, some of the tracks are selected for further analysis, table 3 shows the statistical output for seven of eleven tracks, four tracks are excluded. The table is taken from the EthoVision program. The rejection of a track can be because of a lack of movement by the wasp (figure 17b), too many disruptive elements (figure 17a) or too much missing data within the samples. Track 4 and 11 from table 3 is showed in figure 16a) and b).



a) **b)**

Figure 17. a) A trail with much missing samples and disturbance, from a Sonora leaf at 20 ºC. **b)** A trail where the *E. formosa* have standing still, from a Lilo leaf at 20 ºC.

Table 3. Statistics output for some parameters for seven *E. formosa*. Each track contains the data recorded for one object in one arena during one trial.

Single actor parameters				
30/1-04 lilo 18 degree	Distance moved (cm) Total	Velocity (cm/seconds) Mean	Turn angle (degrees) Mean	Movement (Moving)(seconds) Total duration (%)
track_00001.trk	3,1863	0,0479	19,7555	98,5325
track_00003.trk	8,4574	0,0361	26,2721	94,13
track_00004.trk	1,8911	0,028	36,3636	19,2872
track_00007.trk	2,3115	0,0277	23,5913	37,8407
track_00008.trk	11,0998	0,0661	19,9251	97,3795
track_00010.trk	5,0055	0,0533	16,4851	97,065
track_00011.trk	14,6786	0,0613	22,3945	96,2264

Experiment 3: Parasitization of B. tabaci *nymphs*

Thirty experiments were conducted. Of these four leaves showed no signs of parasitization, no evidence of host feeding or black spots were found. Two leaves died before the experiment was finished. The poinsettia leaves had a wide distribution of *B. tabaci* nymphs, (1^{st} to 4^{th} and pupae); ranging from 14 up to 161. The average percentage of nymphs parasitized by *E. formosa* upon each leaf was 1,769 ± 1,964 (Mean ± StDev) at 18 ºC and 4,333 ± 3,155 at 21 ºC (figure 18) not indicative to the number of nymphs available. This difference is significant (Mann – Whitney $p<0,05$). Dead nymphs and nymphs that had been used for host feeding were registered on 15 out of 28 examining leaves. The average percentage of dead nymphs on each leaf was between 1,33 and 1,53 for both temperatures. Black spots were registered on 8 of 28 examined leaves, i.e. on 28,6 % of the leaves.

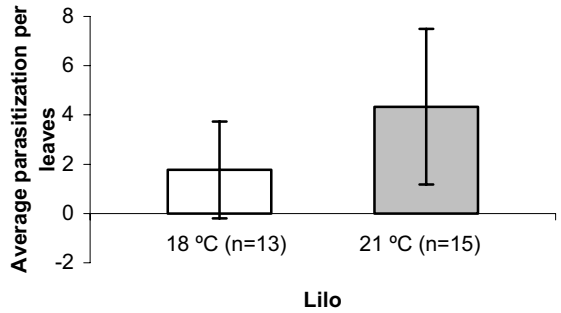


Figure 18. Average number parasitization of *B. tabaci* nymphs per leaf (Lilo), at two different temperatures. n is given in brackets. (Vertical bars: standard: deviations).

Number of nymphs parasitized by *E. formosa* compared to nymphs alive before the experiment began is shown in figure 19 and 20. Number of parasitized nymphs at 18 ºC, shows an exponential growth (figure 19), whilst the parasitization at 21 º C does not show any exponential growth.

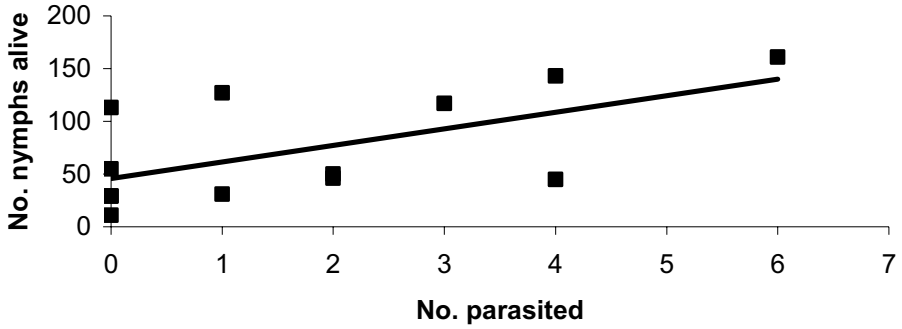


Figure 19. Number of nymphs alive compared to number of nymphs that got parasitized by *E. formosa*, on Lilo leaf at 18 ºC.

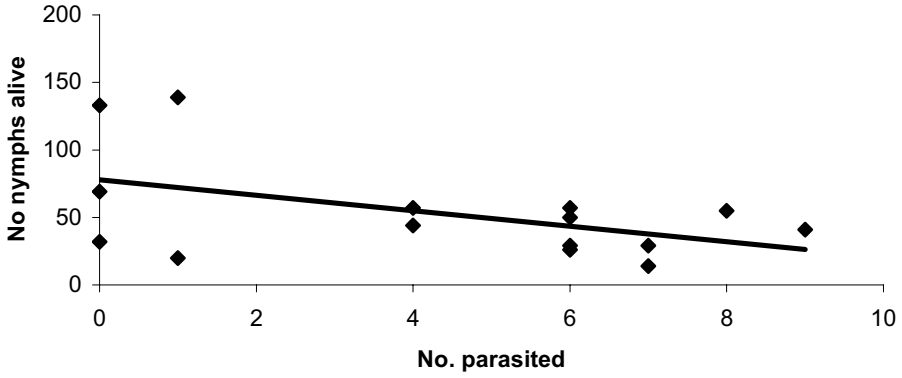


Figure 20. Number of nymphs alive compared to number of nymphs that got parasitized by *E. formosa*, on Lilo leaf at 21 ºC.

Table 1 and 2 in Appendix, shows all the raw material from the parasitization experiments, this was the basis for all the measurements in experiment 3.

5. Discussion

Some of the findings from this study are not in accordance with my hypothesis, that the searching efficiency of *E. formosa* on poinsettia is influenced by the trichome density on the leaves. The only factor that was found to affect the searching and parasitization behaviour was the temperature. Walking velocity and the parasitization ratio of the parasitoid was at its highest at 20 ºC and 21 ºC. In addition to these findings, a lower parasitization rate and walking velocity was found at 18 ºC. Trichome density on the leaf and different temperatures had no affect on walking activity and turn angle.

No significant difference in the walking velocity of *E. formosa* was found between the two poinsettia cultivars (Lilo and Sonora), these had different leaf trichome density. This is consistent with Sütterlin & van Lenteren (1997), who tested the walking velocity of *E. formosa* on ten cultivars of *Gerbera.* The trichome density ranged from 80 to over 1000 hairs cm^{-2}, and they found no differences in walking velocity. The average range of the walking velocity was small, and varied between 0,2 mm/s and 0,3 mm/s at 20 ºC (Sutterlin & van Lenteren, 1997). In my experiments the walking velocity was much higher compared to the findings of Sütterlin & van Lenteren (1996), and varied between 0,52 and 0,64 mm/s. No negative relationship between walking velocity and hair density was found on *Gerbera* and poinsettia, as oppose to the cucumber, where hairless, half – haired and hairy cultivars were tested. van Lenteren *et al.* (1995) found that *E. formosa*'s walking velocity decreased linearly with increasing hair density on cucumber hybrids, with a velocity range beginning at 0,62 and decreasing to 0,20 mm/s at 20 ± 1 ºC. The trichome density on cucumber hybrids used in that experiment ranged from 0 to 157,8 hairs/cm^2. On hairless sweet pepper plants, Sütterlin & van Lenteren (1996) found that the wasp, when tested at 20 ºC had a higher walking velocity, 0,73 mm/s. They also found that the walking velocity for *E. formosa* on tomato had the same velocity (0,31 mm/s) and temperature as on *Gerbera* cultivars. Tomato has a trichome density of above 1900 per cm^2 (Sutterlin & van Lenteren, 1996). Such a huge variation in *E. formosa* velocities between plants with different trichome densities is perhaps due to trichome shape. The cucumber has straight trichomes, whereas *Gerbera* has long trichomes that are curled over the leaves. On a *Gerbera* cultivar with 400 hairs cm^{-2}, the space between the hairs is on average 0,50 mm, which is less than an *E. formosa* (Krips, *et al.*1999). One can deduce from the above mentioned results that the velocity on the *Gerbera* is less than on cucumbers,

tomatoes and poinsettias'. The use of different programs and methods for measurements of walking velocity could be another reason for this. Sütterlin & van Lenteren (1996) have plotted the walking tracks of *E. formosa* on paper, the tracks were read as continuous 10 second interval pieces into a computer with an x-y digitizer. In this paper, all the measurements were set by the EthoVision program, excluding a few that were set manually. When comparing the *E. formosa* velocities on different plants with different trichome density, there is no apparent correlation seen in the results. (table 6). A last proposal was done by Sütterlin & van Lenteren (2000). They suggested that the parasitoids may walk on top of the "hair layer" of cultivars with high trichome density. They may therefore be hampered less than expected (Sütterlin & van Lenteren, 2000), and therefore gain a higher walking velocity.

Table 6. Walking velocity for *E. formosa* on different plant cultivars with different density of hairs.

Plant	Trichome density (cm^2)	Walking velocity for *E. formosa* (mm/s)	References
Poinsettia, Lilo	2029,5	0,604	This paper
Tomato	1900	0,31	Sütterlin & van Lentern (1996)
Gerbera	80 – 1000	0,2 – 0,3	Sütterlin & van Lentern (1996)
Poinsettia, Sonora	449,0	0,519	This paper
Cucumber	157,8	0,2	van Lentern *et al.* (1995)
Cucumber	0	0,62	van Lentern *et al.* (1995)
Sweet pepper	0	0,73	Sütterlin & van Lentern (1996)

I found in my experiments that the temperature influenced the walking velocity on the two poinsettia cultivars. The walking velocity on poinsettia leaves increased with temperature ranging from 18 ºC to 20 ºC. van Roermund & van Lenteren (1995) measured the walking velocity of *E. formosa* on clean tomato leaflets; it increased rapidly from 0,179 mm/s at 15 ºC to 0,307 mm/s at 20 ºC. The same tendency was reported by Sütterlin & van Lenteren (1997) for *Gerbera* leaves, which are hairier than tomato. The walking velocity was significantly different, with 0,23 mm/s at 20 ºC and 0,38 mm/s at 30 ºC. Compared with my results and

those found by other scientists, this seems to correspond. The increase in walking velocity due to a rising temperature may be of importance for biological control in greenhouses where such temperatures occur for several hours almost every day.

In my experiments, *E. formosa's* walking activity was between 80 and 90 % of total time spent on the leaf (7 minute) and did not differ when tested at different temperatures (18 ºC to 20 ºC) or with different leaf hair density. van Roermund & van Lenteren (1995) found that walking activity on clean tomato leaves was relatively constant (70 %), and did not change with temperatures above 20 ºC. Mean time spent residing on the leaf was 17,5 minutes. The activity at temperatures between 15 ºC and 18 ºC was much lower at approximately 10 % on clean tomato leaves. Mean time spent residing on the leaf was longer than one hour (van Roermund & van Lenteren, 1995). The walking activity of *E. formosa* females on 6 different *G. jamesonii* cultivars, measured during a ten minute period lay between 72 to 80 % at 20 ºC (Sütterlin & Lenteren 1997). In other experiments, when the parasitoid were allowed to stay as long as they chose on a *Gerbera* leaf (several hours) the walking activity was found to be 62 % (20 ºC) (Sütterlin & Lenteren 1997). Sütterline & van Lenteren (1999) found that the average walking activity, measured at 1 h and 30min, of the parasitoids on two cultivars of *G. jamesonii* to be 62,8 % on clean leaves at 25 ºC. van Eck-Borsboom (*s. a.*) found in his M.Sc. theses that *E. formosa* had a walking activity on clean cucumber leaves at 57,8 % (van Eck-Borsboom (*s. a.*) in accordance with van Roermund & van Lenteren, 1995). From all these experiments one can suppose that walking activity is affected by the amount of time spent residing on the leaves. Results achieved over a short time span on the leaf, showed a high walking activity, compared to that of a longer time span when a lower walking activity was observed. Therefore time spent on a leaf ought to exceed one hour for walking activity measurements.

The turn angle for *E. formosa* was slightly higher on Sonora than Lilo, though no significant difference was found. It is thought that a higher density of hair on the leaves triggers an increased number of turns in the searching behaviour (van Lenteren *et al.* 1995). In contrast a higher turn angle on Lilo than Sonora was expected due to hair density. This was not found to be the case. Sütterlin & van Lenteren (1997) observed that the walking path of *E. formosa* on *Gerbera* cultivars was relatively straight. It was not altered by the different surfaces of the leaf.

Higher temperatures lead to a higher parasitization rate of *B. tabaci*. An increase in host numbers from 14 to 161 nymphs did not influence the frequency of host encounters at 21 ºC, but showed an exponential growth at 18 ºC. However at 18 ºC and 21 ºC, the percentage host encounters and ovipositions increased with increasing temperatures. The number of hosts parasitized per unit of time depended on the walking velocity of *E. formosa* (van Lenteren *et al.* 1976). The preceding experiments showed that walking velocity increased with the increase of temperature. Walking velocity is a very important factor affecting the numbers of parasitization of the *E. formosa.*

Henter *et al.* (1993) tested the parasitization rate for 37 wasps, reared on *T. vaporariorum* and *B. tabaci* on an unknown plant. The average number of encounters per individual wasp was 5,9 ± 0,66 (x ± SE). When *E. formosa* was first reared on *T. vaporariorum* and then tested on *B. tabaci*, the percentage of host acceptance was 34 %. *E. formosa* first reared on *B. tabaci* and then tested on *B. tabaci* had a parasitization rate of 63 %. I found an average number of 4, 33 parasitizations on each leaf at 21 ºC. This number is a bit higher than the findings of Henter *et al.* (1993). It is difficult to compare my results with those of Henter *et al.* (1993), as I do not know what kind of plants or temperatures they used during their experiments. However they serve as a guide line for the number of nymphs parasitized per leaf under varying conditions.

van Lenteren *et al.* (1995) found that the number of parasitization and host killings of *E. formosa* was higher on half – haired cucumber than on haired cucumber. An increase in the number of hairs on cucumbers leads to a decrease in meeting probability between *E. formosa* and greenhouse whitefly (van Lenteren *et al.* 1995). Several scientists have demonstrated that plant hair hampers small predators and parasitoids on a number of crops (van Lenteren *et al.* 1995 & Krips, 1999). Hairs may drastically hinder the walking velocity and change the walking pattern (van Lenteren *et al.* 1995). van Alphen *et al.* (1976) found that *E. formosa* fed upon 7 % of all hosts (*T.* vaporariorum) encountered, compared to 35 % used for parasitization. In order to be able to compare the leaf hair density proportionately to the ratio of parasitization, this experiment had to be carried out using Sonora as well. On account of a lack of time an experiment with Sonora was not feasible.

During the behavioural experiments, temperature and light intensity was good, but the relative humidity in the experiment room was not realistic with that of RH in a greenhouse. This may have had an impact on my results.

The missing samples within a trial could be predicted if it did not exceeded 5 %. A recording of such a prediction could have resulted in a lower walking velocity, walking activity and/or turn angle. However this was not done due to concern for the overall outcome of the experiments. By predicting the results for the "missing samples" one runs the risk of affecting the possible outcome of the true results.

6. Conclusions

The current study proved the following; walking velocity increased with increasing temperature. Walking velocity, activity and turn angle for *E. formosa* was not hampered by leaf trichome.

It is fair to say that the data presented here is not in accordance with my expectations - that the searching velocity of the parasitoid *E. formosa* would decrease with leaf hair density. However, the temperature did affect the walking velocity and the parasitization rate for *E. formosa*.

The possibility of biologically controlling *B. tabaci* with *E. formosa* on poinsettia seems promising. The increase in walking velocity when placed in a temperature range of 18 ºC – 20 ºC may be of importance for the success of biological control in greenhouse compartments. During the SD period in production of poinsettia plants, this temperature range was evident for several weeks. *E. formosas* walking velocity may be a very important parameter influencing the percentage oviposition in *B. tabaci* nymphs on poinsettia plant.

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Appendix

Table 1. Nymphs alive before parasitization, and number parasitized, dead nymphs and nymphs with black spots after *E. formosa* was released on Lilo leaves with *B. tabaci* nymphs for 48 hour, at 21 ºC.

Nr	Nymphs alive	para. nymphs	dead nymphs	Nymphs with black spots
1	55	8	2	0
2	50	6	1	0
3	57	4	1	0
4	133	0	0	0
5	20	1	1	3
6	57	6	1	1
7	139	1	0	0
8	29	7	2	1
9	44	4	4	1
10	41	9	0	0
11	29	6	2	0
12	14	7	0	0
13	32	0	0	0
14	69	0	5	0
15	26	6	1	1

Table 2. Nymphs alive before parasitization, and number parasitized, dead nymphs and nymphs with black spots after *E. formosa* was released on Lilo leaves with *B. tabaci* nymphs for 48 hour, at 18 ºC.

Nr	Nymphs alive	para. nymphs	dead nymphs	Nymphs with black spots
1	31	1	3	0
2	113	0	0	0
3	46	2	4	0
4	45	4	7	4
5	127	1	0	0
6	161	6	0	0
7	117	3	0	0
8	55	0	0	0
9	50	2	0	0
10	143	4	0	0
11	11	0	1	0
12	29	0	0	1
13	29	0	5	1