CHAPTER 7

BIOCONTROL IN PROTECTED CROPS: IS LACK OF BIODIVERSITY A LIMITING FACTOR?

Annie Enkegaard and Henrik F. Brødsgaard

1. Introduction

Protected crops are a dives entity, ranging from crops grown under very simple plastic or mesh construction to very high-tech glasshouse structures, which have a very high degree of automatisation of e.g. climate control, internal logistics, and robots for plant handling. But in general greenhouse crops are grown under very artificial conditions, where not even soil may be present but the plants are grown in e.g. rock wool or mats of coconut fibres. This makes protected crops very simple ecosystems with very poor biodiversity. On the other hand, once a pest species establish in such systems, it finds itself in an environment of unlimited food availability, a pleasant more or less constant climate that may prevail year round, and no enemies. Basically, biological control aims at provide the protected environment with natural enemies of the pests and thereby increase the biodiversity in the crops in a controlled manner. As implementation of biological control programs becomes widespread, the use of broadspectrum pesticides decrease, and the global trade in plant material increase, the need for more different biological control agents will continue to increase. So, will the research community and commercial insectaries be able to supply this increasing demand for beneficial organisms for the fast growing industry of protected crops?

In this chapter we review the history of biocontrol in greenhouses illustrating the driving forces behind implementation of this plant protection method, providing examples of how new beneficials have been discovered and discussing factors limiting to an increased use of biocontrol. The chapter deals with biological control of arthropod pests, primarily with the use of macroorganisms. Figs. 1-12 show examples of some major pests, as well as some main biological control organisms.

2. Early history of biocontrol in greenhouses

2.1. The use of biocontrol before 1960's

The first record of consistent successful biological control of pests in protected crops by means of natural enemies is from Speyer (1927). He reported that *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) parasitised and controlled the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), on a tomato crop in England. During the subsequent years Speyer developed a mass rearing system and distributed *E. formosa* not only to local growers but to growers and colleagues in several countries (McCleod, 1938). The mass

An Ecological and Societal Approach to Biological Control, 91–122. *J. Eilenberg and H.M.T. (eds.), Hokkanen*© 2006 *Springer.*

Institute of Agricultural Sciences. Figure1: Encarsia formosa *– a parasitoid of whiteflies. Photo F. Lind. Danish*

Institute of Agricultural Sciences. Figure2: Phytoseiulus persimilis *attacks a spider mite. Photo F. Lind. Danish*

KVL, Department of Ecology. Figure 3: Aphid killed by the fungus Verticillium lecanii*. Photo: Leif S. Jensen,*

http://www.forestryimages.org. Figure 4: Bemisia argentifolii*. Photo: Scott Bauer, USDA ARS Image Gallery,*

http://www.biopol.nl/UK/Whiteflies.html. Figure 5: Eretmocerus eremicus *– a parasitoid of* Bemisia*. Photo: BioPol, NL.*

whitefly nymph. Photo: Jack Kelly Clark, University of California, Figure 6: The ladybird beetle Delphastus catalinae *(*D. pusillus*) feeding on a*

http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html. Clark, University of California, Figure 7: Adult Western flower thrips, Frankliniella occidentalis*. Photo: Jack Kelly*

http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html.

California, Figure 8: Peach-potato aphids, Myzus persicae*. Photo: Jack Kelly Clark, University of*

http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html. th January 2005, "Biological Control: A Guide to Natural Enemies in North America, Aphidoletes aphidimyza", Weeden, Shelton, Li & Hoffmann (editors), Cornell University http://www.nysaes.cornell.edu/ent/biocontrol/predators/aphidoletes.html. Figure 9: Larva of the aphid gallmidge Aphidoletes aphidimyza*. Photo: J. Ogrodnick, 5*

Jack Kelly Clark, University of California, Figure 10: A minute pirate bug, Orius *sp. – a polyphagous predator of e.g. thrips. Photo:*

http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html. Figure 11: A leafminer, Liriomyza *sp. Photo: Garta.*

*Clark, University of California, Figure 12: Adult female serpentine leafminer parasite. (*Diglyphus begini*). Photo: Jack Kelly*

http://www.ipm.ucdavis.edu/IPMPROJECT/ADS/manual_naturalenemies.html

rearing and augmentation of *E. formosa* continued until 1949 when growers worldwide turned to the new synthetic pesticides such as DDT and discontinued the use of *E. formosa* (Hussey, 1985).

2.2. Renewed interest in biocontrol in the 1960's

Up through the 1950s growers of protected crops relied exclusively on pesticides for control of pests. Though resistance to DDT quickly was developed in a series of important pests, new groups of pesticides continued to be developed and enabled the growers to overcome resistance problems by shifts and rotation among different pesticide groups. However, by the late 1950s, pesticide resistance in the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), had become so severe that even very frequent pesticide applications did not control the pest. In 1960, Dosse (Bravenboer $\&$ Dosse, 1962) found an effective spider mite predator, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), on a crop of orchids imported from Chile to Germany. The predatory mite proved to be very effective and mass rearing systems were quickly developed. Several research stations and smaller commercial insectaries started mass producing *P. persimilis*, and the vegetable growers in Western Europe and Canada soon found the cost/benefit of the predatory mite so good that many turned to biological control of spider mites within a few years. By 1970, most cucumber growers used *P. persimilis* as their first choice of spider mite control and, by 1980, hardly any of the major cucumber growers in these areas used chemical spider mite control.

 By the end of the 1960's, chemical control of the greenhouse whitefly became increasingly difficult due to build-up of insecticide resistance. Therefore, a British research station collected *E. formosa* from a botanical garden and started a culture. In 1972, a commercial production was re-established and, in the mid 1970, the use of biological control of whiteflies in tomato crops was widely used in Western Europe and Canada (Hussey, 1985). The rapid uptake of this rediscovered beneficial was due, not only to the effectiveness of *E. formosa*, but also to the fact that tomato crops have a rather simple pest species complex. In addition, the product development, where pupae of the parasitoids are glued to cardboard cards, makes *E. formosa* an easy manageable product with a relatively long shelf life. So by 1980, like with the spider mite control in cucumber crops, the greenhouse whitefly in tomato crops was more or less exclusively controlled by biological means in Northern Europe and Canada (van Lenteren *et al.*, 1992).

2.3. Development of biocontrol methods against secondary pests

The widespread use of biological control of spider mites and whiteflies in cucumber and tomato crops, respectively, and hence the termination of the use of broad-spectrum pesticides generated increased problems with former secondary pests. In cucumber crops the onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), and the melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), are such examples and in tomato crops, problems with leaf miners, *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae), increased.

 The first line of action to overcome these "new" severe pests and at the same time preserved the use of biocontrol was to implement IPM-programs incorporating the use of *P. persimilis* and *E. formosa* with the least harmful of the available pesticides, assisted by extensive sideeffect evaluations of pesticides (e.g. Franz *et al*., 1980; Hassan *et al*., 1983, 1987, 1988). In

some cases integrating the use of pesticides with biocontrol could be eased by application of deliberately selected strains of organophosphorous pesticide resistant *P. persimilis* (e.g. Croft & Morse, 1979; Schulten, 1980). Attempts also to select similar strains of *E. formosa* failed (e.g. Walker & Thurling, 1984).

 Concurrent, with the search for pesticide resistant *P. persimilis* and *E. formosa*, researchers throughout Northern Europe looked for new biological control agents to control the secondary pests. This strategy proved to be much more viable, and up through the 1980s a range of new beneficial arthropods was developed and marketed. By the end of 1980s, full biological control programs for glasshouse vegetable crops were developed using e.g. predatory mites (*Amblyseius* spp., *Neoseiulus* spp. (Acari: Phytoseiidae)) and bugs (*Orius* spp. (Heteroptera: Anthocoridae)) against *Thrips tabaci* (e.g. Shipp & Ramakers, 2004), parasitoids (*Aphidius* spp. (Hymenoptera: Braconidae)) and predatory gall midges (*Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae)) against aphids (e.g. Blümel, 2004), and parasitoids against leaf miners (*Dacnusa sibirica* Telenga (Hymenoptera: Braconidae), *Diglyhus isaea* (Walker) (Hymenoptera: Eulophidae)) (e.g. van der Linden, 2004).

 The general method for release was to apply beneficials early in the growing season as soon as the first pests were observed. Sometimes this method did not result in control of the target pest because the pest population had increased too much at the time pest observation and the following application of beneficials. New introduction strategies were therefore invented: pestin-first, preventive introductions (dribble method) and banker plants. In the first method pests are established in low numbers in the culture before release of beneficials to provide an optimal timing of introduction and a more stable foundation for the subsequent build-up of the natural enemies (e.g. Gould *et al*., 1975). However, the practical use of this method has been limited due the growers' understandable reluctance to introduce pests into their crops. In the dribble method beneficials are released already at the time of planting of a new culture in anticipation of later pest infestations (e.g. Parr *et al*., 1976). Banker plants are open rearing systems of beneficials established in the culture on an alternative prey host, e.g. establishment of aphid parasitoids on aphids incapable of attacking the crop reared on a suitable host plant (e.g. Bennison, 1992). Both dribble applications and banker plants are now widely used.

 Biological control was initiated in UK and the Netherlands and from there the use gradually spread first to other North European countries and Canada (van Lenteren & Woets, 1978), and subsequently to more southern regions in Europe, e.g. France, Israel, and Italy (e.g. Woets $\&$ van Lenteren, 1983; Nucifora & Calabretta, 1985, van Lenteren, 1985), and eventually to other regions of the world e.g. USA, New Zealand and Australia (e.g. Woets & van Lenteren, 1984; van Lenteren, 1985; Martin, 1987; Spooner-Hart, 1989).

 It should be noted that there is a noticeable difference between greenhouses of northern cooler climates (glasshouses) and those of warmer Mediterranean climates (plastic greenhouses, screenhouses, plastic tunnels). Glasshouses are rather closed units largely isolated from the outside environment whereas plastic greenhouses are more openly structured creating a constant interchange of pests and beneficials between the greenhouse crops and the neighbouring outdoor crops and weeds (e.g. Avilla *et al*., 2004). In these regions pests therefore constantly re-colonise greenhouse crops via infestation from the outside and released beneficials are more likely to escape from the greenhouses. On the other hand native natural enemies migrate into the greenhouses to a much larger extent than in cooler climates. Therefore they have a major role to play in biological control programs, which emphasise not only releases of beneficials in the greenhouses but also attempts to conserve the local native

population of beneficials in the surroundings (e.g. Gabarra & Besri, 1999). This exploitation of the native fauna in warmer climates have through the years lead to the discovery of a number of natural enemies that subsequently have been mass produced, first with the aim to augment the local populations through releases, but later also for application in northern glasshouses. Examples of such additions to the commercially available arsenal of beneficials for use in greenhouses from this Mediterranean climate reservoir of biodiversity are *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) and *Dicyphus tamaninii* Wagner (Heteroptera: Miridae).

3. Dissemination of biocontrol from vegetables to ornamentals

3.1. Initiation of use of biocontrol in ornamentals

Practical implementation of biological control in ornamentals via IPM programs structured around application of *P. persimilis*, *E. formosa* and/or the fungus *Verticillium lecanii* (Zimm.) Viegas (Deuteromycotina: Hyphomycetes) started already in the late 1970's and early 1980's on a very limited area in UK (Wardlow, 1979), Norway (Stenseth, 1979), Poland (Pruszynski, 1979) and the Netherlands (Woets & van Lenteren, 1982). The area of ornamentals under IPM did, however, not increase noticeably (van Lenteren & Woets, 1979, 1980; Woets & van Lenteren, 1981, 1982). Thus, during the 1970's and early 1980's the notion among researchers and practitioners was that implementation of biocontrol in ornamental cultures, especially pot plants, on a larger scale was unrealistic (van Lenteren & Woets, 1988) primarily because of the low damage threshold of these cultures.

However, like previously in vegetables, ornamental growers started to experience increasing difficulties in controlling pests chemically (Scopes, 1979; van Lenteren, 1988; van Lenteren & Wardlow, 1989) and in the mid 1980's a breakthrough occurred with increasing applications of biocontrol in North European countries in cultures like Chrysanthemum (Gould, 1984), roses (van Lenteren, 1985), Gerbera (van Lenteren, 1985) and Poinsettia, (Wardlow, 1989) initiating a new epoch in the history of biological pest control.

Since then, the use of biocontrol in ornamentals has increased stimulated by the availability of an ever increasing number of beneficial species (Figure 13, Table 1); the usefulness of *V. lecanii* for cleaning cuttings rooting under high humidity conditions (Sopp & Palmer, 1990); the adoption of new strategies for beneficial application (keep-down-strategy (Brødsgaard, 1995)), i.e. inundative releases (see Chapter 1); and increased use of preventive introductions. The uptake of biocontrol among ornamental growers has, however, been slower than among vegetable growers due to factors such as the low damage threshold of ornamentals; zerotolerance for export items; the great diversity of plant species grown as ornamentals (more than 400 species in Europe alone (van Lenteren, 2000)); the frequently more complex production process of ornamentals; the lack of safety periods; and recent marketing of pesticides for which resistance among pest species has not yet evolved. In many cases it is therefore easier for ornamental growers to stick to effective pesticides, when available, as a plant protection measure or to revert to chemical control when new pesticides are marketed.

Despite these limitations implementation of biocontrol in ornamentals, especially in temperate climate regions, in some countries now amounts to up to 10-35% of the area (Enkegaard, 2003). For examples of IPM programs for various ornamental crops see Gullino $\&$ Wardlow (1999).

Table 1: List of commercially available beneficials used (or potentially usable) worldwide for biocontrol of pests on plants in protected crops, interior plant scapes etc. Endemic/exotic is in relation to Western Europe. A ? indicates that the origin of the beneficial species is uncertain

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100 A. ENKEGAARD AND H. F. BRØDSGAARD

Figure 13: Development in number of commercially available beneficial arthropods. Adapted from van Lenteren & Nicoli (2004)

4. Threats to biocontrol in the 1990's

The major threats against the implementation of biological pest control programs have not only been developments of new effective pesticides against the primary pests or development of uncontrolled secondary pests, as mentioned earlier. Accidental introductions of new severe pest species for which there are no biological control agents developed also pose a thread to existing biocontrol programs. So-called zero-tolerance pest species are not tolerated within designated areas and eradication programs will be initiated should such pests be introduced (e.g. EPPO 2004). These eradication programs will almost always be based on applications of broadspectrum pesticides that most certainly will destroy biological control programs already in action. Examples of this are the introductions of the American leafminers, *L. trifolii* (Burgess) and *L. sativa* Blanchard (Hymenoptera: Agromyzidae) into European glasshouse crops (Minkenberg, 1988). The eradication programs of some of these introduced species have not been successful and the pests have established in new areas. Two of these introduced pests that recently have managed to establish themselves as severe pests in protected crops almost worldwide are the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and the cotton whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae).

4.1. The western flower thrips Frankliniella occidentalis

The western flower thrips, *F. occidentalis*, is originally distributed in U.S.A. west of Rocky Mountains, where it for long has been a pest in the cotton agro-ecosystem. However, pesticide resistant populations build up and during 1980s insecticide resistant western flower thrips spread to protected crops worldwide (Brødsgaard, 1989a). In the areas where biological control programs were in function, *F. occidentalis* was a major obstacle to biocontrol because it could only, and with great difficulty, be controlled by broad-spectrum pesticides. This was a twoedged sword. Some growers simply gave up biocontrol while others, who experienced the difficulties in chemical control of this thrips, saw and hoped that biocontrol agents might be able to control *F. occidentalis*. Hence, research efforts in Western Europe and Canada were in the late 1980s and early 1990s put into developing biocontrol against *F. occidentalis*. First, the biocontrol agent, *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), already used against *T. tabaci* in sweet pepper and cucumber crops were tested and used on *F. occidentalis*. However, due to the differences between the biology of the two thrips species such as *F. occidentalis* having a much broader host plant range, a much higher fecundity in flowering crops, and in part different pupation sites compared to *T. tabaci*, the control of *F. occidentalis* by biological means proved to be more difficult than of *T. tabaci*. As with *E. formosa* and *P. persimilis*, *N. cucumeris* was found more or less by chance in a glasshouse crop (Ramakers 1978) and this kick-started biological control of the onion thrips. However, in the case of the western flower thrips coordinated research programs were conducted in many countries on predatory mites and bugs, parasitoids, nematodes, and insect pathogenic fungi (Levis, 1997).

 Within the predatory mites new species were investigated and, in addition, *N. cucumeris* as a biocontrol product was improved. Many of the "new" beneficial species were well known thrips predators but emphasis was put into quantifying their predation potential of *F. occidentalis* and their efficacy potential under growing conditions where *F. occidentalis* is a pest. Focus was on the performance of the mites under dry conditions and with availability of

pollen (Sabelis & van Rijn, 1997). The phytoseiid *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) was found to be a promising candidate (van Houten $\&$ Stratum, 1995) and has been in commercial mass production since then. However, also mites not previously associated with thrips predation were discovered as biocontrol agents of *F. occidentalis*, e.g. the soil dwelling *Hypoaspis miles* Berlese (Acari: Hypoaspididae) that was developed by a Canadian research team and now is an implemented mass-produced thrips control agent in Canada and Europe (Gillespie & Quiring, 1990). But also the well known *N. cucumeris* was greatly improved as a biocontrol product in that a non-diapausing strain was selected from a strain originating from New Zealand (van Houten et al., 1995) and with the development of a slow release system for crops not producing pollen as alternative food for the mites (e.g. parthenocarp cucumbers) (Ramarkers, 1990; Shipp & Wang, 2003).

 Minute pirate bugs of the genus *Orius*, known to be predatory on *F. occidentalis* in cotton, soybean, and strawberry crops in USA, had since the 1970s been investigated in relation to biological pest control in outdoor crops (e.g. Isenhour & Yeargan, 1981). With the spread of *F. occidentalis* to glasshouse crops, interest in *Orius* spp. increased and several research programs were initiated to develop *Orius* species into commercial biocontrol agents for *F. occidentalis* in protected crops. This has been a success and there are presently a handful different species of *Orius* commercially available for biological thrips control in Europe, Canada, and U.S.A. (Sabelis & van Rijn, 1997) (Table 1).

 In many areas where commercial biocontrol agents are used in protected crops, the beneficial arthropods are not endemic to the local fauna. In these areas registration procedures are either lacking or the beneficials are approved based on the assumption that the alien biocontrol agents will not be able to establish permanent populations outside the protected crops due to unfavourable climatic conditions. However, in Australia no non-endemic arthropods are allowed to be imported and, hence, none of the already commercially available biocontrol agents against thrips could be used by the Australian greenhouse growers, when *F. occidentalis* was accidentally introduced in 1993 and thereafter spread throughout the continent. Therefore, to be able to control the highly pesticide resistant *F. occidentalis* biologically, the Australian authorities launched a research program with the aim of finding promising candidates for thrips control within the Australian fauna and developing one or more of these into commercially available biocontrol agents (Goodwin & Steiner, 1996). This quest resulted in hundreds of candidates collected and eventually, after extensive evaluations, two were picked out for mass release experiments (Steiner & Goodwin, 2002). One of these, the phytoseiid *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae)*,* is now in commercial production and available in Australia and Europe (Steiner et al., 2003). Furthermore, a permit for its release in Canada is also currently being sought (Goodwin & Steiner, 2002).

 Driven by the wish to find a selective biological control agent with a high searching efficiency against *F. occidentalis*, a Dutch research program, supported by the European Community, was conducted on parasitoids on thrips. Besides building on earlier Japanese results, the Dutch program was, like the Australian mentioned above, a "full" search for a biocontrol agent starting with a more or less global collection of parasitised thrips. Having collected a range of different parasitoid species and strains, a selection procedure was initiated based on studies of basic bionomics, laboratory experiments, glasshouse evaluations, and then mass production. Based on the results of the basic bionomics and laboratory experiments, a strain of *Ceranisus menes* was selected for the glasshouse and mass rearing experiments. Unfortunately, the parasitoid failed to provide adequate thrips control and mass rearing potential (Loomans, 2003), and, unlike the Australian program, the program was stopped.

4.2. The cotton whitefly Bemisia tabaci

In the mid 1980's a new pest appeared in greenhouses in North America and Europe – the Bbiotype of cotton whitefly *B. tabaci* also known as the silverleaf whitefly *B. argentifolii* Bellows & Perring (Bellows *et al*., 1994). For a review of the *Bemisia* species-complex see Perring (2001).

 This highly adaptable, polyphagous subtropical-tropical species is thought to have originated in Asia or Africa (Brown *et al*., 1995; Campbell *et al*., 1996). The species had formerly been recorded as a pest of especially field crops like cotton, sweet-potato, tomato, cassava, and cowpea (Greathead, 1986) but now the B-biotype began an expansion of its geographical range, attacking new crop species and quickly attaining status as a serious economic pest (e.g. Coudriet *et al*., 1985; Dittrich *et al*., 1986; Gill, 1992; Brown, 1994; Wisler et al., 1998). A range of characteristics accounts for the seriousness of *B. tabaci* as a pest, including its high potential to develop resistance to many pesticides (e.g. Prabhaker et al., 1985; Cahill *et al*., 1996; Horowitz *et al*., 1998, 2002; El-Kady & Devine, 2003); its ability to transmit a multitude of plant pathogenic viruses (e.g. Brown, 1994; de Barro, 1995; Jones, 2003) or induce plant physiological disorders (e.g. Paris, 1993; Baufeld & Unger, 1994; Brown, 1994); and its broad host range (Greathead, 1986; Cock, 1993) that allows it to survive and reproduce – and subsequently disperse between – many crop and weed species both in the field and in greenhouses. In the course of the geographical expansion of the species cross-infestation from field crops to greenhouse crops like Poinsettia occurred and paved the way for a further spread of the species via international trading of greenhouse plants between the continents.

 As a consequence, *B. tabaci* soon became a serious pest in greenhouse crops (e.g. Nedstam, 1988; Baranowski *et al*., 1992; Maisonneuve, 1992). In northern temperate greenhouses infestations occurred primarily in ornamentals like Poinsettia, Begonia, Gerbera and Hibiscus (e.g. Anon., 1989; Broadbent *et al*., 1989; Baker & Cheek, 1993; Fransen, 1994). In southern temperate to subtropical regions also vegetables like tomato, cucumber and pepper were attacked (e.g. Al-Samariee *et al*., 1987; Kring *et al*., 1991; Desbiez *et al*., 2003; Lozano *et al*., 2004; Stansley *et al*., 2004). The reason for this difference presumably lies in the fact that *B. tabaci* in more warm climates established on outdoor crops and weeds from which it easily could penetrate the loose-structured greenhouses dominated by production of vegetables. In cooler climates this cross-infestation pathway was not available due to the lack of outdoor establishment and the spread of *B. tabaci* into and between these regions therefore hinged on international trade of growing plants where ornamentals constitute the major part.

 Already in the beginning of its geographical expansion *B. tabaci* vectored viral diseases in greenhouse vegetables, for instance Tomato Yellow Leaf Curl Virus (TYLCV) (e.g. Sharaf & Allawi, 1981; Berlinger *et al*., 1983; El-Serwiy *et al*., 1987) in e.g. the Middle East – a fact potentially threatening to greenhouse production of vegetables in other regions. Also the prospective for *B. tabaci* to vector diseases potentially infective to greenhouse ornamentals was a cause for serious concern worldwide (e.g. Giustina *et al*., 1989). In the past decades the worst fears has indeed come through with regard to expansion of the range of viral infections in vegetables vectored by *B. tabaci* – TYLCV has broadened it geographical range (e.g. Louro *et al.*, 1996; Moriones & Navas-Castillo, 2000), and new viruses have appeared in formerly uninfested regions, for instance Cucurbit Yellow Stunting Disorder Virus (CYSDV) in greenhouse cucurbits in Spain and France (Berdiales et al., 1999; Desbiez *et al*., 2003), Tomato Chlorosis Virus (ToCV) in greenhouse pepper in Spain (Lozano *et al*., 2004) and Lettuce Infectious Yellow Virus (LIYV) in greenhouse lettuce in Pennsylvania (Brown & Stanghellini, 1988). However, no incidences of transmission of viral diseases in greenhouse ornamentals have yet been reported.

Bemisia tabaci has by now established itself permanently as a greenhouse pest in regions like North Africa, Southern Europe, North America, South America, Australia and Asia (Sukhoruchenko *et al*., 1995; Demichelis *et al*., 2000; Hanafi, 2000; Kajita, 2000; Oliveira et al., 2001; Stansly *et al*., 2004, V.H.P. Bueno, UFLA, Brazil, pers. comm.; M. Steiner, NSW Agriculture, Australia, pers. comm.). In more northern regions for instance in Scandinavia and UK permanent establishment has not occurred but outbreaks of *B. tabaci* occurs annually in greenhouse ornamentals as a result of import of infested plant material (S. Cheek, CSL, UK, pers. comm.; N. S. Johansen, Planteforsk Plantevernet, Norway, pers. comm.).

When *B. tabaci* made its appearance in greenhouses it soon became clear that it was difficult to control with chemicals (e.g. Hamon & Salguero, 1987; Parrella *et al*., 1992) and frequent repeated sprayings became necessary. The use of selective pesticides to avoid side effects on beneficials was not an option and the presence of *B. tabaci* therefore became a serious threat to the recently initiated biocontrol in northern greenhouse ornamentals (Wardlow, 1988; Brødsgaard, 1989b; van Lenteren & Wardlow, 1989). Motivated by the need to effectively control this new whitefly and to some extent also by the wish to preserve the possibility for continued use of biocontrol of other pests, attempts to develop biological control strategies for *B. tabaci* were made.

 Since the problems with control of *B. tabaci* was urgent and since no commercial beneficials at that time was targeted directly against *B. tabaci* attention first focused on beneficials available against the greenhouse whitefly, *T. vaporariorum*, i.e. the familiar *E. formosa* (e.g. Albert & Sautter, 1989; Krebs, 1989; Stenseth, 1990; Parrella *et al*., 1991). However, control of *B. tabaci* with this parasitoid was not satisfactory in many cases (e.g. Parrella *et al.*, 1991; Hoddle & van Driesche, 1999 a, b) and other natural enemies needed investigation. As a consequence the research on *B. tabaci* and on the possibilities for biological control increased in the decades to come as illustrated in Figure 14.

Figure 14: Historical summary of research on B. tabaci/argentifolii and the proportional effort on biological control in both greenhouse and outdoor crops. From Naranjo, (2001)

A number of natural enemies of *B. tabaci* was already known in the 1980's (e.g. Mound & Halsey, 1978; Gerling, 1986; López-Avila, 1986; Cock, 1993). Researchers began investigating some of these for their biocontrol potential (e.g. Gerling, 1987a, b; Kapadia & Puri, 1990) and, in addition, smaller and larger national and international research programmes were launched for worldwide surveys for yet undescribed beneficial species for control of *B. tabaci* (e.g. Faust, 1992; Polaszek, et. al., 1992; Hoelmer, 1996; Henneberry *et al*., 1997, 1998, 1999, 2000; Goolsby *et al*., 2000; Oliveira *et al*., 2001; Nomikou *et al*., 2002). These efforts focused on control of *B. tabaci* with all categories of biocontrol strategies (classic, conservation, inundative, inoculative; see Chapter 1) both in field crops and greenhouses and considerable research efforts have been (and is) undertaken providing information on new beneficial species, their basic biology and behaviour, their interaction with *B. tabaci* and their potential for control. The species of natural enemies investigated includes both extant and imported species. A vast number of natural enemies have been surveyed, and subsequently evaluated in laboratory and greenhouse studies and through release test (e.g. Lacey *et al*., 1993; Goolsby *et al*., 1998; van Lenteren & Martin, 1999, Hoelmer & Goolsby, 2002; Nomikou et al., 2002). As an interesting fact many indigenous parasitoids in the new geographical areas of the expanding *B. tabaci* have been able to attack the pest and to follow with its expansion (Gerling *et al*., 2001) supporting the notion that efficient natural enemies for biological control can indeed be found outside the original geographical source of the pest (e.g. Hokkanen & Pimentel, 1989; Gerling, 1996; van Lenteren & Manzaroli, 1999; van Lenteren & Tommasini, 1999).

 By now the list of known natural enemies of *B. tabaci* encompass 114 predators with species of predatory mites (Phytoseiidae), lady beetles (Coccinellidae), lace wings (Chrysopidae) and mirid bugs (Miridae) dominating (Gerling *et al*., 2001); 54 species parasitoids with the genera *Encarsia* and *Eretmocerus* dominating (Gerling et al., 2001); and 11

species of fungi (Hyphomycetes, Entomophthorales) (Faria & Wright, 2001). Of the known species 21 predators and 3 parasitoids are now commercially available for use in greenhouses. The predators are, however, not necessarily developed or recommended for use against *B. tabaci* (Gerling *et al*., 2001). In addition, 3 of the fungi (*Beauveria bassiana* (Balsamo) Vuill (Deuteromycotina: Hyphomycetes).*, V. lecanii, Paecilomyces fumosoroseus* (Wize) Brown & Smith (Deuteromycotina: Hyphomycetes)) with control efficacy towards whiteflies are on the market (Faria & Wright, 2001). This list will, of course, expand in years to come as a result of continued research, including recently initiated research in geographical areas that are a recent addition to the geographical range of *B. tabaci* e.g. South America and Australia (de Barro et al., 2000; Gerling et al., 2001; V.P.B. Bueno, UFLA, pers. comm.). Provided that sufficient research funding is available it is therefore likely that new potentially important beneficials will be discovered and that these are eventually marketed for use in greenhouses, hereby adding to the existing arsenal.

 Satisfactory control of *B. tabaci* in greenhouse crops can now in some instances be achieved with *E. formosa, Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae), *E. mundus* Mercet (Hymenoptera: Aphelinidae), *M. caliginosus*, *Delphastus catalinae* (Hom) (Coleoptera: Coccinellidae) (previously *D. pusillus* LeConte (Hoelmer & Pickett, 2003)), *Chrysoperla rufilabris* (Brumeister) (Neuroptera: Chrysopidae), *V. lecanii* and *P. fumosoroseus* (e.g. Breene *et al.,* 1992; Stenseth, 1993; Osborne & Landa, 1994; Hoddle *et al*., 1997, 1998; Hoddle & van Driesche, 1999a, b; van Driesche *et al*., 1999: van Lenteren & Martin, 1999; Alomar et al., 2003; Richter et al., 2003; Stansly et al., 2004). However, the impetus to apply biocontrol of *B. tabaci* in practice is limited presently due to availability of pesticides still able to provide adequate control (e.g. Ishaaya *et al*., 2002; Otoidobiga *et al*., 2003; Elzen, 2004; Liu, 2004). In addition, biocontrol of *B. tabaci* still remains difficult in many places and crops and further research and development of new additional beneficials and strategies for use is needed (e.g. Hoelmer, 1996; Gabarra & Besri, 1999; van Lenteren & Martin, 1999; Hoddle, 2004).

4.3. Present status of biocontrol

The overview of the history of biocontrol in greenhouses illustrates that the lack of efficient pesticides has been a major driving force in selection, development and implementation of beneficials for pest control in these crops. It is estimated that biocontrol is used on 15,000 ha of the 300,000 ha with greenhouses worldwide (van Lenteren, 2000). This evolution has resulted in about 115 species of beneficials now being commercially available for biocontrol of pest on the many different plant species grown as vegetable and ornamental crops in greenhouses (Table 1). Growers have therefore become increasingly equipped to cope with the many different pest species in their crops.

However, status quo is not a term that apply to the greenhouse industry. Especially ornamental growers are innovative, constantly trying to adapt to a market craving for new types of products and new plant varieties and species. As a consequence international trade of ornamental plants continues to escalate and markets in new geographical areas like South America, Asia and Africa are developed hereby increasing the risk of introduction of new pest species to areas formerly beyond their natural range (van Driesche, 2002). This threat to the greenhouse industry will continue to exist or may even increase in the future, since phytosanitary measures may prevent establishment of some introduced pests but not all. Thus new pests establish in new regions at rates of e.g. 0.6 (Australia), 1 (the Netherlands), 4 (Japan) or 20 (Hawaii) every year (van Lenteren & Loomans, 2000). A characteristic of invasive arthropod species is their generally high resistance to pesticides (or perhaps herbivore species become invasive *because* they are highly resistant). This creates situations in which growers have to resort to existing biological solutions which may be insufficient towards new pest species, in which case the call goes to the scientific community for rapidly finding of new efficient natural enemies.

5. Factors limiting to bringing new beneficials in use

5.1. Biodiversity – a limiting factor?

The above examples from the history of biocontrol in greenhouses have illustrated that it through time has been possible to find natural enemies of various pest species and to implement their use in practice. That useful natural enemies of pests are available for such exploitation is further illustrated through the numerous examples of successful biocontrol (both classic and otherwise) of both pests and weeds in outdoor crops and landscapes.

No matter the origin of a herbivore species that enters a new geographical area and establish itself as a pest in greenhouses, a number of natural enemies exist that may eventually be adapted as a biocontrol product or in other ways made available for growers for seasonal inoculative or inundative releases in greenhouses.

Previously the notion that exotic pests could only, or at least most efficiently, be controlled by natural enemies of the same geographical origin prevailed (e.g. DeBach, 1964; Huffaker & Messenger, 1976), this notion presumably originating from the many well known examples of classic biological control of pest introduced e.g. to North America from Europe by releases of European natural enemies. However, there are no scientific arguments to support that this notion is an inescapable truth. On the contrary many examples have shown that exotic pests can just as well be controlled by indigenous natural enemies and vice versa (e.g. Hokkanen & Pimentel, 1989; Gerling, 1996; van Lenteren & Manzaroli, 1999; van Lenteren & Tommasini, 1999).

Thus, the biodiversity pool from which natural enemies of a new exotic pest are to be found is not limited to its original geographical area of distribution. The scientific community may look for natural enemies in the local fauna or perhaps even in the fauna of yet another geographical area. The number of insects and mites – which so far have been the most common choice for biocontrol of pests in greenhouses – worldwide is enormous and the proportion of predaceous or parasitic species is proportionally enormous. Add to this a worldwide flora of bacterial and fungal insect pathogenic species together with an equally diverse fauna of entomopathogenic nematodes and it becomes clear that it is not the natural availability of potential beneficials that in any way limits future development of new biocontrol agent. Rather, other factors play a crucial role.

5.2. Finding promising candidates

The above mentioned examples of how new beneficials have been found through times illustrates that the process of finding new promising candidates for biocontrol can take any shape between the two extremes – the empiric approach where a new biocontrol agents are

discovered mainly by chance and the painstaking, yearlong systematic search for and collection of candidates from different geographical regions of the world. No matter the approach research funding is crucial – naturally with no funding, no new natural enemies can be developed and implemented; and equally logic: the more funding the greater the opportunity to scrutinise the biodiversity pool in depth.

5.3. Evaluating and choosing between candidates

Once one or more natural enemies with a potential for controlling the pest in question has been found, a process of evaluation of these candidates sets into motion. This evaluation naturally aims at judging the candidates characteristics as biocontrol agents but assessment of possible unwanted qualities (i.e. potential harm to humans or livestock, polyphagy, hyperparasitism, etc.) and their magnitude and mass production potential are also needed.

Through the history of biocontrol in greenhouses this selection procedure has varied from rather simplistic and superficial tests of biocontrol efficacy to more elaborate and theoretically founded studies of various biological characteristics (rate of population increase, rate of prey kill, influence of climate, etc.) (e.g. van Lenteren, 1986a, 1986b; van Lenteren & Woets, 1988; van Lenteren & Loomans, 2000). The latter approach was developed to counterbalance the empirical procedure aiming at more optimised and efficient evaluation processes. The biological characteristics wanted in a good natural enemy (selection criteria) vary, of course, with the intended introduction strategy $-$ in inoculative strategies focus will be on the synchronisation of the natural enemy with the pest, searching efficiency and reproductive capacity, whereas these aspects are of lesser importance when inundative strategies are used (van Lenteren & Woets, 1988). In the analytical approach several natural enemies are compared with respect to various characteristics in an attempt to time-savingly predict their efficiency (e.g. Drost et al., 1996). It should, however, be kept in mind that the range of enemies tested and compared still inherently is just a more or less random subset of all existing natural enemies of the pest aimed to be controlled.

 Selection criteria should serve as guidelines for wanted and unwanted qualities in a potential beneficial, not as lists that should be followed dogmatically. Thus, it has often been claimed that exotic polyphagous predators should be disregarded as biocontrol agent out of the notion that this characteristic increases the risk that unintentional interactions with other beneficials in the cropping system or with the local fauna (Pimm, 1989; van Lenteren & Loomans, 2000). However, polyphagy might be accepted in cases where the predator in question can clearly be demonstrated to be unable to survive outside the greenhouse environment during unfavourable seasons – herewith establishment and subsequent negative impacts on the local fauna will be negligible (van Lenteren & Loomans, 2000). Interactions with other beneficials in the greenhouse system may still occur (e.g. Rosenheim *et al*., 1995) but if the predator is efficient towards the target pest this may be tolerated and/or managed. In addition, the polyphagous predator may in fact contribute to the control of other pests and through its polyphagous nature sustain itself when target pest populations are low in density (Brødsgaard & Enkegaard, 1997). Several examples of polyphagous predators among the arsenal of beneficials used in greenhouses exist (Table 1), e.g. *Orius* species successfully used for control of thrips and other pests.

 Likewise a natural reaction is to disregard facultative phytophagous species as suitable candidates for biocontrol since these inherently possess the ability to damage the crops in which

they are to function. However, a trait of facultative phytophagy should be evaluated in conjunction with other characteristics and potentials of the species in question before it is deemed useless. *M. caliginosus* is an example of such a facultative phytophagous predator, known indeed to be able to inflict damage to certain crops, e.g. certain tomato varieties and Gerbera (e.g. van Schelt *et al*., 1996; Sampson & Jacobson, 1999). However, *M. caliginosus* is an efficient predator of especially whiteflies used successfully in many countries, often supplementing biocontrol by parasitoids (Lenfant et al., 1998; Muhlberger & Maignet, 1999). The fact that this predator is able to sustain its populations on a diet of plant sap alone (van Schelt *et al.*, 1996) is in some instances beneficiary because it allows it to establish early when pest densities are low.

 Other qualities in a potential beneficial that at first seem disqualifying might likewise be circumvented or managed in ways to make implementation of the species in question possible. The use of personal protection equipment for greenhouse workers might for instance facilitate the use of a new predatory mite that has been shown to provoke allergic reactions in humans.

 A point to be noted with respect to selection of candidates is to keep in mind that successful biocontrol of a certain pest now a days often is based upon the use of more than just one natural enemy. Instead combinations of beneficials are used either in succession (e.g. the introduction of aphid parasitoids followed by later application of gallmidges) or simultaneously but aimed at different niches within the habitat of a greenhouse crop (e.g. the use of soil-dwelling predatory *Hypoaspis* mites for control of thrips pupae in addition to predatory mites and minute pirate bugs for control of nymphal and adult thrips on the above-ground plant parts).

 Finally the theoretically based selection procedure may not be especially appealing to commercial producers wishing, as a competitive strategy, to be able to launch a new suitable beneficial without to much delay after it has been discovered and found efficient.

5.3.1. Registration

In addition to the evaluation of natural enemies with the aim of identifying the most suitable candidate for biocontrol of a specific pest species, other evaluations are becoming increasingly important as more and more countries implement regulation procedures for import and release of natural enemies. The aim is to try to ensure that the use of natural enemies for biocontrol does not have any negative impacts on the environment and the local fauna (see e.g. Hokkanen & Lynch, 1995; Haynes & Lockwood, 1997; van Lenteren *et al*., 2003). Statutory registration of microorganisms has already existed for a number of years in many countries and will not be dealt with further in this chapter (see e.g. Hall & Menn, 1999 for additional information).

However, many countries also apply some form of regulation concerning macroorganisms. As no harmonised system exists yet, requirements for registration of a macroorganism differ between countries – some require documentation that an alien macroorganism is unable to establish itself in nature or at least do not have any harmful impact on the local fauna (e.g. Norway, Nina S. Johansen, Planteforsk Plantevernet, Norway, pers. comm..) while others in addition also require documentation for efficacy in specified crops not only of alien but also of indigenous species (e.g. Switzerland, Serge Fischer, Station Federale de Recherches en Production Vegetale de Changins, Switzerland; pers. comm.).

The procedures of registration have impeded the continued development of new beneficials for biocontrol in the countries in question either by making it unattractive for companies to apply for approval due to the costs involved compared to the anticipated return income, or by

the delayed registration merely due to the bureaucratic evaluation procedure. This is illustrated by the fact that the assortment of commercially available macroorganisms for biocontrol in greenhouses in countries where macroorganism registration is required is much lower (20-25 species (Nina S. Johansen, Planteforsk Plantevernet, Norway; Sylvia Blümel, Austrian Agency for Health and Food Safety; Barbro Nedstam, Swedish Board of Agriculture; Serge Fischer, Station Federale de Recherches en Production Vegetale de Changins, Switzerland; pers. comm.)) than in countries without this legislation (more than 100 species, Table 1).

Attempts to develop a harmonised and relatively simple system of regulation regarding import and release of biocontrol agents is presently underway for Europe (see van Lenteren, 2005). The future will show if the intended simplicity can be achieved herewith pursuing the goal of stimulating the use of biological control.

5.4. Producing and selling the chosen candidate

Once a potential beneficial has been identified an economical method for mass production needs to be developed, either for implementation at a commercial producer or for establishment of local rearings at the growers or cooperatives. The list of presently available beneficials (Table 1) shows that it has indeed been possible to design mass production methods for numerous and very different types of organisms. However, in some instances mass production may not be feasible either because it is too time consuming or too expensive in terms of the material needed to sustain production. A potential candidate that has passed unhindered through the various selection steps might end up being discarded for commercial marketing on grounds of being e.g. too cannibalistic which for rearing would require time consuming efforts to keep this internal mortality factor at a minimum.

Another aspect related to production is quality – an otherwise suitable candidate might be abandoned because it is difficult to produce it in an appropriate quality or to formulate a product with an acceptable shelf life.

For a commercial company to commit itself to production of a new beneficial the company must judge that the beneficial can be sold with an acceptable profit. This means that potential candidates may be disregarded for production if the market is very limited, e.g. because the target pest of the beneficial is of limited importance or because the beneficial has a very limited host range. This necessity for profit making in some cases tends to promote marketing of beneficials with a more broad host range and/or beneficials that can be applied in many different greenhouse crops.

5.5. Making growers use the chosen candidate

That a new beneficial has been made available to the growers does not necessarily imply that it will be applied as a biocontrol agents. Several factors influence the uptake of biocontrol in general by growers, including the status of grower education; the availability of advisory systems; the quality of beneficials; the perceived complexity of applying biocontrol instead of chemical control; the costs; the possibility for overpricing the product (e.g. being organically grown); and – importantly – the availability of pesticides. These matters will not be discussed further, please refer to e.g. Bolckmans (1999), van Lenteren (2003), Bennison (2004).

6. The future

Even though the motivation for the increased use of biocontrol encompasses such factors as idealism among growers, concern for the working environment among greenhouse workers and a wish to avoid phytotoxic effects on plants, the overriding factor influencing the attitude to and willingness to use biocontrol still relates to pesticides issues: growers resort to biocontrol mainly when pesticides are lacking or low in availability (due to legislative regulations and/or limited marketing of new pesticides for the rather small horticultural market) or when existing pesticides are inefficient due to resistance development. A very illustrative example of this is from the tomato industry. In order to produce fruits, the tomato flowers need to be pollinated. This was previously done by hand and as such very time consuming and, thus, expensive. However, after a huge research effort in Belgium and The Netherlands, year-round rearing of bumblebees was developed. Bumblebees are excellent pollinators of tomatoes and when commercial production of bumblebee colonies became available, tomato growers switched away from hand pollination over-night. Besides adding to the biodiversity in tomato crops, bumblebee pollination more or less put a stop for the growers' possibilities to use insecticides on their crops. The result has been that all growers of greenhouse tomatoes in Northern Europe and Canada uses biological pest control.

On the other hand, the present interest among e.g. Danish ornamental growers for using biocontrol, for supporting continued development and innovation of existing and new methods – and for their integration with other plant protection measures – is limited compared with the 1990s due to the recent marketing of e.g. imidachloprid and spinosad for control of phloem suckers and thrips and leaf miners, respectively. Unfortunately, it does not take long for the majority of growers to abandon biocontrol application and revert to chemical control with little or any thought for longer-term resistance-management strategies.

In spite of the fact that the use of biocontrol in greenhouses has been and still mainly is driven by pesticide related motivation it is our belief that biocontrol is here to stay and that biocontrol, possibly in combination with other non-chemical measures, in the long run will be the most sustainable plant protection measure in greenhouses. Biocontrol is a truly sustainable means of control. Once a system is implemented it will be functioning as long as the plant production practices remain unchanged.

Therefore, the need for improved biocontrol and for finding and developing new beneficial agents will continue to exist to allow us to be able to combat not only those pests already harbouring our greenhouse crops but also those that in the future are bound to appear in these crops as a consequence of the incessantly increasing trade of plants and plant parts in a more and more globalised world.

7. Conclusion

Biodiversity is not a limiting factor for a continued expansion of the arsenal of beneficial species used for biocontrol of pests in greenhouses worldwide. New potential candidates can always be found in the local fauna in the geographical origin of the pest, in the area to which the pest has been introduced or in yet other geographical regions *provided* that 1) research

funding for search for and evaluation of natural enemies in terms of their biology, efficacy and mass production possibilities is available; 2) releases of the beneficial in question can be permitted in greenhouses; and 3) the species can be profitably mass produced and sold.

Unfortunately these conditions, especially 1 and 2, are far from fulfilled in most cases: research funding is presently decreasing in many countries and seldom allow thorough exploration and/or evaluations and new beneficials are still in many cases discovered by chance; and registration requirements are costly and many commercial producers may refrain from trying to obtain permits for beneficials, however much wanted, if the intended market is unprofitable.

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