Entomopathogenic fungi against whiteflies

Tritrophic Interactions between *Aschersonia* species, *Trialeurodes vaporariorum* and *Bemisia argentifolii*, and glasshouse crops

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General introduction

The research discussed in this thesis aimed at evaluating entomopathogenic fungi for biological control of whiteflies. In this chapter I first summarize whitefly pests, damage, biology and its control. Secondly, the background concerning entomopathogenic fungi and whitefly will be described. Finally, the aim of the project and the outline of the thesis is presented.

Whitefly pests and damage

About 1200 whitefly species have been described, but only few of them are considered as pests. Among the latter are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) and, more recently, the silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring) (syn. *B. tabaci* biotype B). *B. argentifolii* is part of the *Bemisia tabaci* species complex, of which many biotypes are described. The discussion whether *B. argentifolii* is a separate species or not is still ongoing (*e.g.* De Barro & Hart, 2000; Gerling, 2000; Martin *et al.*, 2000). In this thesis the name *B. argentifolii* is used.

The greenhouse whitefly is a key pest of many greenhouse vegetables and ornamentals, whereas the silverleaf whitefly has been a serious pest in field crops worldwide since the mid seventies (Byrne *et al.*, 1990a). Both species are highly polyphagous, occur world wide and lead to serious economic losses (Gerling, 1990).

The damage to crops is manifold: 1) Direct damage to plant foliage is caused by feeding of whiteflies. Adult and immature whiteflies are phloem feeders, using their piercing-sucking mouthparts to introduce digestive juices and to remove chlorophyll and starch. Infested plants may drop leaves prematurely and have reduced plant growth and vigour (Oetting & Buntin, 1996). 2) A by-product of feeding is the excretion of excess sugars in the form of honeydew. It accumulates on the upper surfaces of plant parts where it serves as a substrate for sooty moulds (*e.g. Capnodium* spp.), thus reducing leaf photosynthesis. More important is the economic damage due to residues of sticky honeydew on fruits, ornamentals and cotton lint (Byrne & Bellows, 1991; Schuster *et al.*, 1996). Sometimes the mere presence of only a few whitefly nymphs and adults can cause problems. 3) The marketability of ornamental plants depends on aesthetic features. The presence of whitefly adults and honeydew will not escape the notice of consumers. For export of plants or plant products a zero-tolerance is valid, especially when the insect is a quarantine pest in the importing country. Infested plants destined for the international market risk rejection. Interception can lead to destruction or re-export of an entire shipment (Fransen, 1993; Schuster *et al.*, 1996). 4) Both

whitefly species serve as vectors of several economically important viral plant pathogens (for overview see *e.g.* Brown *et al.*, 1996; Duffus, 1996). Hence, only a few adults carrying a virus can cause serious damage to a crop.

Whitefly biology

Adult whiteflies are winged, free-flying insects. The females lay their eggs preferably on the abaxial side of young host-plant leaves (van Lenteren & Noldus, 1990). Their eggs are inserted into the leaf tissue by means of a pedicel (Paulson & Beardsley, 1985). Through this pedicel water of the plant can be absorbed to overcome desiccation of the eggs (Byrne *et al.*, 1990b). The hatching crawlers (first-instar nymphs) usually settle in the proximity of the oviposition site following a search for a suitable location and start feeding. Sometimes the crawler can cover large distances, which has been attributed to host-plant suitability (van de Merendonk & van Lenteren, 1978). On rare occasions crawlers could even move between plants (Summers *et al.*, 1996). As soon as a suitable location is found they become sessile by fixing themselves to the leaf with their mouthparts. All four nymphal instars, following the settling of the crawler, will spend their life at the same location, reinserting their mouthparts into the leaf tissue following each molt. The nymphal stages can be distinguished by several morphological characters and easiest by size (Weber, 1931; Lopez-Avila, 1986). The fourth instar transforms into a so-called pupal stage during which the scale-like nymph transforms into the winged adult that emerges through a slit in the dorsum of its shed exoskeleton. The duration of this life-cycle is dependent on whitefly species, temperature and host-plant suitability. For instance, the developmental time of *T. vaporariorum* on poinsettia is faster than that of *B. argentifolii* at 20°C, but at 25 °C it is the other way round (Fransen, 1994). For more detailed information about whitefly morphology, life-history parameters, behaviour or host-plant preference see, for example, van Lenteren & Noldus (1990), Byrne & Bellows (1991), van Roermund & van Lenteren (1992) or Gerling (2000).

Whitefly management strategies

In natural ecosystems and agro-ecosystems where no pesticides are used, an array of natural enemies can keep whitefly at low numbers by a combination of predators, parasitoids and pathogens. Several examples are known of perfect natural control of whitefly, e.g. in tomatoes in the 1960's in California, in cotton during the period 1925 - 1992 in Sudan (van Lenteren *et al.*, 1996), in citrus beginning 1900's in Florida (McCoy, 1985) or in pimento on Jamaica (Börner, 1956). When insecticides and fungicides are applied natural enemies are exterminated and whitefly pests are the result (van Lenteren *et al.*, 1996).

In protected crops, especially in ornamentals, the low tolerance of pest insects is reflected by widespread and frequent use of insecticides. Biological control in floriculture is more difficult compared with biological control in fruit vegetables for several reasons. Firstly, more chemicals are available for ornamentals than for fruit vegetables, because of safety regulations for consumption of vegetables. Secondly, in fruit vegetables only the fruits are harvested, which allows a higher population level of the pest on the leaves. In contrast, most ornamentals are being sold with flowers and leaves, and therefore should be free of injury and presence of insects. Thirdly, the criterion of zero tolerance for both pest and natural enemies has been used as a standard for all products until now, though it is only needed as an export requirement for a few countries (Fransen, 1993). Nowadays, increasing knowledge and concern about the effects of chemical pesticides on the environment and the problem of resistence development against pesticides by insects (Dittrich & Ernst, 1990; Horowitz & Ishaaya, 1996) may cause chemicals to be no longer available in the future. Therefore research has shifted towards other means of control, such as integrated pest management (IPM), including biological control and guided control, and breeding for host-plant resistance, using chemical control only as an ultimate resort. Biological control using predators, parasitoids and/or pathogens, can offer a reliable method to control whitefly.

Predators and parasitoids

About 75 species of whitefly predators have been described. However, many more species prey upon whiteflies, like general predators such as spiders, beetles etc. (van Lenteren *et al.*, 1996). Since whiteflies are minute insects, many of their predators, in particular the ones more specific to their prey, are small arthropods. The positions of the whitefly eggs and nymphs on the underside of leaves also limits the kind of predators to those that occur on leaves (Gerling, 1990). Individual predator species in the families of Anthocoridae, Coccinellidae, Chrysopidae, Hemerobiidae and most of the Miridae are unable to maintain *e.g.* the greenhouse whitefly below damaging levels, although a complex of predators may do so. Only some of the predatory mirid species belonging to the genera *Macrolophus* or *Dicyphus* may be able to reduce whitefly populations sufficiently. Their polyphagy allows them to feed on other prey, like mites, aphids, thrips or noctuid eggs and hence maintain their numbers when whitefly densities are low (Onillon, 1990; van Lenteren *et al.*, 1996).

Circa 100 species of whitefly parasitoids have been identified and more species are expected to be found. Most of the parasitoids are very host specific, but some species are hyperparasitoids and their importation might reduce the efficacy of primary parasitoids (van Lenteren *et al.*, 1996). Many important whitefly parasitoids belong to the genera *Encarsia* and *Eretmocerus* (Hymenoptera: Aphelinidae), and the genus *Amitus* (Hymenoptera:

Platygasteridae), for example, *Encarsia formosa*, *Eretmocerus californicus*, *E. mundus* and *A. fuscipennis* (Gerling, 1990; Fransen, 1994; Manzano, 2000). Biological control of *T. vaporariorum* with *E. formosa* is one of the success stories in biological control, and the parasitoid is a very reliable control agent in crops as tomato, sweet pepper and gherkin, but less so in egg plant and cucumber. In ornamentals, such as gerbera, results are ambiguous, depending mainly on temperature (Roermund, 1995; van Lenteren & Martin, 1999; Sütterlin, 2000).

Pathogens

In general, insect pathogens belong to very diverse taxonomic groups like viruses, bacteria, protozoa, rickettsiae, fungi and entomophagous nematodes. For whiteflies the spectrum of pathogens is more narrow. There are no records of nematodes parasitizing whiteflies and while whiteflies may be killed by viruses or bacteria, this will mainly be due to secondary infection through already existing wounds. So far, the only pathogens reported from Aleyrodidae have been fungi (Fransen, 1990). In general the fungi infecting Aleyrodidae can be divided into two groups: those specific to Aleyrodidae, mainly belonging to the genus *Aschersonia* (teleomorph: *Hypocrella*), and the "broad spectrum fungi" (Fransen, 1990). Fungal species belonging to the latter group, such as *Paecilomyces* spp. and *Beauveria bassiana*, are also able to infect insects belonging to other insect orders, and sometimes they are able to hyperparasitize on other fungi, like for instance *Verticillium lecanii* (Zouba & Kahn, 1992; Verhaar *et al.*, 1998). An overview of fungal species capable of infecting different whitefly species in nature or as biocontrol agent is given in Tab. 1.1 and 1.2.

The mode of infection of various entomopathogenic fungi is basically quite similar. A typical infection cycle involves several steps: conidial attachment, germination, penetration through the insect cuticle, vegetative growth within the host, fungal protrusion outside the insect and conidiogenesis (McCoy *et al.*, 1988). During attachment to the host's body, mucilage associated with conidia and enzymes play an important role. For the subsequent germination of spores a high humidity is mandatory. The next step, penetration of the insect cuticle, which rarely occurs via wounds, is thought to be a combination of mechanical and enzymatic means, in which proteases, lipases and chitinases are the most important enzymes (Butt, 1990). After penetration fungi proliferate within the body of the host. The insect may be killed by some combination of mechanical damage produced by the fungus, nutrient exhaustion and the action of fungal toxins (Gillespie & Claydon, 1989; Butt & Goettel, 2000). Later, hyphae will emerge from the cadaver, dependent on humidity, and produce spores on the exterior of the host (Roberts & Humber, 1981).

Approaches for microbial control

Several approaches have evolved for the use of pathogens and terminology has been adopted primarily from biological control with predators and parasitoids. These include 1) permanent introduction, 2) conservation and 3) augmentation (inoculative and inundative releases) (Hajek, 1993). Permanent introduction is the establishment of an organism in a pest population where it does not naturally occur, which results in more or less permanent suppression (Fuxa, 1987). Environmental manipulation or conservation involves the naturally occurring pest control by means other than direct addition to the pathogen units already present (Fuxa, 1987). However, this control strategy has seldom been utilized, possibly because the epizootiology of host/pathogen systems is too poorly understood to determine likely applications (Hajek, 1993). Augmentative releases of fungal pathogens generally are based on the ability of the fungal pathogen to repeatedly cycle through the host population after introduction. The degree and speed with which fungi are able to increase in prevalence as well as the damage thresholds for the crop determine whether releases should be inoculative (seasonal introduction) or inundative. The latter is frequently described as the use of mycoinsecticides. Among the major strategies of control, inundative releases have been adopted most extensively, especially among the hyphomycetes (Hajek, 1993), frequently using application technology similar to that used for standard chemical pesticides (Hajek & St.-Leger, 1994).

Table 1.1: Records of entomopathogenic fungi on whitefly (revised after Fransen, 1990), their origin and status (n = fungus naturally occurring or i = fungus introduced).

Fungal species	Aleyrodid species	Status	Country	Literature reference
ZYGOMYCOTA				
Zygomycetes: Entomophthorales				
Conidiobolus coronatus	*Bemisia tabaci*	n	Israel	Gindin & Ben-Ze'ev, 1994
Conidiobolus sp.	*Bemisia tabaci*	n	Israel	Gindin & Ben-Ze'ev, 1994
Erynia radicans	*Bemisia tabaci*	n	Israel	Ben-Ze'ev *et al.*, 1988
Orthomyces aleyrodis	*Trialeurodes abutilonea*	n	Alabama	Steinkraus *et al.*, 1998
ASCOMYCOTA				
Pyrenomycetes: Sphaeriales				
Hypocrella spp.			-	see Tab. 1.2
DEUTEROMYCOTA / FUNGI IMPERFECTI				
Coelomycetes: Sphaeropsidales				
Aschersonia spp.			-	see Tab. 1.2
Hyphomycetes: Moniliales				
Acremonium sp.	*Bemisia tabaci*	n	Danmark	Steenberg & Humber, 1999
	Trialeurodes vaporariorum	i	France	Riba & Entcheva, 1984
Aphanocladium album	*Bemisia tabaci*	n	Florida	Humber, 1992
	Trialeurodes vaporariorum	i	Bulgaria	Entcheva, 1979

Table 1.1: Continued.

Fungal species	Aleyrodid species	Status	Country	Literature reference
Hyphomycetes: Moniliales				
Aspergillus sp.	*Bemisia tabaci*	n	India	Humber, 1992
Beauveria bassiana	*Aleurothrixus floccosus*	i	Spain	Santamaria *et al.*, 1998
	Bemisia argentifolii	i	USA	Wraight *et al.*, 1998
	Bemisia tabaci	n	Israel	Ben-Ze'ev *et al.*, 1994
	Trialeurodes vaporariorum	i	USSR	Borisov & Vinokurova, 1983
Cladosporium aphidis	*Aleurochiton aceris*	n	Finland	Huldén, 1986
Cladosporium herbarum	*Aleurodicus cocois*	n	Brazil	Carvalho *et al.*, 1975
Fusarium scirpi[1]	*Dialeurodes* sp.	n	Florida	Fawcett, 1944
Fusarium verticillioides	*Trialeurodes vaporariorum*	i	France	Riba & Entcheva, 1984
Fusarium sp. [2]	*Bemisia tabaci*	n	India	Rao *et al.*, 1989
[2]		n	Florida	Humber, 1992
	Dialeurodes citri	n	Florida	Fawcett, 1908
	Dialeurodes citrifolii	n	Florida	Berger, 1921
Metarhizium anisopliae	*Trialeurodes vaporariorum*	i	Germany	Malsam *et al.*, 1998
Paecilomyces cinnamomeus[3]	*Dialeurodes citri*	n	Florida	Fawcett, 1944
Paecilomyces farinosus[4]	*Aleurocanthus woglumi*	n	Florida	Humber, 1992
	Bemisia tabaci	n	India	Nene, 1973
	Trialeurodes vaporariorum	n	Florida	Humber, 1992
Paecilomyces	*Bemisia tabaci*	n/i	Florida	Osborne *et al.*, 1990a
fumosoroseus[4]	*Trialeurodes vaporariorum*	i	USSR	Borisov & Vinokurova, 1983
Sporotrichum sp.[5]	*Dialeurodes citri*	n	Florida	Fawcett, 1908
Trichothecium roseum	*Bemisia tabaci*	n	Spain	Humber, 1992
	Trialeurodes vaporariorum	i	France	Riba & Entcheva, 1984
Verticillium lamellicola[6]	*Bemisia tabaci*	n	Danmark	Steenberg & Humber, 1999
Verticillium psalliotae	*Bemisia tabaci*	n	Danmark	Steenberg & Humber, 1999
Verticillium fusisporum[4]	*Bemisia tabaci*	i	Danmark	Steenberg & Humber, 1999
	Trialeurodes vaporariorum	n	Sweden	Ekbom & Åhman, 1980
Verticillium lecanii[4]	*Aleyrodidea* sp.	n	Russia	Mor *et al.*, 1996
	Bemisia tabaci	i	USA	Meade & Byrne, 1991
		n	Israel	Mor *et al.*, 1996
	Trialeurodes vaporariorum	n/i	UK	Hussey, 1958; Hall, 1982
Mycelia sterilia				
Aegerita webberi	*Aleurocanthus spiniferus*	n	Taiwan	Chien & Chiu, 1986
		n/i	China	Chen *et al.*, 1994
	Aleurocanthus woglumi	n	Cuba	Börner, 1956
	Aleurothrixus floccosus	n	South/Central America	Börner, 1956
	Dialeurodes citri	n	Florida	Fawcett, 1908
	Dialeurodes citrifolii	n	Florida	Berger, 1921

[1]: Described as *F. aleyrodis* (Fawcett, 1944).
[2]: Described as *Microcera* sp., nowadays *Fusarium* according to Hawksworth *et al.* (1995).
[3]: Described as *Verticillium cinnamomeum* by (Petch, 1932).
[4]: For geographic distribution see Lacey *et al.* (1996).
[5]: Probably *Beauveria* sp. (Fransen, 1990).
[6]: Probably a saprophyte (Steenberg & Humber, 1999).

Table 1.2: *Aschersonia* species (Deuteromycota: Coelomycetes: Sphaeropsidales) described on whitefly according to (Petch, 1921; Petch, 1925; Petch, 1932; Petch, 1939; Mains, 1959a), their teleomorph *Hypocrella* (Ascomycota: Pyrenomycetes: Sphaeriales), whitefly species, their status (n = fungus naturally occurring or i = fungus introduced), and their origin (supplement of Fransen, 1990).

Aschersonia (anamorph) — *Hypocrella* (teleomorph)	Aleyrodid species	Status	Geographic origin of isolate	Reference
Western hemisphere				
A. aleyrodis Webber[1]	*Aleurocanthus woglumi*	n	Costa Rica, El Salvador	Quezada, 1974; Elizondo & Quezada, 1990
	Aleurodicus sp.	n	-	Mains, 1959a
	Aleurothrixus floccosus	n	Brazil	Sanada *et al.*, 1974
		n	Cuba	Morrill & Back, 1912; Mora Morin, 1985
		n	Florida	Watson, 1912
	Aleurothrixus howardii	n	Central/South America	Börner, 1956
	Bemisia argentifolii	n	Colombia	Meekes *et al.*, 1996; Chapter 2
	Bemisia giffardi	n/i	China	Gao & Ouyang, 1981
	Bemisia tabaci	n	Florida	Morrill & Back, 1912
	Dialeurodes citri	n/i	China, Japan	Oho, 1967; Shu, 1996
		n	Cuba, Florida, Texas	Fawcett, 1908; Morrill & Back, 1912; Berger, 1921; Meyerdirk *et al.*, 1980; Samson & McCoy, 1983; Mora Morin, 1985
		i	Louisiana	Morrill & Back, 1912
	Dialeurodes citrifolii	n	Brazil, Florida	Morrill & Back, 1912; Berger, 1921; Fawcett, 1944; Cassino *et al.*, 1984; Nguyen & Hamon, 1985
	Metaleurodicus cardini	n	Jamaica	Börner, 1956
	Metaleurodicus minimus	n	-	Sutton, 1980
	Tetraleurodes acaciae	n	Florida	Dowell, 1982
	Trialeurodes abutiloneus	n	Florida	Morrill & Back, 1912
	Trialeurodes floridensis	n	Florida	Morrill & Back, 1912
	Trialeurodes vaporariorum	i	Colombia	Ramakers & Samson, 1984; Fransen *et al.*, 1987; Vargas Sarmiento *et al.*, 1995; Meekes *et al.*, 1996; Pas *et al.*, 1996; Chapter 2
		i	Cuba	Landa, 1984

Table 1.2: Continued.

Aschersonia (anamorph) — *Hypocrella* (teleomorph)	Aleyrodid species	Status	Geographic origin of isolate	Reference
A. aleyrodis[1] (cont.)	*Trialeurodes vaporariorum*	i	Florida	Primak & Chizhik, 1977; Meekes *et al.*, 1996; Chapter 2
		i	Trinidad	Hirte *et al.*, 1989a
		i	-	Foschi & Deseo, 1987; Pettersson, 1993
	Aleyrodid	n	Brazil, Costa Rica, Cuba, Dominican Republic, Florida, Honduras, Jamaica, Mexico, Mississippi, Panama, Puerto Rico, Texas, Trinidad, Venezuela	Petch, 1921; Mains, 1959a
	-	n	India, Kenya, Solomon Islands	Sutton, 1980
	-	n	Java	Humber, 1992
	Coccus viridis[2]	n	Colombia	Vargas Sarmiento *et al.*, 1995
	Selanaspidus articulatus[3]	n	Brazil	Gravena *et al.*, 1988; Watanabe *et al.*, 1994
A. andropogonis Hennings	Aleyrodid, black Aleyrodid	n	Brazil, Dominican Republic, Florida, Guyana, Puerto Rico, Trinidad	Petch, 1921; Mains, 1959a; Mains, 1959c
— *H. andropogonis* Petch	-			
A. aurantiaca Petch	*Paraleyrodes perseae*	n	Florida	Petch, 1939; Mains, 1959a; Mains, 1959c
— *H. aurantiaca* (Petch) Mains	Aleyrodid	n	Panama, Florida, Surinam	
A. blumenaviensis Hennings	-	n	Brazil	Petch, 1921
A. brunnea Petch	-	n	Brazil	Petch, 1921
A. columnifera Petch	Aleyrodid	n	Florida	Petch, 1921; Mains, 1959a
A. fimbriata Petch — *H. glabrescens* Petch	Aleyrodid	n	Mauritius	Petch, 1939
A. goldiana Saccardo & Ellis	*Bemisia argentifolii*	n/i	Brazil	Lourencao *et al.*, 1999
	Dialeurodes citri	n	Florida	Berger, 1921
	Dialeurodes citrifolii	n	Cuba	Mora Morin, 1985
		n	Florida	Fawcett, 1908; Nguyen & Hamon, 1985
	-	n	Brazil, Cuba, Guyana, Florida, Jamaica, Panama, Puerto Rico, Venezuela	Petch, 1921; Mains, 1959a

Table 1.2: Continued.

Aschersonia (anamorph) — *Hypocrella* (teleomorph)	Aleyrodid species	Status	Geographic origin of isolate	Reference
A. goldiana (cont.)	*Coccus viridis*[2]	n	Puerto Rico	Wolcott, 1955
A. incrasata Mains	Aleyrodid	n	Puerto Rico	Mains, 1959a
A. intermedia Petch	-	n	Chile	Mains, 1959b
A. taitensis Montagne	-	n	Tahiti	Petch, 1921
A. viridans (B. & C.) Patouillard	-	n	Brazil; Cuba; Ecuador; Florida;	Petch, 1921; Mains, 1959a
— *H. viridans* (B. & C.) Petch	black Aleyrodid	n	Mexico; Puerto Rico; Trinidad; Venezuela	
Aschersonia sp.	*Aleurocanthus woglumi*	n	Colombia	Agudelo & Falcon, 1977
	Aleurothrixus floccosus	n	Brazil	Sanada *et al.*, 1974
	Bemisia argentifolii	i	Brazil	Meekes *et al.*, 1996; Chapter 2
	Metaleurodicus minimus	n	Puerto Rico	Wolcott, 1955
	Trialeurodes vaporariorum	i	Brazil, Cuba, Florida, Trinidad	Khristova, 1971; Solovei, 1981; Hirte *et al.*, 1989a; Meekes *et al.*, 1996; Chapter 2
anamorph unknown — *H. castanea* Petch	Aleyrodid		Trinidad	Petch, 1932
anamorph unknown — *H. sloaneae* Patouillard	Aleyrodid		Guadeloupe; Peru; Trinidad	Petch, 1921
Eastern hemisphere				
A. acutispora Petch	Aleyrodid	n	Australia	Petch, 1921
A. australiensis Hennings	Aleyrodid	n	Australia	Petch, 1921
A. badia Patouillard	Aleyrodid	n	Phillippines, Sri Lanka, Vietnam	Petch, 1921
A. crenulata Patouillard & Hariot	pale Aleyrodid	n	West Africa	Petch, 1921
A. confluens Hennings	*Trialeurodes vaporariorum*	i	Vietnam	Hirte *et al.*, 1989a
— *H. mollii* Koorders		i	-	Spassova *et al.*, 1980

Table 1.2: Continued.

Aschersonia (anamorph) — *Hypocrella* (teleomorph)	Aleyrodid species	Status	Geographic origin of isolate	Reference
A. confluens (cont.) — *H. mollii*	black Aleyrodid	n	Burma, India, Java, Phillippines, Sri Lanka, Vietnam	Petch, 1921
A. duplex Berkeley — *H. duplex* (Berk.) Petch	*Ctenochiton viridis*[2]	n	New Zealand	Dingley, 1954
	Ctenochiton sp.[2]	n	New Zealand	Dingley, 1954
	Inglisia sp.[2]	n	New Zealand	Dingley, 1954
	Metaceronema japonica[2]	n	China	Chen, 1988
	-	n	Australia, New Zealand	Petch, 1921
A. flava Petch	*Trialeurodes vaporariorum*	i	Vietnam	Spassova *et al.*, 1980; Hirte *et al.*, 1989a
	Aleyrodid	n	Sri Lanka	Petch, 1921
A. hypocreoidea Cooke & Massee	Aleyrodid	n	Australia, Java, Phillippines, Sri Lanka, Taiwan, West Africa	Petch, 1921
A. papillata Petch	*Dialeurodes citri*	n	India	Gupta & Joshi, 1973
	Trialeurodes vaporariorum	i	China	Fang *et al.*, 1983
	black Aleyrodid	n	Sri Lanka	Petch, 1925
A. placenta Berkeley & Broome — *H. raciborskii* Zimmerman	*Aleurodicus dispersus*	n	Malaysia	Ibrahim & Lee, 1996
	Aleurolobus barodensis	n	India: Gujarat	Thumar & Kapadia, 1994; Parsana *et al.*, 1995
	Aleyrodes proletella	n[5]	Rep. of Georgia[5]	Ponomarenko *et al.*, 1975
	Bemisia argentifolii	i	India	Meekes *et al.*, 1996; Chapter 2
	Dialeurodes cardomomi	n	India	Muraleedharan, 1985; Selvakumaran *et al.*, 1996
	Dialeurodes citri	n	China	Zou, 1988
		i	China, Vietnam	Ponomarenko *et al.*, 1975; Fedorova, 1990
	Pealius azaleae	n[5]	Rep. of Georgia[5]	Ponomarenko *et al.*, 1975
	Trialeurodes vaporariorum	i	China, India, Vietnam	Spassova *et al.*, 1980; Hirte *et al.*, 1989a
		n[5]	Rep. of Georgia[5]	Ponomarenko *et al.*, 1975
	-	n	Africa, India, Java, New Guinea, Phillippines, Singapore, Sri Lanka	Petch, 1921
	-	n	America, Australasia	Evans & Hywel-Jones, 1990

Table 1.2: Continued.

Aschersonia (anamorph) — *Hypocrella* (teleomorph)	Aleyrodid species	Status	Geographic origin of isolate	Reference
A. placenta (cont.) — *H. raciborskii*	*Asterolecanium ungulata*[4]	n	Malaysia	Lim *et al.*, 1991; Ibrahim *et al.*, 1993
	Coccus viridis[2]	n	Malaysia	Ibrahim *et al.*, 1993
A. samoensis Hennings — *H. discoidea* (Berk. & Broome) Sacc.	white Aleyrodid	n	Africa, Burma, India, Java, Malaysia, Samoa, Sri Lanka, Thailand	Petch, 1921; Hywel-Jones & Evans, 1993
	black Aleyrodid	n		
A. tamurai Hennings	*Dialeurodes citri*	n	Japan	Petch, 1921
	Trialeurodes vaporariorum	*i*	-	Spassova *et al.*, 1980
Aschersonia sp.	*Aleurocanthus spiniferus*	n	Taiwan	Chien & Chiu, 1986
	Bemisia argentifolii	i	India, Japan, Thailand	Meekes *et al.*, 1996; Chapter 2
	Dialeurodes citri	*i*	Japan, Vietnam	Uchida, 1970; Aleshina, 1978
	Pealius azalea	n	Japan	Meekes *et al.*, 1996; Chapter 2
	Trialeurodes vaporariorum	i	China, India, Japan, Thailand, Vietnam	Khristova, 1971; Sinadskii & Kozarzhevskaya, 1980; Spassova *et al.*, 1980; Solovei, 1981; Hirte *et al.*, 1989a; Meekes *et al.*, 1996; Chapter 2
anamorph unknown — *H. palmicola* Hennings	Aleyrodid	n	Madagascar; Sri Lanka	Petch, 1921; Petch, 1932
anamorph unknown — *H. tubulata* Petch	Aleyrodid	n	Sri Lanka	Petch, 1921

[1]: *A. aleyrodis* (Western Hemisphere) is morphologically very similar to *A. placenta* (Eastern Hemisphere) and probably conspecific, differing mainly in colour. *A. aleyrodis* is bright orange to red, while *A. placenta* is more yellowish (Mains, 1959a; Rombach & Gillespie, 1988). Petch (1921) distinguishes the species mostly on length of the paraphyses in the pycnidia, which were 40-80 µ for *A. placenta* and 65-100 µ for *A. aleyrodis*. *A. placenta* is considered to be the anamorph of *Hypocrella raciborskii* sensu Petch (Petch, 1921; Petch, 1925; Lim *et al.*, 1991). *A. aleyrodis* is rarely found with a teleomorph, however, Petch (1925) concluded that *A. aleyrodis* is the conidial stage of *H. libera* Sydow. There is doubt about the connection, since *H. libera* is described on coccids and the identity of the pycnidial stage is uncertain, however, *H. libera* is very similar to *H. raciborskii* (Mains, 1959a; Mains, 1959b).

[2]: Not a whitefly species (Homoptera: Aleyrodidae), but a soft scale (Homoptera: Coccoidae: Coccidae).

[3]: Not a whitefly species, but an armoured scale (Homoptera: Coccoidae: Diaspididae).

[4]: Not a whitefly species, but a pit scale (Homoptera: Coccoidae: Asterolecaniidae).

[5]: *A. placenta* was introduced to control *D. citri*, it established and was observed to infect *Aleyrodes proletella*, *Paelius azaleae* and *Trialeurodes vaporariorum* (Ponomarenko *et al.*, 1975).

Abiotic and biotic factors influencing entomopathogenic fungi

The success of biological control using entomopathogenic fungi depends on suitable temperature and humidity conditions. Humidity is often cited as the key abiotic factor influencing the potential of fungi. A high relative humidity or free water is important for various parts of the infection cycle *i.e.* germination, infection and conidiogenesis (McCoy, 1990). The lower limit of relative humidity for spore germination is in the region of 92-93% (Hall & Papierok, 1982). However, infection of *T. vaporariorum* by *A. aleyrodis* was possible at a relative humidity as low as 50% under controlled climate room conditions (Fransen, 1987). It is thought that the microclimate in the phyllosphere largely determines the performance of fungi. This may explain the lack of correlation between infection by *V. lecanii* and greenhouse humidity (Ravensberg *et al.*, 1990). The obstacle of high humidity requirement can be overcome with improvements of formulation and application strategies (Bateman *et al.*, 1993). For fungal growth inside the host and subsequent killing of the insect, the fungus is independent of external conditions.

Compared with the relative humidity, the temperature seems to be of less importance in glasshouses. Many entomopathogenic fungal species grow and sporulate at temperatures between 15 and 30 °C (Osborne *et al.*, 1990a). In general the optimum growth and germination rates on artificial media varied around 25 °C for *A. aleyrodis* and *V. lecanii*, and above 30 °C germination and growth decline rapidly or are impaired (Hall, 1981; Fransen, 1987). The optimum temperature for growth often coincides with optimum temperature for infection. However, temperatures below optimum not necessarily mean that infection is less successful. Although germination and growth of fungi are retarded, the development rate of the host insect may be affected likewise. Thus, the number of insects escaping fungal infection by moulting will not increase as a result of a slower development of the fungus (Hall, 1981).

In addition to abiotic factors, also biotic factors, such as host plant and host insect, play an important role in the efficacy of entomopathogenic fungi. Fungal processes take place in the boundary layer surrounding the leaf or insect in which the air is undisturbed. The humidity in this layer is partly determined by the host-plant characteristics, such as leaf size and shape, leaf hairiness, position of the leaf on a plant (Ferro & Southwick, 1984). The host-plant chemistry may also influence the fungus directly, for instance, by inhibiting or stimulating fungal germination (Blakeman, 1971) or via the insect, influencing its susceptibility or its development rate (*e.g.* Hajek *et al.*, 1995; Elliot *et al.*, 2000; Poprawski *et al.*, 2000).

Host specificity and virulence of entomopathogenic fungi

To evaluate entomopathogenic fungi for the control of insects, several factors have to be considered, such as host specificity, virulence, persistence, epizootic potential and mass

production (Tab. 1.3). The importance of some of these factors depends on the approach which will be used to introduce the pathogen (Fransen, 1987). For instance, when using entomopathogenic fungi in a inundative approach the economics of mass production will be an important factor in selecting a fungal strain. However, when an entomopathogen will be introduced once to control a pest population (*e.g.* permanent introduction, seasonal release), the epizootic capacity and synchronisation with its host are more important criteria (Ferron *et al.*, 1991; Hajek, 1993).

How narrow should the host range of an entomopathogenic fungus be, before it is considered for biological control of a pest? By definition, entomopathogens live by parasitizing insects and it is uncommon that these pathogens also attack organisms outside the Insecta or Acarina (Hajek & Goettel, 2000). One can distinguish between physiological and ecological host range. The former is determined in the laboratory under optimal conditions (*e.g.* high relative humidity) and demonstrates which species can be directly infected by the pathogen. Typical effects include some combinations of mortality, infection and/or pathogen reproduction. The latter is defined as the host range manifested by pathogens under field conditions (Hajek & Butler, 1999). Knowing the physiological host range, an attempt can be made to predict the ecological host range (worst case scenario). However, hosts that can be infected in the laboratory, may never be found infected in the field. This can be due to complex biotic and abiotic interactions in the field, which are difficult to copy in the laboratory. For example a pathogen may be able to kill a sub-optimal host but not be able to reproduce on it

Table 1.3: Topics for evaluation of entomopathogenic fungi for control of insect pests (after Fransen, 1987).

Fungal characteristics:	• host range
	• virulence
	• sporulation ┐
	• persistence ├ epizootic potential
	• dispersal ┘
	• possibilities for mass production
	• suitability for storage and formulation
	• toxicological and safety aspects
	• compatibility with other pest and disease control methods
Host characteristics:	• susceptibility
	• economic injury level
	• age distribution, density and spatial distribution in relation to application or introduction strategy
Environmental characteristics:	
	• *ABIOTIC FACTORS*: humidity, temperature, rain, dew, irrigation, wind, solar radiation
	• *BIOTIC FACTORS*: host-plant quality, crop structure, leaf size and shape, leaf trichomes, microbial interactions

(Hajek & Goettel, 2000). When a strain is specific towards a pest insect it has no direct adversary effects on other natural enemies, like predators and parasitoids, or other beneficials, like bees. *A. aleyrodis*, for instance, is not only unable to infect the parasitoid *E. formosa*, but it is also unable to infect nymphs of *T. vaporariorum* that contain larvae or pupae of *E. formosa*. In addition, the parasitoid will reject nymphs infected with the fungus a few days after fungal infection took place (Fransen & van Lenteren, 1993; 1994). However, being so specific could be a disadvantage for the grower, since specific entomopathogens will not kill other pest insects present in the same crop, which is possible with broad-spectrum entomopathogens such as *V. lecanii* (Schaaf *et al.*, 1990).

Many species of fungal entomopathogens are composed of genetically diverse groups of strains, which usually cannot be differentiated by morphological criteria (Hajek & St.-Leger, 1994). However, these strains may vary widely in virulence towards the target insects (McCoy, 1990). An important consideration in microbial control of insect pests is the selection of highly virulent strains. Species of the genus *Aschersonia*, for instance, are pathogenic to whitefly, but virulence varies considerably, causing for instance 10% to 92% mortality of greenhouse whitefly (Hirte *et al.*, 1989a). Strains of *V. lecanii* originating from different hosts show differences in virulence towards *T. vaporariorum* (Hirte *et al.*, 1989b; Masuda & Kikuchi, 1992), although no relation could be established between virulence of the fungal strains against *B. tabaci* and their original host (Mor *et al.*, 1996). Repeated transfer of entomopathogenic fungi on artificial medium can lead to loss of virulence (McCoy, 1990). Virulence can be influenced by nutrition, and sub-optimum conditions of artificial culture can impair normal physiological development of the fungus (Goral, according to McCoy, 1990), whereas, passage through a host may increase and/or recover the virulence (Hirte *et al.*, 1989a; Butt & Goettel, 2000).

The genus *Aschersonia*

Fungi belonging to the genera *Aschersonia* (anamorph) and *Hypocrella* (teleomorph) are specialized pathogens of whiteflies (Homoptera: Aleyrodidae) and/or scale insects (Homoptera: Coccidae) (Petch, 1921; Evans & Hywel-Jones, 1990). The genera are predominantly (sub-)tropical and are common representatives of entomopathogenic mycobiota in natural forest ecosystems and plantation tree crops (Evans & Hywel-Jones, 1990; Hywel-Jones, 1993).

The geographic distribution of *Aschersonia* and *Hypocrella* species, restricted to whitefly, is given in Tab. 1.2 (see also Tab. 1 in Evans & Hywel-Jones, 1990). Very few species are pantropical and there is a clear distinction between Eastern and Western hemispheres. Since *A. aleyrodis* and *A. placenta* are closely related species (Mains, 1959c;

Samson & Rombach, 1985), the apparently pantropical occurrence might be questionable, although intercontinental movement of host and pathogen on citrus material is a distinct possibility (Evans & Hywel-Jones, 1990; *e.g.* Oho, 1967 and Ponomarenko *et al.*, 1975). The genera are easily recognised by their typically brightly coloured stroma and they can cause spectacular epizootics, implicating them as potent natural control agents of coccids and whiteflies (Evans & Hywel-Jones, 1990). Of the 24 *Aschersonia* species that are described on whitefly, *A. aleyrodis* and *A. placenta* are most commonly observed (Fransen, 1990; Tab. 1.2).

The genus *Aschersonia* is divided into species which are restricted to Aleyrodidae and species restricted to Coccidae (Petch, 1921). Subgenera were proposed based on the host insect and morphological characteristics, *viz.* species restricted to Aleyrodidae having paraphyses within the conidiomata, whereas species restricted to Coccidae are lacking these structures (Petch, 1921; Evans & Hywel-Jones, 1990). However, Dingley (1954) questioned this division in subgenera, since *A. duplex*, with paraphyses in its conidiomata, is consistently associated with Coccidae (see also Tab. 1.2 and Evans & Hywel-Jones, 1990). In addition, most *Aschersonia* descriptions are based on herbarium material only (Samson, 1995) and in many cases host identifications are lacking (Petch, 1921; 1925; 1932; 1939; Mains, 1959a). Host identification was impossible when no living host remained and the complete destruction of infected hosts (Evans & Hywel-Jones, 1990). Hywel-Jones demonstrated that pure cultures of *Aschersonia* can lack paraphyses whereas these were present on the host, indicating that paraphyses are apparently not a very reliable taxonomic feature. The genus is in urgent need of revision and probably many more species remain to be described from tropical forest ecosystems (Evans & Hywel-Jones, 1997)

The utilisation of *Aschersonia* spp. as biocontrol agents has a long history. In the early 1900's, *A. aleyrodis* and *A. goldiana* were mass-produced on sweet potato agar and sold to citrus growers for 75 cents per culture for the control of citrus whitefly, *Dialeurodes citri*, and cloudywinged whitefly, *D. citrifolii* (Berger, 1920b; 1920a; 1921). Today this is a example of successful biological control, where predators, parasitoids and fungi control these pests (McCoy, 1985). Similar results have been obtained in Jamaica, where *A. aleyrodis* successfully controlled guava whitefly, *Metaleurodicus cardini*, (Börner, 1956) and the Azerbaijan region, where several species of *Aschersonia* have been introduced from Southeast Asia and America for the biological control of citrus whitefly (McCoy *et al.*, 1988). Under favourable environmental conditions approximately 80% of nymphal mortality was obtained and the introduced fungus adapted well to new citrus plantations (McCoy *et al.*, 1988). As a consequence, *Aschersonia* spp. were tested against *T. vaporariorum* and in protected cultivation in Europe and China as the only regulatory agent or as a component of an IPM

program in combination with the parasitoid *E. formosa* (Adam, 1978; Solovei, 1981; Fang *et al.*, 1983; Ramakers & Samson, 1984; Fransen, 1987; Hirte *et al.*, 1989a).

Aim and outline of this thesis

As more environmentally responsible crop protection strategies are adopted, natural enemies of both whitefly species, *T. vaporariorum* and *B. argentifolii*, will play an increasing role. Screening for natural enemies which are able to kill both pest insects quickly, without affecting other natural enemies, is an important line of research. Entomopathogenic fungi can meet these requirements and can therefore be a valuable asset to existing biological and chemical control measures (Lacey *et al.*, 1996). Our attention is directed towards the genus *Aschersonia*, because previous research indicated that *Aschersonia aleyrodis* is a promising whitefly control agent because of its high virulence towards greenhouse whitefly, its tolerance to relative humidities as low as 50% (Fransen, 1987), its long persistence on leaf surfaces (Fransen, 1995) and its compatibility with *E. formosa* (Fransen & van Lenteren, 1993; 1994). This project consisted of two components: 1) the identification of virulent isolates of *Aschersonia* spp. for the use against greenhouse and silverleaf whitefly, and 2) the study of factors which influence the effectivity of entomopathogenic fungi, with special reference to host plant, humidity and their interaction.

In **chapter 2** virulence of over 40 isolates of *Aschersonia* spp. against *B. argentifolii* and *T. vaporariorum* is described. Important aspects for microbial control like spore production and correlation between virulence against *B. argentifolii* and virulence against *T. vaporariorum* have been studied. Six isolates are selected for further research on bases of sporulation, virulence and geographical origin. In **chapter 3** dose and time dependent mortality effects of these six isolates on nymphs of *B. argentifolii* and *T. vaporariorum* are described. Median lethal dose and median lethal time are determined and the bioassay procedure is discussed. The germination behaviour and infection strategy of *Aschersonia* spp. on *B. argentifolii* have been studied in **chapter 4** by means of scanning electron microscopy. Also the susceptibility of the various nymphal instars of *B. argentifolii* and *T. vaporariorum* to *A. aleyrodis* and *A. placenta* is studied. This knowledge will add to the understanding of the adaptation of *Aschersonia* spp. towards whitefly species.

The success or failure of biological control with entomopathogenic fungi is often related to the humidity at leaf level which would be favourable or disadvantageous for the fungi. This phyllopshere humidity is determined by ambient humidity, as well as characteristics of the host plant itself. Chapters 5 to 8 focus on the role of the ambient climate and the host plant on efficacy of entomopathogenic fungi.

Research by Fransen (1995) indicated that conidia of *A. aleyrodis* can survive on leaf surfaces of cucumber for at least three weeks and were still able to infect nymphs of *T. vaporariorum*. However, the extend to which the host plant influences persistence of conidia is still unknown. The persistence of *A. aleyrodis* conidia was assessed on three host plants of *T. vaporariorum*, cucumber, gerbera and poinsettia (**chapter 5**). Persistence was determined by assessing (1) germination capacity of conidia and (2) mortality of greenhouse whitefly nymphs caused by conidia after a prolonged period of exposure of these conidia on the leaf surface.

The role of phyllosphere humidity in the effectivity of entomopathogenic fungi against *T. vaporariorum* and *Aphis gossypii* is described in **chapter 6**. The hypothesis that phyllosphere humidity can explain differences in insect mortality due to entomopathogenic fungi was tested on four different crop species, *viz.* cucumber, gerbera, poinsettia and tomato. We measured different climate and host-plant parameters, in order to estimate the humidity in the phyllosphere. By studying more than one insect system, one largely sessile: *T. vaporariorum* and one mobile: *Aphis gossypii*, we tried to elucidate the interactions between the different trophic levels.

The ambient climate together with the host plant determine the phyllospere humidity. However, what is the result of changes in ambient humidity on the efficacy of entomopathogenic fungi within one host-plant species and is this effect comparable for cucumber, gerbera and poinsettia? These questions were answered by studying the influence of ambient humidity on the efficacy of *A. aleyrodis*, *A. placenta* and *V. lecanii* (**chapter 7**).

Within one host-plant species differences in morphology and chemical composition may exist. The ornamental crop gerbera exists of more than 600 different cultivars with large differences in these characteristics (Krips, 2000). A glasshouse experiment was carried out with two cultivars, which differed in morphological characteristics and probably also in chemical composition. The effect of cultivar on the *T. vaporariorum* population and the eventual effect on *A. aleyrodis* and *V. lecanii* was studied (**chapter 8**).

In **chapter 9** all studied aspects of this tritrophic system are discussed. In addition the steps which still have to be taken before *Aschersonia* spp. can be implemented in IPM programmes to control whitefly species. Their advantages and limitations are discussed to answer the question: do *Aschersonia* spp. have a future in biological control?

2

Virulence of *Aschersonia* spp. against whiteflies *Bemisia argentifolii* and *Trialeurodes vaporariorum*[1]

Abstract

Entomopathogenic fungi of the genus *Aschersonia* are specific for whitefly and scale insects. They can be used as biological control agents against silverleaf whitefly, *Bemisia argentifolii* and greenhouse whitefly, *Trialeurodes vaporariorum*. Forty four isolates of *Aschersonia* spp. were tested for their ability to sporulate and germinate on semi-artificial media and to infect the insect host. Seven isolates sporulated poorly (less than 5.10^7 conidia/culture) and ten were not able to infect either of the whitefly species. Several isolates were able to produce capilliconidia. Infection level was not correlated with germination on water agar. After a selection based on spore production and infection, virulence of 31 isolates was evaluated on third instar nymphs of both whitefly species on poinsettia (*Euphorbia pulcherrima*). Infection levels varied between 2% to 70%, and infection percentages of *B. argentifolii* correlated with that of *T. vaporariorum*. However, mortality was higher for *T. vaporariorum* than for *B. argentifolii*, as a result of a higher 'mortality by other causes' of greenhouse whitefly on poinsettia. Several isolates, among which unidentified species of *Aschersonia* originating from Venezuela and Malaysia, *A. aleyrodis* from Colombia, and *A. placenta* from India showed consistent results in their ability to infect both whitefly species.

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Introduction

Control of the greenhouse whitefly, *Trialeurodes vaporariorum*, on glasshouse vegetables with the parasitoid *Encarsia formosa* is one of the success stories in biological control (van Lenteren *et al.*, 1996). In contrast to the use of *E. formosa* in vegetable crops, its use in ornamentals is not that widely accepted, mainly because presence of a few nymphs or small amounts of honeydew is considered as unacceptable in these crops (van Lenteren & Woets, 1988). With the accidental introduction of the silverleaf whitefly *Bemisia argentifolii* (syn. *B. tabaci* - biotype B) on poinsettia cuttings in the Netherlands in 1987 (Fransen, 1994), the situation has become more complicated. Chemical control of *B. argentifolii* is not always effective, as this species has the ability to develop resistance to a wide range of pesticides (Horowitz & Ishaaya, 1996). Biological control of *B. argentifolii* with *E. formosa* is difficult, because it is a less suitable host than *T. vaporariorum* (Szabo *et al.*, 1993; Henter & Lenteren, 1996). Moreover, when the two whitefly species occur together, the parasitoid shows a preference for greenhouse whitefly (Boisclair *et al.*, 1990; Szabo *et al.*, 1993).

Because of the problems *B. argentifolii* is causing in both indoor and outdoor crops, the range of natural enemies being considered for use against whitefly has increased over the last couple of years (Gerling, 1990; van Lenteren *et al.*, 1997). In addition to parasitoids and predators, a range of entomopathogenic fungi are found on whitefly species all over the world. Several fungi are being considered for biological control (*e.g.* Lacey *et al.*, 1996). In order to select fungal pathogens for controlling whiteflies it is necessary to select isolates which combine the best possible characteristics for killing the target insects under glasshouse conditions. Relevant characteristics are: i) good mass production features, such as high sporulation on artificial media, ii) high virulence against target organisms and iii) the ability to withstand the environment in which the pest is occurring (Prior, 1992; Moore & Prior, 1993). Several fungi, like *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii*, have been developed into products for the control of whitefly (Ravensberg *et al.*, 1990; Knauf & Wright, 1994; Bolckmans *et al.*, 1995). Previous research indicated that also *Aschersonia aleyrodis* Webber is a promising whitefly control agent because of its tolerance to relative humidities as low as 50% (Fransen, 1987), its long persistence on leaf surfaces (Fransen, 1995; Meekes *et al.*, 2000) and its compatibility with *E. formosa* (Fransen & Lenteren, 1993; 1994).

Along with *A. aleyrodis* several other species of the genus *Aschersonia* (Deuteromycotina, Coelomycetes; teleomorph: Ascomycota, *Hypocrella*) have been reported on whitefly species all over the world (Petch, 1921; Mains, 1959a, b; Protsenko, 1959). The genus *Aschersonia* is known to cause severe epizootics in whitefly (Aleyrodidae) and scale insects (Coccidae) in the tropics and subtropics (Evans & Hywel-Jones, 1990). The species

attacking whitefly mainly infect the nymphal stage. Although 23 species have been described on whiteflies (for an overview see Fransen, 1990), little is known about their effectivity in controlling both *B. argentifolii* and *T. vaporariorum* in greenhouse crops.

In an attempt to identify more virulent isolates, bioassays were carried out in which spore production, germination and infection of *B. argentifolii* and *T. vaporariorum*, were measured. In this paper we present results of this screening procedure for virulent isolates.

Materials and Methods

Whitefly species and plant material

Bemisia argentifolii was maintained on poinsettia plants (*Euphorbia pulcherrima*, cv. Goldfinger) in cages at 23 °C under greenhouse conditions. *T. vaporariorum* was reared on gerbera plants (*Gerbera jamesonii* hybrids) in cages at 21 °C under greenhouse conditions. The *T. vaporariorum* populations from gerbera were not preconditioned to the experimental host plants, since pre-conditioning of greenhouse whitefly to a less suitable host plant usually does not alter the suitability of that specific host plant (Thomas, 1993).

Bioassays were carried out on young poinsettia plants with 10-15 leaves, in their vegetative phase. Of each plant only the two youngest, full-grown, leaves were used. To obtain third instar whitefly nymphs, 50 to 55 adults, both male and female, were put into clip-cages on poinsettia leaves. The adults were given the opportunity to lay eggs for 24 hours. This resulted for *B. argentifolii* in 259 (± 20.8 se), 91 (± 6.3 se) and 188 (± 20.5 se) nymphs per leaf in experiment 1A, 1B and 3 respectively (see below), and for *T. vaporariorum* in 83 (± 7.0 se) and 218 (± 23.7 se) nymphs per leaf in experiment 2 and 3, respectively (see below). Differences in number of nymphs per experiment are due to seasonal influence on whitefly rearings and fluctuations in sex ratio. The developmental period from egg to third instar nymph at 25 °C was about 14 days for *B. argentifolii* and 19 days for *T. vaporariorum*. For *B. argentifolii* a mixture of second and third instar nymphs were treated, because larval cohorts overlap.

Fungal isolates

Over 40 isolates belonging to the genus *Aschersonia* were collected from all over the world, including isolates from existing collections as well as isolates from fresh material (infected whitefly nymphs). In Table 2.1 an overview is given of the origin of the isolates. Thirty-two isolates are not identified to the species level and are referred to as *Aschersonia* sp.. The majority of isolates are, as far as known, multisporal, except for Aa5. Sporulating colonies on Potato Dextrose Agar (PDA, Difco) were used to make spore suspensions for inoculation of millet-cultures for mass production of spores. Most isolates were cultured on autoclaved

Table 2.1: Origin, growth medium, conidial production, germination of conidia and the capacity to form capilliconidia on water agar of *Aschersonia* isolates, and the ability to infect and the whitefly bioassays in which the isolates were used.

Code	Original code	Collector	Host[a]	Origin	Growth medium	Sporulation[b]	Germination[c] ± SD	Capilli-conidia[d]	Infection of whitefly[e]	Experiment
***Aschersonia* sp.**										
A1	ARSEF-3014	S.R. Sanchez Peña	Aleyrodidae	Mexico	millet	+	96.3 ± 1.6	-	+	1A, 1B, 2
A2	94008	L.A. Lacey	*Dialeurodes citri*	Brazil	millet	+	94.9 ± 0.5	-	+	1B
A3	94010	L.A. Lacey	*D. citri*	Brazil	millet	±	97.6 ± 0.9	-	+	-
A4	94011	L.A. Lacey	*D. citri*	Brazil	millet	+	98.8 ± 1.2	-	+	1B
A5	94021	L.A. Lacey	*D. citri*	Brazil	millet	+	95.6 ± 4.0	-	+	1A, 2
A6	94024	L.A. Lacey	Aleyrodidae	Thailand	millet	+	28.2 ± 1.9	-	+	1B
A7	94025	L.A. Lacey	Aleyrodidae	Thailand	millet	+	27.6 ± 0.7	-	+	1B
A8	94026	L.A. Lacey	Aleyrodidae	Malaysia	millet	+++	96.9 ± 3.1	-	+	1A, 1B, 2
A9	94027	L.A. Lacey	Aleyrodidae	Malaysia	millet	+	99.6 ± 0.5	-	+	1A, 1B, 2
A10	ARSEF-3015	S.R. Sanchez Peña	Homoptera	Mexico	corn	++	8.5 ± 1.7	+	-	-
A11	I89-490	H.C. Evans	-	Mexico	millet/corn	±/+	3.4 ± 2.2	-	+	1B
A12	I92-784	H.C. Evans	-	Brazil	millet/corn	±	61.6 ± 9.8	-	-	-
A13	I92-787	H.C. Evans	-	Brazil	corn	+	20.6 ± 26.2	+	-	-
A14	I92-788	H.C. Evans	-	Madagascar	millet/corn	++	45.2 ± 17.0	+	+	1A, 1B, 2
A15	I93-807	H.C. Evans	-	Guyana	millet	++	94.5 ± 2.3	-	+	1A, 1B, 2
A16	I93-813	H.C. Evans	-	Guyana	corn	++	51.5 ± 22.9	(±)	-	-
A17	I93-815	H.C. Evans	-	Trinidad	millet	++	98.0 ± 1.7	-	+	1A, 1B, 2
A18	I93-856	H.C. Evans	-	Colombia	millet	+++	96.6 ± 1.9	-	+	1A, 1B
A19	I93-858	H.C. Evans	-	Colombia	corn	+	42.0 ± 0.8	+	-	-
A20	I93-860	H.C. Evans	-	Colombia	millet	±	99.6 ± 0.1	-	-	-
A21	I93-860	H.C. Evans	-	Colombia	-[f]	-	- -	-	-	-
A22	I93-861	H.C. Evans	-	Colombia	corn	+	24.4 ± 16.8	+	-	-
A23	I93-901a	H.C. Evans	-	Ghana	millet	+	97.2 ± 2.2	-	+	1A, 1B, 2
A24	I94-908	H.C. Evans	-	Venezuela	millet	+/++	95.3 ± 2.2	-	+	1B, 2
A25	I94-910	H.C. Evans	-	Venezuela	millet/corn	+	97.5 ± 0.9	-	-	-
A26	-	L.A. Lacey	Aleyrodidae	Malaysia	millet	++	98.2 ± 1.3	-	+	1A, 1B, 2
A27	-	L.A. Lacey	Aleyrodidae	Thailand	millet	+	26.4 ± 8.5	-	+	1A, 1B, 2
A28	-	H. Saito	*Pealius azalea*	Japan	millet	++	96.5 ± 1.7	-	+	2, 3
A29	-	S. Balan	*Dialeupora* sp.	India	millet	+	21.6 ± 2.4	-	+	3

Table 2.1: Continued.

Code	Original code	Collector	Host[a]	Origin	Growth medium	Sporulation[b]	Germination[c] ± SD	Capilli-conidia[d]	Infection of whitefly[e]	Experiment
A30	KV-129	R.A. Samson	-	Thailand	millet	++	98.1 ± 1.1	-	+	3
A31	KV-131	R.A. Samson	-	Thailand	millet	+++	95.6 ± 0.7	-	+	3
A32	KV-132	R.A. Samson	-	Thailand	millet	++	96.1 ± 0.4	-	+	3
A. aleyrodis Webber										
Aa1	ARSEF-430	R.S. Soper	Aleyrodidae	Fla., USA	millet	±/+	98.0 ± 1.7	-	+	2, 3
Aa2	ARSEF-992	N. Oho	*D. citri*	Japan	millet	+++	99.5 ± 0.3	(±)	+	1A, 1B, 2
Aa3	ARSEF-2154	M.C. Rombach	Aleyrodidae	Java	millet	±	99.0 ± 0.5	-	+	-
Aa4	ARSEF-2268	W. Gams	Aleyrodidae	Colombia	millet	++	99.3 ± 0.6	-	+	1A, 1B, 2, 3
Aa5	KV-107	W. Gams	Aleyrodidae	Colombia	millet	+++	98.7 ± 0.1	-	+	3
Aa6	KV-108	W. Gams	Aleyrodidae	Colombia	millet	+++	98.0 ± 0.0	-	+	3
A. goldiana Saccardo & Ellis										
Ag1	ARSEF-431	R.S. Soper	Aleyrodidae	Fla., USA	millet	±	92.2 ± 2.2	-	+	-
A. insperata (ined.)[g]										
Ai1	ARSEF-2356	M.C. Rombach	*D. citri*	Phillipines	millet/corn	+	29.9 ± 14.1	-	+	1A, 1B, 2
Ai2	ARSEF-2351	M.C. Rombach	*D. citri*	Java	millet/corn	+++	46.4 ± 38.2	-	+	1A, 1B, 2
A. placenta Berkeley & Broome										
Ap1	-	S. Selvakumaran	*D. cardamomi*	India	millet	+++	91.7 ± 7.0	+	+	1A, 1B, 2, 3
Ap2	CBS-917-79	-	-	SE Asia	millet	++	95.5 ± 0.4	n.d.	+	3
A. turbinata Berkeley										
At1	ARSEF-1030	R.A. Hall	Homoptera	Colombia	corn	±	12.4 ± 2.7	+	-	-

[a]: Host: - = no living hosts were observed; [b]: - = no sporulation, ± = less then 5.10^7, + = 5.10^7 - 5.10^8, ++ = 5.10^8 - 5.10^9, +++ = > 5.10^9 conidia per culture; [c]: Average germination on wateragar; either by formation of a germ tube or by formation of capilliconida; [d]: Formation of capilloconidia: + = yes, - = no, (±) = occasional observation, n.d. = not determined: plates were not kept until 4 days after inoculation; [e]: Infection of whiteflies: both *B. argentifolii* and *T. vaporariorum* were examined; [f]: A21 hardly sporulated on PDA, SDA or these growth media, so conidial suspensions were not available to inoculate medium for assessment of sporulation, germination and infection. [g]: Humber & Rombach, after Humber (1992)

millet, as a solid substrate, in 300 ml Erlenmeyer flasks (10 g millet with 25 ml demineralised water), whereas a few isolates were grown on autoclaved corn flour (Tab. 2.1; Fransen, 1987). The Erlenmeyer flasks were closed with sterile cotton for aeration. The cultures were incubated at 25 °C and L16:D8 (artificial light). Conidia were harvested from three weeks old cultures by rinsing them with sterile demineralised water containing 0.05% (v/v) Tween 80 (Merck).

Sporulation and germination

Based on the total amount of conidia harvested from these cultures, five categories were distinguished, ranging from no sporulation (-) to more than 5 x 10^9 (+++) conidia/culture (Tab. 2.1). In the experiments, the suspensions were standardized to 10^7 conidia/ml by use of a haemocytometer.

To determine germination capacity of conidia, 1 ml of spore suspension (10^7 spores/ml) was sprayed onto water agar plates (15 g/l agar-agar, Merck) using a Potter spray tower (Burkard Manufacturing, UK). After incubation for 24 hours at 25 °C in artificial light (L16:D8), percentage spore germination was determined by observing 300 spores per plate (two plates per run, max. 4 runs). Germination was rated when germ tubes - normal germ tubes as well as those forming capilliconidia - exceeded the width of the conidium.

Virulence

All isolates were passed through *B. argentifolii* once and the reisolates were used for bioassays on virulence. Isolates which could not be reisolated were discarded from these tests.

Fungal virulence was assessed in bioassays with *B. argentifolii* (Exps. 1A and 1B- the experiment was repeated to test more isolates) and with *T. vaporariorum* (Exp. 2). Only a few isolates out of those that did not perform well on *B. argentifolii* were included in the bioassay with *T. vaporariorum*. An additional set of *Aschersonia* isolates were tested on both whitefly species at the same time (Exp. 3). The isolates that were used in the experiments are mentioned in Tab. 2.1; isolates Aa4 and Ap1 were included in every bioassay. For assessment of natural mortality also untreated nymphs (untreated control) and nymphs treated with 0.05% Tween in demineralised water (application control) were included in all experiments. Per treatment, three plants and two leaves per plant were used, resulting in at least 780, 350 and 828 nymphs for a treatment in Exps. 1A, 1B, and 3 respectively, for *B. argentifolii*, and for *T. vaporariorum* in at least 329 and 980 nymphs per treatment in Exps. 2 and 3, respectively.

Two ml of spore suspension was sprayed on the underside of a leaf, attached to the plant, using a Potter spray tower. This resulted in approximately 1.36 x 10^4 conidia/cm^2 poinsettia leaf. After evaporation of water, plants were covered with plastic bags for 48 hours

to maintain a relative humidity (RH) of 95 - 100%. Plants were kept in an air-conditioned greenhouse: RH fluctuated between 40 and 80% depending on season and photoperiod, and the temperature was kept at a constant level of 25 °C (±1 °C).

The final assessment of mortality took place between two to three weeks after application, when all nymphs had turned into pupae and about 30% had developed into adult whiteflies. Mortality can be caused by the fungus or may be due to other causes. A nymph was considered infected when it turned opaque white, orange or brown, depending on the fungal isolate used; a nymph was considered 'dead by other causes' when the insect had desiccated (turned transparent brown) and no apparent cause of death was visible. Egg and first nymphal stage mortality is excluded, since it had occurred before treatment. Overall mortality was used for analysis.

Statistical analysis

The (fungal) treatments were randomly distributed between the plants. For each experiment the proportion of dead nymphs of the total nymphs per plant was compared between fungal treatments. Data were analyzed using Generalized Linear Models, a binomial distribution and logit link function(Genstat 5 release 3.22, Payne *et al.*, 1987) Subsequent differences were compared with t-tests with total significance level of P = 0.05 (RPAIR, PPAIR procedures), in which probability is adjusted to $\alpha = 0.000327$ (Exp. 1A), $\alpha = 0.000198$ (Exp. 1B), $\alpha = 0.000292$ (Exp. 2) and $\alpha = 0.025$ for effect caused by whitefly species or 0.000321 for effect caused by fungal treatment (Exp. 3).

Results

Sporulation

In general conidia of *Aschersonia* spp. were easily produced on millet grains or corn flour. For six isolates the choice of millet or corn medium did not affect the amount of conidia produced (Tab. 2.1). Other isolates produced conidia more abundantly on millet, which may partly be due to an increased surface area for growth. However isolates A10, A13, A16, A22 and At1 showed a higher spore production on corn flour after 3 weeks, despite the smaller surface area.

Of the 44 *Aschersonia* isolates, isolate A21 did not sporulate at all (-) and 7 isolates sporulated poorly (±), providing less than 5×10^7 spores per culture (Tab. 2.1). For this reason these isolates were not included in the bioassays. Seven isolates sporulated very well, producing more than 5×10^9 spores/culture. Three isolates belonged to the species *A. aleyrodis* from Japan and Colombia, one to *A. placenta* from India, one to *A. insperata* from Java and two to unidentified *Aschersonia* isolates, A8 from Malaysia and A18 from Colombia.

Conidial germination

Although most isolates germinated well on water agar (i.e. above 90% germination 24 hrs. post inoculation), a few isolates germinated at a low or intermediate level, varying between 3.4 and 61.6% (Tab. 2.1). Also on nutrient-rich medium like PDA, germination of these isolates was poor (Meekes, unpublished results). After four to eight days on water agar, conidia of the isolates A10, A13, A14, A19, A22, Ap1 and At1, formed one to several long, slender, aerial structures with secondary conidia at their tips (capilliconidia) (Tab. 2.1). In most cases also normal germ tubes were seen, usually a very low percentage. Isolate Ap1, which in general germinated well, formed capillicondia from conidia as well as from the tips of normal germ tubes on water agar (Fig. 2.1). Formation of capilliconidia was rare in isolates A16 and Aa2.

Figure 2.1: Formation of capilliconidia in *A. placenta*, after 6 days on WA. Note the formation of capilliconidia on the germ tubes; 1) primary conidium, 2) capilliconidia, bar = 8 µm.

Virulence

Ten isolates, A10, A12, A13, A16, A19, A20, A21, A22, A25, and At1, did not infect either whitefly species or could not be reisolated from diseased insects (Tab. 2.1). These isolates were excluded from the bioassays on virulence.

On poinsettia mortality of the control treatments (untreated and Tween-treated nymphs) differed considerably between *B. argentifolii* and *T. vaporariorum*. For silverleaf whitefly, mortality varied between 1.9 and 5.8% (Exps. 1 and 3) and for greenhouse whitefly it varied between 6.9 and 15.5% (Exps. 2 and 3). For *B. argentifolii*, the mortality in the control treatments is not significantly different from the percentage 'mortality by other causes'

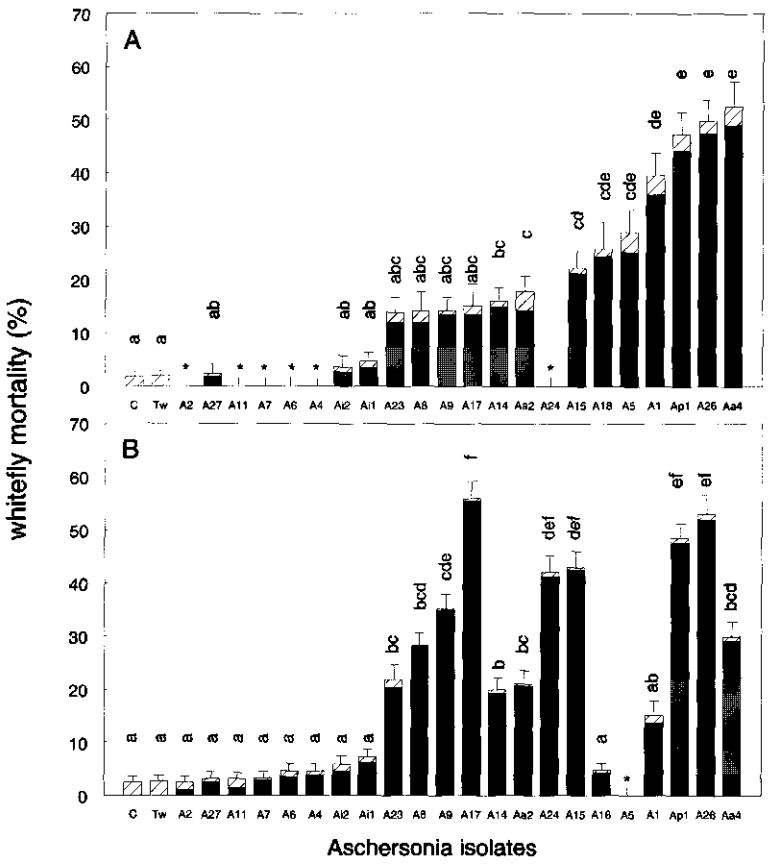

Figure 2.2: Mean percentage mortality of third nymphal instars of *B. argentifolii* by *Aschersonia* spp. (dark grey bars) and other causes (shaded bars), A: Exp. 1A, B: Exp. 1B; error bars represent standard deviation. Bars not followed by the same letter are significantly different (1A: $\alpha = 0.000198$; 1B: $\alpha = 0.000327$).

observed in the treatments with *Aschersonia*. Mortality by other causes varied more with *T. vaporariorum* than with *B. argentifolii*. For isolates A8, Aa2 and Ap1 (Fig. 2.3), and A28, Ap2, Aa6 and A32 (Fig. 2.4), mortality by other causes was significantly higher ($p < 0.05$) than in the control treatments. This could be due to an effect of poinsettia on *T. vaporariorum* or to overlap between natural mortality and mortality caused by the fungus. For this reason the overall to compare the performance of the isolates.

Virulence of the *Aschersonia* spp. for both silverleaf and greenhouse whitefly differed considerably between isolates (Fig. 2.2 - 2.4). In the experiments conducted with *B. argentifolii*, isolates Aa4 (Fig. 2.2A), A17 (Fig. 2.2B), A26 and Ap1 (Fig. 2.2A and 2.2B)

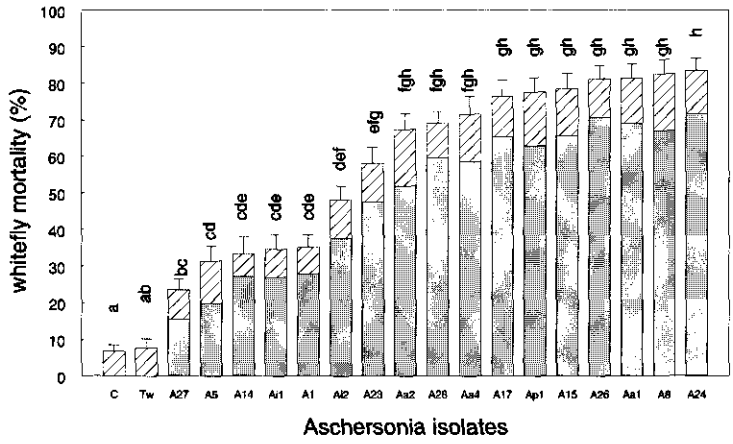

Figure 2.3: Mean percentage mortality of third nymphal instars of *T. vaporariorum* (Exp. 2) by *Aschersonia* spp. (light grey bars) and other causes (shaded bars); error bars represent standard deviation. Bars not followed by the same letter are significantly different ($\alpha = 0.000292$).

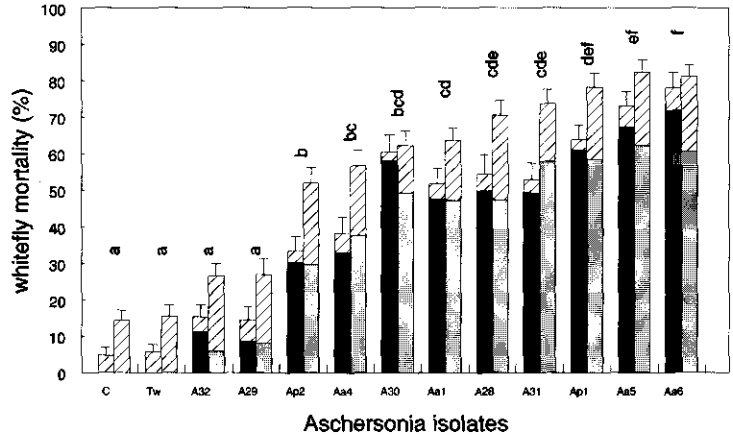

Figure 2.4: Mean percentage mortality of third nymphal instars of *B. argentifolii* (dark grey bars) and *T. vaporariorum* (light grey bars) by *Aschersonia* spp. and other causes (shaded bars) (Exp. 3); error bars represent standard deviation. Bars not followed by the same letter are significantly different ($\alpha = 0.000321$).

caused a significantly higher mortality than the majority of the other *Aschersonia* isolates tested. Several isolates, such as A1, A17, A18 and Aa4 did not perform consistently (Fig. 2.2A vs. 2.2B). On *T. vaporariorum* (Fig. 2.3) many isolates performed well. In Exp. 3 an additional set of isolates was tested on both whitefly species (Fig 2.4). Interaction between whitefly and

fungal treatments was not significantly different (p = 0.66). Overall mortality of *T. vaporariorum* nymphs was significantly higher than overall mortality of *B. argentifolii* nymphs. However, this effect is mainly due to differences in mortality in the control treatments or 'mortality by other causes' between the whitefly *argentifolii* as on *T. vaporariorum*. The isolates Aa6, Aa5 and Ap1 caused a higher mortality on both whitefly species than the majority of the isolates tested in Exp. 3. Four groups of isolates could be distinguished: isolates which were unable to infect either whitefly, those which perform poorly (<10%mortality), an intermediate group (10-50% mortality) and a group which performed well (>50% mortality).

No correlation was found between germination levels on water agar and virulence (Fig. 2.5). When germination of an isolate was low, even under the best circumstances, its virulence was also low. However, when germination was high, the isolate could be avirulent or highly virulent for both whitefly species. For example, germination levels of A14 and Ai2 did not exceed 50%, and mortality levels did not exceed 25%. On the other hand, isolates A5 and A18 showed high germination levels (>92%), but infection levels stayed below 25% (Tab. 2.1, Fig. 2.2 to 2.4), whereas germination levels of A26 or Aa5 also exceeded 92%, but mortality levels were above 50%.

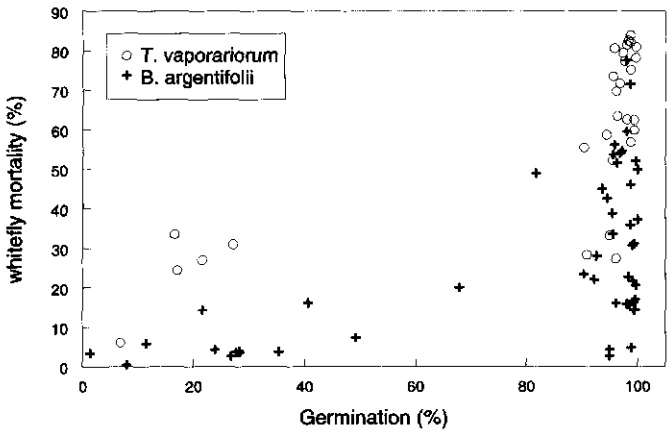

Figure 2.5: Mean percentage whitefly mortality versus mean percentage germination of *Aschersonia* spp. on water agar.

Discussion

Important criteria for development of a mycoinsecticide for biological control are ease of production on a artificial medium, high spore yield and high virulence against target organisms. In Lacey *et al.* (1996) preliminary data on conidial production of *Aschersonia* spp. were given. In Table 2.1 additional data are provided. Differences that exist are based upon choice of medium - millet grains or corn flour -, and data over a prolonged period of time are

included. In general, *Aschersonia* spp. readily produce conidia on (semi-)artificial media in a solid phase or two-phase system (Oho, 1967; Hirte *et al.*, 1989a; Ibrahim *et al.*, 1993; Lacey *et al.*, 1996). Artificial light or daylight enhances conidial production (Rombach & Gillespie, 1988). Sporulation of *Aschersonia* spp. does not occur in liquid culture, or, if so, only at its surface (Ibrahim *et al.*, 1993). This has been a drawback for mass production. However, interest in two-phase and solid phase fermentation technology of fungi has increased in recent years (Lacey *et al.*, 1996) and it offers new perspectives for mass spore production of *Aschersonia* spp. in the future.

Among isolates of *Aschersonia* spp. large differences exist between germination levels on water agar. However there was no clear correlation between germination and infection levels in general. Several isolates were able to form secondary conidia (capilliconidia) on water agar. This phenomenon was first described for *Aschersonia* by Evans (1994), and it is morphologically and probably functionally analogous to the capilliconidia of the Entomophthorales. In the latter, they are thought to be formed when nutritional and/or physical conditions are unsuitable for vegetative growth (Uziel & Kenneth, 1991), for instance in the absence of a suitable host. Through their capacity to inoculate and infect mobile host insects, the Entomophthorales are highly specialized to the entomopathogenic 'behavior' (Glare & Chilvers, 1985). Whether capilliconidia in the genus *Aschersonia* have other virulence characteristics than primary conidia has still to be investigated. Formation of capilliconidia may increase their ability to spread, since primary conidia of *Aschersonia* are produced in mucilage and are mainly dependent on splash dispersal. By formation of one or more capilliconidia dispersal by adult whiteflies or other moving insects to other plants would be enhanced.

Isolates of *Aschersonia* show differences in virulence for whitefly. Although the genus *Aschersonia* is specific to whiteflies (Aleyrodidae) and scale insects (Coccidae), several of the isolates that were tested, were not able to infect *B. argentifolii* or *T. vaporariorum*. This could be due to a number of reasons. Firstly, the history of previous attenuation for some of the stored isolates is unknown. This was partly overcome by passage of the isolates through whitefly. For entomopathogenic fungi in general, it has been demonstrated that successive passage through a host enhances the virulence and repeated subculturing on artificial medium can decrease the virulence. Changes in the ability to infect were thought to be a result of gradual selection of genotypes (in Goettel, 1992). However, St.-Leger *et al.* (1991) stated that environmental conditions during growth were important. They showed that levels of cuticle degrading enzymes were higher on conidia that were directly derived from infected hosts compared to conidia produced on artificial medium and, therefore, the former conidia can preadapt to the pathogenic lifestyle. For *Aschersonia* in particular, Fransen (1987) discovered

that, after subculturing *A. aleyrodis* 12 times on millet, median lethal times were lower than that of isolates that were subcultured three times, although final infection levels were similar. Also Hirte *et al.* (1989a) stressed the importance of passaging isolates through whitefly in order to keep the virulence at a high level. After 1.5 year, infection levels of their *A. placenta* isolate dropped from 75-95% to 20-40%. Some species within the *Aschersonia* genus are very difficult to store and to subculture, and viability is soon lost. It cannot be excluded that attenuation of isolates, before they came in our possession, had an effect on the virulence of the isolates in our experiments.

Secondly, the original host of the isolates is not always known. In several cases no living host remained and destruction of the original host by the fungus was so complete that identification was not possible (Evans & Hywel-Jones, 1990). Petch (1921), in his study on the genera *Hypocrella* and *Aschersonia*, made a clear distinction between species pathogenic to whiteflies (Aleyrodiicolae) and species that are able to infect scale insects (Lecaniicolae). Morphologically, these two groups differ in presence (Aleyrodiicolae) or absence (Lecaniicolae) of paraphyses in the pycnidium (Petch, 1921). Several isolates, which are not described to species level, may have originated from scale insects and are, therefore, not able to infect whitefly. For instance, *A. turbinata* (At1) belonging to the Lecaniicolae did not infect either of the whitefly species. On the other hand, since Petch's monograph of the genus in 1921, several authors described species belonging to the Aleyrodiicolae on scale insects: *A. duplex* (Dingley, 1954), *A. goldiana* (Wolcott, 1955), *A. aleyrodis* (Vargas Sarmiento *et al.*, 1995) and *A. placenta* (Lim *et al.*, 1991). Lim *et al.* (1991) isolated *A. placenta* belonging to the Aleyrodiicolae from *Asterolacium ungulata* (Homoptera: Coccidae) in Malaysia, where the fungus was able to suppress this scale insect considerably. Vargas Sarmiento *et al.* (1995) used an *A. aleyrodis* isolate originating from Coccidae for control of greenhouse whitefly. The possibility exists that a number of species like *A. aleyrodis* and *A. placenta* are less selective to their host as has been presumed thus far. Recently, it has been shown that presence of paraphyses in the pycnidia on the host may lack when the fungus is grown in pure culture. Presence or absence of paraphyses is likely to be phenotypically determined and, therefore, it is not a reliable taxonomic feature (Evans & Hywel-Jones, 1997). The results mentioned above also indicate that specificity of infection within the Aleyrodidae family does not seem to exist for all species. For example an *A. placenta* isolate originating from *Dialeurodes cardamomi* (Ap1) was performing equally well on *B. argentifolii* and *T. vaporariorum* (Fig. 2.2 and 2.3).

The majority of the isolates mentioned in Tab. 2.1 were not identified to species level. It is probable that several of them belong to undescribed species of the genus *Aschersonia*. The taxonomy of this genus is uncertain and urgently in need of revision (Evans & Hywel-Jones, 1997). To investigate relationships among the *Aschersonia* species and isolates, Obornik *et al.*

(1999) reconstructed a phylogeny, using randomly amplified polymorphic DNA (RAPD) markers. In this phylogenetic tree, clustering of isolates coincides with their geographical origin. Isolates A15, A17 and A24 probably belong to *A. aleyrodis*, whereas isolates A28, A30, A31 and A32 are closely related to, or belong to, *A. placenta*. Morphology suggests that the majority of the virulent isolates, such as A17, A24, Aa1, Aa4, Aa5, Aa6 and Ap1 belong to the *A. aleyrodis* - *A. placenta* complex.

Among the isolates that were able to infect whitefly, a positive relationship existed between virulence against *B. argentifolii* and virulence against *T. vaporariorum*. When testing fungal isolates on both species under the same circumstances (Exp. 3), overall mortality on greenhouse whitefly is significantly higher than that on the silverleaf whitefly, due to the higher 'mortality by other causes'. Statistical analysis is carried out on data including 'mortality by other causes'. This mortality may be similar to mortality occurring in the control group of hosts, but is in some cases lower or higher. Most insects are killed by the fungus and as a result cease to be susceptible to other mortality causing factors (Fransen, 1987). Also the possibility of toxins playing a role in the infection process cannot be excluded (Krasnoff *et al.*, 1996). For this reason, we used overall mortality data rather than applying Abbot's formula (Abbott, 1925). Poinsettia, with higher developmental rates for *B. argentifolii* than for *T. vaporariorum* at 25 °C, appears to be a more suitable host plant for the former whitefly species (van Lenteren & Noldus, 1990; Fransen, 1994). Higher infection levels are to be expected when a developmental period of the insect is extended and the host is weakened (Steinhaus, 1958). However, the hypothesis that greenhouse whitefly, with its longer developmental period at 25 °C and its higher control mortality, is more susceptible to infection than silverleaf whitefly is not supported by our results, although this theory is not based on two insect species, but on one species under different circumstances.

The mortality levels of *B. argentifolii* or *T. vaporariorum* by *Aschersonia* spp. seem to be lower than mortality levels by other fungi like *B. bassiana*, *M. anisopliae*, *P. fumosoroseus* and *V. lecanii* found by other authors (Vidal *et al.*, 1997; Malsam *et al.*, 1998; Wraight *et al.*, 1998; Gindin *et al.*, 2000). However, one has to take into account that most bioassays are carried out at different climate settings, with different compositions of the insect population and/or on different host-plant species. Our bioassays, for instance, were carried out in a more practice-related environment and on poinsettia, which seems to have a more hostile environment for entomopathogenic fungi than cucumber and gerbera (Meekes *et al.*, 2000; E. Meekes & E. Beerling, unpubl.). This makes comparison of results of the various bioassays more difficult.

The utilization of *Aschersonia* species as biocontrol agents has a long history. In the early 1900's, *A. aleyrodis* was successfully introduced in Florida for the control of *Dialeurodes*

citri and *D. citrifolii* (Berger, 1921). Today this is a classical example of successful biological control, where predators, parasitoids and fungi control these pests. Similar results have been obtained in the Azerbaijan region, where several species of *Aschersonia* have been introduced from South-East Asia and America for the biological control of citrus whitefly (McCoy *et al.*, 1988). As a consequence, several species of this genus have been tested as a selective control agent against greenhouse whitefly in protected cultivation in Eastern Europe and China, with good results. Several of the *Aschersonia* isolates tested in our study show good potential for biological control of *B. argentifolii* and *T. vaporariorum* through their high spore production on semi-artificial media and high levels of infection of whitefly on poinsettia. Performance of the isolates A24, A26, Ap1, Aa5 and Aa6 will be studied in more detail to get a better insight in (1) their speed of kill and (2) their mortality effects related to concentration of spores. These characteristics are crucial for obtaining effective biological control.

Acknowledgements

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Introduction

The greenhouse whitefly, *T. vaporariorum*, and the silverleaf whitefly, *Bemisia argentifolii* (*B. tabaci* biotype B) are among the major pests in field and greenhouse crops (Byrne *et al.*, 1990a; Becker *et al.*, 1992). Chemical control has often proven to be ineffective, as these species have the ability to develop resistance to a large range of pesticides (Horowitz & Ishaaya, 1996). Due to the negative side-effects of chemical insecticides and consumer concerns, the policy of many countries now aims at a decrease in pesticide use and encourages the development of alternative means of control in the context of integrated pest management (van Lenteren, 2000). One of these methods involves the use of insect pathogenic fungi. The interest for the use of these fungi is high because of, among others, their high virulence, their adaptation to and persistence in the insect habitat, and their compatibility with other natural enemies (Lacey *et al.*, 1996). To develop fungal pathogens into microbial control products it is necessary to select genotypes which combine the best possible characteristics for killing the target insects. In general, these characteristics can be divided into i) good field performance, showing high virulence against target organisms and the ability to withstand the environment in which the pest occurs, and ii) good mass production features, such as high sporulation on artificial media (Prior, 1992).

Over 30 different species of fungi are able to infect whiteflies, including *Aschersonia* spp., *Verticillium lecanii*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* (Hall, 1982; Fransen, 1990; Wraight *et al.*, 1998, Chapter 1) and several have been developed into products for whitefly control (Ravensberg *et al.*, 1990; Knauf & Wright, 1994; Bolckmans *et al.*, 1995; Wraight & Carruthers, 1999). However, results vary with glasshouse climate, culture conditions of the crop and crop species used. Of the above mentioned 30 fungi, 24 belong to the genus *Aschersonia* (Deuteromycotina: Coelomycetes) (Petch, 1921; Mains, 1959a, b). These fungi have been recognized to cause natural epizootics in Aleyrodidae in the tropics and subtropics, and are capable of wiping out entire whitefly populations (Evans & Hywel-Jones, 1990). The utilization of *Aschersonia* spp. as biological control agents has a long history. At the beginning of this century crude inocula of *A. aleyrodis* and *A. goldiana* were already in use to control whitefly populations of *Dialeurodes citri* and *D. citrifolii* in citrus groves in Florida (Berger, 1921). Today this is a classical example of successful biological control, where predators, parasitoids and fungi control these pests (McCoy, 1985). *A. aleyrodis* was also tested as a potential selective control agent against greenhouse whitefly. It was found to be a promising whitefly control agent because of its tolerance to relative humidities as low as 50% (Fransen, 1987; Chapter 7), its long persistence on leaf surfaces (Fransen, 1995; Meekes *et al.*, 2000; Chapter 5) and its compatibility with the parasitoid *Encarsia formosa* (Fransen & van Lenteren, 1993; 1994).

Previous research has focussed on *A. aleyrodis* as a control agent for *T. vaporariorum*, but with the accidental introduction of *B. argentifolii* in Dutch glasshouses in 1987 (Fransen, 1994), a biocontrol agent was needed which would work against both whitefly species equally well. In chapter 2 it was shown that from a total of 44 *Aschersonia* isolates tested several were able to cause high levels of mortality in both *T. vaporariorum* and *B. argentifolii* populations. For this study six *Aschersonia* isolates were selected, based on the quantity of spore production, high mortality levels of both whitefly species and geographical origin (Chapter 2). By taking six isolates from very different regions a wider range of genetic variation was ascertained (Oborník *et al.*, 1999). The virulence of these pathogens was compared by measuring the number of conidia required to kill a certain proportion of a group of insects, expressed as median lethal concentration (LC_{50}, conidia/ml) or median lethal dose (LD_{50}, conidia/cm^2). Virulence was also compared by measuring the time required to kill a certain proportion of a group of insects, expressed as median lethal time (LT_{50}) and recording the nymphal instar in which mortality occurred. Here we report on the results and bioassay procedures for the identification of virulent isolates for further research.

Material and Methods

Whitefly host

The *B. argentifolii* culture was maintained on poinsettia plants (*Euphorbia pulcherrima* 'Goldfinger') in screened cages at 23°C under greenhouse conditions. The *T. vaporariorum* colony was reared on gerbera plants (*Gerbera jamesonii* hybrids) in screened cages at 21°C under greenhouse conditions.

To obtain second instar nymphs, about 50 adults, both male and female, were confined to clip-cages (Ø 2 cm) on the underside of poinsettia leaves (1 clip-cage per leaf). The adults were given the opportunity to lay eggs for 24 hours. This resulted for *B. argentifolii* in approximately 105 (± 2.5 SE) nymphs per leaf in experiment 1 and 2, and for *T. vaporariorum* in 103 (± 2.7 SE) and 48 (± 1.9 SE) nymphs per leaf in respectively experiment 1 and 2 (see below).

The development from egg deposit to second instar nymph was about 25 to 26 days for *B. argentifolii* and 17 to 19 days for *T. vaporariorum* at 20°C on poinsettia. As a result of overlapping nymphal cohorts a mixture of first, second and third instar nymphs were sprayed: first : second : third instar = 35% : 55% : 10% for *B. argentifolii* and 15% : 80% : 5% for *T. vaporariorum* in the first experiment. In the second experiment these percentages were: 10% : 50% : 40% for *B. argentifolii* and 5% : 60% : 35% for *T. vaporariorum*, respectively.

Statistical analysis

Data per set were analyzed using generalized linear models (Genstat 5 release 3.22, Payne *et al.*, 1987) with isolate as independent variable. The proportion dead nymphs was binomially distributed and the logistic-link transformation function was used throughout. An LC_{50} and standard error (based on Fieller's Theorem) was estimated for each isolate, using the Genstat 5 procedure FIELLER.

From the overall mortality over time, the median lethal time was calculated. As every leaf is evaluated over time, these mortalities were not independent. A separate regression analysis (generalised linear model, binomial distribution, logit link function) against time was therefore carried out for each leaf in relation to each of the higher dosages (1×10^7 and 1×10^8 conidia/ml). When significant differences occurred, t-tests were carried out and the probability level was adjusted to the number of t-tests performed.

Fractions mortality in first to third instar, proportional to total whitefly mortality, were arc-sin square-root transformed. This transformed mortality was analysed using a three-way ANOVA with whitefly species, isolate and concentration (10^5 to 10^8 conidia/ml) as independent variables (Genstat 5 release 3.22, Payne *et al.*, 1987). Subsequent differences were compared with t-tests, in which probability (α) was adjusted to the number of t-tests performed (n) (α/n).

Results

Median lethal concentration

In the first set of experiments the median lethal concentrations, varying between 1.89×10^6 (A31, *T. vaporariorum*) and 3.93×10^6 conidia/ml (Aa5, *T. vaporariorum*), were not significantly different for the three isolates. These LC_{50}'s corresponded with a dose of 2.57×10^3 to 5.34×10^3 conidia/cm^2 poinsettia leaf, respectively (Fig. 3.1A and B, Tab. 3.2). Probit slopes ranged from 0.83 (A31 - *B. argentifolii*; Ap1 - *T. vaporariorum*) to 1.32 and 1.44 (Aa5 - *B. argentifolii* and *T. vaporariorum*, respectively).

In the second set the mortality caused by isolate A23 did not exceed 50%. In order to estimate the LC_{50} value, extrapolation outside the experimental data would be necessary. The value itself, however, would be questionable, since an increase in concentration led to a lower mortality (see Fig. 3.1C and D). For this reason isolate A23 was excluded from the LC_{50} calculation. The latter phenomenon was also observed for isolate A24, which caused a lower mortality of both whitefly species at 1×10^8 conidia/ml compared with the mortality at 1×10^7 conidia/ml, hence the highest concentration for isolate A24 was also excluded from analysis. The LC_{50} values for A24 and A26 were not significantly different, varying between 0.52×10^6 (A24, *T. vaporariorum*) and 1.38×10^6 conidia/ml (A24, *B. argentifolii*), which

Table 3.2: The average median lethal dose (LD_{50}), 95% lower and upper confidence intervals (CI) for *Aschersonia* spp. on *B. argentifolii* and *T. vaporariorum*.

			B. argentifolii		*T. vaporariorum*	
Fungal isolate			LD_{50} (x 10^3 con./cm^2)	95% low. & up. CI (x 10^3 con./cm^2)	LD_{50} (x 10^3 con./cm^2)	95% low. & up. CI (x 10^3 con./cm^2)
set 1	*Aschersonia* sp.	A31	3.12 a *[1]	2.17 - 4.50	2.57 a	1.74 - 3.69
	A. aleyrodis	Aa5	4.77 a	3.26 - 7.01	5.34 a	3.67 - 7.72
	A. placenta	Ap1	3.39 a	2.43 - 4.75	4.27 a	3.10 - 5.87
set 2	*Aschersonia* sp.	A23	-	-	-	-
	"	A24	1.87 **a** [2]	1.33 - 2.65	0.71 **a**	0.35 - 1.42
	"	A26	1.57 **a**	1.16 - 2.12	1.54 **a**	0.65 - 3.67

*: numbers followed by the same letters are not significantly different. Statistical analyses were carried out per experiment. Since the same data were used for comparing LD_{50} and LT_{50} values, the probability level was adjusted according to the number of t-test carried out (exp.1: [1]: $\alpha = 0.008$, exp.2: [2]: $\alpha = 0.025$); bold or normal letters indicate different sets of comparisons. *: highest dose excluded for calculations.

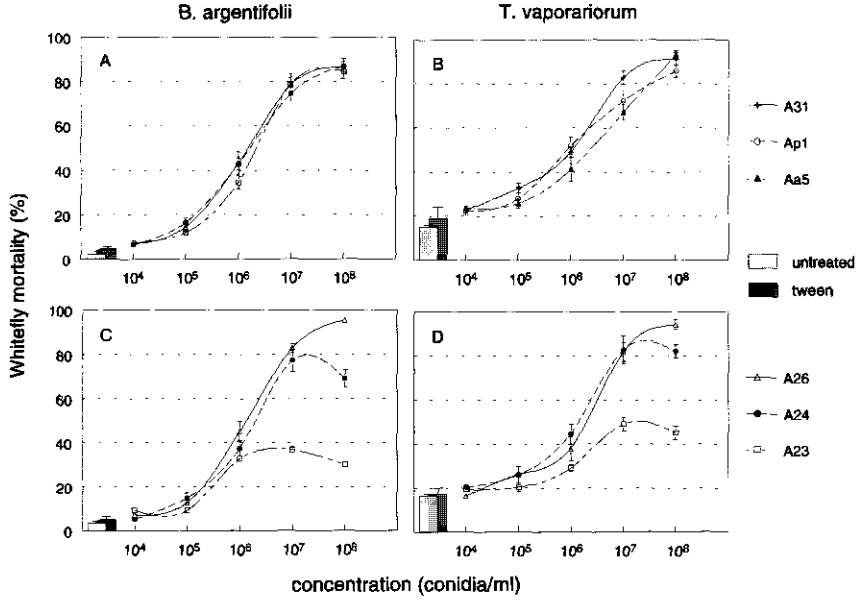

Figure 3.1: Average total mortality of *B. argentifolii* (A: set 1, C: set 2) and *T. vaporariorum* (B: set 1, D: set 2) caused by *Aschersonia* spp. A31, Aa5, Ap1, A23, A24 and A26, applied in concentrations of 10^4 to 10^8 conidia/ml, 28 days after treatment. Vertical lines represent standard error of mean.

correlated with a dose of 0.71 x 10^3 and 1.87 x 10^3 conidia/cm^2, respectively (Tab. 3.2). Probit slopes varied between 0.82 (A24 - *B. argentifolii*) and 1.02 (A26 - *T. vaporariorum*).

In general, control mortalities, *i.e.* untreated and Tween treated whitefly nymphs, were not significantly different ($p > 0.05$) from mortality of nymphs treated with 10^4 conidia/ml (Fig. 3.1) or from each other, hence using Tween as spreader did not affect whitefly mortality. However, mortality of *T. vaporariorum* nymphs exceeded the mortality of *B. argentifolii* nymphs: 17.1% (± 0.7 SE) versus 3.3% (± 0.3) for set 1 and 16.4% (± 0.7) versus 4.3% (± 0.3) for set 2, respectively. Since it was found for some isolates that with increasing concentration of conidia applied, mortality by other causes decreased, we chose not to correct for control mortality.

Median lethal time

The median lethal time was calculated for concentrations 1 x 10^7 and 1 x 10^8 conidia/ml (see above). In the first set of experiments, nymphs of *B. argentifolii* as well as *T. vaporariorum* treated with isolate A31 died significantly earlier than nymphs treated with Aa5 and Ap1 (Tab. 3.3). For *B. argentifolii* no significant difference existed between LT_{50}'s at 1 x 10^7 (4.6 - 7.1 days) and 1 x 10^8 conidia/ml (4.6 - 6.6 days) for all three isolates, whereas for *T. vaporariorum* the LT_{50} at 1 x 10^7 (6.0 - 9.9 days) was significantly higher compared to the LT_{50} at 1 x 10^8 conidia/ml (4.5 - 6.4 days) (Tab. 3.3).

Table 3.3: The average median lethal time (LT_{50}) for *Aschersonia* spp. on *B. argentifolii* and *T. vaporariorum* at concentration of 10^7 and 10^8 conidia/ml.

		LT_{50} (days)					
		B. argentifolii		main effect	*T. vaporariorum*		main effect
Fungal isolate		10^7	10^8	fungal isolate	10^7	10^8	fungal isolate
Set 1 *Aschersonia* sp.	A31	46	46	***a**** [1]	60	45	***a***
A. aleyrodis	Aa5	71	6.6	***b***	96	64	***b***
A. placenta	Ap1	66	59	***b***	99	54	***b***
main effect conc.		A [2]	A		B	A	
Set 2 *Aschersonia* sp.	A23	-	-		-	-	
"	A24	65	87	b [3]	54	56	a
"	A26	50	51	a	53	47	a

*: numbers followed by the same letters are not significantly different. Statistical analyses were carried out per experiment. Since the same data were used for comparing LD_{50} and LT_{50} values, the probability was adjusted according to the number of t-test carried out (exp.1: [1]: $\alpha = 0.004$, lsd = 1.21 [2]: $\alpha = 0.002$, lsd = 1.40; exp.2: [3]: $\alpha = 0.008$, lsd = 1.76); bold or normal letters and capital or lowercase letters indicate different sets of comparisons.

In the second set of experiments isolate A23 was excluded, because extrapolation outside the experimental period (> 28 days) would be meaningless. At 28 days after treatment more than 90% of the control nymphs had already developed into adult whiteflies, which are rarely infected by *Aschersonia* spp. (Fransen *et al.*, 1987). *B. argentifolii* nymphs treated with isolate A24 died significantly more slowly (6.5 and 8.7 days for 10^7 and 10^8, resp.) compared with nymphs of *T. vaporariorum* (5.4 and 5.6 days for 10^7 and 10^8, resp.), whereas nymphs of both whitefly species died equally fast when treated with isolate A26 (5.0 and 5.1 days for *B. argentifolii*, 4.7 and 5.3 days for *T. vaporariorum* at 10^7 and 10^8, resp.) (Tab. 3.3). No significant difference in LT_{50} was found between applied concentrations for either whitefly.

Mortality in first to third instar period

Another method for comparing speed of kill is comparing stages in which mortality is occurs. For instance, when nymphs die in their third instar for one isolate and in their fourth instar for another, isolate one kills its host more quickly than isolate two, when both are applied on the same instar. In Tab. 3.4 the whitefly mortality occurring in first to third instar proportional to overall whitefly mortality, is shown for concentration 1 x 10^5 to 1 x 10^8 conidia/ml. The mortality at 10^4 conidia/ml was not significantly different from control treatments and therefore excluded.

In set 1 for *B. argentifolii* (Tab. 3.4), isolate Aa5 was significantly slower than A31 and Ap1: 67% versus 83% and 84%, respectively, of the total mortality occurred in first to third instar. For *T. vaporariorum* (Tab 3.4) isolate A31 was also significantly slower than Ap1. Furthermore, the higher the concentration, the shorter the infection time.

Table 3.4: Percentage mortality occurring in first to third nymphal instar proportional to the total mortality occurring per set of experiments.

Interactions:		**Isolate**			**Concentration**			
	Whitefly	A31	Aa5	Ap1	10^5	10^6	10^7	10^8
set 1	*B. argentifolii*	83 **a*[1]**	67 **b**	84 **a**	76 **BC**[2]	81 **BC**	76 **BC**	78 **BC**
	T. vaporariorum	74 **b**	71 **b**	83 **a**	66 **C**	74 **BC**	78 **AB**	85 **A**
		A23	A24	A26	10^5	10^6	10^7	10^8
set 2	*B. argentifolii*	35 *b*[3]	40 *b*	64 *a*	53 *A*[4]	49 *AB*	47 *AB*	37 *B*
	T. vaporariorum	31 *b*	58 *a*	60 *a*	52 *AB*	46 *AB*	51 *AB*	51 *AB*

*: numbers followed by the same letters are not significantly different. Statistical analyses were carried out per set of experiments. Probability levels are corrected for the number of t-test carried out, lsd's are only valid when data are arcsin√(fraction) transformed: [1]: $\alpha = 0.003$, lsd = 0.089; [2]: $\alpha = 0.002$, lsd = 0.105; [3]: $\alpha = 0.003$, lsd = 0.125; [4]: $\alpha = 0.002$, lsd = 0.147.

In set 2, a significantly higher proportion of *B. argentifolii* mortality caused by A26 occurred in the first to third instar compared with mortality caused by A24 and A23 (avg. 64% versus 35% and 40% mortality in first to third instar, Tab. 3.4). This meant that isolate A24 and A23 were slower than A26. In contrast to the results in set 1, it was found that the higher the concentration of conidia, the lower the percentage mortality occurring in the first to third instar (Tab. 3.4). For *T. vaporariorum* (Tab 3.4), the percentage mortality occurring in the first to third instar was significantly higher for isolates A24 and A26 in relation to A23 (avg. 58% and 60% versus 31%). The overall mortality caused by isolate A23 did not exceed 50% and only 31% of this mortality occurred in first to third instar of both whitefly species.

Discussion

Median lethal dose

Five of the six *Aschersonia* isolates tested here, were highly infective to nymphs of both whitefly species. Median lethal doses of these five *Aschersonia* isolates varied between 0.7 x 10^3 (A24) to 5.3 x 10^3 conidia/cm^2 (Aa5) for both whitefly species. The high virulence confirms our previous observations in which several *Aschersonia* isolates performed very well (Chapter 2). The findings were comparable with the LD_{50} of *A. aleyrodis* in the experiments of Fransen (1987) which was on average 2.2 x 10^3 conidia/cm^2 for second instar nymphs of *T. vaporariorum* on cucumber plants. Under laboratory conditions, the LD_{50} values observed by Wraight *et al.* (1998) for numerous isolates of *P. fumosoroseus*, *P. farinosus* and *B. bassiana* ranged from 5.0 x 10^3 to 1.5 x 10^4 conidia/cm^2 against 15 days old nymphs of *B. argentifolii* (20°C - 27°C). However, the *P. fumosoroseus* isolates that Vidal *et al.* (1997) used, seem to be more virulent against second instar *B. argentifolii* nymphs (LD_{50} values of 6.2 x 10^2 - 1.3 x 10^3 conidia/cm^2).

When comparing LD_{50} and LT_{50} values of different bioassays, one has to take into account that bioassays are often carried out at different conditions: relative humidity levels and temperatures may not be the same, neither may the composition of the insect population or host plant. For instance, Vidal *et al.* (1997) performed their bioassay on sweet potato at a temperature of 25°C, Wraight *et al.* (1998) used hibiscus plants at 20 - 27°C, and we used poinsettia at 20°C. Furthermore, differences in LC_{50} may be the result of natural variation in insect population and fungus. LC_{50}'s, LD_{50}'s and LT_{50}'s are statistical estimates on a particular set of data collected at a particular time and these values are neither biological constants nor measurements (Robertson *et al.*, 1995), but only relative indicators for virulence, which can be useful in screening for virulent strains.

Bioassay procedure

Many bioassays to test virulence of entomopathogenic fungi against whiteflies have been described, ranging from using fourth instar nymphs detached from the leaf (Landa *et al.*, 1994), leaf discs (Drummond *et al.*, 1987; Vidal *et al.*, 1997), detached leaves (Wraight *et al.*, 1998), rooted, detached leaves (Lacey *et al.*, 1999) to intact plants (Fransen *et al.*, 1987; Malsam *et al.*, 1998). Although the bioassay described above, conducted on intact plants after Fransen *et al.* (1987), is more laborious than a bioassay performed on leaf discs and not always feasible considering space and costs, many advantages exist.

Firstly, side-effects, such as unnatural ageing of leaves and coinciding extra mortality of whitefly nymphs or speeding up of whitefly development, is avoided (Fransen, pers. comm.). Since all nymphal instars, following the settling of the crawlers, will spend their life at the same location (Byrne & Bellows, 1991), leaf discs cannot be replaced. In addition, it is very difficult to maintain leaf discs, especially of poinsettia leaves, fresh for four weeks on artificial medium. The period has to be this long in order to make an accurate estimation of the whitefly mortality, since occasionally signs of infection were only just showing in the pupal stage (Fransen *et al.*, 1987). Rooted leaves might offer the same advantage, but not every host-plant species will root as easily as cabbage (Lacey *et al.*, 1999). Secondly, using intact plants the leaf, and with it the whitefly nymphs, stay in their natural position. Contamination by honeydew and related growth of secondary fungi will not occur on the underside of the leaf, in contrast with leaf discs which are put upside down upon artificial medium. Although, placing the containers upside down, will partly prevent contamination. Thirdly, performing experiments on leaf discs, instead of on whole plants, for instance, may influence the plant chemistry and with it insect behaviour (Risch, 1985; Stamp & Bowers, 1994). In turn, the pest insect can influence its host plant systemically and through this have an effect on other trophic levels, other pest insects (Inbar *et al.*, 1999a; 1999b), predators (Dicke *et al.*, 1990), but also on entomopathogenic fungi (Brown *et al.*, 1995). Finally, the bioassays are conducted under glasshouse conditions and the results should be easier to extrapolate to large glasshouse applications. Thus, in our opinion, using intact plants in climate conditions related to a practical situation will give the most realistic results.

Median lethal time - first to third instar mortality

The time required to kill a certain proportion of a group of insects is usually measured by monitoring the insect mortality daily. Although nymphs infected by *Aschersonia* spp. will turn opaque orange, white or brown, dependent on the *Aschersonia* species, the exact time of death is relatively difficult to determine, since whitefly nymphs are sedentary. The most reliable way to estimate the time of death might be recording of honeydew droppings, because honeydew production is positively correlated with number of nymphs alive (Yasui *et al.*, 1985). However,

Final remarks

In present strategies to develop environmentally safer forms of pest control, entomopathogenic fungi will play an increasing role in the control of *T. vaporariorum* and *B. argentifolii*. Of the six *Aschersonia* isolates tested, isolate A23 was not effective because it killed whitefly nymphs insufficiently. Isolate A24 was also discarded because, like isolate A23, it showed a lower mortality at the highest dose and, in addition, spore production of both isolates was not high (chapter 2) and their virulence did not compensate for that. Although small differences between isolates obtained in carefully manipulated bioassays may not always hold in large-scale experiments, the above data clearly show that *Aschersonia* isolates A26, A31, Aa5 and Ap1 are virulent against *T. vaporariorum* as well as *B. argentifolii* under glasshouse conditions. Their low LD_{50} and LT_{50} values strongly indicate that they can be effective biological control agents.

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4

Germination and infection behaviour of *Aschersonia* spp. on *Bemisia argentifolii* and Trialeurodes vaporariorum on poinsettia[1]

Abstract

Fungi of the genus *Aschersonia* are pathogens of whiteflies, which can be used as biocontrol agents against silverleaf whitefly, *Bemisia argentifolii*, and greenhouse whitefly, *Trialeurodes vaporariorum*. Scanning electron microscopy and bioassays were carried out to obtain better insight into the infection process of *Aschersonia aleyrodis*, *A. placenta* and an unidentified *Aschersonia* sp..

Conidia of *Aschersonia* spp. germinated readily on the cuticula of host insects as well as on water agar. On water agar *A. placenta* also produced capilliconidia. No germination was observed on poinsettia leaf surface, except on the leaf veins. On *B. argentifolii* the fungi formed large amounts of mucilage to attach themselves to the insect. Appressoria were formed before penetration, but also direct penetration was observed. This seemed not related to a specific site on the insect. For both whitefly species, first to third instar nymphs were most susceptible. If the population existed of fourth instar nymphs for more than 50%, infection levels dropped from 90 to 50%. Infected whitefly nymphs usually died in the stage following the treated stage. The fungus protruded from the insect via the margins or via the emergence folds of pupae if humidity levels were high enough. However, sporulation was rarely observed on whiteflies developing on poinsettia plants under the presented conditions.

[1] In preperation as **Meekes, E.T.M, Butt, T.M., Joosten, N.N., Porter, R., Beckett, A., Fransen, J.J. and Lenteren, J.C. van**. Germination and infection behaviour of *Aschersonia* spp. on *Bemisia argentifolii* and Trialeurodes vaporariorum on poinsettia

approximately 1.36 x 10^4 conidia per cm^2 poinsettia leaf. As a control, nymphs were sprayed with 2 ml of 0.05% Tween 80. After evaporation of the water, plants were covered with plastic bags for 48 hours to maintain a relative humidity (RH) of 95 - 100%. Plants were kept in an air-conditioned greenhouse. The RH fluctuated between 40 and 80% depending on season and photoperiod, and the temperature was kept at a constant level of 20 °C (±1 °C). This treatment procedure was used for microscopic studies as well as the bioassays, with the difference that for the bioassays the leaves stayed attached to the plants until final assessment of nymphs, whereas for the microscopic studies the leaves were picked 24, 48, 72 and 96 hrs after inoculation and processed directly or fixed (see electron microscopy).

Microscopic observations

Infection processes of *A. aleyrodis*, *A. placenta* and *Aschersonia* A26 were studied in detail on mostly first to third instar nymphs of *B. argentifolii*. Pieces of poinsettia leaf with infected *B. argentifolii* nymphs were fixed in 3% (v/v) formaldehyde in 0.05 M phosphate buffer pH 7 for 1.5 h at room temperature (ca. 20 °C). Fixation took place 24, 48, 72 and 96 h after inoculation. After fixation, the material was washed twice with phosphate buffer, rinsed with demineralised water and dehydrated through a graded ethanol series culminating in anhydrous acetone (critical point drying, CPD). The leaf pieces with whitefly nymphs were mounted on a copper stub and sputter coated with gold. Specimens were observed with a Philips 501B scanning electron microscope.

Specimens were also observed by low temperature scanning electron microscopy (LTSEM) in a freeze-dried state with a Philips 501B scanning electron microscope. Leaf pieces with infected insects were placed on a thin film of water on a copper stub then processed in a Emscope SP2000 Sputter-Cryo Cryogenic-Preparation System using the procedures described by Beckett and Read (1986).

Germination of conidia of *A. placenta* was also observed by fluorescent microscopy (FM) using the methods described by Butt (1987). In this study 0.02% aqueous Calcofluor (Sigma) was used to stain the fungal cell wall. Specimens were examined with an Olympus BH2 photomicroscope, fluorescence images were recorded on Kodak TriX Pan 400.

Bioassays

For assessment of mortality over a range of life stages of *B. argentifolii* and *T. vaporariorum*, (fungal) treatments were applied every second day starting after egg hatching (Fig. 4.1). Sixteen treatments in total were carried out on separate sets of plants, one leaf per plant, five leaves per treatment.

Since the developmental rate of *B. argentifolii* nymphs at 20 °C was slower than that of *T. vaporariorum* nymphs, oviposition of *B. argentifolii* was scheduled 8 days before the oviposition of *T. vaporariorum*. Treatments of both whitefly species were scheduled on the same day. As a result, suspensions of *A. aleyrodis*, *A. placenta* and 0.05% Tween solutions (spray control) were applied to the infested poinsettia leaves after 21 to 51 (*B. argentifolii*) or 13 to 43 (*T. vaporariorum*) days after oviposition (Fig 4.1).

On the day of conidial application, a pre-count was carried out to determine the number of whiteflies present on a leaf and nymphal stage composition of this whitefly population. The latter was carried out to determine the differential infection probability of the nymphal stages. Here, infection probability of the life stages is a combination of 1) contact probability of conidia with their host (hit-probability), 2) the amount of per nymphal stage: nymphal stages differ in size, hence a first instar nymph will receive less conidia than a third instar nymph, and 3) the actual susceptibility.

For the first sets of treatments, not all whitefly eggs had hatched at the time of pre-count, which could lead to unequal chances of nymphs to come into contact with fungal conidia. This was avoided by removal of these eggs. Suspensions were applied to poinsettia leaves as described above after 13 to 43 (*T. vaporariorum*) or 21 to 51 days (*B. argentifolii*) after oviposition (Fig 4.1).

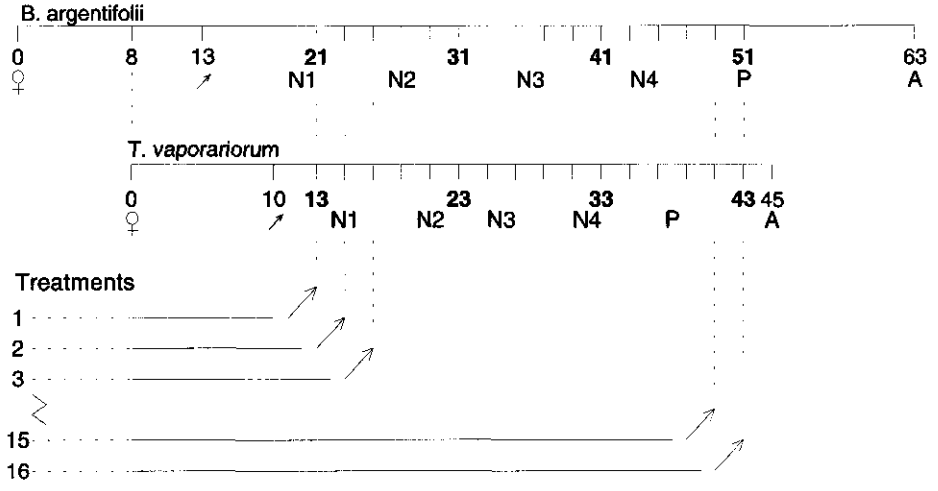
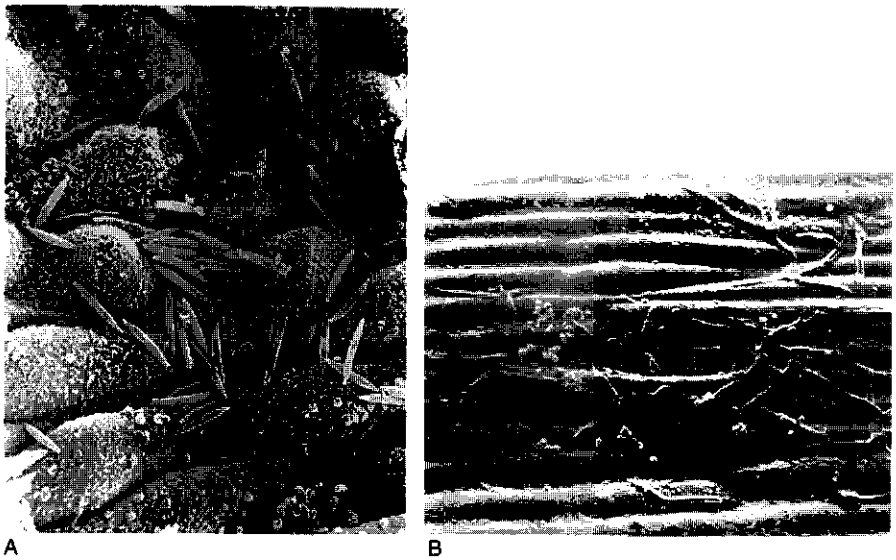


Figure 4.1: Time schedule for treatment of *B. argentifolii* and *T. vaporariorum* nymphs at 20 °C: oviposition by female whiteflies (♀), hatching of first eggs (↗) and the average nymphal stage of whitefly population at the time of treatment (N1- N4 = first to fourth nymphal stage, P = pupae, A = adults).



A B

Figure 4.3: A: Non-germinated conidia of *Aschersonia* A26 on poinsettia leaf lamina on the base of a minor vein 72 hrs p.i., note remnants of mucilage between conidia (778x, CPD). B: germlings of *A. placenta* Aplon major leaf vein, 24 hrs p.i. (414x, LTSEM).

For all *Aschersonia* spp. germination on the leaf rarely occurred (Fig. 4.3A), with exception of the major leaf veins (Fig. 4.3B, Tab. 4.1). The fusiform conidia (for all species 10-16 x 1.5-2.5 µm) could form apical, subapical or lateral germ tubes on the host insect (Fig. 4.4, Tab. 4.1). The majority of *Aschersonia* conidia on the host insect germinated, exhibiting differential germination behaviour, *viz.* some conidia germinated and penetrated directly, others formed appressoria before penetration (within 48 h post inoculation) (Fig. 4.4). Appressorium formation was found on the head-thorax and on the abdomen of the nymphs. On the host insect, the fungus produced mucilage around the conidia and appressoria (Fig. 4.5, Tab. 4.1). Even after the rough CPD treatment, strands of mucilage, attaching the fungus to the host, were clearly visible (Fig. 4.5). No preference for a specific site of entrance on the insect was observed. On a few nymphs extensive mycelial growth was observed, without clear evidence of infection (Fig. 4.6, Tab. 4.1). In some cases, the nymph seemed to be able to escape infection by moulting (Fig. 4.7A), but moulting not always results in escape from infection (Fig. 4.7B).

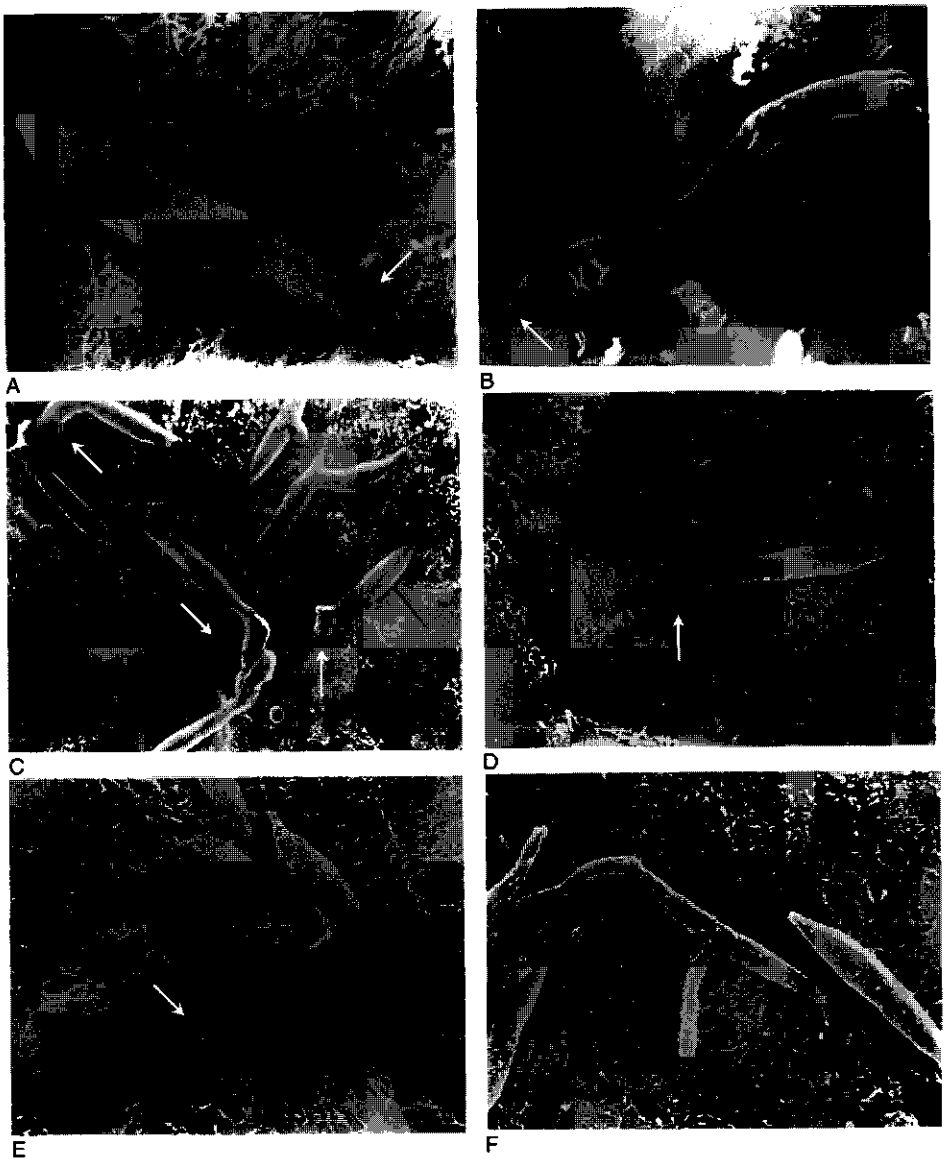


Figure 4.4: Conidia of *Aschersonia* spp. on *B. argentifolii* nymphs: Direct penetration of conidia of *A. placenta*, notice dark zone around penetration site (A: 6250x, 48 h p.i., CPD - B: 3228x, 24 h p.i., LTSEM); C: Appressorium formation by *Aschersonia* sp. A26 (1543x, 48 h p.i., LTSEM); D: Terminal appressorium of *A. placenta* (3230x, 24 h p.i., CPD); E: Penetration after appressorium formation of *A. placenta*, note penetration peg (3230x, 48 h p.i., CPD); F: Apical, subapical and lateral germination by *Aschersonia* sp. A26 (3230x, 48 h p.i., CPD).

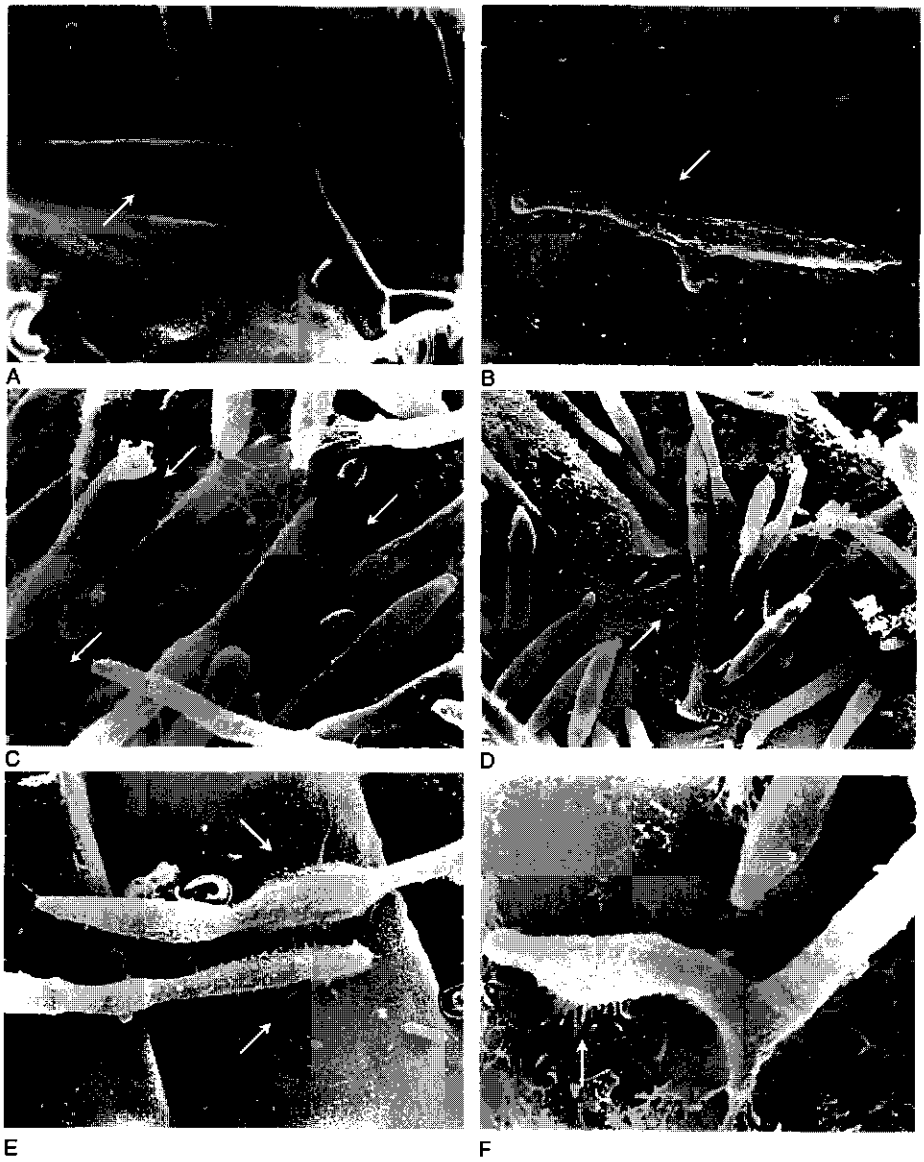
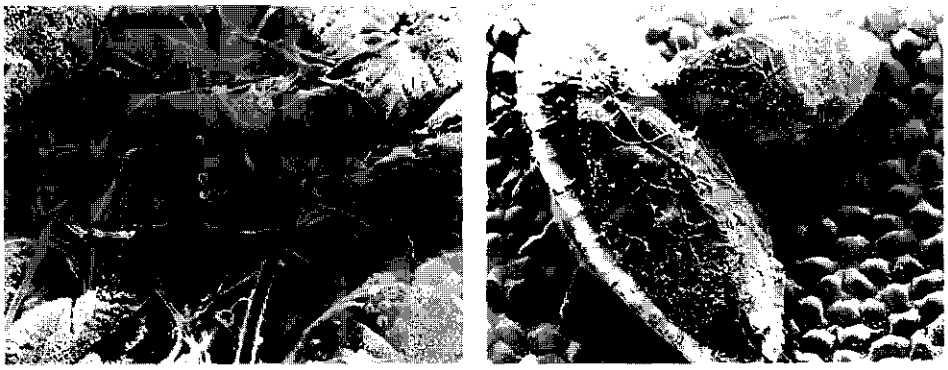
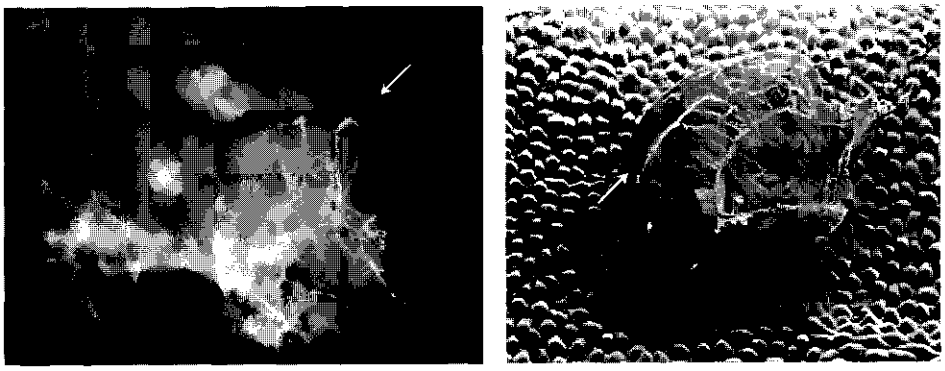


Figure 4.5: Conidia of *Aschersonia* spp. on *B. argentifolii* nymphs: A: Germlings of *Aschersonia* sp. A26 with mucilage pad (3228x, 24 h p.i., LTSEM); B: Germling of *Aschersonia* sp. A26 coated with mucilage, despite rough CPD treatment (3230x, 48 h p.i., CPD); C: Mucilage strands between conidia of *Aschersonia* sp. A26 (3230x, 48 h p.i., CPD); D: Appressorium with mucilage strands (*A. placenta*, 1540x, 48 h p.i., CPD); E: Appressoria attached to the insect with mucilage strands (*A. placenta*, 3230x, 48 h p.i., CPD); F: Germling of with appressorium and maybe a penetration peg, mucilage strands obvious around appressorium (*A. placenta*, 6280x, 48 h p.i., CPD).



A B

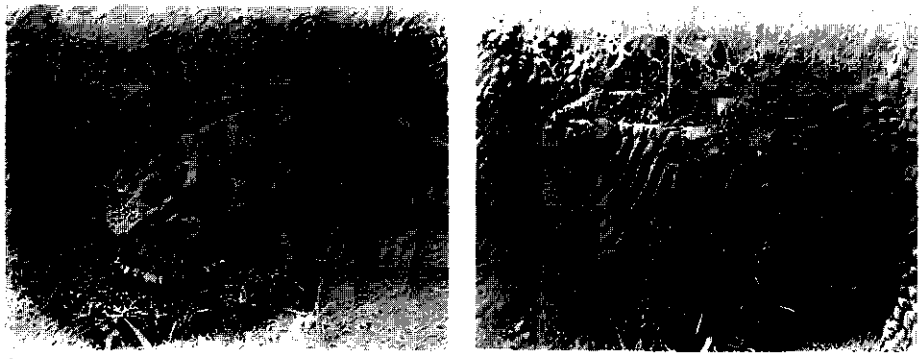
Figure 4.6: A: Mycelial growth on abdomen of second instar nymph of *B. argentifolii* (*A. placenta*, 788x, 48 h p.i., LTSEM); B: First instar nymph of *B. argentifolii* with egg shell, both covered with mycelium of *Aschersonia* sp. A26 (186x, 24 h p.i., LTSEM).



A B

Figure 4.7: A: *B. argentifolii* nymph seems to escape infection by *A. placenta* by moulting (FM); B: *B. argentifolii* nymph just moulted, without escaping infection by *A. placenta* (99x, 48 h p.i., LTSEM).

Four to six days after inoculation, nymphs started to turn orange and when the right conditions were met, mycelial protrusion from the margins and anal opening of nymphs was observed and, depending on developmental stage of the nymph, also via the emergence folds (Fig. 4.8, Tab. 4.1). On poinsettia, however, sporulation of *Aschersonia* spp. on whitefly nymphs hardly took place under conditions varying between of 40-80% RH.



A B

Figure 4.8: A: Fungal protrusion of *Aschersonia* sp. A26 from the margins of a second instar nymph of *B. argentifolii* (advanced stage of infection, 75x, LTSEM); B: *Aschersonia* sp. A26 ruptured though thorax of fourth instar nymph of *B. argentifolii* via emergence folds

Bioassays B. argentifolii

Mortality of *B. argentifolii* nymphs by *A. aleyrodis* and *A. placenta* varied with nymphal composition of the whitefly population at time of treatment (Fig. 4.9A, B and C). In general, *A. aleyrodis* and *A. placenta* caused a significantly higher mortality compared to the control mortality (Tween, on average 3.9% ± 1.8 SE mortality), except when treatment took place after 45 to 51 days for *A. aleyrodis* and after 49 to 51 days for *A. placenta*. For both *Aschersonia* species first to third instar nymphs were most susceptible. When half of the population existed of fourth instar nymphs or pupae (day 37 and further), mortality percentages dropped from > 80% to 50%. This decline in infection probability coincides with the decline of the presence of first to third instar nymphs. First instar nymphs seemed to be slightly less susceptible compared to second and third instar nymphs. However, for the first treatments on average 10% of the nymphs was missing between pre-count and final assessment on the treated as well as on the control leaves. This could be due to crawlers disappearing from the leaf. No significant differences in mortality existed between *A. aleyrodis* and *A. placenta*.

In general, the death of *B. argentifolii* nymphs did not occur in the nymphal stage which was treated, but one stage later (parallel lines Fig. 4.9D). Since it took on average 7.5 days for *B. argentifolii* nymphs to develop to the next stage, it took on average 7.5 days for *Aschersonia* spp. to kill 70-95% of the whitefly nymphs at 20 °C.

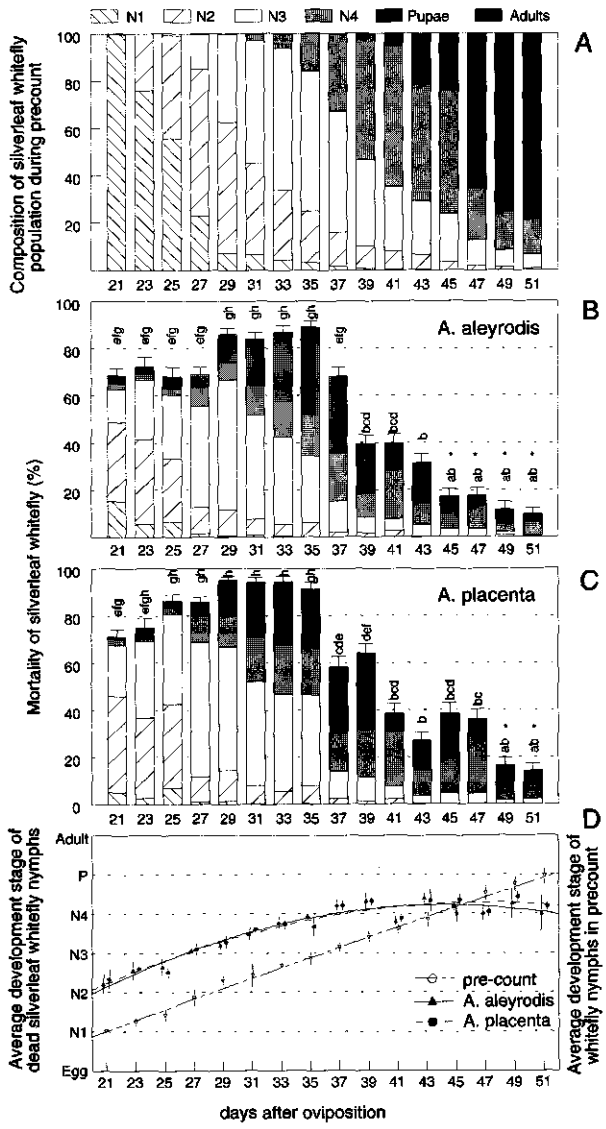


Figure 4.9: A: composition of the *B. argentifolii* population at time of treatment (pre-count) shown as percentage first (N1), second (N2), third (N3), fourth (N4) instar nymphs, pupae or adults of the total population present on a leaf; average whitefly mortality three weeks after pre-count caused by *A. aleyrodis* (B) or *A. placenta* (C), error bars represent standard error of total mortality, stacked bars not followed by the same letter are significantly different (α= 0.0000443), *: not significantly different from control treatment. D: Composition of whitefly population during pre-count and average development stage of the dead *B. argentifolii* nymphs.

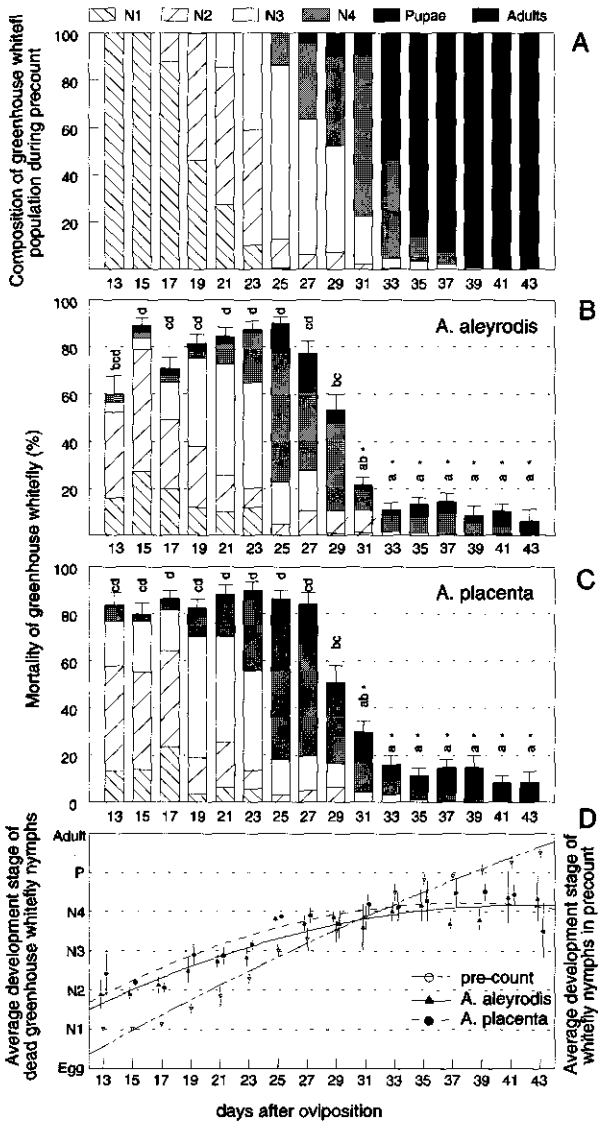


Figure 4.10: A: composition of the *T. vaporariorum* population at time of treatment (pre-count) shown as percentage first (N1), second (N2), third (N3), fourth (N4) instar nymphs, pupae or adults of the total population present on a leaf; average whitefly mortality three weeks after pre-count caused by *A. aleyrodis* (B) or *A. placenta* (C), error bars represent standard error of total mortality, stacked bars not followed by the same letter are significantly different ($\alpha = 0.0000443$), *: not significantly different from control treatment. D: Composition of whitefly population during pre-count and average development stage of the dead *T. vaporariorum* nymphs.

Bioassays T. vaporariorum

Mortality of *T. vaporariorum* nymphs by *A. aleyrodis* and *A. placenta* varied also with nymphal composition of the whitefly population at time of treatment (Fig. 4.10A, B and C). From day 13 to 29 after oviposition *A. aleyrodis* and *A. placenta* caused a significantly higher mortality compared to the control mortality (Tween, on average 13.7% ± 4.8 SE). In this period the majority of the whitefly population existed of first to third instar nymphs. When half of the population existed of fourth instar nymphs or pupae (day 29 and further), mortality percentages dropped from ca. 80% to 50%. This decline in infection probability in the course of the population development coincides with the decline of first to third instar nymphs, as was the case for *B. argentifolii*. No significant differences in mortality existed between *A. aleyrodis* and *A. placenta*.

In general, death of *T. vaporariorum* nymphs also occurred one nymphal stage later than they were treated (parallel lines Fig. 4.10D). Since it took on average 5.5 days for *T. vaporariorum* nymphs to develop to the next stage, it took 5.5 days for *Aschersonia* spp. to kill 70-90% of the whitefly nymphs. Although the development of *T. vaporariorum* population at 20 °C was faster than that of *B. argentifolii* population, *T. vaporariorum* nymphs were apparently unable to escape infection.

Discussion

Germination

In general, these three isolates of *Aschersonia* spp. show very similar germination and infection strategies. The germination of the *Aschersonia* spp. is influenced by the substrate. On the leaf lamina conidia rarely germinate. They seem to be either missing germination cues, or germination is inhibited by fungistatic compounds on the leaf surface. However, on the major leaf veins extensive growth was observed. Leaves can leak nutritious phloem substrates particularly near the veins (Isaac, 1992), which may have stimulated conidia of *Aschersonia* spp. to germinate. It could be more advantageous not to germinate in absence of the host, especially since germlings are more sensitive to unfavourable conditions than non-germinating conidia (Sussman, 1968). Conidia of *A. aleyrodis* and *A. placenta* seem to prefer to 'sit-and-wait' over growth towards their host. In this way they are able to infect hatching or moulting nymphs of *T. vaporariorum* even after a month on a leaf surface (Fransen, 1995; Meekes *et al.*, 2000).

Aschersonia placenta is able to produce capilliconidia on water agar (see also chapter 2), but this has never been observed on poinsettia leaf and only occasionally on gerbera leaf in the pocket between leaf lamina and leaf vein. This indicates that high humidity levels are required for production of capilliconidia. Evans (1994) first described

this phenomenon for *Aschersonia*, and it is morphologically and probably functionally analogous to the capilliconidia of the Entomophthorales. Whether capilliconidia in the genus *Aschersonia* have other virulence characteristics than primary conidia has still to be investigated. Formation of capilliconidia may increase their ability to spread. By formation of capilliconidia, dispersal by adult whiteflies or other moving insects to neighbouring plants would be enhanced.

Mucilage formation

The three *Aschersonia* isolates produce huge amounts of mucilage at several stages of their life cycle. Conidia are formed in pycnidia and are covered with mucilage (Samson & McCoy, 1983; Lim *et al.*, 1991; Osborne & Landa, 1992). Due to the high sugar content the mucus is very hygroscopic (Fransen, unpubl. res.). It facilitates dispersal by free water, like dew and rain, and/or their dispersal by insects (Samson & McCoy, 1983). Osborne and Landa (1992) suggested that *Acalvolia* mites could play an important role in the dissemination of *A. aleyrodis* conidia and occasional transmission of conidia by *Encarsia formosa* has been observed (Fransen & van Lenteren, 1993).

Due to their mucilage layer, conidia of *Aschersonia* spp. would not seem subject to wind dispersal (Samson & McCoy, 1983). Nevertheless, Morrill and Back (1912) showed that *A. aleyrodis* conidia could be spread by wind once they were freed from their mucilaginous matrix after artificial suspension in water (or by rains and dews) and dried. These authors were convinced that wind was the most important factor in long distance dispersal of the fungus from tree to tree and to isolated citrus groves. Rain and dew were considered as an important factor for distribution throughout an individual tree or to closely adjoining trees (Morrill & Back, 1912). Actual establishment of conidia after wind dispersal has not yet been proven.

Conidia borne in mucilage, like those of *Aschersonia* spp. and *Verticillium lecanii*, are hydrophilic and are generally carried in splash droplets (Boucias & Pendland, 1991). The hydrophobic wax layer of insects, which is believed to prevent many fungi to be entomopathogenic, may prevent these conidia from coming into contact with the insect surface. However, conidia of *V. lecanii* were able to adhere to hydrophobic surfaces, such as the insect cuticle or plastic, in the presence of water (St.-Leger, 1991). Evidence presented above indicates that this is also the case for conidia of *Aschersonia* spp.

Although some remnants of the original conidial matrix will still be attached to the conidium after suspension in water (Fig. 4.3A; Fransen, 1987), once on the host *Aschersonia* spp. form again large amounts of mucilage around the conidium, to anchor themselves to their host insect (Fig 4.5; Fransen, 1987). These amounts were never

observed surrounding conidia on the leaf surface (Fig. 4.3 vs. Fig. 4.5). Also appressoria were surrounded by mucilage strands, clearly visible even after the rough CPD treatment. Mucilage produced by appressorial cells is hygroscopic and may create a favourable environment for the extracellular enzymes released by these structures (Boucias & Pendland, 1991).

Infection

Not all areas of the insect cuticle are equally vulnerable to fungal penetration (Butt & Goettel, 2000). The intersegmental membranes (Wraight *et al.*, 1990) and areas under the elytra (Butt *et al.*, 1995) can be preferred sites of infection. Butt *et al.* (1995) showed that germination behaviour of *Metarhizium anisopliae* was dependent on the cuticle, producing numerous appressoria on the cuticle of a flea beetle, whereas direct penetration occurred on aphids. Here, the presence or absence of germination or appressorium formation in *Aschersonia* spp. does not seem to be related to a specific site on the insect, nor to a specific instar (mostly N1 to N3 studied).

Occasionally extensive growth of *Aschersonia* spp. hyphae over *B. argentifolii* nymphs was observed (Fig. 4.7) without clear evidence of penetration through the cuticle. Whether or not infection was successful, could not be verified, since the SEM procedure is detrimental to the insect. Fransen (1987) did observe this phenomenon too, but on older, fourth instar nymphs of *T. vaporariorum*. These nymphs were less susceptible to infection, as was confirmed by our results. This aberrant growth of hyphae was also observed for other fungi on resistant hosts, *e.g.* low pathogenic mutants of *Beauveria bassiana* showed extensive growth on the surface of *Heliothis zeae* larvae with only a limited degree of penetration (Pekrul & Grula, 1979). For *Aschersonia* spp. this might indicate that nymphs of *B. argentifolii* on poinsettia are less susceptible to infection than nymphs on cucumber (Fig. 9.3; chapter 9), as was shown for *T. vaporariorum* (Meekes *et al.*, 2000; Chapter 6 and 7).

Moulting of nymphs can be considered a favourable and unfavourable factor for fungal infection at the same time. When inoculation takes place just before moulting, the insect may be able to shed the fungus together with the old cuticle (Fig. 4.7A). In contrast, the newly moulted cuticle is considered especially vulnerable to fungal attack (Ferron, 1985; McCoy *et al.*, 1988; Butt & Goettel, 2000). Although, the insect may be able to shed the fungus, there is still a possibility it will get infected by settling on *Aschersonia* conidia present on the leaf surface through its increased size after moulting.

Susceptibility

First to third instar nymphs were most susceptible to infection by *Aschersonia* spp. (Fig. 4.9-4.10; Fransen *et al.*, 1987). Some authors report that first instar nymphs of *T. vaporariorum* or *B. argentifolii* were refractory to fungal infection (Hussey, 1958; Hall *et al.*, 1994), others however, found clear symptoms on first instar nymphs of both whitefly species (Fransen *et al.*, 1987; Meade & Byrne, 1991; Lacey *et al.*, 1999). We also found slightly lower infection levels for first instar nymphs, but infection probability will be lower because of their smaller size compared to second or third instar nymphs.

As soon as whitefly nymphs enter their fourth instar, they become less susceptible to infection by *Aschersonia* spp. as already observed by Fransen *et al.* (1987). The lower susceptibility of fourth instar nymphs and particularly of pupae could be related to the fourth nymphal instar developing into the pupa without moulting. The adult develops inside the pupa, so at the pupal stage the fungus has to penetrate not only the pupal case, but the cuticle of the adult as well. *A. aleyrodis* is not able to infect eggs and rarely infects adults of *T. vaporariorum* (Fransen *et al.*, 1987), in contrast to *V. lecanii* which is able to infect adults, or *Paecilomyces fumosoroseus* which is also able to infect adults and eggs (Osborne & Landa, 1992).

The susceptible instars do not always show signs of infection in the treated stage, but these may appear in subsequent development stages. This phenomenon is frequently observed (Fargues & Rodriguez-Rueda, 1980; Fransen *et al.*, 1987; Vidal *et al.*, 1997) and it can be used as an indication for the speed of kill for sessile whitefly nymphs (Vidal *et al.*, 1997; chapter 3). Between 4 to 6 days after inoculation, the infected nymphs turn an orange colour at 20 °C. When the humidity is high enough, the fungus ruptures through the weak spots in the cuticle of the infected insects (Fig. 4.8). The fungus-covered host is first white, but it soon turns to an orange-reddish or luteous colour, depending on the *Aschersonia* spesies, when pycnidia are formed and conidiogenesis begins.

Wax deposits

On the scanning photographs coiled grooved ribbons (Fig. 4.11) were found all over the leaf and nymphs (*e.g.* Fig. 4.3, 4.5A, E). We wondered whether these particles were host-plant or insect derived. These particles, however, have been described before and were attributed to whitefly adults by Baker & Jeffree (1981), since the same structures were present on, for instance, tomato, tobacco, petunia and dwarf bean leaves and corresponded with presence of *T. vaporariorum* adults. Adults of both whiteflies produce a lot of wax extrusions, which provide them with their white powdery appearance. The wax particles are excreted from wax glands on the adult's abdomen, broken off by the hind tibia and

applied over the whole body and wings. In doing so, these particles are also shed in the environment of the insect. In both whitefly species the wax ribbons have the same configuration, since the templates through which they are forced are similar in shape (Byrne & Hadley, 1988).

Figure 4.11: Waxy structures found on first instar nymph of *B. argentifolii*. These waxes are whitefly (adult) derived (3228x, direct print, LTSEM)

Final remarks

Although infection by *Aschersonia* spp. is predominately limited to the first three nymphal stages of *B. argentifolii* and *T. vaporariorum*, the fungi possess a whole range of germination and infection strategies to infect these stages. Together with their long persistence on leaf surfaces (Fransen, 1995; Meekes *et al.*, 2000), their ability to infect whitefly nymphs in less humid environments (avg. 45%, Meekes, unpubl.) and the compatibility with other natural enemies like the parasitoid *Encarsia formosa* (Fransen & van Lenteren, 1993;1994), it makes them potent biocontrol agents.

Acknowledgements

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5

Persistence of the fungal whitefly pathogen *Aschersonia aleyrodis*, on three different plant species[1]

Abstract

Persistence of *Aschersonia aleyrodis*, a fungal pathogen of whitefly, was studied on cucumber (*Cucumis sativus*), gerbera (*Gerbera jamesonii*) and poinsettia (*Euphorbia pulcherrima*). Germination capacity and infectivity of conidia, which stayed on the different plants over a period of up to one month, were assessed. Average germination of conidia on the leaves was low (< 14.3%), whereas it was shown that most of the conidia transferred from the leaf to water agar were viable, even after having been present on the leaf surface for one month. Germination capacity was influenced by host plant species: it was highest on cucumber, followed by poinsettia and lowest on gerbera ($p < 0.05$). On cucumber leaves, conidia stayed viable and were able to infect 90% of the whitefly nymphs, even at 31 days after spore application. On gerbera, germination capacity decreased considerably from 80% (day 0) to 40% (day 31). This was reflected in nymphal mortality, which declined from 75% to 40%. Despite the high germination capacity (60%) of conidia on poinsettia after an exposure of one month, nymphal mortality decreased from 70% at the day of spore application to 10% after three days at leaf surface, and remained low throughout the monitoring period. Relations between germination capacity, infectivity and the host plant environment such as phyllosphere microbes, secondary plant metabolites and microclimate are discussed.

[1] Published in slightly different form as **Meekes, E.T.M, Voorst, S. van, Joosten, N.N., Fransen, J.J. and Lenteren, J.C. van**, 2000. Persistence of whitefly pathogen *Aschersonia aleyrodis*, on three different plant species. *Mycological Research* 104: 1234-1240.

Introduction

As a result of low damage thresholds for pest insects and mites, the present control of whitefly in ornamental crops still relies largely on chemical insecticides (Fransen, 1993). In glasshouse vegetables on the other hand, biological methods to control whitefly are widely used (van Lenteren, 1995). Recognition of negative side-effects of pesticides prompted a shift from exclusive chemical control to integrated pest management and stimulated ongoing research in biological control. In the last two decades entomopathogenic fungi, such as *Verticillium lecanii*, *Paecilomyces* spp., *Beauveria bassiana* and *Aschersonia* spp., have shown potential as control agents against greenhouse whitefly, *Trialeurodes vaporariorum*, and silverleaf whitefly, *Bemisia argentifolii* (*e.g.* Meade & Byrne, 1991; Lacey *et al.*, 1996). Unlike bacteria and viruses, fungi are able to infect phloem-feeding insects naturally by penetration of the insect cuticle, which make them suitable pathogens of whitefly (Fransen, 1990).

Aschersonia aleyrodis causes spectacular epizootics in whitefly populations in the tropics and subtropics (Evans & Hywel-Jones, 1990). The fungus was first used for biological control of whiteflies in citrus groves in Florida in the early 1900's (Berger, 1921), where a combination of predators, parasitoids and *A. aleyrodis* successfully controlled the citrus whiteflies *Dialeurodes citri* and *D. citrifolii* for decades (McCoy, 1985). *Aschersonia aleyrodis* also successfully infects the nymphal instars of the greenhouse whitefly, of which the first three nymphal stages are most susceptible, but it is unable to infect and kill whitefly eggs and only sporadically infects adults (Fransen *et al.*, 1987). Host and conidia may meet in two different ways (i) direct contact, when conidia are sprayed upon the insect host or (ii) indirect contact, when hatching or moulting nymphs settle on conidia present on the leaf surface from an earlier treatment. Hence, good coverage and long persistence of conidia on foliage is important for effective control of whitefly.

A major difficulty in the development of mycopesticides is their relatively short persistence on leaf surfaces. In most studies, ultraviolet light is considered to be an important factor affecting the survival of propagules (Fargues *et al.*, 1996; Moore *et al.*, 1996). Considering microbial control of whitefly pests in protected cultivation, whitefly nymphs occur mainly on the abaxial surface of the leaf, where survival of conidia usually is higher than at the upper side (*e.g.* James *et al.*, 1995; Furlong & Pell, 1997). In addition, glasshouses filter incoming radiation and, therefore, reduce its (detrimental) impact on the survival of conidia compared with open field situations (Jaques, 1972). Fransen (1995) found that conidia of *A. aleyrodis* could survive on leaf surfaces of cucumber for at least three weeks and were still able to infect nymphs of greenhouse whitefly.

Apart from solar radiation, the direct environment of conidia may strongly influence their survival. On different host plants the same fungal strain may show differences in

persistence, as a consequence of chemical and/or morphological differences between plants. Chemicals at the leaf surface can influence the viability of spores (Cooke & Rayner, 1984), but they may also have an effect on the pest organism and its susceptibility to pathogens (Hare & Andreadis, 1983; Ramoska & Todd, 1985). Plant morphology may directly influence the pest species (Bellows Jr. *et al.*, 1994), but their effect on the microclimate at the leaf surface, for example through leaf size, density of hairs on the leaves and crop architecture may be more important (Ferro & Southwick, 1984; Inglis *et al.*, 1993).

The extent to which a host plant influences the survival of conidia of entomopathogens is still unknown. The objective of this study was to assess the persistence of *A. aleyrodis* conidia on several host plants of *T. vaporariorum*. Persistence was determined by assessing (i) germination capacity of conidia and (ii) mortality of greenhouse whitefly nymphs caused by conidia after a prolonged period of exposure of these conidia on the leaf surface.

Materials and Methods

Whitefly host and plant species

Experiments were carried out with greenhouse whitefly, *Trialeurodes vaporariorum*. Whiteflies were reared on gerbera plants (*Gerbera jamesonii* hybrids) in screened cages at 21°C under glasshouse conditions.

The experiments were conducted on three plant species: cucumber (*Cucumis sativus* 'Profito'), gerbera (*Gerbera jamesonii* hybrids) and poinsettia (*Euphorbia pulcherrima* 'Goldfinger'). Conidial suspension were applied only to completely unfolded leaves, in order to avoid alteration in spore coverage, due to growth of leaves. Plants were grown in small glasshouse compartments (30 m^2, 21°C, L16:D8) on tables with an ebb and flow irrigation system.

Fungus

Aschersonia aleyrodis (KV-107), originating from Colombia, was isolated from Aleyrodidae and passaged through greenhouse whitefly once. Sporulating colonies on potato dextrose agar (Difco) were used to prepare a conidial suspension for inoculation of millet-cultures for mass production of conidia. The fungus was cultured on autoclaved millet in 300 ml Erlenmeyer flasks (10 g millet with 25 ml demineralised water), which were closed with sterile cotton for aeration. Cultures were incubated at 25°C and L16:D8 (artificial light). Conidia from 2-3 week old cultures were harvested by rinsing cultures with sterile demineralised water. Conidial densities were determined with a haemocytometer and adjusted to 1×10^7 conidia/ml. After incubation for 24 hrs at 25°C initial germination capacity of conidia on water agar (WA, 15 g/l agar, Merck) exceeded 95%.

Experimental setup

Two ml of conidial suspensions were sprayed on the abaxial surface of leaves, attached to the plant, using a Potter spray tower (pressure 3 x 10^5 Pa, Burkard Manufacturing, UK). This resulted in approximately 1.4 x 10^4 conidia/cm^2. Plants were left to dry for one hour, before they were covered with plastic bags for 48 hours to create a near saturated environment (95-100% RH). Plants were kept in airconditioned glasshouse compartments (30 m^2) at 20 and 25°C (± 2°C); the humidity at 20°C was on average 70%, the humidity at 25°C fluctuated considerably between 40 and 90%, depending on the photoperiod.

Germination capacity of conidia

The germination capacity of conidia on leaf surfaces was determined using a leaf imprint technique (Parbery *et al.*, 1981; Fransen, 1995). Clean leaves, attached to the plant, were treated with conidial suspensions as described earlier. On 0, 3, 10, 17, 24 and 31 days post inoculation (p.i.), leaf discs (Ø 5 cm) were taken from inoculated leaves and the abaxial of the leaves was pressed firmly on WA, using a glass plug of the same diameter. Two leaves per plant and three plants per combination of plant species, time and temperature were used. Germination of conidia on leaf surfaces was determined by microscopic observation of the WA plates directly after the imprint was made. Per plate 300 conidia were evaluated and germination was assumed when the length of the germ-tube exceeded the width of the spore. After 24 hours incubation at 25°C, the plates were evaluated a second time, thus providing information on germination of conidia on leaf surfaces and germination capacity of conidia on water agar after being removed from leaves.

The imprint technique was verified by observation of the conidia on the leaf surface using a Zeiss fluorescent microscope. Conidia on leaves were stained with 0.01% Fluorescent Brightener (Merck). The majority of the conidia (80-90%), both germinated as well as ungerminated conidia, were transferred to the water agar by the imprint technique used.

Whitefly nymphal mortality

Fifty whitefly adults, confined to clip cages, were allowed to oviposit for 24 hours on the abaxial surface of leaves. This procedure was carried out every three to four days on another set of leaves, starting 10 days before inoculation with the fungus until 21 days after inoculation (Fig. 5.1); two leaves per plant and five plants per treatment were used. Egg to first instar development of *T. vaporariorum* took approximately 10 days at 20°C and 8 days at 25°C, so the first set of eggs (day -10) had already developed into first instar nymphs at day 0, when application of conidia took place. For the second until the fourth set of eggs, conidia were applied to the eggs, since hatching took place after spore treatment at day 0. From the fifth set

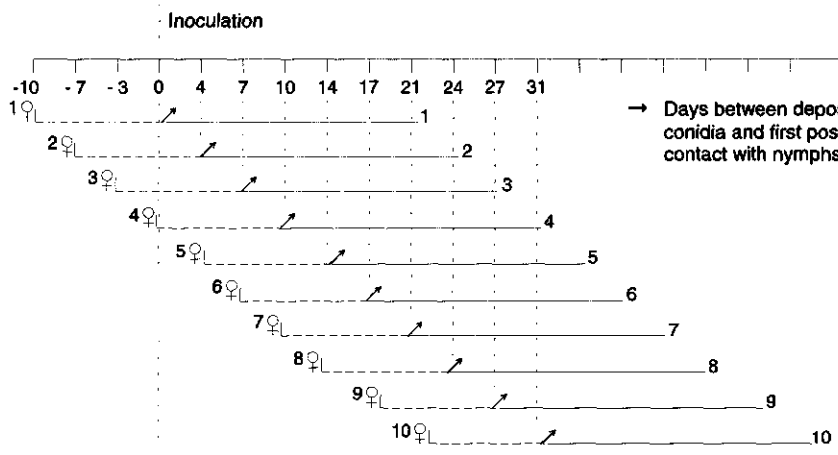

Figure 5.1: Time schedule for assessment of persistence of *A. aleyrodis* conidia by nymphal mortality: egg deposit by female whiteflies (♀), eggs present on leaf (L - - -), egg hatching (↗ = first possible contact of nymphs with conidia) and presence of nymphs on leaf until final assessment (——). Conidia have to persist from day 0 (= application) until egg hatching (↗) to be able to infect *T. vaporariorum* nymphs (period between vertical dotted lines).

of eggs onwards, eggs were deposited on leaves inoculated on day 0 with *A. aleyrodis* conidia. About two to three weeks after egg hatching the first adults started to emerge and final assessment for nymphal mortality was carried out. A nymph was considered dead when it turned orange or when the insect had desiccated and turned transparent brown.

Statistical analysis

Data were analysed using generalised linear models (Genstat 5 release 3.22, Payne *et al.*, 1987), with y representing the number of germinated conidia (germination capacity) or number of infected nymphs (nymphal mortality) per plant with parameters n, total amount of conidia or nymphs and p, probability of germination or infection. The proportions of germinated conidia or infected nymphs was binomially distributed and the logistic-link transformation function was used throughout.

Although the effect of temperature should be tested against variation between glasshouses with the same treatment, we were unable to do so, as the experiment was not repeated at glasshouse level (whole plots). An alternative would be to perform the test using rest variation at a lower stratum. But this is a progressive test and effects are more likely to be significant. Significant differences were therefore treated as trends.

Results

Germination capacity of conidia

The germination of conidia on the leaf surface, although low (max. 14.3%), was influenced by host plant species ($p < 0.001$) (triangles in Fig. 5.2). Overall average germination was highest on cucumber (9.8 ± 2.5% se), followed by gerbera (5.2 ± 1.3%) and was lowest on poinsettia (3.2 ± 0.7%). Germination levels tended to be higher at 25°C than at 20°C for each plant species.

After 24 hours incubation on water agar, the majority of conidia had germinated (Fig. 5.2). Germination capacity of conidia was influenced by plant species ($p < 0.001$), highest for conidia transferred from cucumber leaves and lowest for conidia originating from gerbera leaves. Germination levels decreased in time, with the strongest decline for conidia originating from gerbera leaves ($p < 0.05$). Germination tended to be higher at 20°C than at 25°C, although in general the decrease of germination in time tended to be higher at 20°C than at 25°C ($p < 0.1$). After having been present on a cucumber plant for 31 days, 66 to 90% of conidia were still able to germinate. For conidia transferred from poinsettia and gerbera plants, germination levels were on average 62 - 64% and 27 - 45%, respectively, after 31 days.

Whitefly nymphal mortality

Whitefly nymphal mortality differed significantly between host plant species ($p < 0.001$). Mortality on cucumber was very high and remained high during the course of the experiment, in contrast with mortality on gerbera or poinsettia (Fig. 5.3). The percentage dead nymphs was highest on cucumber plants, decreasing only from about 99% (day 0) to 90% on day 31 (20°C) or 88% on day 29 (25°C). On gerbera mortality decreased from 73% (day 0) to 37% on day 31 (20°C) or decreased from 96% to 69% on day 29 (25°C). Mortality was lowest on poinsettia leaves, decreasing from on average 56% (day 0) to 4% on day 31 (20°C) or to 17% (25°C). The initial mortality of *T. vaporariorum* on poinsettia was low (52 - 59%) compared to initial mortality on cucumber (99%) or gerbera (72 - 95%) ($p < 0.001$). The decrease over time in mortality of nymphs on cucumber leaves is significantly smaller than on poinsettia or gerbera. It seems that mortality at 25°C was higher than mortality at 20°C, although differences are small, but due to the lack of replicates at glasshouse scale, this could not be tested

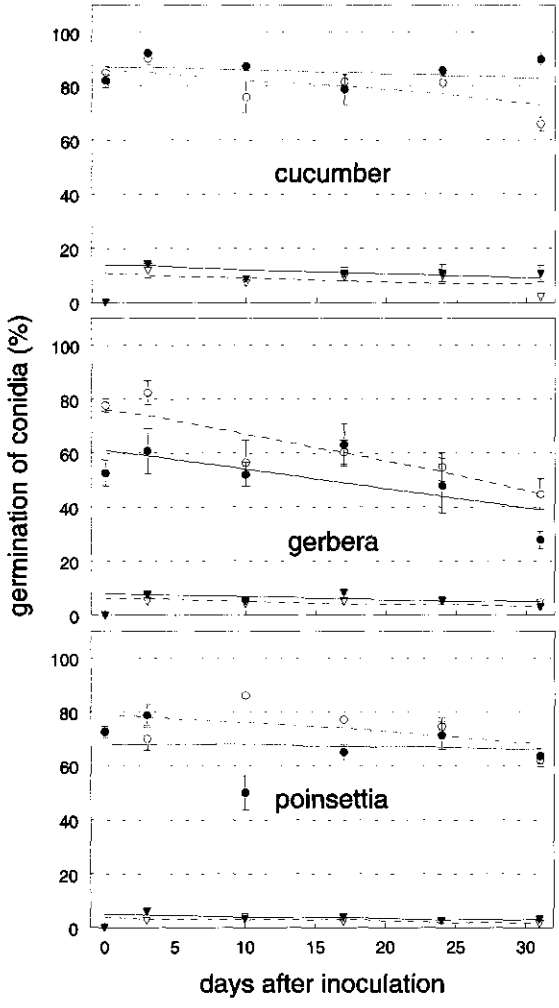

Figure 5.2: Mean percentage germination of *A. aleyrodis* conidia from 0 days until 31 days on leaf surface of cucumber, gerbera and poinsettia measured at two temperatures; by direct observation of agar imprint of leaf (= germination on leaf surface): - -▽- -: 20°C, —▼—: 25°C or observation after 24 hours incubation on WA at 25°C (= germination capacity): - - ○ - -: 20°C, —●—: 25°C. Vertical lines represent standard errors of means.

Discussion

The ability of an entomopathogen to persist in the habitat of its host is important for the effectiveness of naturally occurring and introduced pathogens (Jaques, 1983). Our results indicate that *A. aleyrodis* is able to survive well in the host habitat and to kill its host over a

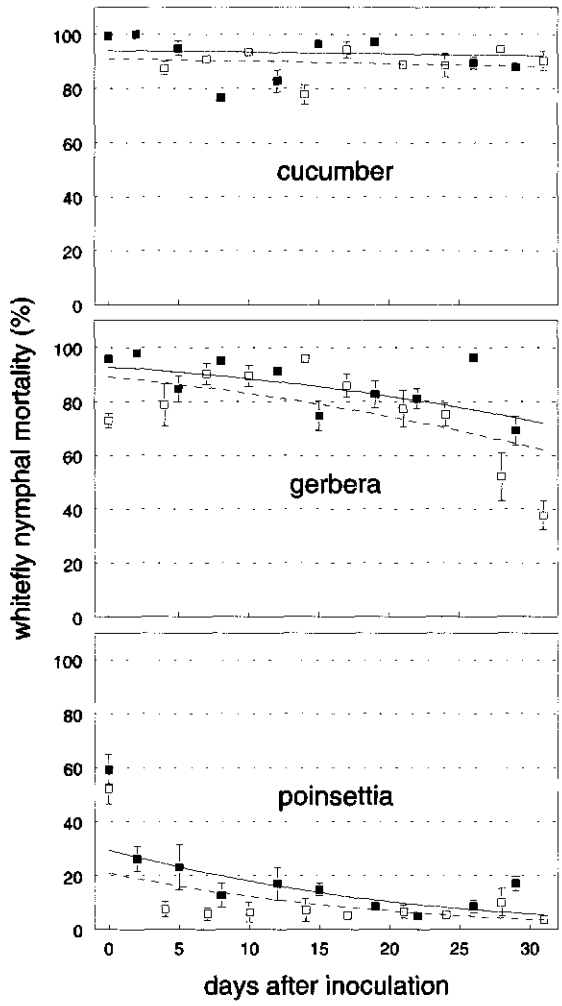

Figure 5.3: Mean percentage whitefly mortality by *Aschersonia aleyrodis* (- -□- - : 20°C, —■—: 25°C) after presence of conidia on abaxial leaf surface of three whitefly host plants (cucumber, gerbera and poinsettia) in days between inoculation (day 0) and first possible contact. Vertical lines represent standard errors of means.

period of at least one month. The persistence of conidia, determined in this study by germination capacity and nymphal mortality is, however, largely influenced by host plant species. Nymphal mortality and germination capacity of conidia seemed to be positively correlated, as has been suggested by Fargues *et al.* (1988). In our study on cucumber conidial germination capacity and whitefly mortality remained high for at least one month. On gerbera

both declined, suggesting a less favourable environment for persistence of conidia. When poinsettia was used as a host plant for *T. vaporariorum*, nymphal mortality declined considerably in time, despite the high germination capacity of conidia. Since the initial mortality (day 0) on poinsettia was also low, other factors than survival of conidia are likely to play a role. The results on poinsettia are not only limited to *T. vaporariorum*, since comparable results were obtained for silverleaf whitefly, *B. argentifolii* (S. van Voorst & E. Meekes, unpubl. results). Additionally, not only did conidia of *A. aleyrodis* remain viable for over a month on poinsettia, but also conidia of *A. placenta* showed the same ability and the same trend: a slow decline in viability on poinsettia and a steeper decline on gerbera (A. Tapaninen & E. Meekes, unpubl. results).

To assess germination capacity of conidia on the leaf surface, an agar imprint technique was used, rather than a leaf washing technique. The latter method involves counting of colony forming units. Other microorganisms may colonize the agar before the presence of the slow growing *A. aleyrodis* is noted, so the leaf washing technique is not very accurate (Fransen, 1987). In addition, the agar imprint method provides information about conidial 'behaviour' on the leaf surface. It is remarkable that conidia did not germinate in high numbers on the leaf surface, although they were exposed to a near saturated environment for 48 hours. On artificial substrates like paraffin wax or cellophane, high percentages of conidia do germinate at 93.9 - 100% and free water was not necessary (Fransen, 1995), and when conidia are sprayed upon whitefly nymphs, they germinate readily (personal observation, E. Meekes). It is not yet known whether low germination on leaf surface is due to lack of stimulation or due to inhibition by the presence of secondary chemicals on the leaf surface and/or physical factors. In contrast to *A. aleyrodis* conidia, up to 56% of those of *V. lecanii* germinated on cucumber leaves when incubated for 24 hrs at high humidity (>95%) (Verhaar, 1998). In general, *A. aleyrodis* seems to 'sit-and-wait' rather than germinate and grow towards its host. For this fungus, which is specialized on Aleyrodidae (Evans & Hywel-Jones, 1990), it could be advantageous not to germinate in absence of its host, especially since germlings are more sensitive to unfavourable conditions than non-germinating conidia (Sussman, 1968). In addition, conidia of *Aschersonia* spp. are mainly dispersed by water, so if they germinate under these circumstances, they can loose their capacity to infect their host.

The same fungal strain differed in its persistence on the three plant species, which could be due to a number of reasons. First, the composition of the phyllosphere microbiota differ among and within host plant species. Some microorganisms are exclusively associated with one plant, others are more opportunistic (Sinha, 1971). When leaves age, differences in microflora occur: the number and composition of the phylloplane biota increases to reach a maximum at senescence (Cooke & Rayner, 1984). Several interactions exist between microbes

on the leaf surface, such as competition for nutrients, antibiosis and mycoparasitism (Elad *et al.*, 1996), and the leaf microbes may outcompete the entomopathogens. Information about the microorganisms occurring on cucumber, gerbera and poinsettia is limited to fungal plant pathogens and introduced microbes. Cucumber, and to a lesser extent gerbera, are susceptible to a great number of foliar diseases (Smith *et al.*, 1988; Asselberg *et al.*, 1996). In this respect, they differ from poinsettia, of which only one foliar disease is known, powdery mildew (*Oidium* sp.) (Motte & Unger, 1995; Koike & Saenz, 1998), indicating that either the microorganisms are successfully outcompeting the pathogens or, more likely, that the physical and/or chemical characteristics of the leaf create an environment unsuitable for growth of many fungi.

Secondly, the chemical composition of the host plant species can differ substantially. Chemical substrates leached from the leaf may act as a nutrient source for fungi, while leaf cuticles contain components that can also be exploited (Cooke & Rayner, 1984). On the other hand, leaves of certain plants may exude fungistatic substances, which can inhibit germination or restrict germ-tube growth. Cuticular waxes can cause these effects also (Blakeman, 1971). Leaves of poinsettia contain cytotoxic triterpenoids (Smith-Kielland *et al.*, 1996) and leaf extracts have been used as a seed treatment against nematodes and fungi (Khan *et al.*, 1996; Sebastian & Gupta, 1996). Differences in chemical plant substances may also have an effect on the host insect, thus influencing fungal infection indirectly. The insect diet can influence infection of the Colorado potato beetle by *B. bassiana*, rendering the insect more susceptible (Hare & Andreadis, 1983). Fungal inhibitors also produced by the plant may protect the insect (Ramoska & Todd, 1985). Legaspi *et al.* (1996) showed that lacewinged flies feeding on silverleaf whitefly reared on poinsettia did not reach the pupal stage, which was suggested to be due to accumulation of detrimental plant compounds in the whitefly nymphs.

Thirdly, the morphology of the host plant and its canopy characteristics may play a role. Leaf surface features, such as size and shape, surface topography and canopy characteristics vary among plant species. Cucumber has large, hairy leaves, whereas poinsettia has smaller, rather smooth leaves. The cucumber canopy is open as compared with gerbera, which is a rosette type plant. These features affect the leaf boundary layer, in which insect - fungus interactions take place. Broad and/or hairy leaves have a thicker boundary layer than narrow leaves and leaves with a smooth surface. But also temperature, placement of leaves on a plant (sunny or shady) and turbulence will influence the thickness of this layer. The humidity in this layer is a gradient controlled by the stomata and the thickness of this layer, since exchange of gases depends on diffusion processes (Burrage, 1971; Ferro & Southwick, 1984). The microenvironment of the fungus, and especially relative humidity, is of great importance to its longevity. For many fungi the intermediate humidities (50-70 % RH) are supposed to be

more damaging to their survival than both extremes, for conidia as well as germlings (Clerk & Madelin, 1965; Sussman, 1968; Diem, 1971).

In contrast to the results of Fransen (1995), temperature did not greatly affect the germination capacity and nymphal mortality by *A. aleyrodis*. Fransen (1995) showed that germination capacity of conidia on cucumber leaf at 25 °C decreased from 90% on day nine to 20% on day 15 p.i. and this was thought to be due to increased microbiota on the leaf surface caused by early senescence. In our experiments, senescence of poinsettia and gerbera leaves hardly occurred during the experiments. In cucumber, senescence of leaves was observed and the leaves aged earlier at 25 °C than at 20 °C. This did not occur before 35 days p.i., which could explain the differences in germination capacity between Fransen's (1995) experiments and ours. In our experiment a slightly higher germination capacity and whitefly mortality was observed at 25 °C compared with 20 °C.

A. aleyrodis is being evaluated for the control of whitefly in glasshouses, where the environment is less extreme than in open field situations. In its natural environment, (sub)tropical forest ecosystems, where bright sunshine alternates with clouds and rain, *A. aleyrodis* has to overcome extreme circumstances. Conidia of *A. aleyrodis* are bright orange due to pigments, mainly carotenoids (Eijk *et al.*, 1979). Resistance of spores to solar radiation is often related to presence of these pigments, which may enable pigmented spores to withstand short periods of exposure to radiation better than hyaline spores, although variation among and within species exists (Fargues *et al.*, 1996). *Aschersonia aleyrodis* is not known to produce resting spores or other structures to overcome unfavourable circumstances, such as those produced by several entomophthoralean species (Uziel & Kenneth, 1991; Gindin & Ben-Ze'ev, 1994), but other strategies may be involved. *Verticillium lecanii* is able to infect aphids on glasshouse chrysanthemums up to 14 days p.i., with a conidial half-life of 4 days (Gardner *et al.*, 1984), but it survives substantially longer on sporulating aphid cadavers (Hall, 1981).

In conclusion, the persistence of *A. aleyrodis* conidia on cucumber and gerbera leaves over long periods of time is a positive aspect of this biocontrol agent. Since newly emerged whitefly adults move upwards from the old canopy to younger leaves for feeding and oviposition (van Lenteren & Noldus, 1990), reapplication of the entomopathogen to new canopy will still be needed. It will be interesting to know if other entomopathogenic fungi are able to survive in similar situations and will use the same strategy to do so. Future research may show if other aspects in the fungal infection process, as well as persistence, are influenced by host plant species.

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6

Role of phyllosphere climate on different host plants in the interaction between entomopathogenic fungi and the two pest species *Trialeurodes vaporariorum* and *Aphis gossypii* [1]

Abstract

Can phyllosphere humidity explain differences in insect mortality due to entomopathogenic fungi on different crops? This was tested for cucumber, gerbera, tomato and poinsettia on which mycosis of greenhouse whitefly *(Trialeurodes vaporariorum)* and cotton aphid *(Aphis gossypii)* were determined in relation to host-plant climate. Phyllosphere humidity was estimated using climate and host-plant parameters. Hydrophobicity of the leaves and crop density were also taken into account.

On cucumber, gerbera and tomato, the fungi *Aschersonia aleyrodis* and *A. placenta* caused over 90% mortality in whitefly, while *Verticillium lecanii* caused 50% mortality. On poinsettia, whitefly mortality was significantly lower for all three fungi (ca. 20%). The percentage of aphids infected by *V. lecanii* or *M. anisopliae* was significantly higher on gerbera than on cucumber. With *V. lecanii* the highest percentage mycosis was obtained.

The cucumber phyllosphere was more humid than the one on the other crops. This cannot explain higher aphid mycosis on gerbera compared to cucumber, nor lower whitefly mortality on poinsettia. The fact that poinsettia leaves were more hydrophobic than the other leaves may offer an explanation for the observed lower whitefly mortality, but chemical host-plant aspects may also play a role. Our results underline the importance of the first trophic level (plant) for entomopathogenic fungi in integrated pest management programs.

[1] Submitted as **Meekes, E.T.M. / Beerling, E.A.M., Joosten, N.N., and Fransen, J.J.** Role of phyllosphere climate on different host plants in the interaction between entomopathogenic fungi and the two pest species *Trialeurodes vaporariorum* and *Aphis gossypii*.

Introduction

The efficacy of entomopathogenic fungi as microbial control agents may vary with the crop species on which these mycopesticides are applied (*e.g.* Hall, 1985; van der Schaaf *et al.*, 1991; Bolckmans *et al.*, 1995; Meekes *et al.*, 2000). This may be a consequence of chemistry, physical structure and/or growth characteristics of the host plants and their canopies. When testing mycopesticides in glasshouses, it is often assumed that the variation in efficacy on different crops is primarily caused by crop-dependent phyllosphere humidity (van der Schaaf *et al.*, 1991; Bolckmans *et al.*, 1995). The phyllosphere environment of the fungus, and especially the relative humidity, is of paramount importance to its effectivity and persistence (Diem, 1971; Drummond *et al.*, 1987). Ambient relative humidity, temperature and air turbulence will influence the boundary layer in which the insect-fungus interaction takes place (Burrage, 1971; Ferro & Southwick, 1984). However, phyllosphere humidity and thickness of the leaf boundary layer are also affected by crop-specific features such as size, shape and position of leaves, leaf surface topography (hairs, waxes, stomata, veins), leaf area index and photosynthetic activity (transpiration) (Ferro & Southwick, 1984). Physical plant features may also influence insect behaviour (Dixon, 1987; Klingauf, 1987) which may affect the interaction between insect and fungus (Furlong & Pell, 1996). Furthermore, plant chemistry may affect, positively or negatively, the insect-fungus interaction (Elliot *et al.*, 2000).

We determined insect mortality due to entomopathogenic fungi on four glasshouse crop species: cucumber, gerbera, tomato and poinsettia. The fungi *Aschersonia aleyrodis* Webber, *A. placenta* Berkeley & Broome (deuteromycetes: coelomycetes) and a whitefly-pathogenic strain of *Verticillium lecanii* (Zimm.) Viégas (deuteromycetes: hyphomycetes) were tested against the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) on these four crops. On cucumber and gerbera, an aphid-pathogenic strain of *V. lecanii* and *Metarhizium anisopliae* (Metschnikoff) Sorokin (deuteromycotina: hyphomycetes) were used against cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae). Both insect species are extremely polyphagous and amongst the principal pests in a range of crops (Byrne *et al.*, 1990a; Blackman & Eastop, 1984). Greenhouse whiteflies and cotton aphids differ in their relationship with their host plant, as a result of differences in behaviour and life history. Whitefly nymphs are sedentary, except for the first stage, while all aphid stages are mobile. In general, the more suitable the host plant, the less mobile the insect will be, which is valid for both insect species (van de Merendonk & van Lenteren, 1978; Knudsen *et al.*, 1994). Another difference is that the generation time of viviparous aphids is usually shorter than that of whiteflies. On cucumber for instance the generation time of greenhouse whitefly at 20 °C was ca. 36 days (Meekes, unpubl. res.), whereas for cotton aphid at 20 °C the developmental time on cucumber was 5 to 7 days (van Steenis, 1995). Efficacy

of entomopathogenic fungi is likely to be influenced differently by these contrasting insect - host plant relationships.

With *A. aleyrodis* and *A. placenta,* fungi specialised on Aleyrodidae, good results have been obtained in controlling whiteflies (Fransen, 1987; Meekes *et al.*, 1996), as with a whitefly-pathogenic strain of *V. lecanii* (van der Schaaf *et al.*, 1991). An aphid-pathogenic strain of *V. lecanii* has been extensively tested as a control agent of aphids, including cotton aphids, with good results depending on ambient relative humidity (*e.g.* Hall & Papierok, 1982; Helyer *et al.*, 1992). *M. anisopliae* is able to infect many aphid species (Butt *et al.*, 1994; Milner, 1997).

Here we tested the hypothesis that phyllosphere humidity can explain differences in insect mortality due to insect-pathogenic fungi on four different crop species. We measured different climate and host-plant parameters of four different crop species, in order to calculate the vapour pressure deficit (VPD) in their phyllosphere. By measuring humidity and temperature in the proximity of the leaf and the leaf temperature itself, the phyllosphere VPD can be estimated (*e.g.* Goudriaan & van Laar, 1994). Direct measurement of VPD within the leaf boundary layer is impossible, as the measurement itself would disturb this layer (Ferro & Southwick, 1984). Simultaneously with the climate measurements, greenhouse whitefly and cotton aphid mortality due to entomopathogenic fungi was determined on the host plants, and correlated to VPD. In addition, hydrophobicity of the leaves and crop density were determined and related to VPD. By studying more than one insect-fungus interaction in the same experiment, we aimed at exceeding the limits of one insect or fungus species, to obtain a better understanding of the role of phyllosphere climate in their interaction.

Materials and Methods

Host plants

The host plants used were the ornamentals gerbera (*Gerbera jamesonii* cv. Serena) and poinsettia (*Euphorbia pulcherrima* cv. Goldfinger) and the vegetables cucumber (*Cucumis sativus* cv. Profito) and tomato (*Lycopersicon esculentum* cv. Moneydor). The plants were individually grown in pots and placed together on ebb and flow tables to form a crop. For poinsettia this resulted in 8.3 plants/m^2, for the other crop species in 7.2 plants/m^2. The gerbera plants were flowering during the experiment, while others plants were vegetative. For practical reasons, the two vegetables were lopped twice above the fourth leaf and lateral shoots were pinched out. This resulted in plants with a maximum height of one metre, in contrast to growers' common practice, where plants can grow to several metres. All plants were insecticide- and fungicide-free.

Insects

Trialeurodes vaporariorum, the greenhouse whitefly, was maintained on gerbera plants (*Gerbera jamesonii* hybrids). *Aphis gossypii*, the cotton aphid, originated from tomato and was reared on cucumber and gerbera plants for many generations (ca. 2 months). All insect cultures were kept in screened cages at 21 ± 2 °C in glasshouses.

For the experiment, 50 whitefly adults of both sexes were confined in clip cages (Ø 2 cm) on young leaves. The females were given the opportunity to oviposit for 24 hours and were then removed, which resulted in 50 (± 1.8 se) first and second instar nymphs after 16 and 18 days at 20 °C (on tomato and gerbera respectively). The whitefly populations from gerbera were not preconditioned to the experimental host plants, since pre-conditioning of greenhouse whitefly to a less suitable host plant usually does not alter the suitability of that specific host plant (Thomas, 1993). Cotton-aphid infested leaves were picked four and three weeks prior to the experiment. Cucumber-reared aphids were put on experimental cucumber plants, and gerbera-reared aphids were used on experimental gerbera plants, 5-10 aphids per plant.

Fungi

Three fungal strains, *Aschersonia aleyrodis* KV107, *A. placenta* Ap1 and *Verticillium lecanii* KV01, were applied against whitefly. *Aschersonia aleyrodis* originated from Aleyrodidae in Colombia (W. Gams, The Netherlands) and *A. placenta* was isolated from *Dialeurodes cardamomi* (Homoptera: Aleyrodidae) from India (S. Selvakumaran, India) respectively. These two *Aschersonia* strains were passaged through whitefly hosts twice. Sporulating colonies from pure cultures on potato dextrose agar (PDA, Difco) were used to inoculate millet cultures for mass production of conidia (Fransen, 1987). Cultures were incubated at 25 °C and L16:D8 (artificial light), and conidia were harvested from 2-3 week old cultures. Cultures of *V. lecanii* KV01 originated from the commercial product Mycotal (Koppert BV, the Netherlands), which contained 10^{10} conidia/gram formulated product. The product was suspended in demineralised water which was plated on PDA and incubated at 23 °C for 10 days. Conidia of all three fungi were suspended in demineralised water containing 0.05% v/v Tween 80 (Merck) as a spreader (Fransen, unpubl. res.) and suspensions were diluted to 10^7 conidia/ml. On average conidial germination exceeded 90% on water agar (Agar-agar, Merck).

Aphids were treated with two fungal species: *V. lecanii* KV71 and *Metarhizium anisopliae* 299981. Cultures of *V. lecanii* originated from the commercial product Vertalec (Koppert BV, the Netherlands) and contained 10^9 blastospores/gram formulated product. *M. anisopliae* was isolated from Homoptera (Trinidad) (A.K. Charnley, UK). Both fungi were passaged through the cotton aphid twice, and the fungi were subsequently cultured on PDA and incubated at 23 °C. Conidia were harvested from one to two week old plates and diluted

to 1 x 10^7 conidia/ml with 0.03% Tween 80 (Butt *et al.*, 1992). On average conidial germination exceeded 80% on water agar.

Experimental design

The experiment was carried out in four 150 m^2 glasshouse compartments, each containing 16 ebb and flow tables (4.0 m x 1.8 m) as commonly used for potted plants. Each compartment in this split-plot design experiment was divided into two sections (A and B) with two blocks of four (A) and two (B) tables. Crops were randomly assigned to tables within a block. In section "A", an experiment was carried out with greenhouse whitefly on four pairs of tables (= four plant species), each with 52 plants, except for poinsettia where there were 60 plants. In section "B", an experiment was carried out with cotton aphids on two pairs of tables (cucumber and gerbera) again with 52 plants. All tables were divided into four plots for different treatments, which were randomly allocated. These plots consisted of four experimental plants, surrounded by buffer plants of the same species.

Treatment procedure

Each experimental plant was sprayed once with a De Vilbiss hand sprayer (Van der Kuip Ltd., Utrecht, the Netherlands) using compressed air (ca. 3 x 10^3 hPa); run-off of suspensions was avoided. For whitefly-infested plants, one whitefly-infested leaf per plant was sprayed with one of the following applications: a) 1 x 10^7 conidia/ml *A. aleyrodis*, b) 1 x 10^7 conidia/ml *A. placenta*, c) 1 x 10^7 conidia/ml *V. lecanii* KV01, or d) 0.05% Tween (application control), respectively. Poinsettia formed almost no new leaves during the experiment. Hence, whitefly-infested leaves were found in the top of the poinsettia plant, whereas in the other crops, whitefly-infested leaves were found in the lower parts (see also under 'phyllosphere climate'). Whitefly mortality on the treated leaf was assessed after two weeks, by which time surviving insects had become pupae (one leaf per plant, four plants per plot). Numbers of desiccated nymphs and mycosed nymphs (orange nymphs by *A. aleyrodis* or *A. placenta* or opaque white nymphs by *V. lecanii*) were determined. Because of possible overlap between natural mortality and mycosis, the overall mortality was used for analysis.

Aphid-infested plants were sprayed entirely either with: a) 1 x 10^7 conidia/ml *V. lecanii* KV71, b) 1 x 10^7 conidia/ml *M. anisopliae*, or c) 0.03% Tween (application control), or d) were left unsprayed. After one week, one upper and one lower leaf per plant (see also under 'phyllosphere climate') were collected and stored in plastic bags at 4 °C. At this temperature, no new cases of mycosis will develop and aphid reproduction will be negligible, making it possible to examine leaves at a later date. Unfortunately, many aphids died under these

circumstances and therefore only the percentage aphids with visible signs of mycosis could be used for analysis.

Ambient climate

The climate within the four glasshouses was monitored using aspiration boxes (Fluxon) with an electronic humidity sensor and a PT100 element for air temperature measurement. Temperature and humidity levels were recorded every 5 minutes during the course of the experiment and expressed as two-hour averages. The ebb and flow tables were equipped with a heating system (common practice for potted plants (Vogelezang, 1993), which was set at 20 °C, giving an average temperature of 19.6 °C (range 18.9 - 20.9 °C). The relative humidity (RH) ranged from a minimum of 54.8% to a maximum of 73.1%. Experiments were carried out under a regime of 10 hours light and 14 hours dark.

Phyllosphere climate

In November and December, relative humidity (RH) and temperature were measured at the first fully grown leaf and one of the oldest green leaves, using a handheld thermohygroscope (Rotronic Hygroskop DV-2) containing an electronic humidity sensor and a PT100 element for air temperature measurement. The sensor was fixed in a stand, which was adjusted to a distance of 1 cm from the underside of a leaf. On consecutive days the climate in gerbera, poinsettia, tomato and cucumber was determined, the latter was used as the reference. Measurements were carried out on the reference crop and one of the other crops on one table per crop in all four glasshouse compartments and on two plots per table. This was repeated three times on different days. Leaf temperatures were measured using an infra-red camera (Heimann, S925). The air VPD was estimated from the measured air RH and temperature (*e.g.* Goudriaan & van Laar, 1994). The phyllosphere VPD was estimated from leaf temperature, leaf width and air VPD (Goudriaan & van Laar, 1994; Goudriaan pers. comm.). Formulas and assumptions are given in the appendix.

Plant morphology

As a measure for crop architecture and density, the decrease in photosynthetically active radiation (PAR) was determined (Sunfleck Ceptometer CEP40, Decagon Devices Inc., US). The larger the difference in PAR above (set at 100%) and below the canopy, the denser the crop. The PAR above and below the canopies of gerbera, poinsettia, tomato and cucumber were determined; the latter was used as reference. This was repeated three times during the experiment for each comparison (two replicates per glasshouse compartment, four compartments per day).

The hydrophobicity of leaves from the four plant species was assessed qualitatively by placing 10 µl of water or 0.05% v/v Tween (spreader) in demineralised water on the abaxial side of a leaf. Leaves were of similar age to those used in the experiment. Immediately after placing a droplet, photographs were taken. The shape of water droplets on a leaf surface reflects the hydrophobicity of the leaves (Holloway, 1970). The rounder the droplet, the smaller the contact between the droplet and the leaf surface, and the more hydrophobic the leaf surface. By adding a spreader, the contact between droplet and the leaf surface will become larger.

Statistical analysis

Fractions whitefly mortality (four leaves per plot) and fractions aphid mycosis (two upper and two lower leaves per plot) were arc-sin square-root transformed. Transformed mortality and mycosis were analysed using a two-way ANOVA with (fungal) treatment and host-plant species as independent variables, and glasshouse compartments, blocks, tables and plots as block treatments (Genstat 5 release 3.22, Payne *et al.*, 1987). Subsequent differences were compared with t-tests, in which probability (α) was adjusted to the number of t-tests performed (n) (α/n).

The calculated VPD's at different leaf levels were analysed for each crop individually with cucumber as reference, using a two-way ANOVA with leaf level and crop species as independent variables, and dates, glasshouse compartments, and plots as block treatments (Genstat, Payne *et al.*, 1987). Subsequent differences were compared with t-tests, in which probability (α) was adjusted to the number of t-tests performed (n) (α/n).

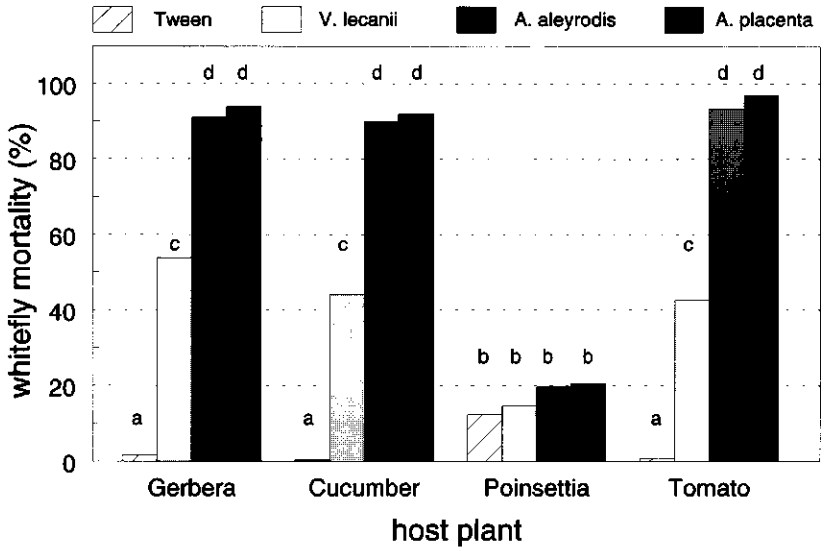

Figure 6.1: Whitefly mortality on cucumber, gerbera, poinsettia and tomato caused by application control (0.05% Tween), *V. lecanii* KV01, *A. aleyrodis* and *A. placenta*. Bars followed by the same letter are not significantly different (comparisons can only be made within the same treatment or host plant species; for a = 0.001, lsd for arcsin√(fraction) transformed data = 0.145.

Results

Greenhouse whitefly

Whitefly mortality varied according to host plant (Fig. 6.1; adjusted percentages, back-transformed from the arc-sin square root transformed fractions). All fungi caused a significantly lower mortality on poinsettia (< 21%, and not significantly different from the control) than the other three crop plants. Whitefly mortality in the control was significantly higher on poinsettia than on other host plants. Both *Aschersonia* species performed significantly better than *V. lecanii* on cucumber, gerbera and tomato. All fungal treatments caused a significantly higher mortality than the control treatments on all crops, except for poinsettia.

Cotton aphid

Aphid mycosis on upper and lower leaves of gerbera and cucumber was generally low, but significant host-plant differences did exist (Fig. 6.2, adjusted percentages, back-transformed from the arc-sin square root transformed fractions). On the upper leaves, both crop and treatment had significant effects on aphid mycosis; no significant interaction between these

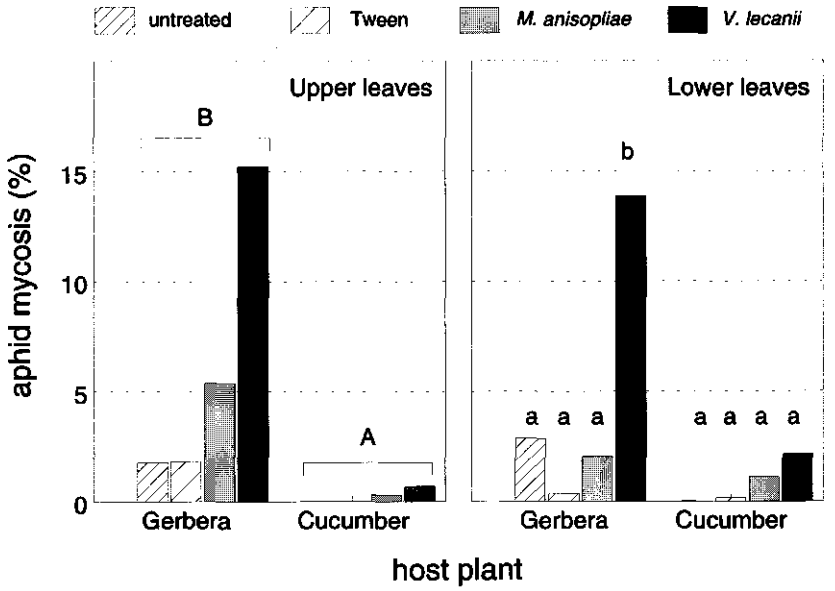

Figure 6.2: Cotton aphid mycosis on upper and lower leaves of cucumber and gerbera caused by control (untreated), application control (0.03% Tween), *M. anisopliae* and *V. lecanii* KV71. Upper leaves, main effect 'crop': cucumber (A) and gerbera (B); for a = 0.025, lsd for arcsin √(fraction) transformed data = 0.123. main effect 'treatment': control (a), application control (a), *M. anisopliae* (ab) and *V. lecanii* KV71 (b); for a = 0.004, lsd for arcsin √(fraction) transformed data = 0.141. Lower leaves, interaction between 'crop' and 'treatment': gerbera/control (a), gerbera/application control (a), gerbera/*M. anisopliae* (a), gerbera/*V. lecanii* (b), cucumber/control (a), cucumber/application control (a), cucumber/*M. anisopliae* (a), cucumber/*V. lecanii* (a); for a = 0.003, lsd for arcsin√(fraction) transformed data = 0.173

effects was found. In general, the percentage of mycosed aphids on upper leaves of gerbera was significantly higher than on upper leaves of cucumber. Furthermore, *V. lecanii* induced significantly higher mycosis than both control treatments, whereas *M. anisopliae* resulted in an intermediate percentage of mycosed aphids. On the lower leaves, we did find a significant interaction between crop and treatment. A higher percentage of mycosed aphids was found on gerbera plants treated with *V. lecanii* compared to the other treatments and compared to cucumber plants. Mycosis found in both control groups is probably the result of contamination during the experiment and natural infection of the population.

Phyllosphere climate

The phyllosphere of cucumber was more humid than that of gerbera and tomato (Tab. 1a). Of these crops, only the humidity of the cucumber phyllosphere (VPD = 1.48 - 1.73 hPa, RH = 93.6 - 92.6% at 20 °C) exceeded 92%, the value critical for germination of fungal conidia

(Walstad *et al.*, 1970; Ferron, 1977; Fransen, 1987; Chandler *et al.*, 1994). The upper leaves of all three plants were more humid than the lower leaves due to heating of the tables (Vogelezang, 1993).

A significant interaction between crop and leaf level was found when comparing phyllosphere VPD of poinsettia and cucumber (Tab. 1b). Lower leaves of poinsettia were less humid than upper leaves, and both were less humid than the cucumber leaves. The upper leaves of poinsettia were infested with whitefly and were used for assessing whitefly mortality. The humidity of the poinsettia upper-leaf phyllosphere (VPD = 1.76 hPa, RH = 92.7% at 20 °C) exceeded 92%, the value critical for germination of fungal conidia (Walstad *et al.*, 1970; Ferron, 1977; Fransen, 1987; Chandler *et al.*, 1994).

Table 1: Estimated vapour pressure deficit and matching RH (at 20 °C) in the phyllospere at upper and lower leaf level; a: only main effects, that is host-plant species and leaf level, are significant, b: interaction between both main effects is significant.

a		Main effects				
Comparison	Host-plant species	VPD [hPa]	*RH [%]*	Leaf level	VPD [hPa]	*RH [%]*
Cucumber -	Cucumber:	1.73 a*[1]	*92.6*	Upper	1.85 A[2]	*92.0*
Gerbera	Gerbera:	2.42 b	*89.8*	Lower	2.30 B	*90.4*
Cucumber -	Cucumber:	1.48 *a*[3]	*93.6*	Upper	1.74 *A*[4]	*92.4*
Tomato	Tomato:	2.41 *b*	*89.8*	Lower	2.16 *B*	*90.8*
b		Upper leaf:			Lower leaf:	
Comparison	Host-plant species	VPD [hPa]	*RH [%]*		VPD [hPa]	*RH [%]*
Cucumber -	Cucumber:	1.10 *a*[5]	*95.3*		1.35 *a*	*94.4*
Poinsettia	Poinsettia:	1.76 *b*	*92.7*		2.46 *c*	*90.2*

*numbers followed by the same letters are not significantly different; typographically different letters indicate different sets of comparisons; α's are corrected for the number of comparisons made resulting in the following lsd's: [1] 0.117, [2] 0.117, [3] 0.143, [4] 0.143 and [5] 0.240.

Relationship between phyllosphere climate and mortality/mycosis

The mortality or mycosis data for each plant - insect - fungus combination were related to the calculated phyllosphere VPDs (Fig. 6.3). Below 1.9 hPa almost no germination of fungal conidia will take place (Walstad *et al.*, 1970; Ferron, 1977; Fransen, 1987; Chandler *et al.*, 1994), and therefore we divided VPD in low, ranging from 0 - 1.9 hPa (92 - 100% RH at 20 °C), and high, when larger than 1.9 hPa (vertical line in Fig. 6.3). Fifty percent mortality was used as a rough distinction between high and low mortality and mycosis (horizontal line in Fig. 6.3). If phyllosphere climate explains differences in insect mortality and mycosis, then

low VPD relates to high mortality and high VPD to low mortality, and data points will be within the quadrants H_0. For more than half of the data sets this is not the case (Fig. 6.3).

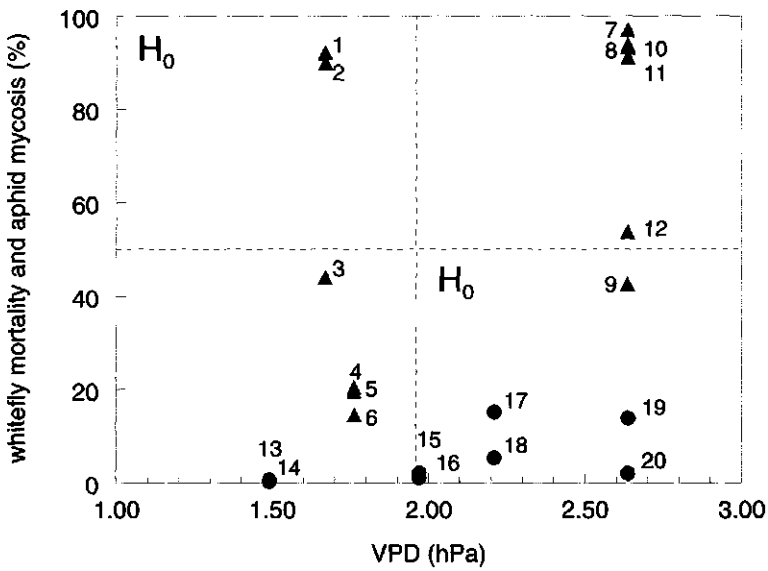

Figure 6.3: Relationship between phyllosphere vapour pressure deficit (VPD) and whitefly mortality or aphid mycosis. Numbers indicate different plant - insect - fungus combination (see table below). H_0 = mortality or mycosis > 50% and VPD < 1.9 hPa, or mortality or mycosis < 50% and VPD > 1.9 hPa.

Insect species	Fungal species	Cucumber leaf higher	Cucumber leaf lower	Poinsettia leaf higher	Poinsettia leaf lower	Tomato leaf higher	Tomato leaf lower	Gerbera leaf higher	Gerbera leaf lower
whitefly ▲	*A. placenta*		1	4			7		10
	A. aleyrodis		2	5			8		11
	V. lecanii KV01		3	6			9		12
aphid ●	*V. lecanii* KV71	13	15					17	19
	M. anisopliae	14	16					18	20

Plant morphology

In this experiment more light was transmitted through the cucumber crop than through the gerbera and poinsettia crops, meaning that the cucumber crop was less dense (Fig. 6.4). No difference in light transmission between cucumber and tomato crops was found.

The hydrophobicity of leaves from the four different plant species was studied by the shape of water droplets placed on the leaf surface (Fig. 6.5). The roundest droplet was found

on poinsettia, indicating that it has the most hydrophobic leaves of all four host plants. No large differences existed in shape of the droplets between tomato, gerbera and cucumber. Also the effect of a spreader on the shape of water droplets was studied (results not shown). All droplets with spreader were less round than their counterparts without spreader, but the same differences existed in shape of the droplets between the crop species.

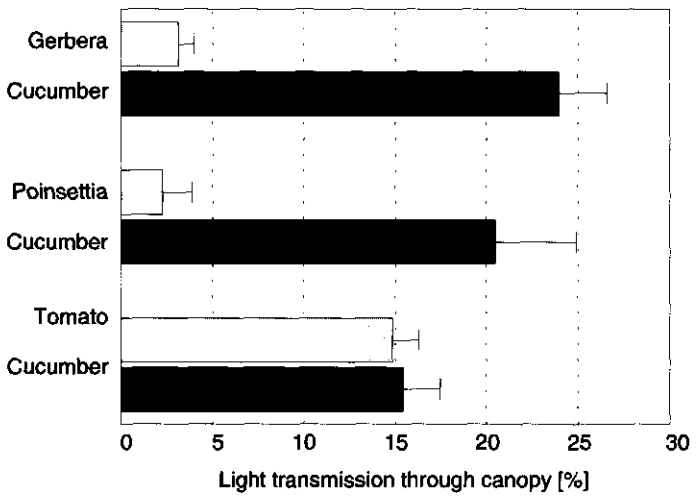

Figure 6.4: Percentage transmitted light (photosynthetically active radiation, PAR) through the canopy: cucumber (dark grey) compared to gerbera, poinsettia, and tomato. Vertical lines represent standard errors of mean.

Discussion

Whitefly mortality and aphid mycosis clearly varied according to host-plant species (Fig. 6.1 and 2). We also found significant differences in phyllosphere VPD between those host plants (Tab. 1). The question is whether these differences in phyllosphere humidity between host plants can explain differences in mortality and mycosis. If this would be the case, one would expect a high mortality or mycosis to be associated with to a high humidity, and a low mortality or mycosis with a low humidity (H_0 in Fig. 6.3). Low levels of whitefly mortality were found on poinsettia (4,5,6 in Fig. 6.3), versus high mortality levels on tomato (7,8,9 in Fig. 6.3) and gerbera (10,11,12 in Fig. 6.3), while on poinsettia a more humid phyllosphere climate was found than on the other crops. For aphids, but not for whitefly, the mycosis on gerbera (17,18,19,20 in Fig. 6.3) was higher compared with mycosis on cucumber (13,14,15,16

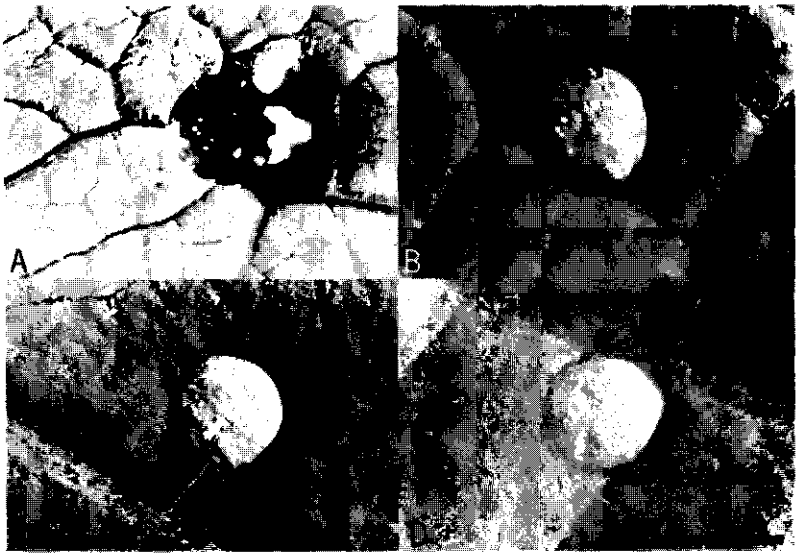

Figure 6.5: Shape of water droplets on leaf surface of A: poinsettia, B: cucumber, C: tomato, and D: gerbera, indicating in hydrophobicity of these leaves. Bar = 0.34 mm.

in Fig. 6.3), whereas cucumber had a more humid phyllosphere climate than gerbera. From these results we conclude that VPD differences between host plants cannot explain differences in mortality and mycosis. Phyllosphere climate in a more open crop is commonly believed to be less humid than that in a dense crop (van der Schaaf *et al.*, 1991; Bolckmans *et al.*, 1995). In our *between host-plant* experiments the latter argument does not hold, since cucumber had a more humid phyllosphere climate despite a more open crop structure, while poinsettia and gerbera, with a more dense crop structure, had a lower humid phyllosphere climate (Fig. 6.4).

The counter-intuitive result that VPD differences between host plants cannot explain differences in mortality and mycosis, is discussed below. Physical and chemical aspects of host plants may clarify our results.

Physical aspects of the host plant

A phyllosphere VPD of 2.4 hPa (~ 90% RH at 20 °C) on gerbera and tomato was apparently sufficient for both *Aschersonia* species to cause a high whitefly mortality (7, 8, 10, 11 in Fig. 6.3), comparable to mortality levels on cucumber (VPD < 1.9 hPa or RH > 92%; 1, 2 in Fig. 6.3). Although many entomopathogenic fungi need a VPD of ca.1.9 hPa or lower, that is an RH of more than 92% (at 20 °C), optimal germination and growth take place at 0 hPa (100% RH; (Gillespie & Crawford, 1986; Milner & Lutton, 1986; Fransen, 1987). The high whitefly

mortality on gerbera and tomato suggests that the calculated VPD is an overestimation of the real phyllosphere VPD. One should realise that the VPD data shown here are an average of a whole leaf and thus obscuring differences within a leaf caused by roughness (*e.g.* hairiness, venation), stomata, and other leaf characteristics (Holloway, 1970). Furthermore, the influence of the insect on the phyllosphere climate and the microclimate of the insect itself (Kramer, 1980; Ramoska, 1984) which could affect fungal germination and growth, are not taken into account. Vidal *et al.* (1998) also did not find differences in whitefly *(Bemisia argentifolii)* mortality on cucumber and tomato either when testing *Paecilomyces fumosoroseus*. Further, whitefly mortality on cabbage was not different from cucumber and tomato (Vidal *et al.*, 1998). In those experiments, however, the highly favourable greenhouse climate, which has a positive effect on phyllosphere humidity (Ferro *et al.*, 1979; Gaede, 1992), may have blurred differences between crops.

For poinsettia, the calculated phyllosphere VPD may have been underestimated (= phyllosphere humidity overestimated). On this crop, all three fungi (4, 5, 6 in Fig. 6.3) caused whitefly mortality no higher than 21%, whereas both *Aschersonia* species were able to cause high whitefly mortalities on cucumber, gerbera and tomato under similar or less favourable phyllosphere VPDs. (Baille *et al.*, 1994a; 1994b) found that the transpiration rate for poinsettia is very low compared with other ornamentals, and leaf stomatal resistance was considerably higher for poinsettia (higher than r_s = 100 s/m, as was the assumption in the VPD formula, see Appendix). This results in a higher VPD than we calculated, which may inhibit fungal growth or may even be detrimental to the fungus. In this respect the hydrophobicity of the leaf, which is influenced by its wax layer and hairiness, is also likely to play a considerable role in the actual phyllosphere humidity. Of all plant species used here, poinsettia had the most hydrophobic leaf surface. Water droplets on this leaf stayed compact, even when a spreader was used, in contrast to droplets on gerbera, cucumber or tomato leaves (see Fig. 6.5). Apart from negatively influencing the distribution of conidia on the leaf surface of poinsettia, it will also negatively affect the formation of a thin film of fluid on this leaf surface after spraying or condensation. This could in turn decrease the likelihood that conidia get into contact with the insect host and could negatively affect germination of conidia.

Less virulent fungi will never give rise to high mortality regardless of the humidity conditions, and this may have been the case for *M. anisopliae* against aphids on cucumber (14 in Fig. 6.3), *V. lecanii* against aphids (13 in Fig. 3) and *V. lecanii* against whitefly (3 in Fig. 6.3). Differences in virulence can explain the difference in whitefly mortality between the *Aschersonia* species and *V. lecanii* (van der Pas *et al.*, 1996; Fransen unpubl. res.).

Entomopathogenic fungi differ considerably in their humidity requirements for germination. Some fungi may need a saturated environment, like *V. lecanii* (Hall, 1981).

Others, such as *M. anisopliae* (Walstad *et al.*, 1970) and *A. aleyrodis* (Fransen, 1995), are able to germinate under slightly dryer conditions. When humidity conditions are unfavourable, the fungus remains inside the insect (Ferron, 1985). Since aphid mycosis was detected using external cues, the actual mycosis was probably underestimated. As a consequence, one would expect to observe a higher aphid mycosis on cucumber than on gerbera because of the higher phyllosphere humidity of cucumber. However, the observed mycosis on gerbera was higher than on cucumber, which suggests that a difference in phyllosphere humidity was not the main source of difference in aphid mycosis found between these two crops. The low observed aphid mycosis caused by *M. anisopliae* compared to that caused by *V. lecanii* may be explained by different humidity requirements of these fungi for protrusion outside the insect, along with the slower killing and sporulation of *M. anisopliae* compared with *V. lecanii* (Hall, 1980). In general, successful control of aphid populations is known to depend largely on the epizootic potential of the fungus (Hall & Papierok, 1982; Hall, 1985) and thus on sporulation, which may explain the low aphid mycosis in this short-term experiment.

Chemical aspects of the host plant

Chemical aspects of host plants are known to play a role in insect-fungus interaction as well as physical aspects (Hare & Andreadis, 1983; Costa & Gaugler, 1989; Brown *et al.*, 1995; Hajek *et al.*, 1995). Plant species differ substantially in their chemical composition, among which the nature and quantity of toxic substances in their phloem sap (Harborne *et al.*, 1999). For instance, some tomato cultivars produce tomatine which was found to be toxic to *Beauveria bassiana* (Costa & Gaugler, 1989). Also, leaves of poinsettia contain cytotoxic triterpenoids (Smith-Kielland *et al.*, 1996) and extracts of these leaves were used against fungi and nematodes (Khan *et al.*, 1996; Sebastian & Gupta, 1996). However, the viability of *A. aleyrodis* conidia was not affected by a prolonged stay on poinsettia or cucumber leaves, so no direct effect of toxins on conidia was apparent (Meekes *et al.*, 2000). Persistence of *V. lecanii* conidia on gerbera leaves is consistently higher than on cucumber (Beerling, unpubl. res.), which may explain the lower performance of *V. lecanii* against aphids on cucumber compared with gerbera. Since infection of aphids depends largely on pick up of conidia (Hall & Papierok, 1982), phyllosphere chemicals may play a larger role in persistence of fungi, in contrast to infection of a principally sessile insect like whitefly.

Also, the influence of host plant via the insect is important (for overview, see Berenbaum, 1988). Fruits, roots or seeds of the Cucurbitaceae contain cucurbitacins which were found to protect the spotted cucumber beetle against *M. anisopliae* (Tallamy *et al.*, 1998), but we used a less bitter cucumber cultivar which contains very low amounts of cucurbitacins (A. Janssen, pers. comm.). Whiteflies (*B. argentifolii*) reared on poinsettia are unsuitable as

food for lacewings, which has been attributed to nutritional inadequacy or to accumulation of detrimental plant compounds in the whitefly (Legaspi *et al.*, 1996). A similar interaction between first and third trophic level may exist in entomopathogenic fungi on this host plant, hence explaining the low whitefly mortality on poinsettia.

Developmental rate, reproduction and mortality of whitefly and aphids are also influenced by host-plant species (Minks & Harrewijn, 1987; van Lenteren & Noldus, 1990). The control mortality of greenhouse whitefly was significantly higher on poinsettia relative to cucumber, gerbera or tomato (see also van Lenteren & Noldus, 1990). A less suitable host plant, as reflected by higher natural mortality, could result in an increased susceptibility to entomopathogens, due to stress of the host insect (Steinhaus, 1958). In contrast, this could also result in an decreased susceptibility to entomopathogens compared to insects from a more suitable host plant, since these 'healthier' insects better support growth of natural enemies (Schultz & Keating, 1991; Hoover *et al.*, 1998). However, here low fungal infection is probably not linked to host-plant quality, since another whitefly (*B. argentifolii*) exhibited the same low infection levels on poinsettia as greenhouse whitefly (E. Meekes, unpubl. res.), while poinsettia is a very suitable host plant for *B. argentifolii* (van Lenteren & Noldus, 1990; Fransen, 1994).

The developmental and reproduction rate of cotton aphid was higher on cucumber than on gerbera (Beerling, pers. obs.). Aphids were more likely to escape fungal infection on cucumber due to a shorter exposure time (Clancy & Price, 1987), which involves also the loss of inoculum by moulting. In addition, a higher reproduction had consequences for the percentage surviving aphids, especially when one realises that even infected aphids give birth (Hall, 1976). Furthermore, a more favourable host plant does not encourage mobility of the insect and therefore pick up of conidia (Hall & Papierok, 1982). These aspects combined may explain the higher aphid mycosis on gerbera than on cucumber, in spite of the higher phyllosphere humidity on cucumber.

Concluding remarks

Poinsettia in its vegetative phase is attacked by few pest species and only one foliar pathogen: powdery mildew (*Oidium* sp.) (Motte & Unger, 1995; Koike & Saenz, 1998). Cucumber, gerbera and tomato carry many more pests and diseases (Anonymous, 1999). The leaf surface of poinsettia seems therefore a more hostile environment for pests and plant pathogens, and maybe also for entomopathogenic fungi. From an evolutionary perspective, the differences found in efficacy of fungi on different plant species support the theory that some plant species may have invested in creating an environment which fosters entomopathogens, thereby obtaining bodyguards to protect them against herbivores as an indirect defence. This contrasts

with others, like poinsettia, which may have invested rather in defending themselves directly against plant pathogens at the cost of potential bodyguards (Price *et al.*, 1980; Dicke & Sabelis, 1988; Sabelis *et al.*, 1999; Elliot *et al.*, 2000).

Our results demonstrate that the efficacy of entomopathogenic fungi does not depend only on the interaction between the entomopathogenic fungus and the target insect. Of overriding importance is the tritrophic interaction with the host-plant species, as shown by variation in success of entomopathogenic fungi in greenhouse pest control in different crops. Differences in phyllosphere humidity between the crops cannot explain our results. Direct chemical effect of the host plant on fungi and indirect chemical effect via the insect probably account largely for the observed differences in efficacy of entomopathogenic fungi on different host plants. These findings are of significance for (development of) efficient microbial control. As is the case with predators and parasitoids (Barbosa *et al.*, 1982; Sabelis & Jong, 1988; Vet & Dicke, 1992), our results underline the importance of considering the first trophic level (the plant) for entomopathogenic fungi in integrated pest management programmes.

Acknowledgements

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Appendix

The vapour pressure deficit in the phyllosphere, VPD_{leaf} is based on Goudriaan and van Laar (1994) and Goudriaan (pers. comm.).

$$VPD_{leaf} = VPD_{air} - r_{bv}\,\gamma R_n / \rho_{Cp} + (s + \gamma\, r_{bv} / r_{bh})\Delta T \qquad \text{[hPa]}$$

where [i]:

- vapour pressure deficit in air, $VPD_{air} = (1 - \mathbf{RH_{air}} / 100)\, e_{s(Tair)}$ [hPa]
  saturation vapour pressure, $e_{s(Tair)} = 6.107\, exp(17.4\, \mathbf{T_{air}} / (239.0 + \mathbf{T_{air}}))$ [hPa]
- leaf boundary layer resistance to heat, $r_{bh} = 100\sqrt{(w/v)}$ [s/m]
  leaf width[ii], $w = 0.23$ (cucumber), 0.11 (gerbera), 0.09 (poinsettia), 0.04 (tomato) [m]
- local windspeed, $v = 0.1$ (in glasshouse only convection; Gates (1980)) [m/s]
- psychrometric coefficient, $\gamma = 0.67$ (at 20 °C) [hPa/°C]
- volumetric heat capacity of air, $\rho_{Cp} = 1200$ [J/m^3 °C]
- slope of the saturation vapour pressure curve, $s = 1.45$ (at 20 °C) [hPa/°C]
- resistance to exchange of water vapour [iii], $r_{bv} = 2\,(0.93\, r_{bh})$ [s/m]
- temperature difference, $\Delta T = \mathbf{T_{leaf}} - \mathbf{T_{air}}$ [°C]
- net radiation absorbed per leaf area, $R_n = \lambda E + H$ [W/m^2]
  sensible heat loss per leaf area, $H = \Delta T\, \rho_{Cp} / r_{bh}$ [W/m^2]
  latent heat loss, $\lambda E = (sH + \delta) / \gamma^*$ [W/m^2]
  drying power, $\delta = VPD_{air}\, \rho_{Cp} / r_{bh}$ [hPa W/m^2 °C]
  $\gamma^* = \gamma\,(r_l + r_{bv}) / r_{bh}$ [hPa/°C]
  total leaf resistance, $r_l = r_s \quad r_c / (r_s + r_c)$ [s/m]
  $\rightarrow r_l = r_s$ (cuticular resistance, r_c negligible) [s/m]
  leaf stomatal resistance, $r_s = 100$ (when stomata are open) [s/m]

[i] Bold: measured parameters.

[ii] Leaf width was measured on leaves of similar age as those used in the experiment.

[iii] The resistance to water vapour (r_{bv}) is twice as big as the resistance to heat (r_{bh}), since water vapour can only diffuse from the underside of the leaf (stomata), whereas heat can diffuse from both sides of the leaf

7

Relative humidity and host-plant species influence mycosis of whitefly, *Trialeurodes vaporariorum*, by *Aschersonia aleyrodis*, *A. placenta* and *Verticillium lecanii*[1]

Abstract

The influence of relative humidity (RH), host-plant species and their interaction on mycosis of greenhouse whitefly, *Trialeurodes vaporariorum*, by entomopathogenic fungi *Aschersonia aleyrodis*, *A. placenta* and *Verticillium lecanii* was studied. Experiments, conducted on poinsettia, gerbera and cucumber at 50% and 80% RH, showed a clear host-plant effect. On cucumber and gerbera all fungi caused significantly higher mortality compared with the control, whereas whitefly mortality on poinsettia remained low and was not significantly different from the control. A higher ambient RH resulted in a higher mortality caused by either of the three fungi. Both *Aschersonia* spp. performed significantly better than *V. lecanii*. In subsequent experiments an additional period of 0, 3, 6, 12, 24 or 48 hours of high RH was applied after treatment with *A. aleyrodis* and *V. lecanii*. Mortality caused by the fungus increased with a longer additional period of high RH in combination with a higher ambient humidity. However, the host-plant effect exceeded the effect of ambient or additional high RH period; whitefly mortality was highest on cucumber, intermediate on gerbera and lowest on poinsettia. On poinsettia both fungi only had a significant effect on whitefly mortality after a 48 hrs high RH period. On gerbera and cucumber *A. aleyrodis* performed better under dryer conditions than *V. lecanii*. Relations between ambient RH, fungal species and the host-plant environment are discussed.

[1] Submitted as **Meekes, E.T.M, Joosten, N.N., Fransen, J.J. and Lenteren, J.C. van.** Relative humidity and host-plant species influence mycosis of whitefly, *Trialeurodes vaporariorum*, by *Aschersonia aleyrodis*, *A. placenta* and *Verticillium*

Introduction

Various abiotic environmental conditions influence fungal pathogen - host insect interactions. The fungus is highly dependent on suitable environmental conditions during different phases in its life cycle, *viz.* the initial part of the infection cycle, spore germination on the insect host and penetration of its cuticle, and, later on, during protrusion of the fungus from the insect's body and conidiogenesis (Ferron, 1977).

Very high RH levels (≥ 95%) are required for spore germination and sporulation outside the host insect (Hallsworth & Magan, 1999). However, entomopathogenic fungi differ considerably in their humidity requirements for germination. Some fungi may need a saturated environment or free water, like *Verticillium lecanii* or *Hirsutella thompsonii* (Hall, 1981; McCoy, 1981). Others, such as *Beauveria bassiana, Metarhizium anisopliae* (Walstad *et al.*, 1970) or *Aschersonia aleyrodis* (Fransen, 1995), can germinate at lower RH levels. Moreover, ambient RH is less critical than presumed previously, since the RH in the micro-environment surrounding the spore influences germination more than ambient RH (Marcandier & Khachatourians, 1987; Ravensberg *et al.*, 1990).

Fungal processes take place in the boundary layer surrounding the leaf (or insect) in which the air is undisturbed. The RH at the leaf surface is dependent on the diffusion rate at which water vapour is transferred through the boundary layer. The humidity in the phyllosphere is therefore determined by stomatal aperture, the thickness of the boundary layer and their interaction (Burrage, 1971). The aperture of the stomata is mainly influenced by environmental factors, such as light, CO_2 concentration, water status of the plant, differences in ambient humidity and leaf temperature (Jones, 1986). The thickness of the boundary layer is largely determined by host-plant characteristics, such as leaf size and shape, hairiness of the leaf and placement of the leaf on the plant, but also by turbulence and ambient temperature (Ferro & Southwick, 1984). Thus ambient and phyllosphere RH are correlated.

In the tritrophic system, host plant - whitefly - entomopathogenic fungus, the host plant can influence the efficacy of the fungus not only via the micro-environment, but also by production of allelochemicals or a combination of both. Allelochemicals can stimulate or inhibit the fungus directly (Blakeman, 1971) or effects may occur via the insect (Gallardo *et al.*, 1990; Hajek *et al.*, 1995).

Previous research showed varying results on poinsettia, gerbera and cucumber (previous chapters; Meekes *et al.*, 2000), which may be explained by tritrophic interactions. The fungal species studied in this paper, *A. aleyrodis*, *A. placenta* and *V. lecanii*, differ in their host spectrum and humidity requirements. Species of the genus *Aschersonia* are restricted to whiteflies and scale insects (Evans & Hywel-Jones, 1990), whereas *V. lecanii* is a common soil inhabiting fungus, which is able to survive saprophytically and infect a broader range of

insects, like aphids, thrips and whiteflies (Hall, 1981). It is also known as a mycoparasite on powdery mildew (Verhaar *et al.*, 1998) or rust (Zouba & Kahn, 1992). In its natural habitat, (sub)tropical forest ecosystems, *A. aleyrodis* only causes epizootics under very humid conditions (Wolcott, 1955), since it is dependent on rain for splash dispersal (Samson & Rombach, 1985). However, when applied as a mycoinsecticide against greenhouse whitefly on cucumber, *A. aleyrodis* was able to control the whitefly population at a ambient RH of 50% (Fransen, 1987b). When *V. lecanii* was applied against whiteflies on cucumber or tomato, the eventual success seemed not to be correlated with ambient RH (Ravensberg *et al.*, 1990).

In this paper we describe the influence of ambient RH on mycosis of whitefly on the host plants cucumber, gerbera and poinsettia at continues high or low RH, and at levels fluctuating around a high or low average RH, with additional periods of high RH after fungal treatment.

Materials and Methods

Plant material

The effect of host plant on efficacy of entomopathogenic fungi was studied on three plant species: cucumber (*Cucumis sativus* cv. Profito) gerbera (*Gerbera jamesonii* hybrids) and poinsettia (*Euphorbia pulcherrima* cv. Goldfinger). All plants were free of insecticides and fungicides. The two youngest, almost unfolded leaves on each poinsettia, gerbera and cucumber plant were infested with greenhouse whitefly (see below). After infestation with whitefly, only the cucumber plants were topped twice above the third leaf and lateral shoots were pinched out.

Whitefly host

Trialeurodes vaporariorum was maintained on gerbera plants in screened cages at 21°C under greenhouse conditions. To obtain second/third instar whitefly nymphs, 50 adults, both male and female, were confined to clip-cages on leaves. The adults were given the opportunity to oviposit for 24 hrs, which resulted in 188 ± 6 (SE), 99 ± 4 and 137 ± 6 nymphs per leaf for cucumber, gerbera and poinsettia, respectively. The average developmental period from egg to second/third instar nymph varied between 18 days on cucumber and 22 days on gerbera. The relative contribution of the different nymphal instars to the population was approximately 1 first : 6 second : 10 third instar whitefly nymphs for all plant species at the time of fungal application.

Fungi

Three fungal strains, *Aschersonia aleyrodis* KV107, *A. placenta* Ap1 and *Verticillium lecanii* KV01, were applied against whitefly. *Aschersonia aleyrodis* originated from Aleyrodidae in Colombia (W. Gams, The Netherlands) and *A. placenta* was isolated from *Dialeurodes cardamomi* (Homoptera: Aleyrodidae) from India (S. Selvakumaran, India) respectively (chapter 2). These two *Aschersonia* isolates were passed through greenhouse whitefly three times before augmentation. Sporulating colonies from potato dextrose agar (PDA) were used to inoculate millet-cultures for mass production of conidia. The fungi were cultured on autoclaved millet in Erlenmeyer flasks, which were closed with cotton, to allow aeration. Cultures were incubated at 25 °C and L16:D8 (artificial light). Conidia from 2-3 week old cultures were harvested by rinsing cultures with sterile demineralised water containing 0.05% (v/v) Tween 80 (Merck) as a spreader (Fransen, unpubl.). Suspensions were diluted to 10^7 conidia/ml.

Cultures of *V. lecanii* KV01 originated from the commercial product Mycotal® (Koppert BV, the Netherlands), which contained 10^{10} conidia/gram formulated product. The product was suspended in demineralised water which was plated on PDA and incubated at 23 °C for 10 days. Conidia were suspended in demineralised water containing 0.05% Tween. Suspensions were filtered (Wattmann filter no. 1) and diluted to 10^7 conidia/ml.

Treatment procedure

Two ml of the *A. aleyrodis*, *A. placenta*, *V. lecanii* or 0.05% Tween suspensions were sprayed on the undersides of the leaves using a Potter spray tower (Burkard Manufacturing, UK). The 0.05% Tween acted as control. Leaves stayed attached to the plant until final assessment for mortality. After evaporation of the water, plants were moved to the climate box or glasshouse (see below).

Assessment of mortality took place after two (exp. 1) or three (exps.2 and 3) weeks. Mortality was divided into mortality caused by the fungus and that by other causes. A nymph was considered infected by the fungus when it turned opaque orange (*A. aleyrodis*, *A. placenta*) or white (*V. lecanii*). A nymph was considered 'dead by unknown causes', when the insect had desiccated and no apparent sign of fungal infection was visible. Overall mortality was used for analysis.

Experiment 1: Continuous high or low RH

Aschersonia aleyrodis, *A. placenta*, *V. lecanii* or Tween suspensions were applied to the leaves. After spraying, plants were placed in a climate box (Fitoclima 801, Snijders Scientific, NL) at a high (80 ± 4%) or low (50 ± 6%) RH level and a temperature of 20°C (± 1 °C).

Temperature and RH were measured continuously. Per treatment combination, two leaves per plant and five plants were used; plants were left uncovered. The experiment was repeated twice. Final assessment of whitefly mortality took place two weeks after fungal application.

Experiment 2 and 3: Variable period of high RH

Aschersonia aleyrodis, *V. lecanii* or Tween suspensions were applied to the leaves. After spraying, plants were returned to the glasshouse and were either covered with plastic bags for 3, 6, 12, 24 or 48 hrs to create a condition of 95 - 100% RH or left uncovered (0 hrs high RH). Per treatment combination, two leaves per plant and six plants were used. After removal of the plastic bags, plants were kept on tables with an ebb - flow irrigation system in airconditioned glasshouse compartments (30 m^2) set at 20°C. The temperature and RH in the crops were measured every 5 minutes and averaged over two hour periods.

The experiment was carried out twice: once in spring and once during the summer, both times in two glasshouse compartments. During the spring experiment (exp. 2) the average ambient RH was 45.1% (± 7.1% SD; min.: 31.7%, max.: 60.6%) and during the summer experiment (exp. 3) the average ambient humidity was 88.7% (± 8.9 %; min.: 80.1%, max.: 96.0%). The temperature was on average 20.0 ± 0.2 °C (SD) during the spring experiment and 20.3 ± 1.0 °C during the summer experiment. Final assessment of whitefly mortality took place three weeks after fungal application.

Statistical analysis

Fractions whitefly mortality per plant were arc-sin square-root transformed. Transformed mortality data were analysed using a three-way ANOVA with (fungal) treatment, host-plant species and ambient RH (high/low - exp. 1, short/long - exp. 2 or 3) as independent variables and climate box or glasshouse compartment as block treatment (Genstat 5 release 3.22, Payne *et al.*, 1987). Subsequent differences were compared with t-tests, in which probability was adjusted to the number of t-tests performed (0.05/n).

Results

Experiment 1: Continuous high or low RH

In general, at high ambient RH whitefly mortality caused by the fungi was higher than at low ambient RH, but not in the control (Tab. 7.1; Fig. 7.1). *A. aleyrodis* and *A. placenta* caused a higher mortality than *V. lecanii* at 50% as well as at 80% RH.

A clear host plant effect on whitefly mortality was found. On poinsettia, all fungi caused a significantly lower whitefly mortality (≤ 25% mortality and not significantly different

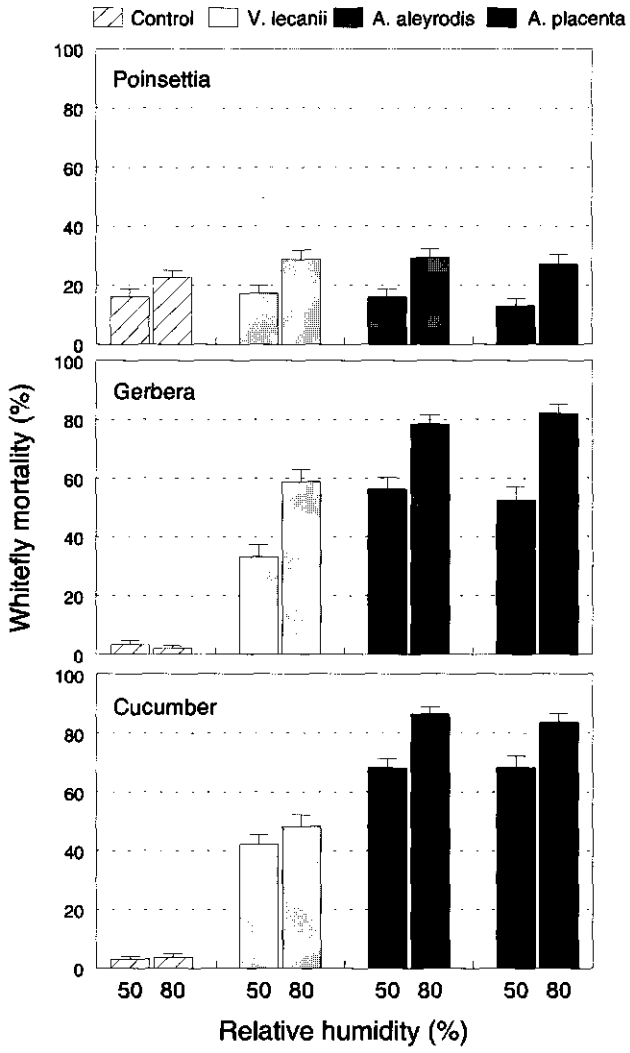

Figure 7.1: Average overall mortality of *T. vaporariorum* in control (0.05% Tween), or caused by *V. lecanii*, *A. aleyrodis* or *A. placenta* on poinsettia, gerbera and cucumber at 50% and 80% ambient RH. Error bars represent standard deviation.

from control) than on cucumber and gerbera (Tab. 7.1, Fig. 7.1). The control mortality however, was significantly higher on poinsettia than on the other host plants. *A. aleyrodis* and *A. placenta* performed significantly better than *V. lecanii*, on cucumber and gerbera. All fungal treatments caused a significantly higher mortality than the control treatments on all crops, except for poinsettia.

Table 7.1: The average whitefly mortality (%*) per treatment and ambient RH level or per treatment and host plant species ($p < 0.001$).

Main-effects	*Treatments*			
RH level	Control	*V. lecanii*	*A. aleyrodis*	*A. placenta*
50%	6.1 *a***[1]	32.2 *b*	49.6 *c*	47.4 *c*
80%	7.0 *a*	51.7 *c*	75.6 *d*	72.8 *d*
Host plant				
poinsettia	19.7 **b****[2]	25.3 **b**	24 **b**	22.5 **b**
gerbera	1.9 **a**	52.5 **c**	79.1 **d**	80.7 **d**
cucumber	3.3 **a**	49.2 **c**	86.4 **d**	80.0 **d**

*: Adjusted percentages, back-transformed from the arc-sin square root transformed fractions;
**: Numbers followed by the same letter are not significantly different, comparisons can only be made between treatments and within RH level or host-plant species; bold and normal letters indicate different comparisons; [1]: $\alpha = 0.0016$, lsd = 0.116; [2]: $\alpha = 0.0008$, lsd = 0.149; lsd's are only valid when data are arcsin√(fraction) transformed.

Experiment 2: Variable period of high RH

This experiment performed in spring at low ambient RH (ca. 45%) showed that the longer the additional period of high RH, the higher the probability was for whitefly nymphs to die of infection. A host-plant effect was also evident here. On poinsettia, both *V. lecanii* and *A. aleyrodis* infected whitefly nymphs at low levels only. Mortality caused by *V. lecanii* was not significantly different from the control treatment, even after 48 hrs additional high RH. In contrast, mortality caused by *A. aleyrodis* was significantly different from the control treatment after 24 and 48 hrs high RH (Fig. 7.2). On gerbera, both fungi caused a higher mortality after 12 hrs high RH compared to the control mortality, *A. aleyrodis* causing a slightly higher mortality than *V. lecanii* (48 hrs high RH). On cucumber both fungi caused the highest mortality compared to poinsettia and gerbera. The whitefly mortality caused by the fungi was significantly higher than the control mortality in all cases, even without an extra period of high RH, with the exception of the *V. lecanii* at 12 hrs additional high RH. Especially *A. aleyrodis* performed well against *T. vaporariorum* and caused 55% (0 hrs high RH) to 95% (48 hrs high RH) mortality at an average ambient RH of 45% in the glasshouse.

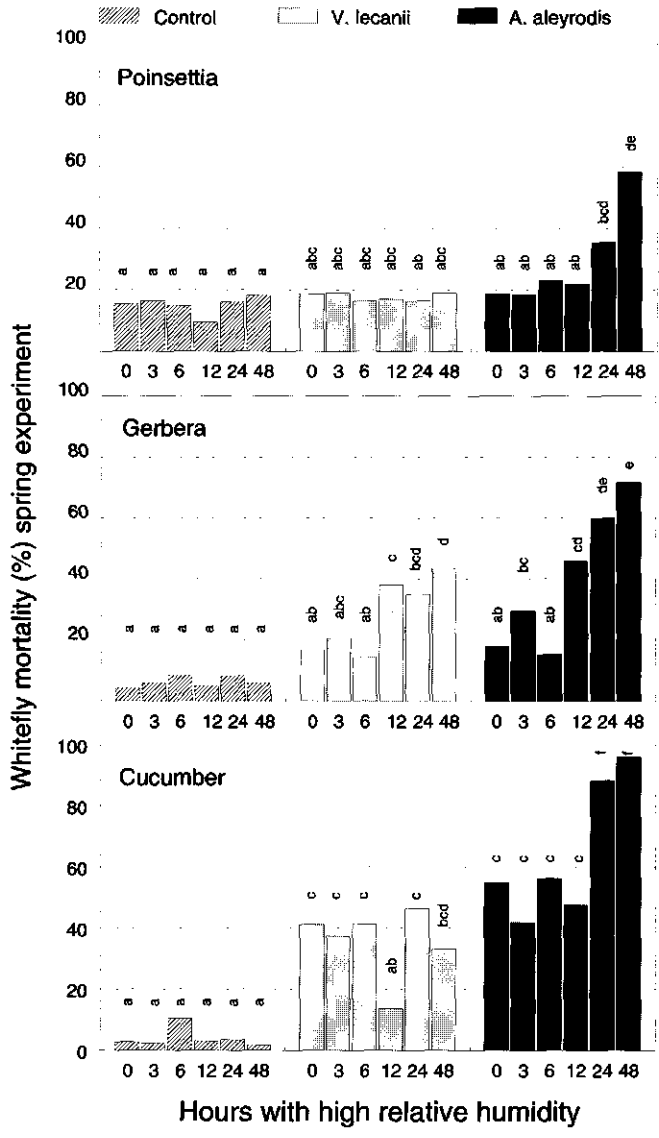

Figure 7.2: Average whitefly mortality in control (0.05% Tween) or caused by *V. lecanii* and *A. aleyrodis* on poinsettia, gerbera and cucumber at six different periods of high RH: 0, 3, 6, 12, 24 and 48 hrs in spring (ambient RH 45%). Percentages are back-transformed from the arc-sin square root transformed fractions. Bars followed by the same letter are not significantly different (comparisons can only be made within the same treatment, host plant species or RH level; $\alpha = 0.0002$, lsd = 0.264; lsd is only valid when data are arcsin$\sqrt{}$(fraction) transformed).

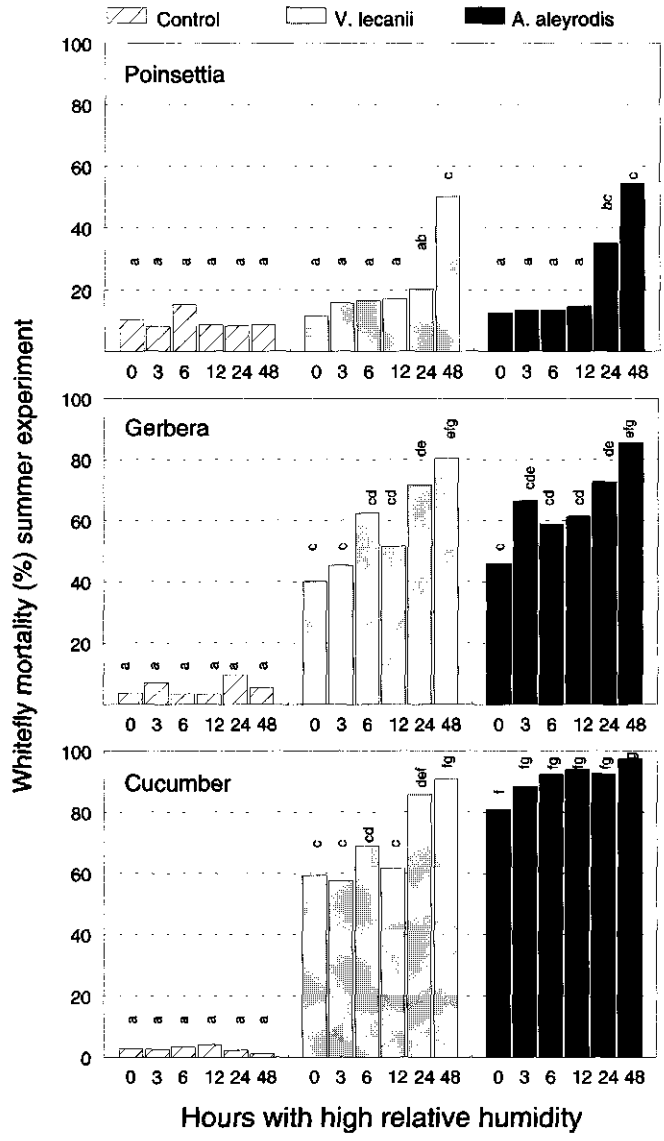

Figure 7.3: Average whitefly mortality in control (0.05% Tween) or caused by *V. lecanii* or *A. aleyrodis* on poinsettia, gerbera and cucumber at six different periods of high RH: 0, 3, 6, 12, 24 and 48 hrs in summer (ambient RH 85%). Percentages are back-transformed from the arc-sin square root transformed fractions. Bars followed by the same letter are not significantly different (comparisons can only be made within the same treatment, host plant species or RH level; α = 0.0002, lsd = 0.232; lsd is only valid when data are arcsin$\sqrt{}$(fraction) transformed).

Experiment 3: Variable period of high RH

During the experiment performed in summer, the ambient RH was around 85%. Also here we found that the longer the additional period of high RH, the higher the mortality from infection by *V. lecanii* and *A. aleyrodis*. As in the spring experiment, a host-plant effect was found (Fig. 7.3). On poinsettia both fungi had hardly any effect on whitefly mortality, although now *V. lecanii* caused a significant increase in mortality after a period of 48 hrs high ambient RH, whereas *A. aleyrodis* caused a significantly higher mortality after 24 and 48 hrs high RH. On gerbera the two fungi performed equally well against *T. vaporariorum*, this in contrast with the spring experiment. On cucumber *A. aleyrodis* and *V. lecanii* caused the highest whitefly mortality compared to gerbera and poinsettia. *A. aleyrodis*, causing 81 to 97% mortality, performed again significantly better than *V. lecanii*, causing 59 to 90% mortality.

Discussion

Host plant - ambient humidity

In this tritrophic system of host plant - whitefly - entomopathogenic fungus, the host-plant species appeared to be of overriding importance in the eventual success of microbial control. On poinsettia the period of high ambient RH (>95%) had to exceed 24 hrs to reach 50% mortality of *T. vaporariorum*, whereas on cucumber 50% to 70% mortality was reached without an additional period of high RH and even under relatively dry conditions (avg. 45% - 50% RH) (see Fig. 7.1 - 7.3). Rovesti *et al.* (1997) also found that only partial control of whitefly (*Bemisia tabaci*) on poinsettia was achieved, although environmental conditions were very favourable for fungal growth. Vidal *et al.* (1998) found no host-plant effects when *Paecilomyces fumosoroseus* was applied against the silverleaf whitefly (*B. argentifolii)* on cucumber, tomato and cabbage. However, since Vidal's experiments were carried out at high ambient RH (near 100% ambient RH for 10 hrs) host-plant effects, if present, could be inconspicuous. On poinsettia *P. fumosoroseus* (Pakistan strain, courtesy of L.A. Lacey) caused similar low mortality levels as *A. aleyrodis* and *V. lecanii* (Fransen, unpubl.) and those mortality levels of both *T. vaporariorum* and *B. argentifolii* were comparable to this study.

The differences in mortality mentioned above could be related to differences in humidity at the host plant's leaf surfaces, since phyllosphere humidity is partly determined by the host plant itself and by its response on environmental conditions (Burrage, 1971; Jones, 1986; Baille *et al.*, 1994a). Cucumber, with its larger, hairy leaves (van Lenteren *et al.*, 1995), and gerbera, with its rosette type of plant, may have a thicker boundary layer than poinsettia. In addition, the influx of humidity into the boundary layer of poinsettia may be low, since the leaf stomatal resistance was considerably higher for poinsettia than for other ornamentals (factor 3 difference). Poinsettia also appeared to have a more hydrophobic leaf surface

compared to cucumber and gerbera (chapter 6). All these differences in physical host-plant characteristics suggest that the phyllosphere humidity on poinsettia is lower than on gerbera or cucumber. Under humid conditions mycosed whitefly nymphs on poinsettia only showed fungal protrusion from the insect's body at some humid places in the crop, in contrast with infected nymphs on gerbera or cucumber (Meekes, pers. obs.). However, when phyllosphere humidity was calculated for these host-plant species, the observed differences did not explain the low whitefly mycosis on poinsettia compared to cucumber or gerbera (chapter 6) and even under very suitable RH levels (89% RH, with additional 48 hrs 95 - 100% RH) whitefly mortality on poinsettia did not reach the same levels as on cucumber or gerbera (Fig. 7.3). So, although the above differences in plant characteristics will influence the effectivity of entomopathogenic fungi, they are probably not the sole explanation for the poor performance of these fungi on poinsettia.

Host plant - chemical aspects

Chemical aspects of host plants are known to play a role in insect-fungus interaction as well (Elliot *et al.*, 2000; Poprawski *et al.*, 2000). Plant species differ substantially in their chemical composition, among which the nature and quantity of toxic substances in their phloem sap (Harborne *et al.*, 1999). Poinsettia leaves, for instance, contain cytotoxic triterpenoids (Smith-Kielland *et al.*, 1996) and extracts of these leaves were used against fungi (Khan *et al.*, 1996). However, since conidia of *A. aleyrodis* stayed viable for over a month on poinsettia leaves, direct toxic effects on conidia can likely be excluded (Meekes *et al.*, 2000; chapter 5).

Secondary plant substances may also affect the insect's fitness and through that its susceptibility to pathogens (Hare & Andreadis, 1983; Boucias *et al.*, 1984; Ramoska & Todd, 1985; Gallardo *et al.*, 1990). For instance, fruits, roots or seeds of the Cucurbitaceae contain cucurbitacins which were found to protect the spotted cucumber beetle against *M. anisopliae* (Tallamy *et al.*, 1998), but we used a less bitter cucumber cultivar which contains very low amounts of cucurbitacins (A. Janssen, pers. comm.). Lacewings (*Chrysoperla* sp.) feeding on poinsettia reared whitefly (*B. argentifolii*) did not reach the pupal stage, which was attributed to consumption and accumulation of detrimental plant compounds in the whitefly nymphs (Legaspi *et al.*, 1996). A similar interaction between first and third trophic level may exist in entomopathogenic fungi on this host plant, hence explaining the low whitefly mortality on poinsettia. The higher control mortality of greenhouse whitefly on poinsettia relative to cucumber or gerbera (Fig. 7.1), is not likely to have affected the infection levels here, because another whitefly (*B. argentifolii*) exhibited the same low infection levels on poinsettia as greenhouse whitefly, while its control mortality on poinsettia is similar to that on cucumber (Fig. 9.3; chapter 9).

Fungal species - ambient humidity

As with most other biological control agents, entomopathogenic fungi are limited by an array of biotic and abiotic factors. Traditionally the ambient RH has been considered the most serious constraint on the use of fungi for the control of insects, as germination of conidia requires a RH above 90 to 95% (Gillespie & Crawford, 1986; Hallsworth & Magan, 1999). Our results showed that a high ambient RH positively influenced mycosis of *T. vaporariorum*. It is also not surprising that short periods of near saturated humidity increased insect mycosis, since optimal germination and growth of many entomopathogenic fungi take place at near 100% RH (Schneider, 1954; Gillespie & Crawford, 1986; Milner & Lutton, 1986; Fransen, 1987a; Hallsworth & Magan, 1999).

Aschersonia aleyrodis and also *A. placenta* were more virulent on cucumber, gerbera and poinsettia than *V. lecanii*, especially under relatively dry conditions. *V. lecanii* seemed to be more sensitive to low ambient RH, as was found by Riba and Entcheva (1984). For *V. lecanii* the daily periods of high RH had to exceed 16 hrs to enable the fungus to grow and successfully infect greenhouse whitefly on cucumber and tomato and at least 20 hrs of high RH were needed to cause more than 50% mortality (Ekbom, 1981). However, *V. lecanii* isolates were found to vary in their humidity requirements (Drummond *et al.*, 1987). Exposure to 12 hrs of high humidity on cucumber (Fig. 7.2) resulted in lower infection levels for *V. lecanii* than 0 to 6 hrs high humidity. *V. lecanii* colonizes the host cuticle before penetration will take place (Schreiter *et al.*, 1994; Askary *et al.*, 1999) and may be more sensitive at this stage to the sudden drop from >95% to 45% humidity by removal of the plastic bags.

However, in spite of high humidity requirements for germination, fungal infection occurs at a wide range of ambient RH levels (Kramer, 1980; Ramoska, 1984; Marcandier & Khachatourians, 1987, Fig 7.1-7.3), suggesting that the humidity at the surface of the host integument or on the leaf surface is sufficient for spore germination and host penetration (Ravensberg *et al.*, 1990; St.-Leger, 1991).

Practical considerations

In glasshouses in the Netherlands where poinsettia is grown during late summer and autumn, the ambient RH is kept between 70-85%, but ambient humidity may be higher when black-out shields are used to obtain short days for initiation of flowering (Fransen, pers. comm.). However, even at an average ambient RH of 89% (Exp. 3), whitefly mortality by fungi was low (< 30%). In glasshouses with year round production of cucumber or gerbera, growers employ a range of climate regimes, and climate conditions vary with season and from year to year (*e.g.* Kerssies, 1994; Dik & Elad, 1999). Ambient humidity may vary between 35 - 95%, but at night ambient RH levels of 80 - 95% are easily reached. This should be sufficient for

whitefly control with fungi; maybe with exception of periods of frost and on bright sunny days when ambient RH can drop to very low levels (van Houten *et al.*, 1995). In our experiments we applied the fungi only once, whereas for commercial products repeated applications are recommended (*e.g.* Wraight & Carruthers, 1999). Repetitive application would increase the mortality levels that we found. With a diurnal fluctuation in ambient RH in glasshouses, high during the night - low during day, repetitive application of high ambient RH periods would be another option (Ekbom, 1981; Fransen, 1987a). However, artificially increasing the ambient RH in glasshouses, which has been suggested by some authors (Helyer *et al.*, 1992) would be unacceptable to most growers, because of the risk to stimulate plant-disease development.

Our results are based on conidial suspensions amended with 0.05% Tween as spreader. More recent developments in formulation of conidia has led to the use of oil carriers. Oils are compatible with hydrophobic surfaces, such as insect and leaf cuticles, eliminating the need for wetting, sticking or spreading agents (Wraight & Bradley, 1996) and they improve efficacy of, for instance, *Metarhizium flavoviride* under low moisture conditions (Bateman *et al.*, 1993). Against the whitefly *B. tabaci* the use of oil formulations has been successful, increasing the efficacy of *P. fumosoroseus* on cucumber plants from no apparent infection (Tween formulation) to 80-100% infection (oil formulations) (Smith, 1994). Also oil formulations of *V. lecanii* show promising results (Pas *et al.*, 1998). If a low RH at the leaf surface is one of the main reasons why control of whitefly on poinsettia is low, oil formulation can be an option. In addition, oils can also be insecticidal (Helyer, 1993). In fact, some oils are registered as insecticides (Wraight & Bradley, 1996). Possible sublethal effects of oils on the target insect could enhance the eventual effect of entomopathogenic fungi (Ferron, 1985; Quintella & McCoy, 1997).

Our results clearly indicate that *A. aleyrodis* and *A. placenta* are less dependent on ambient RH and perform better against the greenhouse whitefly than *V. lecanii*. *A. aleyrodis* and *A. placenta* will be a valuable addition to biological control of whiteflies on crops like gerbera (chapter 8) and cucumber, especially with the long persistence of these fungi on leaf surfaces (Fransen, 1995; Meekes *et al.*, 2000; chapter 9) and the compatibility of *A. aleyrodis* with the whitefly parasitoid *Encarsia formosa* (Fransen & van Lenteren, 1993; 1994).

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8

Effect of gerbera cultivar on the efficacy of entomopathogenic fungi against *Trialeurodes vaporariorum*[1]

Abstract

Can host-plant characteristics influence the efficacy of entomopathogenic fungi? This was tested using two gerbera cultivars with different plant architecture and trichome density on the leaf: 'Bianca' having more and larger leaves and fewer trichomes/cm^2 than 'Bourgogne'. Two entomopathogenic fungi, *Aschersonia aleyrodis* and *Verticillium lecanii*, were tested for the control of greenhouse whitefly, *Trialeurodes vaporariorum*. The fungi were applied in two concentrations, 10^6 and 10^7 conidia/ml, in a gerbera crop in a glasshouse situation.

In 'Bianca', both fungi caused a whitefly mortality up to 80%. Whitefly mortality was higher for 10^7 than for 10^6 conidia/ml. In 'Bourgogne', *V. lecanii* caused a significantly lower mortality than *A aleyrodis*. Although no cultivar differences in whitefly development time were found, other characteristics, like natural mortality and build-up of the whitefly population, seemed to differ. Differences in mortality by the fungi in relation to whitefly characteristics and cultivar, including spray droplet distribution, are discussed.

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Introduction

Biological control in floriculture is still difficult compared with biological control in fruit vegetables, due to several reasons. First, more chemicals are available for ornamentals than for fruit vegetables, because of safety regulations for vegetables. In addition, due to the high number of cultivated ornamental species and cultivars, compared to vegetables, a high diversity in pest and disease problems, and a variety of different environmental and cultural conditions exist. Secondly, in fruit vegetables only the fruits are harvested, which allows a higher population level of the pest, and some leaf injury can be tolerated. In contrast, most ornamentals are being sold with flowers and leaves, and therefore should be free of injury and presence of insects. Thirdly, the criterion of zero tolerance for both pest and beneficials has been used as a standard for all ornamental products until now, though it is only needed as an export requirement for a few countries (Fransen, 1993; Gullino & Wardlow, 1999).

Currently, development of Integrated Pest Management (IPM) programs for ornamental crops have a high priority, because of insecticide resistance problems and demand of the general public. In the ornamental crop gerbera (*Gerbera jamesonii*), 80-85% of the growers works according to IPM guidelines, while it was only 10% 5 years ago (M.J. van der Mey; F.R. van Noort, pers. comm.). In this crop only flowers are harvested and therefore cosmetic damage to leaves is acceptable within certain limits, and biological control is more feasible. Several parasitoids and predators are used commercially against greenhouse whitefly (*Trialeurodes vaporariorum*) in gerbera. However, for treatment of hot spots or when population levels exceed the action threshold, chemical control is imminent. Screening for natural enemies which are able to kill pest insects quickly, without affecting other natural enemies, is therefore an important line of research. Entomopathogenic fungi can be a valuable asset to existing biological and chemical control measures (Lacey *et al.*, 1996). In the last two decades several entomopathogenic fungi, such as *Verticillium lecanii*, *Paecilomyces* spp., *Beauveria bassiana*, *Metarhizium anisopliae* and *Aschersonia* spp. (Fransen, 1987; Ravensberg *et al.*, 1990; Malsam *et al.*, 1998; Wraight *et al.*, 1998), have been tested for their potential to control *T. vaporariorum,* with good results. Their ability to infect insects with sucking mouthparts by penetration of the cuticle, which other entomopathogens cannot do, make them suitable pathogens of whitefly species (Fransen, 1990).

In the Netherlands, gerbera is an economically important crop (> 200,000 ha) of which over 600 cultivars are grown commercially (F.R. van Noort, pers. comm.). Currently, cultivars are mainly selected for colour and shape of their flowers and their post-harvest quality. However, they differ largely in other characteristics such as plant chemistry and plant morphology, like leaf shape, leaf size and trichome density (Sütterlin & van Lenteren, 1996; Krips *et al.*, 1999; Krips, 2000). These characteristics can influence the efficacy of natural

enemies. For example, volatiles that are produced by spider mite-damaged plants of four gerbera cultivars differed in chemical composition and attractiveness to the predatory mite *Phytoseiulus persimilis* (Krips, 2000). The host plant is also known to affect the efficacy of entomopathogenic fungi (*e.g.* Gallardo *et al.*, 1990; Hajek *et al.*, 1995). Chemicals at the leaf surface can influence viability of fungal spores (Cooke & Rayner, 1984), but chemical composition may also have an effect on the pest species and its susceptibility to pathogens (Hare & Andreadis, 1983; Ramoska & Todd, 1985). In addition, with an increasing amount of trichomes on a gerbera leaf, the searching activity of *P. persimilis* was hampered, as was its predation rate of spider mites (Krips *et al.*, 1999). Also *Encarsia formosa*, a parasitoid of whitefly species, is influenced by trichome density on different cucumber cultivars (van Lenteren *et al.*, 1995). Morphological factors also define the microclimate at leaf surface, in which fungal processes take place, through leaf size, density of trichomes on the leaves and crop architecture (Ferro & Southwick, 1984). To what extent the architecture of a host plant is able to influence the efficacy of entomopathogens is still unknown. Here, we report on glasshouse experiments with *A. aleyrodis* and *V. lecanii* for the control of *T. vaporariorum* in gerbera carried out in two morphologically and possibly also chemically different cultivars.

Materials & Methods

Whitefly-stock rearing

Trialeurodes vaporariorum was reared on gerbera plants (*Gerbera jamesonii* hybrids) for many generations over several years, in screened cages at 21°C (± 2 °C) under greenhouse conditions.

Gerbera crop and glasshouse conditions

In a glasshouse of 150 m^2 two gerbera cultivars were grown: 'Bourgogne' and 'Bianca'. The Bourgogne cultivar produced red flowers and had 5-7 leaves per plant at the start of the experiment; 'Bianca' produced white flowers and had 8-15 leaves per plant. The leaves of 'Bourgogne' were relatively small compared to leaves of 'Bianca': ca. 35 x 10 cm versus ca. 45 x 16 cm respectively, but leaves of 'Bianca' were rather smooth compared with leaves of 'Bourgogne': 105 trichomes/cm^2 versus ca. 730 trichomes/cm^2 on the underside of the leaves (Krips *et al.*, 1999). The number of abaxial stomata/mm^2 varied for 'Bourgogne' between 70 - 75 and for 'Bianca' between 65 - 68.

The plants were cultivated on rockwool and were provided with water and nutrients via drip irrigation, comparable to commercial growing systems. The cultivars were grown in 9 rows, which were two plants wide and 29 plants long. Every row was divided in five plots of 10 (2 x 5) plants, which were separated by an open space (1 plant wide). On every side four

(2 x 2) plants were left as buffer, as well as one row at the beginning, near the entrance. The eight rows were divided in 4 blocks in north-south direction. Every block consisted of one row 'Bianca' and one row 'Bourgogne'.

The temperature and relative humidity (RH) in the glasshouse were measured every 5 minutes and averaged over one hour periods. Glasshouse temperature was on average 19.2 °C (± 2.5°C SD), however on bright sunny days temperatures could rise up to 29 °C. The average humidity was 61.1% RH ± 14.1% SD, but during the second half of the experiment the RH fluctuated considerably (Fig. 8.1), between 20.2% and 80.4% RH (minimum and maximum during 1 hour), as a result of the low humidity levels outside which were on average 30% RH. Only during the day the misting system was operated, so that the crop would not be wet during the night and growth of plant pathogenic fungi was avoided. This led to a diurnal rhythm of higher RH during the day than during the night.

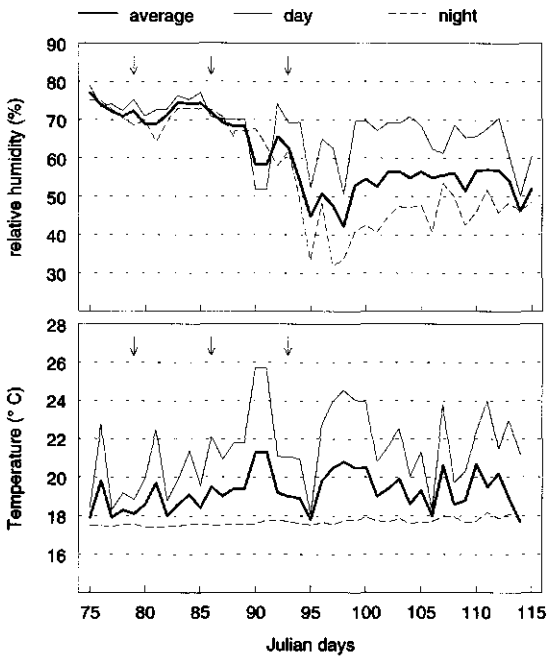

Figure 8.1: Average day and night temperatures (°C) and humidity levels (% RH) in glasshouse; →: indicates time of treatment.

Whitefly infestation in glasshouse

Once per two weeks, 7, 5 and 3 weeks before the first treatment, ca. 3000 whitefly adults, both male and female, were distributed over the gerbera plants. Every week the number of adult whiteflies per plant were counted, starting 4 weeks before the first treatment. However, during these pre-assessments of the whitefly population, the cultivar Bianca seemed more attractive than the cultivar Bourgogne: young leaves of 'Bianca' contained up to 40 adults per leaf, whereas 'Bourgogne' had almost none. To obtain an even distribution among the cultivars, 5 to 10 adults of both sexes were confined to clip-cages (Ø 2 cm), one clip-cage per 'Bourgogne' plant. The clip-cages were removed after 24 hrs and care was taken not to disturb the adults. This procedure was repeated once a week, for 4 weeks.

Fungi

Aschersonia aleyrodis originated from Aleyrodidae in Colombia (W. Gams, CBS, The Netherlands) and was passaged through whitefly twice. Sporulating colonies from pure cultures on potato dextrose agar (PDA, Difco) were used to inoculate millet-cultures for mass production of conidia (Fransen, 1987). Cultures were incubated at 25 °C and L16:D8 (artificial light). Conidia from 2-3 week old cultures were harvested by rinsing cultures with sterile demineralised water and suspensions were diluted to 1 x 10^6 or 1 x 10^7 conidia/ml with 0.05% (v/v) Tween 80 solution (Merck). Initial viability of conidia on water agar (15 g/l agar, Merck) after 24 hrs at 25°C exceeded 95% germination.

V. lecanii KV01 was obtained as commercial formulation Mycotal® (Koppert Biological Systems BV, the Netherlands), which contains 10^{10} conidia/gram. The product was suspended in sterile water to obtain 10^7 conidia/ml, of which 0.1 ml was plated on PDA and incubated at 23°C for 10 days. Subsequently, a conidial suspension was made, filtered (Wattmann filter no. 1) and diluted to 1 x 10^6 or 1 x 10^7 conidia/ml with 0.05% Tween 80 solution. Initial viability of *V. lecanii* conidia exceeded 98% germination after incubation for 24 hrs. at 25 °C on water agar.

Treatment procedure

Five different treatments were used, allotted to the different plots within a row (4 replicates per cultivar). The fungi of both species were applied at two concentrations: 1 x 10^6 and 1 x 10^7 conidia/ml and one plot per row was sprayed with 0.05% Tween suspension (control treatment). The suspensions were applied with a high volume hand sprayer (nozzle 55, pressure 4 x 10^5 Pa). Care was taken to hit the underside of the leaves and 'run-off' of suspensions was avoided. The amount of spray suspension was 1300 l/ha (converted from 150 m^2) and 1.3 x 10^{12} to 1.3 x 10^{13} conidia per ha. This is comparable to amounts used in

commercial practice (Wraight & Carruthers, 1999). The treatments were applied once a week in three consecutive weeks. Four weeks after the last treatment 40 leaves per plot were harvested and examined for total number of dead nymphs and overall number of whitefly nymphs and pupal cases present. For each treatment, the number of dead nymphs were divided in two categories (i) mycosed nymphs (orange nymphs [*A. aleyrodis*] or opaque white nymphs [*V. lecanii*]) and (ii) desiccated nymphs (no apparent sign of infection: "mortality cause unknown")

Whitefly development time

The development time of *T. vaporariorum* was monitored for the cultivars 'Bianca', 'Bourgogne' and 'Fame'. 'Fame' was used to be able to compare the results of this experiment to results of other studies (Dorsman & Vrie, 1987; Roermund & van Lenteren, 1992; Sütterlin, 2000) and had ca. 111 trichomes/cm^2 (Sütterlin & van Lenteren, 1997). Six plants of each cultivar were grown in pots and placed together on ebb and flow tables in a glasshouse compartment (30 m^2 at 20 ± 2 °C (SD) and 65 ± 10% RH). Plants were three months old and had at least five leaves, of which one leaf was used. For the experiment 50 whitefly adults of both sexes were confined in clip cages (Ø 2 cm) on young, completely unfolded leaves. The females were given the opportunity to oviposit for 24 hrs and were removed afterwards. Every one or two days egg hatching (day 2 to 17), adult emergence (day 28 to 42) and nymphal mortality were scored, until 80 to 90% of the adults had emerged.

Statistical analysis

Fractions whitefly mortality in the glasshouse experiment were arc-sin square-root transformed. Transformed total mortality was analysed using a two-way ANOVA with (fungal) treatment and host-plant cultivar as independent variables, and blocks as block treatments (Genstat 5 release 3.22, Payne *et al.*, 1987). Transformed "mortality by infection" and transformed "mortality cause unknown" were analyzed in the same way. Tests were performed at a probability level of 0.025, since the data of total mortality on one hand, and "mortality by infection" and "mortality cause unknown" on the other hand, are linked. The subsequent differences were compared with t-tests, in which probability was adjusted to the number of t-tests (n) performed (0.025/n).

The median hatching time and median emergence time were calculated from the hatching of nymphs and emerging of adults over time. As every leaf is evaluated over time, these hatching and emergence data were not independent. A separate regression analysis against time was therefore carried out for each leaf in relation to cultivar (general linear model, binomial distribution, logit link function) and the 50% hatching and 50% emergence time were

calculated. These values were compared using a one-way ANOVA with cultivar as independent variable (Genstat 5 release 3.22, Payne *et al.*, 1987).

Results

Glasshouse experiment

In both cultivars the fungal treatments caused significantly higher total mortality compared to the control treatment (Fig. 8.2). In general, the higher the concentration of conidia applied, the higher the whitefly mortality. On 'Bianca', both fungi caused a whitefly mortality up to 80% (Fig. 8.2). On 'Bourgogne' *V. lecanii* caused a significantly lower total mortality compared to *A. aleyrodis* and compared to 'Bianca'. For both fungal treatments more nymphs on 'Bianca' died as fourth instar/pupa than on 'Bourgogne' (Fig. 8.2). 'Bourgogne' was less attractive to whitefly adults than 'Bianca', therefore different whitefly infestation strategies were applied (see materials & methods). This could have resulted in a younger whitefly population on 'Bourgogne'.

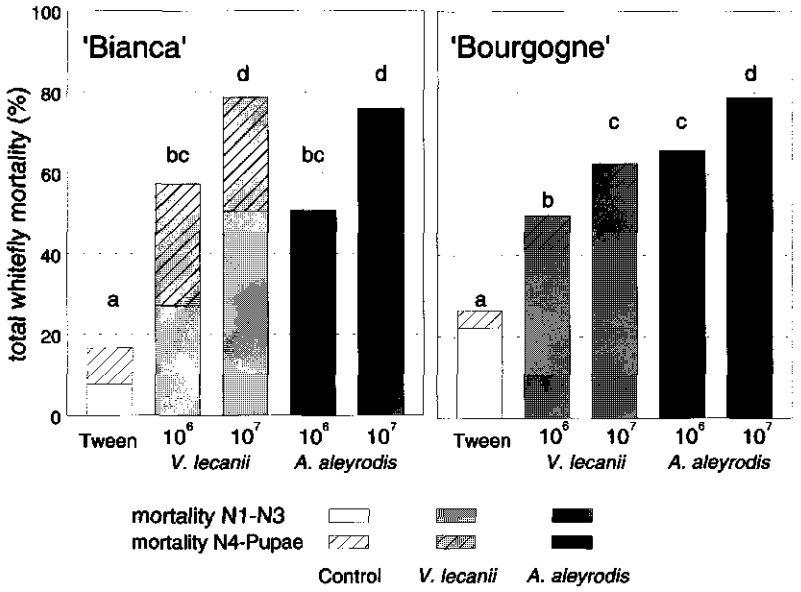

Figure 8.2: Average total mortality in gerbera cultivars Bianca and Bourgogne in control treatment (Tween 0.05%, white) or caused by *V. lecanii* (light grey) and *A. aleyrodis* (dark grey) applied in two concentrations: 10^6 and 10^7 conidia/ml. Total mortality is divided according to the development stage in which mortality took place: uniform = first to third nymphal instar, shaded = fourth instar - pupa. Bars followed by the same letter are not significantly different, lsd = 0.178 (lsd is only valid when data are arcsin√(fraction) transformed).

When mortality by infection and mortality by unknown causes were tested separately, the cultivar differences were even more pronounced. On 'Bianca' the infection levels were comparable to the total mortality levels (Fig. 8.2 and 8.3-I), whereas on 'Bourgogne' a more pronounced difference between the two fungi in infection levels was found. Both *V. lecanii* treatments were significantly lower than either *A. aleyrodis* treatment (Fig. 8.3-I). A significant interaction between treatment and cultivar was found, indicating a cultivar influence on the outcome of the treatment data.

Mortality by unknown causes showed a significant difference between the cultivars. On 'Bourgogne' the whitefly mortality by unknown causes was significantly higher than on 'Bianca' (Fig. 8.3-II) and the majority of these nymphs died in their first to third instar (data not shown). This effect is not very clear in the control treatments, as the differences are masked by accidental infection. This infection was caused by drift of conidial suspensions during spraying and was correlated with the treatments applied in the connected plots.

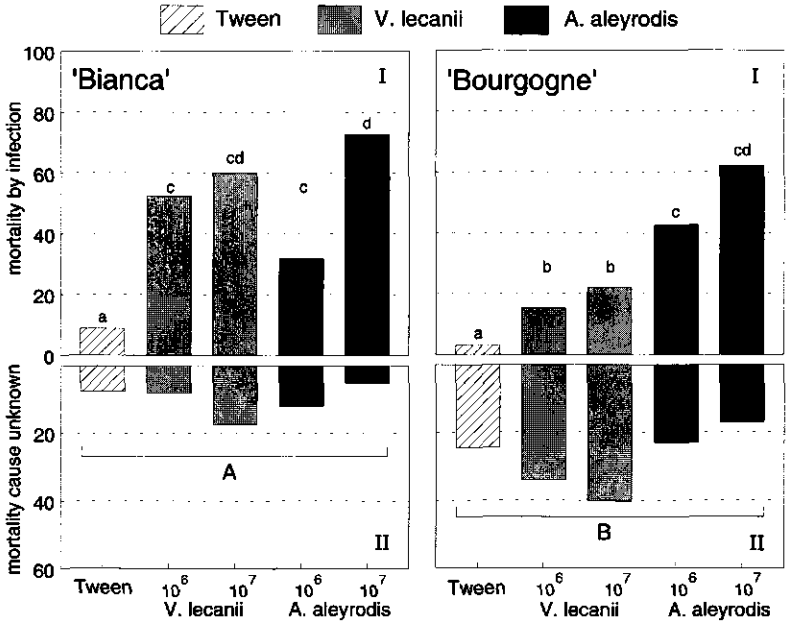

Figure 8.3: Average whitefly mortality in gerbera cultivars Bianca and Bourgogne in control treatment (Tween 0.05%) or caused by *A. aleyrodis* and *V. lecanii* applied in two concentrations. Mortality is divided in I: Mortality by infection; II: Mortality by causes unknown. Bars followed by the same letter are not significantly different; typographically different symbols indicate different sets of comparisons. Comparisons can only be made between different treatments within the same cultivar and between the cultivars for the same treatment; I: $lsd^{a-d} = 0.256$; II: cultivar effect: $lsd^{A-B} = 0.234$, treatment effect (*) lsd = 0.167: Tween - *ab*, *V. lecanii* 10^6 - *ab* and 10^7 - *b*, *A. aleyrodis* 10^6 - *ab* and 10^7 - *a*; lsd is only valid when data are arcsin√(fraction) transformed.

Mortality by unknown causes was significantly higher for *V. lecanii* (conc. 10^7 conidia/ml) compared with *A. aleyrodis* (conc. 10^7 conidia/ml), but not significantly different from the control treatment (Fig. 8.3-II).

Development time whitefly

The number of eggs deposited by the females in this 'no choice' situation was not significantly different for the three cultivars ($p = 0.465$). Also the development time of *T. vaporariorum* from egg deposit to 50% hatching ($p = 0.425$) and the median adult emergence time ($p = 0.267$) were not significantly different for the three gerbera cultivars (Tab. 8.1).

The total immature mortality for 'Bianca' was on average 9.2%, whereas for 'Fame' and 'Bourgogne' this was 11.6% and 13.8% respectively, but this was not significantly different ($p = 0.564$). Immature mortality was calculated using dead nymphs still present on the leaf surface. On 'Fame' and 'Bourgogne' many more nymphs went, however, missing than on 'Bianca' (Tab. 8.1). These nymphs could have moved out of the observation area, which might have resulted in higher mortality there. It was not possible to observe the whole leaf, because the bioassays were carried out on intact plants and leaves easily became detached. In addition, dead nymphs will not be visible inevitably. On 'Bourgogne', for instance, more crawlers got stuck in the trichomes and subsequently died than on 'Bianca' (Meekes, pers. obs.), but these were not traceable anymore after a few weeks.

Table 8.1: Number of eggs per leaf and development time (days) from egg deposit* to 50% egg hatching or to 50% adult emergence of *Trialeurodes vaporariorum* on different gerbera cultivars at 20°C

Gerbera cultivar	number of eggs per leaf ± SD	50% hatched eggs [days] ± SD	50% adult emergence [days] ± SD	total immature mortality [%] ± SD	missing nymphs [%]
'Bianca'	140 ± 24 a	9.9 ± 0.2 *a*	35.9 ± 1.3 **a**	9.2 ± 5.9 A	0.3
'Bourgogne'	135 ± 33 *a*	9.7 ± 0.4 *a*	37 ± 2.5 **a**	13.9 ± 7.3 A	44.1
'Fame'	168 ± 52 a	9.8 ± 1.5 *a*	35.6 ± 3.2 **a**	11.6 ± 2.5 A	48.3

*: Egg laying completed within 24 hrs.

Discussion

Entomopathogenic fungi are able to cause up to 80% whitefly mortality in a gerbera crop grown under semi-commercial conditions. The results, however, showed that gerbera cultivars influence the efficacy of entomopathogenic fungi, depending on which fungal pathogen was used. Both fungi performed equally well on 'Bianca', whereas on 'Bourgogne' *A. aleyrodis* caused a higher whitefly mortality than *V. lecanii* (Fig. 8.2 and 8.3). These differences could

be a result of direct interactions between fungus and cultivar, or indirect interactions with the insect as intermediate.

The host-plant cultivars differed in plant architecture, leaf size and trichome density on a leaf, and probably also in leaf chemical composition (Krips, 2000). The morphological features can influence the humidity at leaf level (Burrage, 1971; Ferro & Southwick, 1984; Chapter 6). 'Bourgogne' has fewer and smaller leaves than 'Bianca', which could enhance the exchange of water vapour on 'Bourgogne'. On the other hand, 'Bourgogne' has seven times more trichomes per leaf area than 'Bianca' (Krips *et al.*, 1999), which could counteract the enhanced exchange (Ferro & Southwick, 1984). However, not only the amount, also the shape of trichomes can differ substantially, *e.g.* single erect trichomes versus trichomes which entangle each other, forming a web over the leaf surface (Sütterlin & van Lenteren, 1997). The amount of trichomes, their shape and the composition of epicuticular waxes also influence the hydrophobicity of a leaf (Holloway, 1970). Leaves with few trichomes (like 'Bianca') enhance wetting, whereas leaves with many web-forming trichomes (like 'Bourgogne') produce a highly water repellent surface (Holloway, 1970). On leaves of 'Bianca' droplets tend to be larger and merge more easily than on 'Bourgogne', indicating that this might be the case. The higher wetting possibility combined with a denser crop possibly provided a more suitable climate for entomopathogenic fungi on 'Bianca' than on 'Bourgogne'. This would reduce the efficacy of *V. lecanii* on 'Bourgogne' more than *A. aleyrodis*, since *V. lecanii* is more sensitive to lower humidity levels (chapter 7). On 'Bourgogne', the higher "mortality by unknown causes" for *V. lecanii* (Fig 8.3-II) compared to *A. aleyrodis* could indicate that the relative humidity at the leaf level was not suitable for the fungus to protrude outside the insect's body, nor show signs of infection inside the host's body (white discoloration). When no protrusion takes place, infection by *A. aleyrodis* is easier to observe, since infected nymphs turn an orange colour, whereas nymphs, especially the young ones, infected by *V. lecanii* may not always turn white.

With increasing hydrophobicity the droplet retention decreases (Brunskill, 1956). Since the web-forming trichomes on a 'Bourgogne' leaf produce a hydrophobe surface (Holloway, 1970), the amount of inoculum present on a 'Bourgogne' leaf may be less, or is not as uniformly distributed compared with a 'Bianca' leaf. Besides, nymphs at the leaf plane are shielded by the leaf trichomes on 'Bourgogne' and less conidia may reach them. Since whitefly nymphs are sessile, but for a part of the first development stage (crawlers), direct hit of nymphs is the most important route of infection. The "lesser coverage" on 'Bourgogne' should affect both fungi equally, but *A. aleyrodis* conidia are far more persistent (half-life of >25 days, Meekes *et al.*, 2000) than conidia of *V. lecanii* (half-life of 4 days on chrysanthemum, Gardner *et al.*, 1984). This enables *A. aleyrodis* to profit more from a second route of infection *viz.* via spray residue (Meekes *et al.*, 2000). In general it is suggested that, after hatching of the eggs,

crawlers move only a few millimetres before settling on a suitable spot on the leaf, where they remain for the rest of their nymphal development (Byrne & Bellows, 1991). On both gerbera cultivars nymphs moved considerably more - up to many centimetres from the oviposition site -, indicating that they had ample opportunity to come into contact with spray residue.

Differences between cultivars may also affect entomopathogenic fungi via the pest insect e.g. development time or insect susceptibility. Development time and fecundity of *T. vaporariorum* differs between gerbera cultivars (Sütterlin, 2000) and the faster the insect develops, the smaller the time frame will be in which the insect can be infected (Clancy & Price, 1987). No difference, however, in developmental time and number of eggs between the cultivars used in our experiments was observed (Tab. 8.1). Nevertheless, these parameters do not include preference of whitefly adults for one cultivar over the other. In the glasshouse experiment, where adults did have a choice, 'Bianca' was preferred over 'Bourgogne'. Also, the "disappearance" of nymphs from cv's Bourgogne and Fame versus Bianca suggests that gerbera cultivar did affect the whitefly population. The preference of whitefly for 'Bianca' required a different infestation strategy (see materials & methods), which led eventually to a younger whitefly population on 'Bourgogne' (see Fig. 8.2). If, for instance, a larger part of the whitefly population on 'Bourgogne' still consisted of eggs, which are not susceptible to either fungus, the chance that a hatched nymph will get infected by *A. aleyrodis* is higher than by *V. lecanii*, due to the longer persistence of *A. aleyrodis* conidia. Insect susceptibility to fungal pathogens is also known to be affected by host-plant differences in secondary metabolites (Costa & Gaugler, 1989; Gallardo *et al.*, 1990; Poprawski *et al.*, 2000). However, so far no secondary metabolites that may affect fungi through the pest insect have been reported on gerbera leaves (Harborne *et al.*, 1999). Although various gerbera cultivars differ with respect to their chemical composition, for instance, spider mite-induced volatiles of various cultivars differed in chemical composition and attractiveness to the predatory mite *P. persimilis* (Krips, 2000), it is unclear at the moment if this can influence entomopathogens.

The above results show that whitefly populations in gerbera can be suppressed by using entomopathogenic fungi in a glasshouse situation under conditions which are common during commercial gerbera production (19 °C and 65% RH, Kerssies, 1994). Cultivar differences seem to be important: both fungi performed equally well on 'Bianca', but in 'Bourgogne' *A. aleyrodis* caused a higher whitefly mortality than *V. lecanii*. These differences seem to be a result of fungal characteristics in relation to physical plant factors and/or insect characteristics, indicating that *A. aleyrodis* is better equipped to differential circumstances than *V. lecanii*.

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9

General discussion

Microbial control of whitefly with *Aschersonia* spp. comprises many aspects, of which several are addressed in this thesis. The previous chapters will be summarized and discussed in the framework of the topics for evaluation of entomopathogenic fungi for the control of insect pests (chapter 1, Tab. 1.3). Furthermore, the implications for *Aschersonia* spp. as microbial control agents for the silverleaf and greenhouse whitefly are given and suggestions for future research are made.

Fungal characteristics

Host specificity and virulence

The specificity of entomopathogenic fungi has been described as "the expression of reciprocal adaptations and affinities between a pathogenic organism and the entirety of its host species" (Fargues & Remaudiere, 1977). However, literature on entomopathogenic fungi concerning specificity does not provide a basis to discern a general principle, *viz.* some fungal isolates are specific to one insect species or genus, others show high virulence against species of various insect orders (Ferron *et al.*, 1972; Fargues, 1976; Mor *et al.*, 1996; Vidal *et al.*, 1997). It is difficult to make a statement on specificity and virulence for the genus *Aschersonia* on basis of the results in chapter 2. The question whether some *Aschersonia* species are specific to whitefly only and others to soft scales only, as was described by Petch (1921), has still to be answered. All isolates originating from Aleyrodidae were able to infect *Bemisia argentifolii* and *Trialeurodes vaporariorum*, so it seems that those isolates have similar virulence characteristics. However, of 22 isolates, out of a total of 44 isolates studied, the original host insect was unknown or was not identified to family level, in this case Coccidae or Aleyrodidae (Tab. 2.1, chapter 2). Nine out of these 22 isolates were unable to infect either whitefly. These isolates could be specific for soft scales, especially since one of these isolates is *A. turbinata*, which has been described from coccids. It is possible that some species and/or isolates of *Aschersonia* may have broader host ranges than others and than previously was presumed (Petch, 1921), for instance, *A. aleyrodis* and *A. placenta* have been described from many Aleyrodidae species, but also from Coccidae and occasionally Diaspididae (Tab. 1.2, chapter 1). The morphological characteristic on which *Aschersonia* spp. are described as whitefly pathogens or soft-scale pathogens, seems to be phenotypically determined, and therefore not a reliable taxonomic feature (Evans & Hywel-Jones, 1997). The aspect of taxonomy requires an urgent update, in which one may consider testing against a range of possible host insect.

Selection of an optimal strain is crucial to successful application of entomopathogenic fungi (Hajek & St.-Leger, 1994). To evaluate entomopathogenic fungi for the control of insect pests, several fungal characteristics can be considered (Tab. 1.3, chapter 1). High virulence of the pathogen towards the target insect species is one of the most important selection criteria. A fungal entomopathogen used as a biocontrol agent of whitefly in glasshouses should show high virulence against *B. argentifolii* as well as *T. vaporariorum*, since both whitefly species can occur at the same time. In general, the virulence of the different *Aschersonia* isolates against *B. argentifolii* was positively related to the virulence against *T. vaporariorum* (chapter 2, Fig. 9.1). Several *Aschersonia* isolates were not only highly virulent towards both whiteflies, but also showed a high spore production on semi-artificial medium, another prerequisite for microbial control.

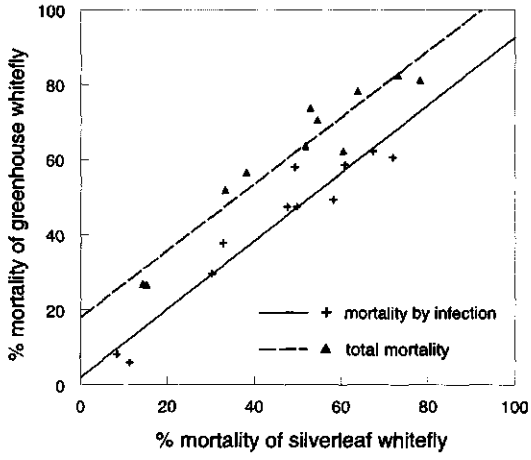

Figure 9.1: Relation between total mortality and mortality by infection of silverleaf whitefly, *B. argentifolii*, and greenhouse whitefly, *T. vaporariorum*, by eleven isolates of *Aschersonia* spp. (see also Fig. 2.5, chapter 2). Correlation coefficients: mortality by infection $r^2 = 0.92$, total mortality $r^2 = 0.91$.

Median lethal doses for five isolates of *Aschersonia* spp. varied between 1650 to 4770 conidia/cm^2 for *B. argentifolii*, and between 710 to 5340 conidia/cm^2 for *T. vaporariorum* on poinsettia (chapter 3). These values are higher than those found by Vidal *et al.* (1997) for *Paecilomyces fumosoroseus*, but lower than the values Wraight *et al.* (1998) found for *B. bassiana*, *P. fumosoroseus* and *P. farinosus* (see also chapter 3). Regression coefficients (probit-slopes) of these five *Aschersonia* isolates varied between 0.82 and 1.32 for *B. argentifolii* and between 0.83 and 1.44 for *T. vaporariorum*, which is in the same range as the coefficients found by Vidal *et al.* (1997) and Wraight *et al.* (1998). For fungal dose-host mortality responses these coefficients typically vary between 0.5 and 1.5 (Wraight & Carruthers, 1999), and are low compared to chemical compounds. In this thesis *A. aleyrodis*

and *A. placenta* showed a higher virulence towards *T. vaporariorum* and gave more consistent results than *V. lecanii* (chapter 6, 7 and 8), although Meade & Byrne (1991) and Gindin *et al.* (2000) found high infection levels of *B. argentifolii* and *T. vaporariorum* by *V. lecanii*. Unfortunately these results are difficult to compare since no LD_{50} values are given.

At high doses, conidia of *Aschersonia* spp. seem to posses self-inhibitors (chapter 3). These self-inhibitors could be advantageous for the fungi, to prevent germination during conidiogenesis and germination *en masse*. It is unlikely that this will interfere with microbial pest control, since this phenomenon only occurs at very high dosages (1.4×10^5 conidia/cm^2), which are unnecessary and uneconomical for microbial control.

Median lethal time of the five *Aschersonia* isolates varied between 5 to 7 days for *B. argentifolii*, and 5 and 10 days for *T. vaporariorum* (chapter 3). Since whitefly nymphs are sessile, the exact time of death is relatively difficult to determine compared to mobile insects. The LT_{50} values are determined using signs of infection, such as discolouration, will probably underestimate the actual time of death, because actual death will have taken place before signs of infection occur. When the nymphal stage is used to determine time of death, death will occur on average one nymphal stage later than the nymphal stage treated at 20 °C, which means that 5 to 7 days after inoculation 80-90% of the whitefly population dies of infection by *A. aleyrodis* and *A. placenta* (chapter 4). This is faster compared to the LT_{50} values found for these *Aschersonia* isolates (chapter 3).

Bioassay methods depend on the consistency of the method when comparing different isolates of fungal pathogens. In the case of *B. argentifolii* and *T. vaporariorum* the use of intact plants appears to be a suitable system, since consistent results were found over time. The bioassay did need some adjusting compared to Fransen (1987), *viz.* Fransen (1987) used 24 hrs of high humidity for cucumber plants, whereas the standard bioassay used in this thesis was 48 hrs of high humidity for poinsettia plants. It was necessary to create a more favourable climate, since poinsettia seems to have a more hostile environment for entomopathogenic fungi (chapter 5, 6 and 7). Once a more favourable situation is created on poinsettia LD_{50} and LT_{50} values of *A. aleyrodis* on this crop (chapter 3) are comparable to results obtained with *A. aleyrodis* on cucumber (Fransen, 1987). However, since on poinsettia mortality levels are rarely above 85 - 90% (chapter 2 - 4), the LD_{95} values will probably differ.

Although bioassays carried out on intact plants are more labourious than a bioassay performed on leaf discs and are not always feasible considering space and costs, many advantages exist. Side effects like premature ageing of leaves do not occur, leaves stay in their natural position and systemic effects on all trophic levels can be studied. Spraying leaves with a fungal suspension, instead of dipping leaves, and performing the bioassays under glasshouse conditions makes the results easier to extrapolate to large glasshouse applications.

Sporulation, dispersal and persistence

Whitefly nymphs infected with *Aschersonia* spp. may show extensive sporulation if the environmental conditions are favourable. However, on poinsettia sporulation only occurred in humid spots within a crop, whereas on gerbera it was often observed (E.T.M. Meekes, pers. obs.). Even when extensive sporulation is observed, secondary infection will be limited and is not expected to contribute to effective whitefly control in a glasshouse situation (Fransen, 1987). Conidia which are produced in slimy heads usually are dispersed by water or by insects (Samson & McCoy, 1983; Osborne & Landa, 1992), although *Aschersonia* conidia might also be subject to wind dispersal in nature when freed of their mucilage matrix and dried by wind (Morrill & Back, 1912). Since it does not rain in a glasshouse and overhead irrigation is rare, only condensation of water on the leaf surface may enable conidia to be transported from a sporulating insect to a susceptible one on the same leaf. Transmission of conidia by other insects like *Encarsia formosa* (Fransen & van Lenteren, 1993) or mites (Osborne & Landa, 1992) will probably not contribute to a high rate of infection either, especially within the short time period available for control of a whitefly population in a glasshouse crop (Fransen, 1987).

In a glasshouse situation conidia of *A. aleyrodis* were able to stay viable for over a month and infect hatching *T. vaporariorum* nymphs (chapter 5); 60-80% of *A. aleyrodis* and *A. placenta* conidia were still able to germinate on water agar after 40 days stay on a poinsettia

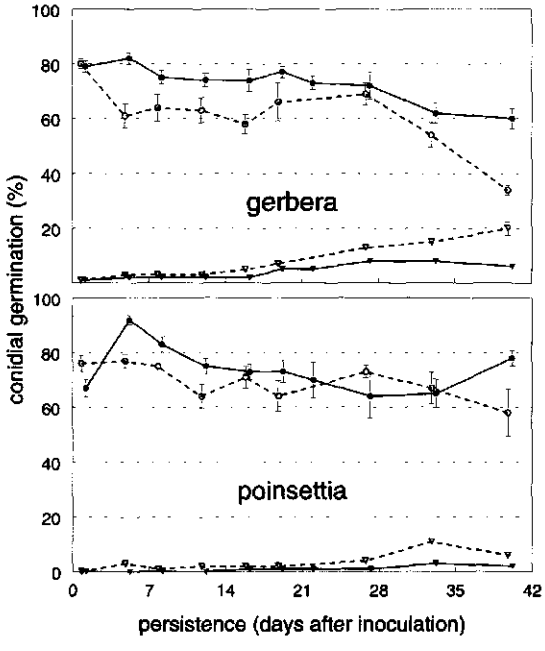

Figure 9.2: Mean percentage germination of *A. aleyrodis* and *A. placenta* conidia from 0 days until 40 days on leaf surface of gerbera and poinsettia at 20 °C; by direct observation of agar imprint of leaf (= germination on leaf surface): - -▽- - : *A. aleyrodis*, —▼—: *A. placenta*, or observation after 24 hours incubation on WA at 25°C (= germination capacity): - -○- - : *A. aleyrodis*, —●—: *A. placenta*. Vertical lines represent standard error of mean (for more details see chapter 5). Tapaninen & Meekes, unpublished results.

leaf surface (Fig. 9.2; Tapaninen & Meekes, unpubl.). Although *Aschersonia* spp. were evaluated for the control of whitefly species in glasshouses, where the environment is less extreme than in open field situations, conidia of *Aschersonia* spp. are also likely to persist in field situations. In (sub)tropical forest ecosystems, its natural environment, where bright sunshine alternates with clouds and rain, *Aschersonia* spp. have to overcome extreme circumstances. Conidia of many *Aschersonia* spp. contain pigments, for instance *A. aleyrodis* conidia contain carotenoids (Eijk *et al.*, 1979), which may enable them to withstand short periods of exposure to radiation better than hyaline spores, although variation between and within species exists (Fargues *et al.*, 1996). Moreover, conidia are directed against whitefly nymphs which are mainly present at the undersides of leaves and thus protected from direct sunlight. Also the mucilage matrix of conidia may be able to act as an antidesiccant (Boucias & Pendland, 1991) and protect the conidia against environmental stress (Louis & Cooke, 1985). The long persistence of *Aschersonia* conidia is probably a phenomenon that is common within the genus *Aschersonia*, since species of *Aschersonia* and their teleomorph *Hypocrella* were capable to withstanding long periods of drought in their natural environment (up to 5-6 months) (Evans & Hywel-Jones, 1997). The absence of germination at the leaf lamina (chapter 4 and 5), may also be a strategy to stay viable over a prolonged period. However, viability and especially infectivity are host-plant related (chapter 5, 6 and 7) and the extent of this residual effect on whitefly control is dependent of whitefly distribution over the plants and plant growth.

Culture and mass production

In general, the majority of *Aschersonia* spp. grows and sporulates well on most conventional mycological media and semi-artificial media, like brown rice, soybeans or millet (Ramakers *et al.*, 1982; Samson *et al.*, 1982; Ramakers, 1983; E.T.M. Meekes, unpubl. res.), but notable differences in nutritional requirements are apparent; some isolates had a higher conidial production on millet, whereas others sporulated better on corn medium (chapter 1, E.T.M. Meekes unpubl. res.). Optimum temperature for growth and sporulation of *Aschersonia* spp. varies between 25 to 30 °C (Spassova, 1980; Rombach & Samson, 1982) and no mycelial growth is observed at temperatures exceeding 31 - 35 °C, depending on the isolate (Rombach & Samson, 1982; Samson & Rombach, 1985). Sporulation can be strongly enhanced by light, of which wavelengths between blue and black light are most favourable (Samson *et al.*, 1982; Rombach & Gillespie, 1988). After subculturing *A. aleyrodis* 12 times on millet, final infection levels of *T. vaporariorum* were similar to that of isolates that were subcultured three times (Fransen, 1987), however, one has to be careful extrapolating these results to other *Aschersonia* isolates. Malt extract agar is most favourable for isolation and maintenance in the

laboratory, although growth is slower and sporulation is less abundant compared with media containing peptone (Rombach & Gillespie, 1988; E.T.M. Meekes, unpubl. res.). On several other media (*e.g.* yeast/peptone/glucose), a sudden deterioration of colonies may occur and valuable cultures can easily be lost (Rombach & Gillespie, 1988). This deterioration seems to be common among entomopathogenic fungi, therefore, regular passage (*e.g.* once a year) through a suitable host insect followed by subsequent reisolation, or suitable storage of the original isolate is recommended in order to maintain viability and infectivity (Kogan & Seryapin, 1978; Samson & Rombach, 1985; Hirte *et al.*, 1989a; Butt & Goettel, 2000).

The majority of control programs utilizing fungi rely on inundative releases (McCoy, 1990; Hajek & St.-Leger, 1994) and require large-scale application of the fungus each time the pest population exceeds its action threshold. Small scale mass-production of the fungus already started at the beginning of the 1900's, when *A. aleyrodis* and *A. goldiana* were grown in wide-mouth one-pint bottles on sweet potato agar (Berger, 1920b; 1920a; 1921). In the 1970's in Eastern Europe, several *Aschersonia* spp. were mass-produced on beer wort with a sugar content of 10%, at 24-26 °C and 80-100% RH in luminescent light (Kogan & Seryapin, 1978; Spassova, 1980). After 25-30 days, the cultures could be used or refrigerated (< 4 °C) for up to one year (Kogan & Seryapin, 1978). The advised fungal dose per ha varied between 10^{12} and 10^{14} conidia, which could kill up to 98% of the whitefly nymphs after three to four treatments (Kogan & Seryapin, 1978).

Standard technology for mass production of several entomopathogenic fungi has employed a substrate of cooked rice or other grains on trays, in autoclavable bags or in glass jars (Wraight & Carruthers, 1999). Cereals form attractive solid substrates, being cheap, nutritious, and easy to sterilize (Hall & Papierok, 1982). However, average yields of approximately 1-5 x 10^9 conidia/g dry weight of substrate are achievable, and is generally adequate to support application rates of 1 - 5 x 10^{12} conidia/ha (Wraight & Carruthers, 1999). Commercial scale production capacity necessary to support multiple applications to crops at a high rate of 10^{13} conidia/ha at costs competitive with synthetic chemical insecticides has long represented a major production barrier (Wraight & Carruthers, 1999). The standard technology used by industry for production of fungi and bacteria is submerge culture, by which the microbe is produced in a standardized liquid medium (McCoy *et al.*, 1988). Harvesting spores from submerged fermentations is much easier because the spores can be readily collected and concentrated by centrifugation or filtration and then dried (Bartlett & Jaronski, 1988). *Aschersonia* spp. do not produce conidia in liquid culture, or only at its surface (Hirte *et al.*, 1989a; Ibrahim *et al.*, 1993), which has been a draw back for their mass production. Many other entomopathogenic fungi, such as *B. bassiana*, *P. fumosoroseus* and *V. lecanii* produce "blastospores" in submerge culture (Humphreys *et al.*, 1986; Hirte *et al.*, 1989b; Feng *et al.*,

1994). Although in some cases virulence of these blastospores is higher than that of conidia, they are more sensitive to adverse conditions (Hall, 1981; Shimizu & Aizawa, 1988; Lacey & Mercadier, 1998) and therefore attention is shifted to production of conidia on solid phase systems. To mass produce *Aschersonia* spp. cost-effectively, two barriers have to be taken. Firstly, conidia are produced in mucilage which asks for a different approach for harvesting conidia, in contrast to *e.g. B. bassiana* or *P. fumosoroseus* which produce hydrophobic conidia. Secondly, *Aschersonia* spp. need at least two to three weeks to reach an optimal production on semi-solid media, in contrast to *B. bassiana*, which starts sporulating within a few days after inoculation of growth medium (Wraight & Carruthers, 1999). Each organism has its own particular requirements – substrate type, nutrients, etc. – and mass-production efforts must be individually tailored for optimal yields (Bartlett & Jaronski, 1988). Research into the optimum growth medium to mass produce *Aschersonia* spp. is needed, but the recent interest in two-phase and solid-phase fermentation technology will offer new perspectives for future mass production (Fransen, 1990; Lacey *et al.*, 1996; Wraight & Carruthers, 1999; Butt & Goettel, 2000).

Formulation

Formulation can greatly improve the efficacy of entomopathogens both in protected and field crops. There are four basic functions of formulations: 1) to stabilize the organism during production, distribution and/or storage, 2) to aid handling and application, 3) to protect the biocontrol agent from harmful environmental factors and 4) to enhance activity of the organism (Jones & Burges, 1998). It is generally accepted that stability (shelf-life) for 12-18 months without refrigeration would be required to service general agricultural markets, although stability of 3-6 months would be sufficient for products produced on contract for applications at a specific time (McCoy, 1990; Wraight & Carruthers, 1999). The type of formulation and selection of additives depends on the biology and physical properties of the pathogen, host insect and target crop (Soper & Ward, 1981). The basic components of most formulations include, in addition of fungal conidia, one or more of the following: a carrier, diluent, binder, dispersant, UV protectants and virulence-enhancing factors (Butt & Goettel, 2000).

Additives that have hygroscopic and adhesive properties are important to a formulation since the mode of action for fungi generally involves integumental contact of the host by the infective unit under conditions of high moisture. As with other pesticides, wetting agents that reduce the surface tension of the spray droplet are important in maximizing distribution of the infective unit, especially considering the hydrophobic cuticle of the insect hosts (chapter 4) and differences in epicuticular wax layers of the various host plants (Fig 6.5, chapter 6). Conidia

have been generally formulated as a wettable powder or dust for those fungi that have been successfully mass produced. In a wettable powder, conidia are generally diluted with inert fillers, wetting agents and spreaders. Water is often used as the carrier. Where conidia germinate quickly in water a dust is preferred. Talc, flour and milk powder have been used as suitable diluents for dusts (McCoy, 1990).

The most important recent development in formulation of conidia has been the use of oil carriers. Oils are relatively effective in sticking conidia to insect and plant surfaces, thus eliminating the need for wetting, sticking, or spreading agents. The use of oils also circumvents the dust problems associated with wettable powders and oils are more effective carriers of low-volume applications than water (Wraight & Bradley, 1996). In addition, they can enhance fungal performance at low humidity levels (Bateman *et al.*, 1993). However, oils are more expensive and can be phytotoxic, hence should be applied at low rates, and many are insecticidal themselves (Wraight & Bradley, 1996; Butt & Goettel, 2000). They do not necessarily enhance the performance of entomopathogenic fungi, since in field tests of *B. bassiana* against *B. argentifolii* emulsifiable oil formulations of conidia performed no better than aqueous suspensions prepared with a highly effective wetting agent (Wraight & Carruthers, 1999). Regarding *Aschersonia* spp. considerable research input is still needed before a product will be available to growers. As with mass production, every fungus has its own particular requirements and the formulation should not interfere with the infection process (Butt & Goettel, 2000). However, fungal formulation is probably the key factor to past failure and future successes (McCoy, 1990).

Safety

Potential safety issues have to be addressed when microbial biocontrol agents are intended to control insect pests (or diseases). Unintended adverse effects may be fourfold (Cook *et al.*, 1996): 1) *Competitive displacement.* Microorganisms could displace naturally occurring microorganisms through competition for nutrients (Cook *et al.*, 1996). Since *Aschersonia* spp. originate from the (sub)tropical areas and are specialized on Aleyrodidae (Evans & Hywel-Jones, 1990), it is, therefore, unlikely that they will establish themselves in temperate regions outside the glasshouse and eradicate their host insect or displace other entomopathogenic fungi.

2) *Allergenicity* Certain kinds of pollen and airborne fungal spores are inevitably present in the air we breath and cause allergies in sensitive people. Potentially, a biocontrol micro-organism released in the air could elicit allergic reactions (Cook *et al.*, 1996). Since conidia of *Aschersonia* spp. are produced in mucus and conidia are normally not airborne (Evans & Hywel-Jones, 1990), this risk is relatively small. Thereby, only a very small

proportion of fungal species produce spores that cause allergies (Latgé & Paris, 1991). In addition, any microbial product of which is claimed that it suppresses pests (or diseases) needs to be registered on the Dutch market under the pesticide law. Such products should be applied with the necessary precautions (*e.g.* wearing protective clothing and mask). Exposure to allergenic particles of all types is common in agricultural settings, and allergies will not be a new problem because of the use of biocontrol agents, but should be addressed as a safety issue during development and application (Latgé & Paris, 1991).

3) *Toxicity of biologically active metabolites.* Fungi secrete a wide range of metabolites, some of which are important medicines or research tools, some are toxic or carcinogenic (Strasser *et al.*, 2000b). Chemically diverse, toxic metabolites have been described in several entomopathogenic fungal genera, such as *Beauveria, Paecilomyces* and *Verticillium* (Khachatourians, 1991; Gindin *et al.*, 1994; Boucias, 1998; Strasser *et al.*, 1998), and also *A. aleyrodis* and *A. insperata* seem to produce exotoxins *in vitro* on nutrient-rich media (Kurbatskaya *et al.*, 1983; Krasnoff *et al.*, 1996). Although some of these secondary metabolites are known to be important pathogenicity determinants, the role of others remains unclear (Strasser *et al.*, 2000a; 2000b). There is concern that these toxic fungal metabolites may enter the food chain and form a risk to humans and animals. However toxin quantities produced *in vivo* are usually far lower than those produced in nutrient-rich media, and inter- and intra-specific variation in the production of fungal metabolites exits (Strasser *et al.*, 2000b). It is also important to put the relative amounts of toxins produces by entomopathogenic fungi into perspective, since the majority of fungal toxins which enter the food chain in significant levels and frequency to cause concern are produced by common saprophytic fungi, like *Aspergillus*, *Fusarium* and *Penicillium* (Steyn, 1995). Entomopathogenic fungi, unlike saprophytic fungi, do not secrete metabolites on plant material in sufficient quantities to cause a health risk (Strasser *et al.*, 2000b).

4) *Pathogenicity.* Microbial biocontrol agents used as pathogens could also be pathogenic to nontarget organisms. However, *Aschersonia* spp. are not expected to cause any negative effects to the environment because of their high specificity towards whiteflies and/or soft scales (Evans & Hywel-Jones, 1990) and are unable to infect beneficial insects.

Compatibility with other control methods

The application of *Aschersonia* spp. has to be integrated into a management system of other control measures against pests and diseases. The use of fungicides especially may impair the efficacy of these fungi. At the beginning of this century, Fawcett (1908) already remarked that applications of *A. aleyrodis* conidia with spray equipment which was earlier used for spraying pesticides or which contained copper, were not successful. Occasional tests concerning mixing

of *A. aleyrodis* conidia with fungicides and insecticides were made. Oho (1967) found that conidia did not germinate in solutions of any fungicide tested. However in reduced concentrations of maneb, germination was not affected. Zibul'skaya *et al.* (1975) found that sensitivity to fungicides was correlated with the age of the *Aschersonia* culture, cultures of 30 days old were less sensitive compared to cultures of 60 days old. Application of the detrimental fungicide mancozeb did not impair infection of third instar nymphs of *T. vaporariorum* when applied three days later than the spore suspension, when the fungus had already infected the insect successfully (Fransen, 1987).Thus after a certain safety period fungicides can be applied. Some of the insecticides tested by Oho (1967) suppressed germination (*e.g.* dichlorvos), whereas the majority of insecticides had no adverse affect at their recommended dose. Also miticide Avermectin B1 was not harmful to *A. aleyrodis* (McCoy *et al.*, 1982). The application of *Aschersonia* spp. together with new fungicides or insecticides still has to be tested. Considering the use of fungicides or insecticides together with entomopathogenic fungi, they should not actually be mixed together, but applications should be scheduled at certain time intervals.

The combined use of natural enemies (parasitoids and *Aschersonia* spp.) of whitefly is feasible. For instance, *E. formosa* is able to distinguish infected hosts from noninfected hosts (Fransen & van Lenteren, 1993). In addition *E. formosa* prefers older instar nymphs (Fransen & Montfort, 1987), which are less susceptible to *A. aleyrodis* and *A. placenta* (chapter 4). Only whitefly hosts parasitized by *E. formosa* less than four days before inoculation with *A. aleyrodis* may become infected and the parasitoid may die (Fransen & van Lenteren, 1994). Due to their high specificity, *Aschersonia* spp. do not pose a risk to other natural enemies and beneficial organisms.

Host characteristics

Susceptibility

First, second and third instar nymphs of both whitefly species are highly susceptible to *A. aleyrodis* and *A. placenta* (chapter 4), whereas whitefly eggs are not infected. Whitefly adults are rarely infected, but only in an environment with 100% RH (Fransen *et al.*, 1987). The fourth instar nymphs became less susceptible over time when they develop into pupa. This phenomenon is remarkably similar for *B. argentifolii* and *T. vaporariorum* (chapter 4). This maturation immunity of nymphal stages is a common feature, which has been described for many insect-fungus combinations (Tanada & Kaya, 1993).

For many years it was believed that development of resistance to pathogens used for pest control would be unlikely. This was based on pathogens being living organisms that are continually coevolving with their host, unlike chemical pesticides, and have been able to

coexist with their hosts over eons (Briese, 1986). However, resistance has developed in some combinations of pest and bacterial or viral entomopathogens and/or their products, but so far for fungal entomopathogens no cases of true resistance of target insects are known, although this may be due to the relatively limited use of entomopathogenic fungi as control agents or a lack of follow-up studies. There is considerable intraspecific variance in fungal species resulting in differences in pathogenicity, and likewise, there is simultaneously variability in the host (Shelton & Roush, 2000). Diversification of the flora in tropical forests led to increased specialization of plant-sucking homopterans and a coevolving host specialization of their pathogens (Evans, 1988). Although pathogens, which are well adapted to their host, were considered to be relatively benign, this is not necessarily the case. A pathogen might evolve to relatively high virulence when its dependence on the infected host mobility is reduced by using other transmission modes, for instance, dispersal by water or wind. Pathogens may also reduce their reliance on host mobility by a 'sit-and-wait' transmission, which means reliance on mobility of susceptible hosts. In general, these category of pathogens are very persistent (Ewald, 1995). This may explain the high virulence of *Aschersonia* spp., which have to rely on other modes of transmission, since their host is sessile, but other host and pathogen characteristic are also likely to play a role. With the application of microbial insecticides in glasshouses one would expect a high selection pressure, but since *Aschersonia* spp. are not endemic pathogens constantly present in the whitefly population, this is unlikely to happen. Combined use of, for instance, the parasitoid *E. formosa* reduces this probability even more (Fransen, 1987). In general, successful laboratory selection for resistance against fungal entomopathogens does not appear to have taken place, and there are no documented cases of resistance in the field (Shelton & Roush, 2000).

Whitefly distribution and its implications

Within a plant, adults of *T. vaporariorum* prefer young leaves for feeding and oviposition. A typical vertical distribution of development stages within the plant arises, because of the combination of preferred oviposition sites, plant growth and nymphs being largely sessile (van Lenteren & Noldus, 1990). The distribution of the greenhouse whitefly is largely aggregated, on a leaf, within a plant, between plants or on glasshouse scale (depending on the size of the glasshouse). Vertical distributions of *B. argentifolii* within a plant resemble those described for *T. vaporariorum*, but are probably less pronounced (van Lenteren & Noldus, 1990) probably due to their slower developmental time and resulting overlapping nymphal cohorts (chapter 4; Fransen, 1994; Drost *et al.*, 1998). The differential susceptibility of the various life stages of whitefly (chapter 4) will require reapplication of the fungus to achieve a good control of a whitefly population over time. However, this also offers perspectives for the use of

E. formosa, which prefers older instar nymphs for oviposition (Fransen & Montfort, 1987), in combination with the pathogen.

Environmental influences

Host plant

In this thesis it was shown that the host plant is an important factor in the efficacy of entomopathogenic fungi in tritrophic systems. It influences the efficacy of entomopathogenic fungi directly, *e.g.* by affecting persistence of *A. aleyrodis*, and indirectly by influencing the development rate of insect and/or their susceptibility as well as the phyllosphere humidity and conidial deposit.

The persistence of *A. aleyrodis* conidia was directly affected by the host-plant species. In the study on cucumber, the germination capacity and whitefly mortality exhibited by *A. aleyrodis* remained high over a period of at least one month, but on gerbera both germination capacity and whitefly mortality declined, suggesting a less favourable environment for persistence of conidia on gerbera (chapter 5). This could be due to differences in microflora, differences in chemical composition of the host plant, *e.g.* availability of nutrients of fungal-static compounds, and/or differences in phyllosphere humidity. On poinsettia nymphal mortality declined considerably in time, despite the high germination capacity of conidia (chapter 5). This could mean that conidia on poinsettia leaves are still viable, but lost their ability to infect, but it could also mean that low infection levels on poinsettia are not related to fungal persistence. Besides the direct effects mentioned above, also indirect effects via the host insect may play a role.

Developmental rate, reproduction and mortality of whitefly are influenced by host-plant species (van Lenteren & Noldus, 1990). The control mortality of greenhouse whitefly was significantly higher on poinsettia relative to cucumber, gerbera or tomato (chapter 6; see also van Lenteren & Noldus, 1990). A less suitable host plant, as reflected by higher control mortality, could result in an increased susceptibility to entomopathogens, due to stress of the host insect (Steinhaus, 1958). In contrast, this could also result in a decreased susceptibility to entomopathogens compared to insects from a more suitable host plant, since these 'healthier' insects better support growth of natural enemies (Schultz & Keating, 1991; Hoover *et al.*, 1998). However, here low fungal infection is probably not directly linked to plant quality for the host insect, since *B. argentifolii* exhibited the same low infection levels on poinsettia as *T. vaporariorum* even after 48 hrs high humidity (>95% RH, Fig. 9.3), while on poinsettia *B. argentifolii* shows a low natural mortality (Fig 9.3; van Lenteren & Noldus, 1990; Fransen, 1994). Nevertheless, aberrant growth of germinated *Aschersonia* conidia was occasionally observed on the cuticle of *B. argentifolii* nymphs (chapter 4), which may indicate

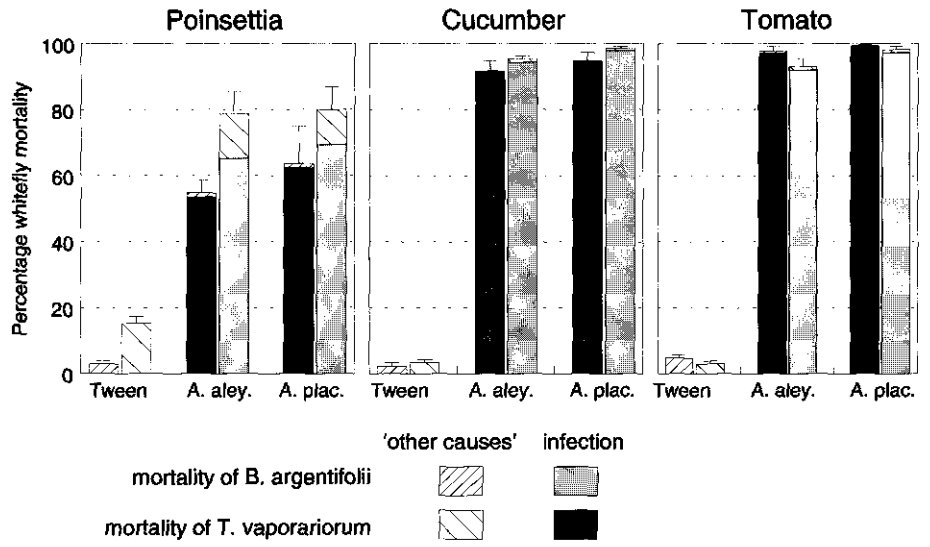

Figure 9.3: Mean percentage mortality of third nymphal instars of *B. argentifolii* (dark grey bars) and *T. vaporariorum* (light grey bars) by *A. aleyrodis* and *A. placenta* (10^7 conidia/ml) and other causes (shaded bars) on poinsettia ('Goldfinger'), cucumber ('Profito') and tomato ('Moneydor') after 48 hrs high humidity (>95% RH, see also Materials and Methods of chapter 2 and 3); error bars represent standard error of mean.

possible lower susceptibility in a part of the host population. The epicuticle, the first barrier the fungus has to breach, contains large amounts of free lipids, which are able to influence the fungus, by for instance inhibiting or stimulating germination or formation of non-penetrating germ tubes (Boucias & Pendland, 1991). For *B. argentifolii* the composition of these lipids is directly related to host-plant species, but for *T. vaporariorum* this composition is mainly determined by the insect itself and only partly by the host plant (Neal *et al.*, 1994).

The development rate of *T. vaporariorum* was highest on cucumber and lowest on gerbera, however, differences at 20 °C are small (ca. 4 days between 50% emergence adults, E.T.M. Meekes, pers. obs.). Hence, the chances of escaping fungal infection, which involves also the loss of inoculum by moulting, are about the same for whitefly nymphs on the different host plants due to equal exposure times (window of opportunity; Clancy & Price, 1987). This is a general phenomenon in many insect - fungal pathogen interactions (Ferron, 1985). The cotton aphid strain used in chapter 6, which did have a higher development and reproduction rate on cucumber than on gerbera (E.A.M. Beerling, pers. obs.), is more likely to escape fungal infection on cucumber (Clancy & Price, 1987). In addition, a higher reproduction had consequences for the percentage surviving aphids, especially when one realises that even

infected aphids give birth (Hall, 1976). Furthermore, a more favourable host plant does not encourage mobility of the insect and therefore pick up of conidia (Hall & Papierok, 1982).

Plant species differ substantially in their chemical composition, among which the nature and quantity of toxic substances in their phloem sap (Harborne *et al.*, 1999). For instance, some tomato cultivars produce tomatine which was found to be toxic to *Beauveria bassiana* (Costa & Gaugler, 1989) and inhibited germination of *P. fumosoroseus* at high levels (Poprawski *et al.*, 2000). Also leaves of poinsettia contain cytotoxic triterpenoids (Smith-Kielland *et al.*, 1996) and extracts of these leaves were used against fungi and nematodes (Khan *et al.*, 1996; Sebastian & Gupta, 1996). These substances may also affect the susceptibility of a pest organism to pathogens (Hare & Andreadis, 1983; Ramoska & Todd, 1985). Fruits, roots or seeds of the Cucurbitaceae contain cucurbitacins which were found to protect the spotted cucumber beetle against *M. anisopliae* (Tallamy *et al.*, 1998), but we used a less bitter cucumber cultivar which contains very low amounts of cucurbitacins (A. Janssen, pers. comm.). Both *B. bassiana* and *P. fumosoroseus* performed better against *B. argentifolii* on cucumber than on tomato plants (Poprawski *et al.*, 2000). *B. argentifolii* nymphs reared on poinsettia are unsuitable as food for lacewings, which has been attributed to accumulation of detrimental plant compounds in the whitefly (Legaspi *et al.*, 1996). With the application of entomopathogenic fungi against whitefly on poinsettia a similar interaction between first and third trophic level may exist, hence explaining the low whitefly mortality on this host plant (chapter 5 - 7).

Another way the host plant influences the efficacy of entomopathogenic fungi is by its leaf structure. This will influence the way spray droplets, and with them the fungal spores, are deposited. The amount of trichomes and the composition of epicuticular waxes can influence the hydrophobicity of a leaf. Venation, epidermis cells and ornamentation of a leaf enhances the roughness of a leaf and with it the hydrophobicity (Holloway, 1970). For instance hirsute leaves produce a more repellent surface than smooth leaves (Holloway, 1970) and droplet retention increases with decreasing surface tension (Brunskill, 1956). On relatively smooth leaves spray droplets tended to be larger and merged easier, than on hirsute leaves (chapter 8). In addition, nymphs at the leaf plane are shielded by the leaf trichomes and less conidia will reach them. Although poinsettia is relatively smooth compared to leaves of certain gerbera cultivars, it had the most hydrophobic leaf surface. Water droplets on this leaf stayed compact, even when a spreader was used, in contrast to droplets on gerbera, cucumber or tomato leaves (chapter 6). This will lead to a less uniform distribution of conidia on the poinsettia leaf surface compared to leaves of the other plant species. A more uniform distribution will provide greater opportunity for insects to come into contact with conidia on the leaf surface (Ebert *et al.*, 1999) during the crawler stage or during moulting.

Humidity

One of the major limitations of entomopathogenic fungi is their requirement for high humidity for the germination and infection processes. The phyllosphere environment of the fungus, and especially the relative humidity, is of paramount importance to its effectivity and persistence (Diem, 1971; Drummond *et al.*, 1987). Ambient relative humidity, temperature and air turbulence will influence the boundary layer in which the insect-fungus interaction takes place (Burrage, 1971; Ferro & Southwick, 1984). However, the influence of humidity on success of entomopathogenic fungi is intertwined with host-plant characteristics, since phyllosphere humidity and thickness of the leaf boundary layer are also affected by crop-specific features such as size, shape and position of leaves, leaf surface topography (hairs, waxes, stomata, veins), leaf area index and photosynthetic activity (transpiration) (Ferro & Southwick, 1984).

In general a higher ambient humidity will lead to a higher mortality due to infection by entomopathogenic fungi (chapter 7). However, to reach a certain level of whitefly mortality the duration and the level of high humidity differs per host-plant species. On poinsettia, mortality levels of *T. vaporariorum* varied between 50-85% at high ambient humidity levels (with 48 hrs > 95% RH; chapter 4 & 7) and at low ambient humidity levels (45%) infection levels did not exceed 25%. In contrast, on cucumber mortality levels at low ambient humidity were still 50% even without any additional period of high humidity (chapter 7).

The duration and the level of high humidity to reach a certain level of whitefly mortality also differs per fungal species. Entomopathogenic fungi differ considerably in their humidity requirements, some fungi may need a saturated environment to germinate, like *V. lecanii* (Hall, 1981), while others, such as *M. anisopliae* (Walstad *et al.*, 1970) and *A. aleyrodis* (Fransen, 1995), are able to germinate under slightly dryer conditions. For instance, under dry conditions (avg 45% RH) *V. lecanii* does not perform as well as *A. aleyrodis* even with an additional 48 hrs > 95% RH, whereas under humid conditions (avg 85% RH) *V. lecanii* eventually reaches the same level of whitefly infection as *A. aleyrodis* (chapter 7).

Although many entomopathogenic fungi need a RH of at least 92% or more, optimal germination and growth will take place at 100% RH (Gillespie & Crawford, 1986; Milner & Lutton, 1986; Fransen, 1987). However, a phyllosphere humidity of 90% on gerbera and tomato was apparently sufficient for both *Aschersonia* species to cause a high whitefly mortality (chapter 6), comparable to mortality levels on cucumber (phyllopshere RH > 92%). The high whitefly mortality on gerbera and tomato suggests that the calculated humidity in chapter 6 is an underestimation of the real phyllosphere humidity. One should realise that the humidity data shown in chapter 6 are an average of a whole leaf and thus obscuring differences within a leaf caused by roughness (*e.g.* hairiness, venation), stomata, and other leaf

characteristics (Holloway, 1970). Furthermore, the influence of the insect on the phyllosphere climate and the microclimate of the insect itself (Kramer, 1980; Ramoska, 1984) which could affect fungal germination and growth, are not taken into account.

On the other hand for poinsettia the calculated phyllosphere humidity may have been overestimated. On this crop, all three fungi caused whitefly mortality no higher than 21%, whereas both *Aschersonia* species were able to cause high whitefly mortalities on cucumber, gerbera and tomato under similar or less favourable phyllosphere humidities (chapter 6). Baille *et al.* (1994a; 1994b) found that the transpiration rate for poinsettia is low compared with other ornamentals, and leaf stomatal resistance was considerably higher for poinsettia (higher than r_s = 100 s/m, as was the assumption in the vapour pressure deficit formula, see Appendix chapter 6). This results in a lower humidity level than was calculated, which may inhibit fungal growth or may even be detrimental to the fungus. In this respect the hydrophobicity of the leaf, which is influenced by its wax layer and hairiness, is also likely to play a considerable role in the actual phyllosphere humidity. Of all plant species used here, poinsettia had the most hydrophobic leaf surface. Water droplets on this leaf stayed compact, even when a spreader was used, in contrast to droplets on gerbera, cucumber or tomato leaves. The hydrophobicity of poinsettia leaves together with a (possible) higher stomatal resistance and 'sunken' stomata might indicate that poinsettia is adapted to a relatively dry environment by reducing its water loss by transpiration as much as possible. This might also affect germination and infection of whitefly by entomopathogenic fungi.

The phyllosphere humidity on cucumber (RH ≥ 93%) should be high enough for conidia of *A. aleyrodis* to germinate on the leaf surface (chapter 6), but this did not happen (Chapter 5). Also on gerbera and poinsettia leaf conidia of *Aschersonia* spp. did not germinate (chapter 4 and 5), indicating that they need additional triggers for germination, since they germinated readily on nymphs of *B. argentifolii* (chapter 4). Although phyllosphere humidity may not explain the differences in mortality between host-plant species in its own, it does explain differences within host-plant species and between fungal species.

Commercial application

Mycopesticides against whitefly have been introduced in several ways. At the beginning of this century, when spray application was not yet successful (because spray equipment contained traces of insecticides and fungicides of earlier applications), transplanting of young citrus trees with infected whitefly nymphs into citrus plantations not yet infested by *A. aleyrodis* was recommended. Another way to introduce the fungus was pinning leaves with infected nymphs into trees infested with *D. citri* (Fawcett, 1908).

Later, spray applications were recommended and care was taken to hit the underside of the leaves, where whitefly nymphs are most common. Conidial suspensions were made by mashing up the cultures in water and sieving them through cheese cloth. One culture bottle provided sufficient spores to treat 70-100 citrus trees against *D. citri*. In citrus, application from April to October were possible (Florida), but the effect was most certain during the rainy summer period from July to mid-September (Berger, 1920b; 1921). The recommended dose in glasshouses is 10^{13} conidia/ha (10^{6} – 10^{8} conidia/ml) to reach an almost complete kill of *T. vaporariorum* in a cucumber crop (Kogan & Seryapin, 1978; Ramakers & Samson, 1984). Control is little affected by outdoor conditions, because the naturally occurring relative humidity in a full grown cucumber crop is sufficiently high for infection (Ramakers *et al.*, 1982; Ramakers, 1983). Also in a gerbera crop, application of 10^{12} – 10^{13} conidia/ha was sufficient to reach 80% mortality of *T. vaporariorum* (Chapter 8).

At the moment several fungal products against whitefly are on the market. These products are based on, for instance, *B. bassiana* (Naturalis, Botanigard), *P. fumosoroseus* (PreFeRal or PFR) and *V. lecanii* (Mycotal). Only Mycotal is registered on the Dutch market, whereas other products are registered abroad for the control of whiteflies, but are not yet available to Dutch growers. Results with Mycotal have been variable, depending on crop species and humidity levels in the glasshouse (Ravensberg *et al.*, 1990). The best results were obtained when humidity levels were at least several hours above 80%. At lower humidities the performance can be improved by adding a vegetable oil (Addit). *Aschersonia* spp. have shown to be highly effective in killing whiteflies, more so than *V. lecanii* (Tween formulated), more consistently and at lower humidity levels (chapters 6 - 8). *B. bassiana* and *P. fumosoroseus* seem to have the same humidity requirements as *Aschersonia* spp. (Wraight *et al.*, 2000). However, since the interaction between host plant and ambient humidity has a significant impact on the obtained whitefly mortality (chapter 7), one should be careful comparing fungi tested in different bioassays and on different host plants. The experiments of Wraight *et al.* (2000), for instance, were carried out on hibiscus, whereas in this thesis experiments were conducted on cucumber, gerbera and poinsettia. *Aschersonia* spp. seem to prefer a 'sit-and-wait' strategy. Hatching and moulting nymphs will settle on them. This strategy enables *Aschersonia* spp. to survive and cause mortality levels up to 90% 31 days after application (chapter 5). In contrast, *V. lecanii* germinates on the leaf surface (Verhaar, 1998), which could compromise its persistence, since germlings are more susceptible to adverse circumstances than ungerminated conidia (Sussman, 1968). Its half-life is considerably shorter than that of *A. aleyrodis*, *viz.* 4 days on chrysanthemum (Gardner *et al.*, 1984). *B. bassiana* generally provides control for 3-4 days after application (Vandenberg *et al.*, 1998), although in some cases 10% from the original dose was still present in the field after 28 days (James *et al.*,

Table 9.1: continued.

Aspects for evaluation	Results	Reference
Environmental characteristics:		
• ***Biotic factors***		
host plant		
• host-plant chemistry	• not tested, but likely influences whitefly development, and maybe fungal infection	• chapter 8 chapter 5-7
• crop structure, leaf size and shape, trichomes density, cuticular waxes	– influence phyllosphere humidity – no correlation between crop architecture and phyllosphere humidity between host-plant species – cultivar effects on mycosis, dependent on fungal species	– chapter 6 – chapter 6 – chapter 8
• microbial interactions	• not tested	

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Summary

Many horticultural and agricultural crops are good host plants for the greenhouse whitefly, *Trialeurodes vaporariorum,* and the silverleaf whitefly, *Bemisia argentifolii*. Their damage to crops is manifold. When present in sufficient numbers they can cause leaf drop and inhibit fruit maturation. They are efficient vectors of economically important plant viruses. Whiteflies produce honeydew, which soils and damages crops and hampers the processing of products such as cotton. It serves as a substrate for sooty moulds, thus reducing leaf photosynthesis and renders plants and fruits unsightly. For export of plants or plant products a zero-tolerance is valid, especially when the insect is a quarantine pest in the importing country, therefore the simple presence of whitefly can lead to destruction or re-export of an entire shipment.

As more environmentally responsible agricultural strategies are adopted, natural enemies of both whitefly species, will play an increasing role. Screening for natural enemies which are able to kill both pest insects quickly, without affecting other natural enemies, is an important line of research. Entomopathogenic fungi can meet these requirements and can therefore be a valuable asset to existing biological and chemical control measures. Our attention is directed towards the genus *Aschersonia*, because previous research indicated that *Aschersonia aleyrodis* is a promising whitefly control agent because of its tolerance to relative humidities as low as 50%, its long persistence on leaf surfaces and its compatibility with the parasitoid *Encarsia formosa*.

This project consisted of two components: 1) to identify virulent isolates of *Aschersonia* spp. for the use against greenhouse and silverleaf whitefly, and 2) to study factors which influence the effectivity of entomopathogenic fungi, with special reference to host plant, humidity and their interaction.

Entomopathogenic fungi of the genus *Aschersonia* are specialized on whitefly and scale insects and can be used as a biological control agent against silverleaf whitefly, *B. argentifolii* and greenhouse whitefly, *T. vaporariorum*. In **chapter 2** 44 isolates of *Aschersonia* spp. were tested for their ability to sporulate and germinate on semi-artificial media and to infect the insect host. Seven isolates sporulated poorly (less than 5.10^7 conidia/culture) and ten were not able to infect either of the whitefly species. Several isolates were able to produce capilliconidia on water agar. The Infection level was not correlated with germination on water agar. After a selection based on spore production and infection, virulence of 31 isolates was evaluated on third instar nymphs of both whitefly species on poinsettia (*Euphorbia pulcherrima*). Infection levels varied between 2% to 70%, and infection percentages of *B. argentifolii* correlated with that of *T. vaporariorum*. However, mortality was

higher for *T. vaporariorum* than for *B. argentifolii*, as a result of a higher 'mortality by other causes' of greenhouse whitefly on poinsettia. Several isolates, among which unidentified species of *Aschersonia* originating from Venezuela and Malaysia, *A. aleyrodis* from Colombia, and *A. placenta* from India showed consistent results in their ability to infect both whitefly species.

Out of the above 44 isolates, six isolates were selected based on virulence, spore production and geographical origin. In **chapter 3** the LD_{50} and LT_{50} values of *A. aleyrodis*, *A. placenta* and four *Aschersonia* spp. isolates were compared to select the most virulent isolate for control of *B. argentifolii* and *T. vaporariorum*. Bioassays were carried out on predominantly second instar nymphs on intact poinsettia plants under glasshouse conditions and the dosage ranged from 14 to 1.4 x 10^5 conidia/cm^2. Five isolates were highly pathogenic to both whitefly species; mortality by one isolate did not exceed 50%, therefore no LD_{50}'s or LT_{50}'s were calculated. On *B. argentifolii* LD_{50}'s varied between 1600 and 4800 conidia/cm^2 and LT_{50}'s between 4.6 - 8.7 days and on *T. vaporariorum* LD_{50}'s varied between 700 and 5300 conidia/cm^2 and LT_{50}'s between 4.5 and 9.9 days. LT_{50}'s based on visual signs are not very accurate for sedentary insects, like whitefly nymphs, therefore we also looked at the nymphal instar in which the infected nymphs died. For four isolates the majority of nymphs died in the first to third instar period. However, the differences found between isolates did not always agree with differences found comparing LT_{50}'s. For two isolates the optimum mortality did not occur at the highest dosage, which was also reflected in the speed of kill.

In **chapter 4** scanning electron microscopy and bioassays were carried out to obtain better insight into the infection process of *A. aleyrodis*, *A. placenta* and an unidentified *Aschersonia* sp.. Conidia of *Aschersonia* spp. germinated readily on the cuticula of host insects as well as on water agar. On water agar *A. placenta* also produced capilliconidia. No germination was observed on poinsettia leaf surface, except on the leaf veins. On *B. argentifolii* the fungi formed large amounts of mucilage to attach themselves to the insect. Appressoria were formed before penetration, but also direct penetration was observed. This seemed not related to a specific site on the insect. For both whitefly species, first to third instar nymphs were most susceptible. If the population existed of fourth instar nymphs for more than 50%, infection levels dropped from 90 to 50%. Infected whitefly nymphs usually died in the stage following the treated stage. The fungus protruded from the insect via the margins or via the emergence folds of pupae, if humidity levels were high enough. However, sporulation was rarely observed on whiteflies developing on poinsettia plants under the prevailing conditions.

Persistence of *A. aleyrodis* was studied in **chapter 5** on cucumber (*Cucumis sativus*), gerbera (*Gerbera jamesonii*) and poinsettia. Germination capacity and infectivity of conidia, which stayed on the different plants over a period of up to one month, were assessed. Average

germination of conidia on the leaves was low (< 14.3%), whereas it was shown that most of the conidia transferred from the leaf to water agar were viable, even after having been present on the leaf surface for one month. Germination capacity was influenced by host plant species: it was highest on cucumber, followed by poinsettia and lowest on gerbera. On cucumber leaves, conidia stayed viable and were able to infect 90% of the whitefly nymphs, even at 31 days after spore application. On gerbera, germination capacity decreased considerably from 80% (day 0) to 40% (day 31). This was reflected in nymphal mortality, which declined from 75% to 40%. Despite the high germination capacity (60%) of conidia on poinsettia after an exposure of one month, nymphal mortality decreased from 70% at the day of spore application to 10% after three days at leaf surface, and remained low throughout the monitoring period. The phyllosphere microflora, secondary plant metabolites and microclimate can play a role in these findings.

Can phyllosphere humidity explain differences in insect mortality due to entomopathogenic fungi on different crops? This was tested in **chapter 6** for cucumber, gerbera, tomato and poinsettia on which mycosis of *T. vaporariorum* and cotton aphid (*Aphis gossypii*) were determined in relation to host-plant climate. Phyllosphere humidity was estimated using climate and host-plant parameters. Hydrophobicity of the leaves and crop density were also taken into account. On cucumber, gerbera and tomato, the fungi *A. aleyrodis* and *A. placenta* caused over 90% mortality in whitefly, while *V. lecanii* caused 50% mortality. On poinsettia, whitefly mortality was significantly lower for all three fungi (ca. 20%). The percentage of aphids infected by *V. lecanii* or *Metarhizium anisopliae* was significantly higher on gerbera than on cucumber. With *V. lecanii* the highest percentage mycosis was obtained. The phyllosphere of cucumber was more humid than that of the other crops. This cannot explain higher aphid mycosis on gerbera compared to cucumber, nor lower whitefly mortality on poinsettia. The fact that poinsettia leaves were more hydrophobic than the other leaves may offer an explanation for the observed lower whitefly mortality, but chemical host-plant aspects may also play a role. Our results underline the importance of the first trophic level (plant) for entomopathogenic fungi in integrated pest management programs.

The influence of relative humidity (RH) and host-plant species and their interaction on mycoses of *T. vaporariorum* by entomopathogenic fungi *A. aleyrodis*, *A. placenta* and *V. lecanii* was studied in **chapter 7**. Experiments conducted on poinsettia, gerbera and cucumber at 50% and 80% RH showed a clear host-plant effect. On cucumber and gerbera all fungi caused significantly higher mortality compared with the control, whereas whitefly mortality on poinsettia remained low and was not significantly different from the control (0.05% Tween). A higher RH resulted in a higher mortality caused by the fungi. Both *Aschersonia* spp. performed significantly better than *V. lecanii*. In following experiments an

additional period of 0, 3, 6, 12, 24 or 48 hours of high RH was applied after treatment with *A. aleyrodis* and *V. lecanii*. Mortality caused by the fungus increased with a longer additional period of high RH and higher ambient humidity. However, the host-plant species effect exceeded the effect of ambient or additional high RH period; whitefly mortality was highest on cucumber and lowest on poinsettia. On poinsettia both fungi had hardly any effect on whitefly mortality, except after a 48 hrs high RH period. On gerbera and cucumber all fungal treatments were significantly different from the control and *A. aleyrodis* performed better than *V. lecanii*.

The influence of cultivar differences on the efficacy of entomopathogenic fungi is tested in **chapter 8**. The experiment was conducted in a glasshouse (semi-practice) using two gerbera cultivars, 'Bourgogne' and 'Bianca' with different plant structure and trichome density on the leaf: 'Bianca' having more and larger leaves and fewer trichomes/cm^2 than 'Bourgogne'. Two entomopathogenic fungi, *A. aleyrodis* and *V. lecanii* were applied for the control of *T. vaporariorum*. The fungi were applied in two concentrations, 10^6 and 10^7 conidia/ml. In 'Bianca', both fungi caused a whitefly mortality up to 80%. Whitefly mortality was higher for 10^7 than for 10^6 conidia/ml. In 'Bourgogne', *V. lecanii* caused a significantly lower mortality than *A aleyrodis*. Although no cultivar differences in whitefly development time were found, other characteristics, like natural mortality and build-up of the whitefly population, seemed to differ. Differences between results in mortality in relation to cultivar (humidity, hit-probability) and whitefly characteristics may explain the differences between the efficacy of the fungal species.

The host plant is the most important factor in this tritrophic system, exceeding the influence of humidity. However, although entomopathogenic fungi are less successful in controlling whiteflies on poinsettia, these problems may be circumvented by use of formulation. *Aschersonia* spp. are highly virulent against whitefly pests, can cause natural epizootics in the field, can be grown on artificial media and applied with conventional insecticide application equipment, are well adapted to survive in the canopy environment, and are compatible to/or even complementary with other natural enemies.

Samenvatting

Witte vlieg: biologie, plaag, schade en bestrijding

In tegenstelling tot wat de naam doet vermoeden zijn witte vliegen geen vliegen, maar behoren ze tot de sapzuigende insecten (Homoptera), waartoe ook de bladluizen behoren. Er zijn meer dan 1200 witte vlieg soorten beschreven, maar slechts enkele soorten vormen een probleem binnen de landbouw. In kasteelten zijn twee soorten schadelijk, nl. de kaswittevlieg, *Trialeurodes vaporariorum*, en de tabakswittevlieg, *Bemisia argentifolii*. De kaswittevlieg vormt al enkele decenia een probleem, de tabakswittevlieg is per ongeluk geïntroduceerd in 1987.

Witte vlieg heeft gevleugelde adulten. De vrouwtjes leggen hun eieren bij voorkeur op de onderzijde van jonge bladeren. Het eerste larvale stadium is nog mobiel, maar zodra het een geschikte voedingsplek op een blad gevonden heeft, zal de larve tijdens zijn hele larvale stadium op dezelfde plek blijven. De larve steekt z'n stylet in het blad om zich te voeden met plantensappen en zal deze alleen terugtrekken tijdens de vervelling naar het volgende stadium. Witte vlieg heeft vier larvale stadia die steeds in grootte toenemen. Het vierde stadium ontwikkelt zich tot pop, wat betekent dat er geen afzonderlijk pop-stadium is, maar dat de adult zich binnen de huid van het vierde stadium ontwikkelt. Uiteindelijk zal de nieuwe adult door scheuren in het exoskelet naar buiten komen. De duur van deze levenscyclus is afhankelijk van de plantensoort en de temperatuur.

Witte vlieg kan op verschillende manieren schade veroorzaken aan gewassen. Ten eerste, als witte vliegen zich massaal aan een plant voeden, kan de plant zich minder goed ontwikkelen, wat kan leiden tot opbrengstderving, het laten vallen van bladeren, etc. Ten tweede scheidt de witte vlieg de overmaat aan suiker weer uit, de zogenaamde honingdauw. Dit stimuleert de groei van roetdauwschimmels, wat de plant kan remmen, maar belangrijker is de economische schade: vruchten, bloemen en katoenpluis worden bedekt met een plakkerig laagje. Ten derde kan witte vlieg verschillende plantenvirussen overbrengen. Eén enkele witte vlieg besmet met een plantenvirus kan grote problemen veroorzaken binnen een teelt. Ten vierde gelden voor export van planten of plantenproducten vaak nultoleranties, er mag dan geen enkele witte vlieg op het product aanwezig zijn. Als de wittevliegsoort in een importerend land een quarantaine insect is, kan dit leiden tot vernietiging van de totale lading.

In natuurlijke ecosystemen en landbouwsystemen waar geen pesticiden gebruikt worden vormt witte vlieg een veel minder groot probleem, omdat ze op een laag niveau gehouden worden door verschillende natuurlijke vijanden: predatoren, parasitoiden en pathogenen. Predatoren doden hun prooi door deze op te eten of leeg te zuigen. Parasitoiden,

bv. sluipwespen, leggen eieren in het insect (gastheer) en de ontwikkelende parasitoidlarve consumeert het insect tot dat het sterft. Pathogenen zijn ziekteverwekkers zoals bacteriën, schimmels en virussen, die vaak hun gastheer doden. Door het gebruik van bestrijdingsmiddelen zijn deze natuurlijke vijanden vaak uitgeroeid en kan witte vlieg een plaag worden. Verder hebben verandering in gewasrotatie, verkorting van braakperiodes en opeenvolgende teelten van wittevlieggevoelige gewassen ertoe geleid dat natuurlijke vijanden niet langer in staat waren witte vlieg op een voldoende laag niveau te houden.

Chemische bestrijdingsmiddelen zijn na de Tweede Wereld Oorlog de belangrijkste methode geworden voor de bestrijding van insectenplagen. Ze waren eenvoudig toe te passen, vormden een goede bescherming tegen plagen en waren betrouwbaar. Pas later werden de nadelige gevolgen duidelijk: risico's voor mens en milieu en resistentie van insecten tegen deze middelen. Dit gaf aanzet tot onderzoek naar andere bestrijdingsmethodes. Het huidige beleid is erop gericht de afhankelijkheid en het gebruik van chemische bestrijdingsmiddelen terug te brengen. Een belangrijk alternatief is biologische bestrijding waarbij predatoren, parasitoiden en pathogenen worden ingezet om een plaag onder controle te krijgen.

Pathogenen

In dit proefschrift staan insectpathogene schimmels ter bestrijding van witte vlieg centraal. Ze werken relatief snel t.o.v. predatoren en parasitoiden en zijn met conventionele spuitappartuur toe te dienen. Het infectie proces ziet er als volgt uit: een spore die op het insect belandt kiemt, penetreert de cuticula en betrekt zijn voedsel uit het het insect. Het insect zal hieraan sterven en de schimmel groeit vervolgens weer naar buiten om te sporuleren. Na verspreiding van de sporen door bv. wind of water zal het proces opnieuw beginnen.

Deze schimmels kunnen onderverdeeld worden in twee groepen, specifieke schimmels die alleen witte vlieg infecteren, zoals *Aschersonia*-soorten en breed werkende schimmels, die insecten uit verschillende ordes kunnen infecteren en soms ook andere schimmels, voorbeelden hiervan zijn *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii*. Het voordeel van het gebruik van specifieke schimmels is dat het andere natuurlijke vijanden niet zal infecteren, ze zullen echter ook andere plaaginsecten niet infecteren.

Verschillende criteria kunnen worden gebruikt voor het selecteren van een succesvolle schimmel: gastheerspecificiteit, virulentie, persistentie, het gemak waarmee sporen (de infectieuse eenheid van schimmels) kunnen worden geproduceerd, etc.. Schimmels worden vaak op dezelfde manier toegepast als chemische bestrijdingsmiddelen. De sporen die door schimmels worden geproduceerd worden op het aangetaste gewas gespoten, vaak in hoge doses en de behandeling wordt enkele keren herhaald, vandaar dat er gesproken wordt over myco(=schimmel)pesticiden. Selectiecriteria als virulentie, nl. een zo hoog mogelijke sterfte

bereiken met een zo laag mogelijke dosis in een zo kort mogelijke tijd, en productiegemak zijn erg belangrijk.

Een schimmelsoort kan bestaan uit genetisch zeer diverse isolaten/stammen, die aanzienlijk kunnen verschillen in virulentie t.o.v. een plaag. De virulentie kan door verschillende factoren worden beïnvloed. Zo kan een schimmel die vaak op kunstmatig medium is gekweekt zijn virulentie verliezen. Ook omgevingsfactoren kunnen de efficiëntie van een schimmel beïnvloeden. Over het algemeen hebben schimmelsporen een zeer hoge relatieve luchtvochtigheid nodig om te kunnen kiemen (meer dan 92%), wat lang een bottleneck is geweest voor het gebruik van schimmels voor biologische bestrijding. Daarnaast kan het insect zelf en/of de plant waarop het insect voorkomt, de waardplant, een grote rol spelen.

Aschersonia

Schimmelsoorten uit het geslacht *Aschersonia* infecteren alleen wittevliegsoorten en/of dopluissoorten. De schimmels zijn afkomstig uit (sub)tropisch gebied, zijn vaak opvallend van kleur, ze kleuren bv. hun gastheer oranje, en zijn in staat hun gastheer volledig uit te roeien. Sommige *Aschersonia*-soorten zijn specifiek voor witte vlieg, andere soorten infecteren alleen dopluis. Doordat de larvale stadia van dopluis en witte vlieg sterk op elkaar lijken, is er discussie over de specificiteit van de schimmel, omdat de gastheersoort (witte vlieg of dopluis) na infectie vaak niet meer te herkennen is.

Verschillende *Aschersonia* soorten werden al aan het begin van de vorige eeuw ingezet voor biologische bestrijding van citruswittevlieg in Florida. De schimmel komt daar van nature voor, maar werd kunstmatig gekweekt zodat citrustelers de schimmel in overmaat in hun boomgaarden konden inzetten. In de jaren 60 en 70 werden verschillende soorten ingezet ter bestrijding van citruswittevlieg en kaswittevlieg in verschillende Oost Europese landen. Doordat deze schimmels zeer specifiek zijn, dus te gebruiken naast andere natuurlijke vijanden, ze in staat zijn lang op bladoppervlaktes te overleven en minder afhankelijk zijn van hoge luchtvochtigheid, is er nog steeds interesse voor deze schimmels.

Doel en opbouw van het proefschrift

Het doel van dit onderzoek was tweeledig. Ten eerste, het selecteren van een virulente *Aschersonia* stam die zowel tabaks- als kaswittevlieg kan onderdrukken (hoofdstuk 2 t/m 4). Ten tweede, het bepalen van factoren die de efficiëntie van entomopathogene schimmels beïnvloeden, in het bijzonder de invloed van luchtvochtigheid en waardplant (hoofdstuk 5 t/m 8).

In hoofdstuk 2 worden verschillende *Aschersonia* isolaten getest op hun vermogen goed te sporuleren op kunstmatig medium en hun virulentie tegen kas- en tabakswittevlieg. Hieruit worden 6 isolaten geselecteerd die in hoofdstuk 3 nader bekeken worden, gezocht wordt naar een stam die met een zo laag mogelijke dosis in een zo kort mogelijke tijd in staat is beide wittevliegsoorten te onderdrukken. In hoofdstuk 4 wordt het infectieproces van enkele isolaten nader beschreven aan de hand van scanningelectronenmicroscoopfoto's en gevoeligheid van de verschillende wittevlieg stadia. In hoofdstuk 5 is de persistentie van *A. aleyrodis* onderzocht op verschillende gewassen. Of de gevonden verschillen tussen de gewassen gerelateerd kunnen worden aan de luchtvochtigheid op bladniveau vlak rond het blad is onderzocht in hoofdstuk 6. In hoofdstuk 7 wordt de invloed van de omgevingsluchtvochtigheid en het gewas op de effectiviteit van insectpathogene schimmels beschreven. Uiteindelijk zijn 2 schimmels in hoofdstuk 8 op kasniveau met elkaar vergeleken, waarbij tevens de invloed van rasverschillen onderzocht is. In hoofdstuk 9 worden de belangrijkste resultaten in het kader gezet van biologische bestrijding met behulp van schimmels.

Hoofdstuk 2:

Vierenveertig isolaten van verschillende *Aschersonia*-soorten zijn getest op hun vermogen om goed te sporuleren op kunstmatig medium en hun virulentie tegen kas- en tabakswittevlieg, beide belangrijke voorwaarden voor het succesvol gebruik van schimmels ter bestrijding van witte vlieg. De 44 isolaten waren afkomstig uit verschillende schimmelcollecties, daarnaast zijn enkele stammen door de onderzoekster zelf geïsoleerd uit geïnfecteerd wittevliegmateriaal. De isolaten komen oorspronkelijk uit (sub)tropische gebieden van Amerika (25 isolaten), Azië (17 isolaten) en Afrika (2 isolaten).

Met uitzondering van één isolaat, sporuleerden alle isolaten in meer of mindere mate op geautoclveerde gierst, hoewel sommige isolaten beter sporuleerden op maismedium. Acht isolaten sporuleerden zeer goed met ruim meer dan 5 x 10^9 sporen per culture. Kiemingspercentages op wateragar verschilden sterk, variërend tussen 3.4 en 99.5%, waarbij enkele isolaten naast gewone kiembuizen ook capilliconidia produceerden. Capillicondia zijn conidia die gevormd worden op een haarachtige structuur. Bij andere entomopathogene schimmels dienen deze stucturen voor het infecteren van passerende insecten. Welke functie deze sporen bij *Aschersonia*-soorten vervullen is nog niet bekend.

Van de sporulerende isolaten waren er negen niet in staat kas- en tabakswittevlieg te infecteren, of konden niet opnieuw geïsoleerd worden uit de insecten. Dit kan te maken hebben met de specificiteit van de isolaten. Als de oorspronkelijke gastheer niet bekend is, kunnen insolaten van dopluis afkomstig zijn. Deze isolaten zijn waarschijnlijk niet in staat om witte

vlieg te infecteren. Het kan ook zijn dat de isolaten al zo lang op kunstmatig medium gekweekt zijn dat ze het vermogen om witte vlieg te infecteren kwijt zijn geraakt. De herisolaten zijn getoetst op hun virulentie tegen beide wittevliegsoorten. Meerdere isolaten waren in staat een hoge sterfte onder beide wittevliegsoorten te veroorzaken. Virulentie tegen kaswittevlieg was dan ook positief gecorreleerd met virulentie tegen tabakswittevlieg. Er was echter geen relatie tussen goed kiemende en virulente isolaten. Uiteindelijk zijn zes isolaten gekozen op basis van sporulatie (A31, Aa5 en Ap1), virulentie (A24, A26, A31, Aa5 en Ap1) en geografische oorsprong (A23).

Hoofdstuk 3

Deze zes isolaten zijn vergeleken op basis van hun LD_{50}: de dosis waarbij 50% van de wittevliegpopulatie overlijdt, en hun LT_{50}: de tijd die nodig is om 50% van de populatie te doden. Hoe lager de LD_{50} en hoe korter de LT_{50} hoe virulenter het isolaat zal zijn. Omdat A23 vaak niet in staat was meer dan 50% van de populatie te doden zijn de LD_{50} en LT_{50} niet berekend voor dit isolaat. De LD_{50} varieerde voor tabakswittevlieg van 16 (A26) tot 48 sporen/mm^2 (Aa5) en voor kaswittevlieg van 7 (A24) tot 53 sporen/mm^2 (Aa5), deze waarden waren echter niet significant verschillend van elkaar. Bij de hoogste toegepaste dosis (10^8 sporen/ml) lag het infectiepercentage voor isolaten A23 en A24 lager in vergelijking met de op één na hoogste dosis (10^7 sporen/ml). Deze zelfinhibitie is een fenomeen dat vaker geconstateerd is bij schimmels en dient om kieming *en masse* te voorkomen. De dosis waarbij dit fenomeen optreedt is echter zo hoog dat het geen weerslag zal hebben op biologische bestrijding van plagen.

De LT_{50} kan bij niet-mobiele insecten alleen bepaald worden aan de hand van uitwendige infectiesymptomen, in het geval van *Aschersonia* door het verkleuren van de larven van doorzichtig naar parelmoerachtig oranje (A24, A26, Aa5 of Ap1), wit (A23) of bruin-oranje (A31). Voor tabakswittevlieg varieerde de LT_{50} van 5 (A31) tot 8 dagen (A24) en voor kaswittevlieg van 5 (A31/A26) tot 10 dagen (Ap1). Het gbruik van uitwendige symptomen kan leiden tot een onderschatting van de LT_{50}, omdat sterfte vaak al optreedt voordat symptomen zichtbaar worden. Er is daarom ook een tweede methode gebruikt, nl. het tijdstip van sterfte aan de hand van het larvestadium waarin sterfte plaatsvindt bepalen. Deze gegevens kwamen globaal overeen met de gegevens van de LT_{50} waardes, echter isolaat Ap1 overtrof hierbij andere isolaten i.t.t. de LT_{50} waarden. Het verkleuren van de larven na infectie door Ap1 zou wel eens trager kunnen verlopen dan bij de andere isolaten.

Uit hoofdstuk 2 en 3 blijkt dat isolaten A26, A31, Aa5 en Ap1 goede kandidaten zijn voor biologische bestrijding van witte vlieg.

Hoofdstuk 4

Het infectieproces van tabakswittevlieg door *A. aleyrodis* (Aa5), *A. placenta* (Ap1) en *Aschersonia* sp. A26, is in meer detail bekeken d.m.v. scanning electronen microscopie. Daarnaast is de gevoeligheid van de verschillende stadia van kas- en tabakswittevlieg voor Ap1 en Aa5 onderzocht.

Uit bovenstaande waarnemingen bleek dat de drie isolaten nauwelijks kiemen op de bladschijf van kerstster (poinsettia), maar wel op de bladnerven en op de insecten zelf. De sporen op het insect produceerden een slijmlaag waardoor ze zich beter kunnen hechten aan het insect. Voordat de kiembuizen de huid van het insect penetreerden werden vaak appressoria gevormd. Dit zijn structuren waarmee de schimmel zich extra goed aan de ondergrond kan hechten, wat uiteindelijk penetratie vergemakkelijkt. Echter, in even zoveel gevallen bleef bij penetratie de vorming van appressoria achterwege. De penetratieplaats en het al dan niet vormen van appressoria leek niet gerelateerd aan een specifieke plek op de huid van het insect. Soms werden door de schimmels een aanzienlijke hoeveelheid schimmeldraden op het insect gevormd zonder dat infectie plaats leek te vinden. Eerste tot en met derde stadium larven van zowel kas- als tabakswittevlieg zijn zeer vatbaar voor infectie van *A. aleyrodis* en *A. placenta*, vierde stadium larven en poppen zijn veel minder vatbaar. Deze verminderde vatbaarheid heeft waarschijnlijk te maken met het ontwikkelen van de adulte witte vlieg binnen de huid van de vierde stadium larve. Voor beide wittevliegsoorten is er opmerkelijk veel overeenkomst in vatbaarheid van de verschillende larvestadia. In het algemeen vond sterfte plaats in een stadium later dan het behandelde stadium, d.w.z. dat 80-90% van de populatie binnen 5 à 7 dagen stierf. Onder gunstige omstandigheden, lees hoge luchtvochtigheid, komt de schimmel via de zwakke plekken van het insect weer naar buiten om te sporuleren.

Hoofdstuk 5

Voor een succesvolle infectie van witte vlieg zal een spore in contact moeten komen met het insect. Echter bij bespuitingen van planten zullen maar weinig sporen direct in contact komen met het insect. Doordat het eerste stadium van witte vlieg beweeglijk is en doordat bij opeenvolgende vervellingen de larven steeds in grootte toenemen kunnen deze sporen alsnog in contact komen met hun gastheer. Hiervoor moeten ze echter wel lange tijd levensvatbaar blijven. Of sporen na een lang verblijf op het blad nog in staat zijn te kiemen op wateragar (een maat voor levensvatbaarheid), daarnaast in staat zijn hun gastheer te infecteren en wat de rol van de plant in deze is, is in dit hoofdstuk onderzocht.

De kiemkracht van sporen van *A. aleyrodis* bleef erg lang behouden, nl. na 31 dagen op een komkommerblad bleek 80 à 90% van de sporen nog steeds in staat om te kiemen; op

kerststerblad bleek dit gemiddeld 70% te zijn en op gerberablad 'slechts' gemiddeld 50%. Voor komkommer en gerbera bleek dit een goede maat voor de infectiekans van kaswittevlieglarven, nl. 31 dagen na bespuiting werd op komkommer nog steeds 90% van de larven gedood door infectie, op gerbera varieerde het dodingpercentage tussen 50 en 70%. Op kerstster bleef het dodingspercentage ver achter bij de verwachting, het zakte nl. van 70% op de dag van bespuiting naar 20% op dag 3 tot en met dag 31 na bespuiting. Op bladeren van de drie gewassen bleek er nauwlijks kieming te zijn opgetreden, wat kan wijzen op een "zit en wacht" strategie van *A. aleyrodis* sporen in plaats van te kiemen en naar hun gastheer toe te groeien.

Dat de plantensoort de kiemkracht en infectiekans sterk beïnvloedt, kan te maken hebben met verschillende factoren. Ten eerste, de samenstelling van microörganismen op het blad verschilt sterk van plant tot plant, deze kunnen inwerken op insectpathogene schimmels via bv. competitie om voedingsstoffen op het blad, etc.. Zo blijkt dat zowel komkommer- als gerberablad aangetast kunnen worden door verscheidene schimmelziektes, terwijl bij kerstster slechts één bladpathogeen bekend is. Ten tweede, de chemische samenstelling van de planten verschilt aanzienlijk, wat kan leiden tot directe remming door de plant danwel beïnvloeding van de vatbaarheid van het insect. Zo is van kerstster bekend dat deze plant giftige stoffen bevat die celdodend kunnen werken. Ten derde, de planten verschillen aanzienlijk in morfologie, bv. komkommerbladeren zijn sterk behaard en aanzienlijk groter in vergelijking met bladeren van kerstster. Deze morfologische kenmerken kunnen de luchtvochtigheid in de laag op het blad, waarin het infectieproces zich afspeelt, beïnvloeden. Ondanks de grote invloed van de plantensoort op de persistentie van *A. aleyrodis* is de lange periode dat deze schimmel in staat is kaswittevlieg te infecteren zeer opmerkelijk.

Hoofdstuk 6

De infectieprocessen vinden plaats in een dunne laag op het blad en de luchtvochtigheid in deze laag is van groot belang voor de effectiviteit van insectpathogene schimmels. Deze fyllosfeerluchtvochtigheid wordt bepaald door omgevingstemperatuur, -luchtvochtigheid en wind, maar ook door eigenschappen van de plant, zoals bladvorm en -grootte, bladbeharing, dichtheid van het gewas, etc. De fyllosfeerluchtvochtigheid kan echter niet direct gemeten worden zonder deze te beïnvloeden en kan alleen benaderd worden door ander factoren als omgevingstemperatuur, bladtemperatuur e.d. te meten en vervolgens de luchtvochtigheid te berekenen. De fyllosfeerluchtvochtigheid is berekend voor gerbera, komkommer, kerstster en tomaat, waarbij kaswittevlieg op alle gewassen was uitgezet, terwijl katoenluis alleen op komkommer en gerbera was uitgezet. De fyllosfeerluchtvochtigheid is vervolgens gerelateerd aan de effectiviteit van verschillende insectpathogene schimmels op deze gewassen. De schimmels *A. aleyrodis*, *A. placenta* en *Verticillium lecanii* zijn gebruikt tegen witte vlieg, en

Metarhizium anisopliae en *V. lecanii* zijn gebruikt tegen katoenluis.

De fyllosfeerluchtvochtigheid op komkommer was significant hoger dan op de andere gewassen en lag gemiddeld rond de 94%. De meeste schimmelsporen hebben een minimale luchtvochtigheid van 92 à 93% nodig om te kiemen. Beide *Aschersonia* soorten veroorzaakten meer dan 90% sterfte, *V. lecanii* slechts 44%. Ondanks de lagere fyllosfeerluchtvochtigheid op tomaat- en gerberablad (90%), veroorzaakten ook hier beide *Aschersonia* soorten een sterfte van 90% of meer, terwijl *V. lecanii* het minder goed deed. Hoewel schimmels in staat zijn om te kiemen bij 92%, gaat het sneller als de luchtvochtigheid hoger is, wat kan betekenen dat de berekende luchtvochtigheid hier een onderschatting is van de werkelijke luchtvochtigheid op bladniveau.

Hoewel op kerststerblad de luchtvochtigheid op hetzelfde niveau lag als op gerbera en tomaat, bleek hier geen van de schimmels in staat een hoge wittevliegsterfte te veroorzaken, nl. 21%, wat niet verschilde van de sterfte in de controlebehandeling. De bladeren van kerstster zijn sterk waterafstotend in vergelijking met de andere planten, wat gevolgen kan hebben voor de verdeling van de sporen over het bladoppervlak. Dat de fyllosfeerluchtvochtigheid de gevonden verschillen in sterfte niet kan verklaren wordt bevestigd door de bladluisgegevens; de bladluissterfte veroorzaakt door schimmels lag hoger op gerbera dan op komkommer.

Naast de fysische eigenschappen van de planten kunnen ook chemische eigenschappen een rol gespeeld hebben in de verschillen. Zo kunnen toxische stoffen van de plant de schimmel direct beïnvloeden, maar ook insecten beschermen tegen schimmelinfecties. Het verschil tussen de schimmels kan veroorzaakt zijn door een verschil in 'droogte' tolerantie. Zo heeft *V. lecanii* een hogere luchtvochtigheid nodig dan *A. aleyrodis*. Echter, het verschil in mortaliteit tussen de twee schimmels kan ook veroozaakt zijn door een verschil in virulentie.

Hoofdstuk 7

De rol van de omgevingsluchtvochtigheid, plantensoort en hun interactie in het succes van schimmelinfecties is hier nader onderzocht. Komkommer- gerbera- en kerststerplanten geïnfesteerd met kaswittevlieg werden behandeld met *A. aleyrodis*, *A. placenta* en *V. lecanii* onder droge en vochtige omstandigheden al dan niet met een extra periode van zeer hoge luchtvochtigheid (> 95%) variërend van 3 to 48 uur. Dit laatste werd alleen met *A. aleyrodis* en *V. lecanii* uitgevoerd.

Over het algemeen bleek dat een hogere omgevingsluchtvochtigheid leidde tot een hogere infectie, echter met uitzondering van kerstster. Dat de plantensoort van groot belang is blijkt wel uit het feit dat op kerstster de sterfte veroorzaakt door de schimmels alleen verschilt van de controle wanneer de periode met extra hoge luchtvochtigheid 48 uur of langer is, terwijl op komkommer bij een omgevingsluchtvochtigheid van gemiddeld 45% zonder

extra periode hoge luchtvochtigheid *A. aleyrodis* al 50% sterfte veroorzaakte. Verder bleek dat *A. alyerodis* (en *A. placenta*) effectiever zijn bij lage luchtvochtigheden dan *V. lecanii*.

Uit deze gegevens blijkt dat wanneer de omgevingsluchtvochtigheid maar gunstig genoeg is, de grote verschillen die gevonden zijn in effectiviteit van de schimmels op de verschillende plantensoorten, verminderen. Door schimmels te formuleren tot een product, waardoor ze minder afhankelijk worden van luchtvochtigheid, kan dit probleem omzeild worden. Omgevingsluchtvochtigheid beïnvloedt het infectieproces en kan evt. verschillen in effectiviteit van schimmels binnen een plantensoort verklaren, maar de plantensoort overtreft het effect van omgevingsluchtvochtigheid.

Hoofdstuk 8

Twee schimmels, nl. *A. aleyrodis* en *V. lecanii* zijn toegepast onder semipraktijkomstandigheden in een gerberagewas met een gemiddelde omgevingsluchtvochtigheid van 70%. Er zijn vele gerberarassen op de markt (> 600) en deze kunnen aanzienlijk verschillen in morfologische danwel chemische eigenschappen. In dit hoofdstuk is gewerkt met twee rassen die gekozen zijn op basis van hun morfologische verschillen. 'Bianca' vormt een zeer dicht gewas met gemiddeld 8-15 relatief grote bladeren per plant en een lichte bladbeharing, terwijl 'Bourgogne' een relatief open gewas vormt met gemiddeld 5-7 relatief kleinere bladeren per plant met een zeer dichte bladbeharing. Naast effectiviteit van de schimmels, die toegepast zijn in twee doses, te weten 10^6 en 10^7 sporen per ml, in drie opeenvolgende weken, is ook gekeken naar de ontwikkeling van kaswittevlieg op beide rassen.

Uit het kasexperiment blijkt dat hoe hoger de toegepaste dosis hoe hoger de wittevliegsterfte door schimmelinfecties. Op 'Bianca' veroorzaken zowel *A. aleyrodis* als *V. lecanii* een hoge wittevliegsterfte, nl. 80%. Dat beide schimmels even goed presteren, dit in tegenstelling tot voorgaande resultaten, kan te maken hebben met het meervoudig toepassen van de schimmel in dit experiment. Op 'Bourgogne' echter, doet *V. lecanii* het significant slechter in vergelijking met *A. aleyrodis* en in vergelijking met 'Bianca'. Hiervoor zijn verschillende verklaringen aan te voeren.

Ten eerste, een sterk behaard blad ('Bourgogne') is meer waterafstotend dan een licht behaard blad ('Bianca'). In het laatste geval vloeien de druppels verder uit, wat de luchtvochtigheid positief kan beïnvloeden. Samen met de dichtere structuur van het gewas kan de luchtvochtigheid op het 'Bianca'blad hoger zijn dan op 'Bougogne'. Gezien *A. aleyrodis* minder afhankelijk is van hoge luchtvochtigheid kan dit de hogere sterfte, in vergelijk met *V. lecanii*, verklaren.

Ten tweede, door de slechtere verdeling van sporen op 'Bourgogne'-blad (waterafstotendheid v/h blad) en doordat wittevlieglarven beter beschermd worden door de beharing, zal de raakkans op 'Bourgogne' kleiner zijn dan op 'Bianca'. Doordat *A. aleyrodis* in

staat is langer te overleven op een blad dan *V. lecanii*, zou de infectie van *A. aleyrodis* uiteindelijk hoger kunnen zijn.

Ten derde, hoewel de wittevliegontwikkeling op beide rassen niet verschilt, hebben de adulte witte vliegen een voorkeur voor 'Bianca'. Om toch een vergelijkbare wittevliegpopulatie op 'Bourgogne' te creëren, is er vaker uitgezet in 'Bourgogne' dan in 'Bianca'. Dit heeft geleid tot een 'jongere' populatieopbouw in 'Bourgogne'. Op het moment van bespuiting waren er waarschijnlijk meer eieren op 'Bourgogne' aanwezig en gezien *A. aleyrodis* langer overleeft op bladoppervlaktes, kan deze schimmel hiervan geprofiteerd hebben.

Uit dit experiment komt duidelijk naar voren dat insectpathogene schimmels in staat zijn een wittevliegpopulatie in gerbera te onderdrukken, waarbij *A. aleyrodis* beter uitgerust lijkt te zijn dan *V. lecanii* om onder wisselende omstandigheden goed te functioneren.

Hoofdstuk 9

Voor het gebruik van *Aschersonia*-soorten in de praktijk zullen eerst nog enkele hindernissen genomen moeten worden. Voor grootschalig gebruik van schimmels is een effieciënte massakweek nodig. Iedere schimmel heeft zo zijn specifieke eisen en hiervoor zal meer onderzoek noodzakelijk zijn. Nieuwe systemen die de laatste jaren ontwikkeld zijn voor het effectief kweken van andere schimmels zouden hierbij uitkomst kunnen bieden. Daarnaast is voor gebruik in de praktijk een product nodig dat een half tot anderhalf jaar houdbaar moet zijn, zonder z'n effectiviteit te verliezen. Het formuleren van een schimmel tot een product heeft voordelen, zo kan bv. de schimmel beter beschermd worden tegen ongunstige omstandigheden, bovendien kan het gebruiksgemak verbeterd worden. Echter het infectieproces mag niet gehinderd worden door de formulering.

De interesse voor het gebruik van biologische bestrijding in het algemeen is groeiende vanwege resistentieontwikkeling bij plaaginsecten tegen chemische bestrijdingsmiddelen en toenemende druk vanuit de maatschappij voor een milieuvriendelijkere aanpak van ziektes en plagen. Uit bovenstaande resultaten en uit voorgaand onderzoek blijkt dat verschillende *Aschersonia*-soorten zeer virulent zijn tegen beide wittevliegsoorten, kunnen worden gekweek op kunstmatig medium, zeer goed zijn aangepast aan het bladsysteem, zeer persistent zijn en door hun hoge mate van specificiteit goed te combineren zijn met andere natuurlijke vijanden. Steeds minder nieuwe, selectievere chemische bestrijdingsmiddelen komen op de markt vanwegen hoge kosten voor ontwikkeling en registratie, en dit geldt nog sterker voor een kleine markt als de glastuinbouw. Doordat insectpathogene schimmels in zoverre vergelijkbaar zijn met chemische bestrijdingsmiddelen dat ze op eenzelfde manier kunnen worden toegepast en relatief snel werken in vergelijking met bv. parasitoiden en predatoren is er meer en meer belangstelling voor deze categorie natuurlijke vijanden.

Nawoord

Het heeft heel wat zweetdruppeltjes gekost, maar eindelijk is het zover: het proefschrift is af !! Het grootste deel van het werk, dat hier wordt beschreven, is uitgevoerd op het Proefstation voor Bloemisterij en Glasgroente, in Aalsmeer (nu Praktijkonderzoek Plant en Omgeving, sector glastuinbouw), in nauwe samenwerking met het Laboratorium voor Entomologie van de Wageningen Universiteit. Hoewel alleen mijn naam op de kaft staat, was dit onderzoek niet mogelijk geweest zonder de inzet van vele mensen. Een aantal van hen wil ik hierbij met name bedanken.

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Wageningen,
Mei 2001

Ellis

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Meekes, E.T.M. , Fransen, J.J. & Lenteren, J.C. van, 1994. The use of entomopathogenic fungi for the control of whiteflies. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **59/2a,** 371-377.

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Curriculum Vitae

Elisabeth (Ellis) Truus Maria Meekes werd op 8 November 1967 geboren te Eibergen. In 1986 behaalde zij het VWO-diploma aan de scholengemeenschap 'het Marianum' te Groenlo en in datzelfde jaar begon zij met de studie plantenziektenkunde aan de Landbouwuniversiteit te Wageningen. Tijdens de doctoraalfase van deze studie deed zij onderzoek naar de invloed van waarplantvariëteit, waardplantras en gastheerdichtheid in het tritrofisch systeem kool - *Pieris brassicae* - *Cotesia glomerata* bij de vakgroep Entomologie en naar de herinplantingsziekte in asperge bij de vakgroep Fytopathologie. Een stageperiode werd doorgebracht bij Royal Sluis, San Juan Bautista/Salinas (Californië, USA), waar ze onderzoek deed naar methoden voor het toevoegen van microorganismen aan zaad ter bestrijding van omvalziekte (*Pytium ultimum*). In 1992 behaalde zij haar doctoraalexamen en in datzelfde jaar werkte ze bij Incotec (Enkhuizen). In 1994 werd ze aangesteld als AIO bij de vakgroep Entomologie in een samenwerkingsproject met het Proefstation voor Bloemisterij en Glasgroente (Aalsmeer), een project gefinancieerd door het Productschap Tuinbouw. Dit werk heeft geleid tot het proefschrift getiteld: 'Entomopathogenic fungi against whiteflies: tritrophic interactions between *Aschersonia* species, *Trialeurodes vaporariorum* and *Bemisia argentifolii*, and glasshouse crops'. In 1999 - 2000 werkte ze als toegevoegd onderzoeker bij de vakgroep Fytopathologie aan witte roest (*Albugo candida*) op kool en andere kruisbloemigen. Sinds mei 2001 is ze werkzaam als ecologisch fytopatholoog bij Plant Research International.

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On the front cover:
- right: whitefly nymphs infected with *Aschersonia* sp. A15 and *A. placenta* Ap1
- left: whitefly adult, healthy whitefly nymphs
- top: poinsettia crop and gerbera cv Bourgogne

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