Biological control of pests in GM plant experiments: risks, benefits and consequences for containment

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Biological control of pests in GM plant experiments: risks, benefits and consequences for containment

Kees Booij[1] & Gerben Messelink[2]

1 Plant Research International
2 Wageningen UR Greenhouse Horticulture

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Address

Wageningen UR Plant Research International
P.O. Box 16
6700 AA Wageningen
The Netherlands
+31 (0) 317 480670

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Preface

Experiments in which genetically modified plants or animals are grown and studied under laboratory or greenhouse conditions have to comply with safety regulations. These regulations prescribe amongst others that genetically modified materials may not disperse outside the contained greenhouse or culture cabinet. Containment regulations also stipulate that no other organisms that the genetically modified study species may be present in the growth cabinet or greenhouse. One such group of organisms are pest species infesting the study plant, such as trips, spider mite, aphids or other herbivorous insects. It is common practice to suppress such pests by chemical means. However, there are situations in which the use of chemical pesticides is undesirable, e.g. when such chemicals influence the physiological functioning of the plant in a way that is incompatible with the experiment. The use of biological control (predatory mites, parasitoid wasps, predatory bugs) may be a good option in such cases. The question addressed in this report is: when biological control is used in greenhouses or growth cabinets will this require additional containment measures?

The report explores the conditions that could increase the risk of dispersal of genetically modified plant material. Most attention is paid to pollen, and to the arthropod complexes that are associated with pollen. This includes not only the classical pollen collectors such as bees and bumblebees, but many species of plant pests, as well as natural enemies of pests.

The report concludes that additional risk of biological control seems almost negligible if biological control is very effective, i.e. when there is hardly any pest population in the greenhouse and the biological control agent itself is not associated with pollen. The report also identifies conditions of increased risk (plants producing pollen, pests and antagonist associated with flowers). Such conditions require additional containment measures.

We are expecting that scientists will increasingly opt for biological control in greenhouse experiments. The use of natural enemies could also be considered in experiments with gm-plants. The present report constitutes a practical and useful help if COGEM is asked to advise on applications for contained use under such conditions.

Nico M. van Straalen,
Chair of the Advisory Committee

Summary

When growing genetically modified plants under contained experimental conditions such as greenhouses or climate rooms often insect pests are establishing on the plants even though care is taken to avoid contamination at the start of the experiments. As insect pests are often disturbing experiments and they are potential carriers of genetic plant material, in particular pollen, they have to be controlled. Mostly this is done by chemical control as this is assumed to be most effective to keep pest levels low and minimize the risks for escape of insects (and genetic plant material) from the facilities. Chemical pest control, however, has its drawbacks such as health risks for the applicants, development of pesticide resistance, failing effectiveness, and sometimes plant toxicity. In order to reduce pesticide use there is a wish to apply biological control with natural enemies.

This study analyses the potential effects of biocontrol versus chemical control systems on the spread of transgenic plant material by pests and natural enemies. It is assumed that by introducing new (bio) control systems the average population densities may change and it should be realized that both predators as well as pests can spread plant genetic material such as pollen. Reviewing current effective biocontrol systems for protected crops shows that thrips, aphids, whiteflies, spider mites and fungus gnats are the most common infestations found in greenhouses and other protected cultures Biocontrol systems have been developed for all of them, though the efficacy varies from crop to crop. Predatory mites, *Orius* flower bugs and parasitoid wasps are most frequently used as biocontrol agents.

If the crop is genetically modified transgenic plant material such as pollen, seeds, nectar and DNA (in microbes associated with the plant) can be ingested or carried for dispersal of genetically modified plant DNA by the pests and natural enemies. The question addressed in this study is: is the risk of dispersal of gm-plant material significantly altered by the introduction of biological pest control under contained conditions? It is assumed that pollen is the most significant risk factor for the current study. In principle all pests and natural enemies mentioned above are able to carry pollen to some extent.

After analysis of factors involved, it is concluded that the risk of gene transport is affected by the effectiveness of the (bio)control system and the resulting the average density of pests and natural enemies, their association with flowers and physical contact with pollen, their tendency to feed on pollen as well as prey, the size and adherence of pollen to the arthropods, and their mobility. In case the GM plant is not producing pollen or infertile pollen, the risk drops to zero, but when fertile pollen is present, pests and natural enemies are abundant and their pollen spreading capacity is high, risks may be significant.

In order to judge whether biological control option enhances the risk of dispersal of gm-plant material above the risk incurred with to chemical control all these factors have to be taken into account. Unfortunately very little is known about the pollen spreading capacity of the organisms in greenhouses other than from the real pollinators such as bees, bumblebees and hover flies. Given current knowledge, our preliminary opinion is that additional risks of biocontrol compared to chemical control is negligible when effectivity of the biocontrol system is similar to the chemical control system and the pest and the natural enemies are not strongly associated with flowers with abundant pollen and when their mobility is limited. When the association of the natural enemies with flowers is high or when levels of pollen carrying pests are considerably higher under biocontrol systems, extra containment measures are applicable depending on the situation. Better knowledge on pollen spread capacity of pests and natural enemies could be very helpful for risk assessment in this case.

Samenvatting

Bij de kweek van genetische modificeerde planten in kassen en klimaatkamers worden veel maatregelen genomen om deze vrij van plagen te houden. Desondanks worden veel van deze plantenkweken toch door insecten geïnfecteerd en worden bestrijdingsmiddelen ingezet om de teelt weer schoon te krijgen. Los van het feite dat de aanwezigheid van insecten in transgene planten volgens de regelgeving voorkomen dient te worden is aantasting door plagen ongewenst omdat ze de experimenten verstoren. Daarnaast is de vrees dat ze transgeen plantmateriaal zoals pollen verspreiden wanneer ze uit de kas of klimaatkamer kunnen ontsnappen. Naast de gebruikelijke inperkingsmaatregelen worden vaak chemische middelen ingezet om de plagen te bestrijden en op een zo laag mogelijk niveau te houden. Het gebruik van chemische middelen heeft echter nadelen. Naast de risico's voor resistentieontwikkelingen gezondheidsrisico's voor de gebruiker, zijn ze soms ook proeftechnisch onwenselijk en is de effectiviteit niet altijd optimaal. Vaak is het wenselijk het gebruik van deze chemische middelen terug te dringen en in plaats daarvan biologische bestrijding met natuurlijke vijanden toe te passen.

In deze studie is onderzocht of de toepassing van biologische bestrijding extra risico's met zich meebrengt in vergelijking met chemische bestrijding. Het idee hierachter is dat naast de plaag ook de natuurlijke vijanden zouden kunnen bijdragen aan de verspreiding van transgeen materiaal en dat bij biologische bestrijding de aantallen plaaginsecten mogelijk hoger zouden kunnen zijn. Literatuuronderzoek over de gangbare biologische bestrijdingssystemen in bedekte teelten laat zien dat trips, luis, spintmijt, witte vlieg en rouwmuggen de meest voorkomende plagen zijn waarvoor ook biologische bestrijding is ontwikkeld, hoewel de effectiviteit varieert van gewas tot gewas. Tegen deze plagen worden vooral roofmijten, roofwantsen, en parasitaire wespen ingezet.

Transgeen plantmateriaal zoals stuifmeel, zaad en los DNA in nectar of micro-organismen kunnen in theorie alle worden gegeten en getransporteerd door plaaginsecten en natuurlijk vijanden die ermee in contact komen. De kernvraag bij deze studie was of the introductie van biologische bestrijding extra risico met zich meebrengt met betrekking tot de verspreiding van transgeen materiaal bij ingeperkt gebruik van genetisch gemodificeerde planten. Daarbij wordt verondersteld dat de aanwezigheid en transport stuifmeel de belangrijkste factor is bij dit risico, hoewel ook andere ontsnappingsroutes van transgeen werden onderzocht.

Op basis van deze studie is geconcludeerd dat het verspreidingsrisico van transgeen materiaal wordt bepaald door de effectiviteit van het (biologische) bestrijdingssysteem en de daarvan afgeleide gemiddelde dichtheid van plagen en natuurlijke vijanden, het contact van beide en de neiging om pollen te eten, de mate waarin stuifmeel zich aan de organismen hecht, en hun beweeglijkheid.

Bij planten met weinig of alleen steriel stuifmeel is er geen risico en kan biologische bestrijding prima worden toegepast. Naarmate er meer stuifmeel aanwezig en de plagen en natuurlijke vijanden talrijker zijn en hun pollenverspreidingscapaciteit hoger is kan het risico op verspreiding van transgeen materiaal aanzienlijk zijn.

Om te kunnen beoordelen in welke mate de toepassing van biologische bestrijding het risico op verspreiding van transgene verhoogd moeten dus alle bovenstaande factoren worden meegenomen. Een bottleneck hierbij is dat de kennis over stuifmeeltransport door insecten anders dan de bekende plantenbestuivers zoals bijen en zweefvliegen, heel beperkt is. Op basis van de huidige kennis lijkt het echter gerechtvaardigd aan te nemen dat toepassing van biologische in veel gevallen weinig extra risico oplevert wanneer de bestrijding effectief is en noch de plaag noch de natuurlijke vijanden een sterke binding met stuifmeel hebben. Wanneer de natuurlijke vijanden een sterkere binding met stuifmeel hebben of stuifmeel consumerende plagen hogere dichtheden hebben bij biologische bestrijding kan het risico hoger worden en is het aan te bevelen de inperkingsmaatregelen aan te scherpen of toch te kiezen voor microbiële of chemische bestrijding. Meer kennis over de

stuifmeelverspreiding van verschillende organismen zou helpen m tot een betere risicobeoordeling te komen voor toepassing van biologische bestrijding.

1 Introduction

The growing of genetically modified crops is strictly regulated. Not only for commercial growing under field conditions but also in the experimental stage before they are released to the market. This is typically valid for scientific experiments where modified plants are used for fundamental genetic studies and in case new crop varieties are developed that have altered genetic properties for pest and disease resistance, food quality or content of high value substances.

In all cases, requirements for containment are necessary to avoid the spread of genetic material from the experimental or development conditions. In many cases genetically modified plants are grown in non-sterile facilities such as greenhouses and are prone to be affected by a range of insect pests. In the framework of GMO containment, measures should guarantee that no other organisms should be present other than those for which a license is given. Within this constraint, often some form of control of pest insects is necessary, also to avoid disturbance or failure of experiments and to reduce risks that pest insects cause spread of material from the experimental facility. According to tradition and regulation, chemical control is applied to control such insect pests based on the idea that this is most reliable.

Apart from the risks for the applicants, chemical control of pests may have drawbacks as it is not always fully effective, it may lead to pesticide resistance when frequently applied and the treatments may negatively affect the growing conditions as well as the outcomes of experiments. Application of modern biocontrol strategies as an alternative for chemical applications has several advantages, in particular in greenhouse conditions where many biocontrol tools have been developed and proven to be effective. Therefore it is worthwhile to see whether biocontrol can also be applied in greenhouses with contained growing of genetically modified plants. In that case it is necessary to analyse the potential change in the risks for spread of plant material due to the presence of both the pest insects as well as their natural enemies. This risk is related to the effectiveness of biocontrol compared to chemical control in preventing the potential spreading of plant material under both conditions. For example when insect pest as well as the biocontrol agent are able to take-up and spread pollen, the pollen-spreading risk depends on the numbers and spreading capacity of both. It can be imagined that the risk can either increase or decrease when biocontrol is applied instead of chemical control. In particular when chemical control is not fully effective (which is often the case), pest populations tend to build up quickly again, while effective biocontrol may keep them continuous at a low level.

This report identifies the potential spread of genetically modified material from greenhouses when biocontrol is applied and in particular the pollen-vector potential of common pests and natural enemies in some representative GM crops. The report is partly based on theoretical considerations using known characteristics of the pests and natural enemies such as pollen adherence and dispersal capacity, from which the risk in different biocontrol settings is estimated. Based on this, criteria and guidelines are given when biocontrol can be considered as applicable and how containment measures can be used to reduce the spreading risk of GM plant material.

2 Work plan

For the evaluation of potential risks associated with applying biological pest control in greenhouses where genetically modified crops are grown, the following steps have been taken. By reviewing recent literature and acquiring additional information from experts where necessary an inventory was made of:

- Current crops that are genetically modified for research or economic reasons and crops of high value that are likely to be so in the future. (keywords: GM-crops, transgenic crops, model crops)
- Cropping systems in greenhouses that have commonly applied systems of biological control, including their pest species and natural enemies applied. (based on reviews from biocontrol in greenhouses)
- Role of arthropods in spreading (trans)genes including the mode of uptake and transport. (keywords: gene flow, pollen transport, gene spread, dissemination)
- Information that is required to estimate gene spread based on crop type, the role of natural enemies as gene-vector, and the escape potential of the natural enemies.

Focus of this study is on biocontrol systems for plants/crops that are currently or likely to be applied experimentally, using greenhouse facilities in the Netherlands. The natural enemies that are or will be used as a biocontrol agent for the major pests in those crops are included and evaluated in this study. The study focused on some species that are representative for biocontrol systems and form a hazard because of their capacity to transport GM material and capacity to escape from the greenhouse facilities. An overview of some characteristic crop-pests-natural enemy combinations that are currently used in Europe is provided in Annex 1.

The case studies can serve as a baseline from which future new crops / crops and new biocontrol agents can be judged. The question remains if it is possible to characterize a system as a whole base on the organisms present according to what is acceptable or not and/or what containment measures should be taken in specific situations.

3 Uptake and transport routes of modified genes

3.1 Introduction

Potentially new genes incorporated in plants by genetic modification can be spread into the environment when plant parts are transported by wind/ air currents or by animal that feed on GM plant parts or by animals when plant parts can adhere to their bodies. Within the context of this project we focus on the potential transport of plant material by arthropods in conditions where the insect and mite pests are controlled by natural enemies.

It is necessary to consider the potential transport by both the pest as well as the natural enemies because their abundances are linked and containment measures will affect the escape potential of both. The different potential uptake and transport routes of modified genes will be discussed one by one below.

3.2 Pollen

The most obvious risk of transmission of GM plant material by arthropods is through transport of pollen. Apart from notorious pollinators and flower visitors numerous insect and mite species feed on the nectar, pollen and/or other plant exudates that are associated with flowers (Wäckers et al. 2005). As a result of this feeding activity, pollen becomes attached to them (Figure 3.1 and 3.2). Insects that do not actively feed on nectar, pollen or plant exudates also can become ‘dusted’ with pollen if they walk around or enter the flowers in search of food, mates, prey or shelter (Jones 2012).

Not only pollen from entomophilous plants (insect pollination) but also pollen from anemophilous plants (wind pollination) are consumed by insects and mites such as the predatory mite *Amblyseius swirskii* (Goleva and Zebitz 2013). Pollen of anemophilous plants is often produced abundantly and because of the absence of strong air flows in greenhouses, these types of pollen are often found abundantly on the leaves of plants when they are released from the flowers (Messelink, personal observations). The outer hardened wall of pollen, called exine, often bears spines or warts, or is variously sculptured (Figure 3.3). Strongly sculptured grains (Figure 3.4), are often observed in entomophilous plants and it is assumed that this sculpturing aids in attaching pollen to the pollinator (Walker 1976); (Tanaka et al. 2004). But also pollen species that do not have these clear spines on the exine attach to insects (Figure 3.5). Mostly pollen from entomophilous plants (actually the majority of plant species) are larger, often richly structured and sticky whereas pollen of anemophilous species tend to smaller and smooth.

A recent study from China studied the dispersal of pollen from transgenic varieties of rice (a self-pollinating plant with pollen with a smooth outside layer) by insects (Pu et al. 2014). They identified an impressive number of more than 510 insect species that visited rice flowers and approximately half of the collected insects had rice pollen on their bodies. Among them were also natural enemies such as the syrphid *Episyrphus balteatus*, *Coenosia* sp. (hunter fly), *Harmonia axyridis* (lady beetle), *Geocoris ochropterus* (a predatory bug) and *Meteorus gyrator* (a parasitoid of caterpillars) (Table 3.1). This shows that in principal all types of pollen can be attached to almost all types of insects and mites. For the moment there is no information on the relationship between pollen characteristics and the change of being transported by various insects (apart from specific pollinators). It seems likely that pollen from entomophilous plants are likely to adhere more easily to insects in genera because they are often more sticky and have evolved to adhere to insects (Schoonhoven et al 2015). As shown in the study of Pu et al (2014), the dispersal of pollen may occur easily by many insects that are able to fly and actively visit flowers with pollen. The number of pollen grains per specimen is strongly different among arthropod species. The pollen load per specimen in general is rather low for most species compared to the flower visiting bees and hoover flies (Syrphidae , Table 3.1). Apart from the syrphid flies also flies from several other families are frequent flower visitors and likely to transport lots of pollen (Howles et al. 2009) but those rarely include pest species or natural enemies. More relevant in this context is that several thrips species are pests as well as pollen feeding. And it was thrips shown that individual thrips can carry an average of 15 pollen grains (Mound and Terry 2001).

A special case concerns the Lepidoptera. In the caterpillar stage several species are considered as pest and often occurring in greenhouses. In the adult stage, as moth, several species do feed on nectar and are known to carry pollen and can act as pollinators (Devoto et al. 2011). As they are generally good flyers they are potential pollen transporters.

Table 3.1

Number of observed rice pollen grains carried by different insects in a field study of Pu et al. (2014).

Order	Family	species	pollen grains
Coleoptera	Coccinellidae	*Harmonia axyridis* (Pallas)	1.2±0.4
Coleoptera	Coccinellidae	*Propylea japonica* (Thunberg)	2.8±1.0
Diptera	Muscidae	*Coenosia* sp.	4.1±2.1
Diptera	Syrphidae	*Episyrphus balteatus* (De Geer)	5.9±2.0
Diptera	Syrphidae	*Eristalis arbustorum* (Linnaeus)	192.8±191.8
Hemiptera	Anthocoridae	*Orius* sp.	5.7±5.1
Hemiptera	Lygaeidae	*Geocoris ochropterus* (Fieber)	2
Hymenoptera	Apidae	*Apis mellifera* Linnaeus	716.8±82.5
Hymenoptera	Braconidae	*Cotesia ruficrus* (Haliday)	4.0±2.0
Hymenoptera	Braconidae	*Meteorus gyrator* (Thunberg)	1
Lepidoptera	Pieridae	*Pieris rapae* (Linnaeus)	0.6±0.3
Thysanoptera	Thripidae	*Frankliniella intonsa* (Trybom)	5.2±1.4

Figure 3.1 *Pollen attached to the legs and head of the syrphid Episyrphus balteatus on a flower of Cistus incanus (photo credits: André Karwath).*

Figure 3.2 *Predatory mites (Amblyseius swirskii, left) and predatory bugs (Macrolophus pygmaeus, right) flowers of sweet pepper plants.*

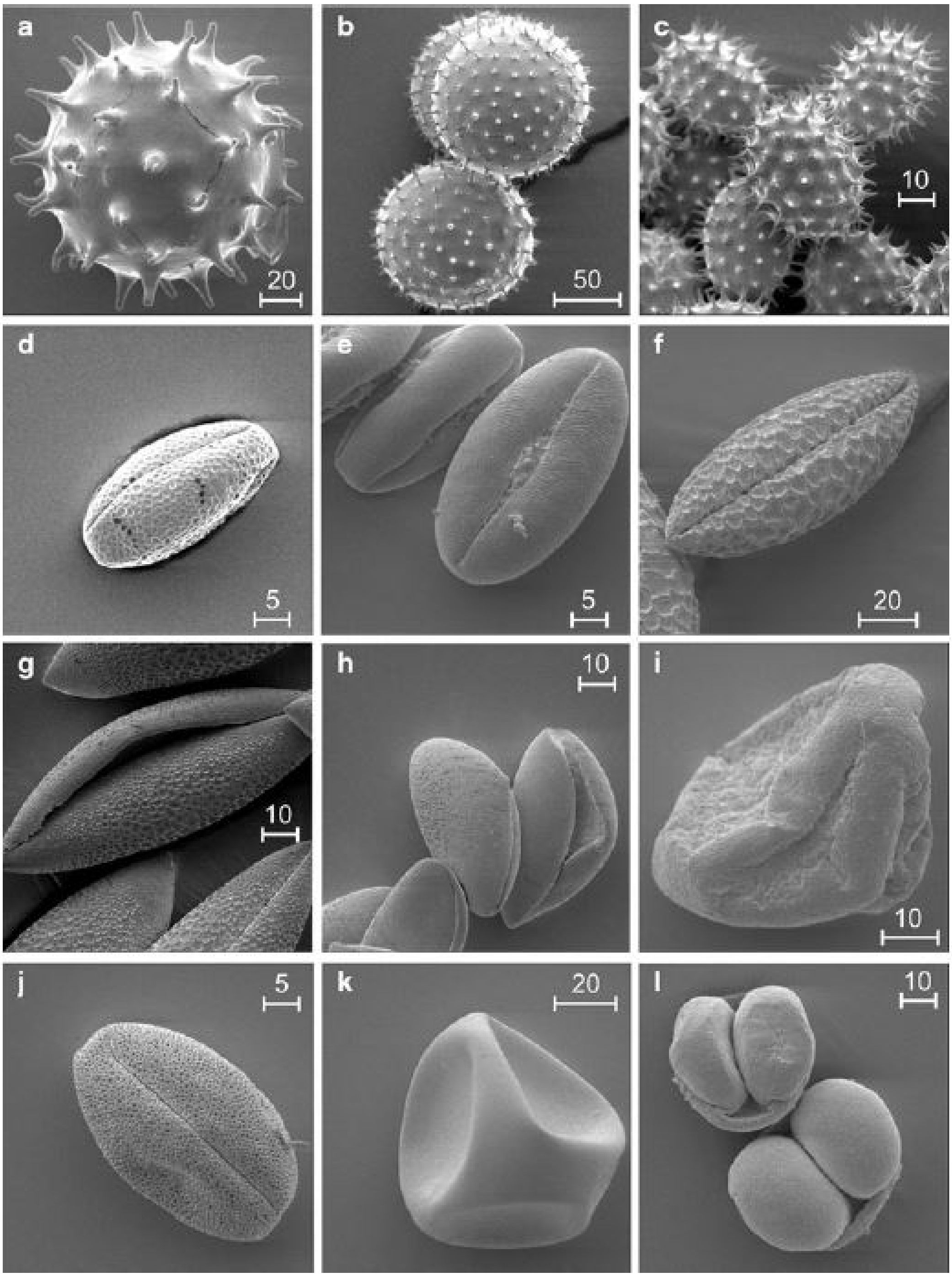

Figure 3.3 *SEM-photographs of entomophilous plant pollen: a Hibiscus syriacus (shrub Althea), b Cucurbita pepo (pumkin), c Helianthus annuus (sunflower), d Paulownia tomentosa (princess tree), e Aesculus hippocastanum (horse chestnut), f Lilium martagon (lilies), g Hippeastrum sp. (amaryllis), h Narcissus pseudonarcissus (narcissus), i Tulipa gesneriana (tulip); anemophilous plant pollen: j Ricinus communis (castor bean), k Zea mays (maize), l Pinus sylvestris (Scots pine (unit of size bars = µm). Photo credits: Irina Goleva and Claus Zebitz (Goleva and Zebitz 2013).*

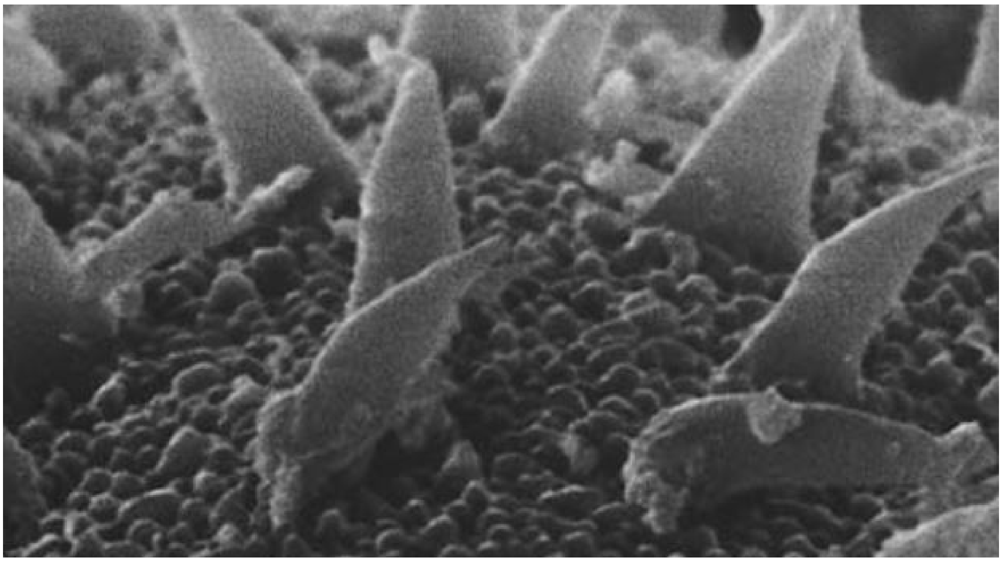

Figure 3.4 *SEM-photograph of the exine sculpture of Blyxa japonica. Photo paper (Tanaka et al. 2004).*

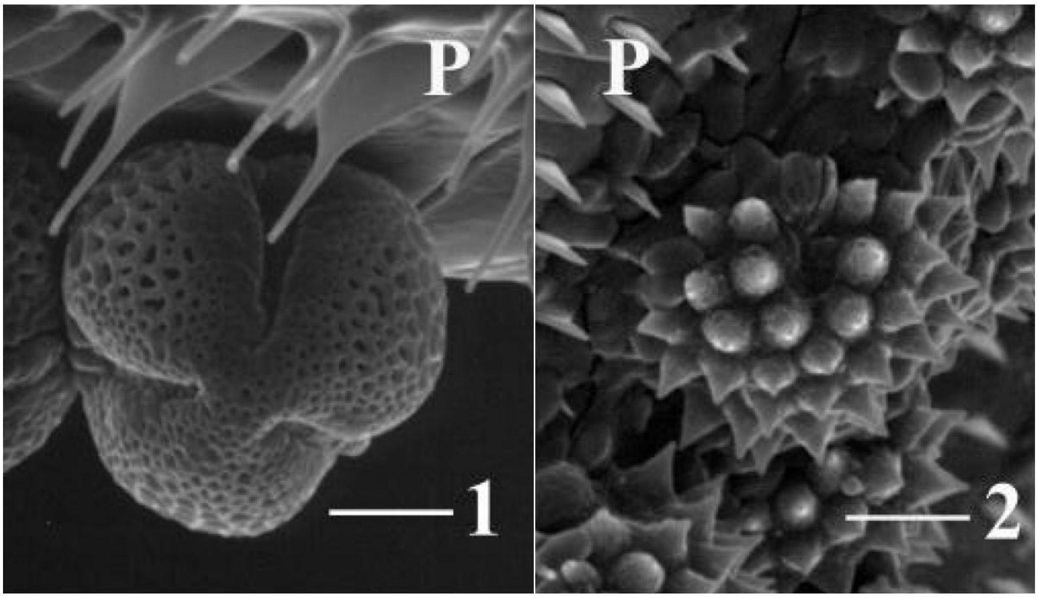

Figure 3.5 *SEM-photograph of pollen grains with a smooth outside layer (left) and with spines (right), that are both attached to an adult of the noctuid pest Spodoptera exigua. Bar 1 indicates 5 µm and 2 10 µm (Jones and Jones 2001).*

3.3 Seed

Seeds of plants can be dispersed by animals, mainly birds and mammals (Richardson *et al*. 2000). Seed dispersal by insects is rare and only documented for ants and dung beetles (Vander Wall et al. 2005). Whether this can occur in greenhouses is not known. Seed dispersal by natural enemies used in greenhouses has not been documented and seems unlikely to occur as most seeds are large compared to the generally small sized natural enemies. However, it cannot be excluded that some of the larger natural enemies, such as *Episyrphus balteatus* also can get contaminated with small seeds when visiting flowers. In the case aphids are not effectively controlled, excessive excreted honeydew may attract ants that are able to actively transport seeds. A list of transgenic plants for which potential seed spread (though not by insects) is relevant is published by the COGEM (2014).

3.4 Plant material in guts of herbivores and omnivores

Arthropod pest species consume plant material which can either be phloem sap (by phloem feeders such as leafhoppers, whiteflies and aphids) or plant tissue (plant chewing herbivores such as beetles and caterpillars) seeds and pollen. Not only pests, but also several omnivorous natural enemies consume plant tissue, such as the type IV predatory mites (Adar. 2012) and mirid predatory bugs (Coll and Guershon 2002). In addition, natural enemies can take up DNA or RNA fragments of plants after consuming herbivores.

Whether this plant material in the insect or mite gut remains intact and act as a source of DNA spread is unknown, but normally the digestive system will fully break down all fragments. The transmission of functional DNA fragments through food webs, including microbes is assumed to be insignificant and not further examined in this report. A first preliminary literature search indicates that very little is known about and fate of DNA in food chains.

3.5 Viruses

A further theoretical route that should be considered is the presence of plant viruses that may absorb plant DA including transgenes in their genome. However, as far known only sucking pest species such as aphids, white flies, thrips and leafhoppers are able to transmit viruses from plant to plant. Even though natural enemies may ingest plant viruses by feeding on such prey, it is not likely that those viruses can be released again from a predator or parasitoid. No information about this was found in the literature.

3.6 Microbial control agents and nematodes

Microbial control agents for pest are living micro-organisms such as bacteria and fungi are used to infect and kill pest species. Entomopathogenic fungi can actively disperse conidia in the air, but attachment of plant material into these conidia has, to our knowledge, never been observed. Bacterial control such as with Bacillus thuringiensis can be very effective. It seems very unlikely however that they will play a role in spreading any plant genes. Also nematodes are used in biocontrol but active dispersal of nematodes is very limited. Hence, it is not likely that these microbials or nematodes are able to transmit genes of GM crops from facilities to related crops outside.

3.7 Preliminary conclusion about gene spread routes

For now, we assume that pollen will be the main (and possibly the only) route for potential dissemination of transgenes. Actually all risk studies concerning gene-spread by insects thus far, focus on pollen transport. Recent strategies to address gene flow problems from GM plants are based on gene confinement by flower sterility, parthenocarpy or to mechanisms that avoid transgenes to be present in pollen (Ding *et al.* 2014). However, several GM crops may still produce fertile pollen which is a potential risk for gene-spread. In all those cases it should be realized that some pest species are also able to transport pollen (for example flower visiting moths or western flower thrips). So when biocontrol is effective, the additional transport by the natural enemies may be counterbalanced by a reduced transport by the pest species that are controlled. Hence to compare gene spread risks in biological or chemical control systems can only be done when the contribution of pests and biocontrol agents to gene transfer can be estimated under different conditions. Complex interactions may be part of these estimations. For example, it is known that some species of natural enemies can induce winged morphs of aphids (Müller *et al.* 2001), which will increase the potential risk of pollen transport.

Apart from insects and mites, also human beings can transmit modified plant genes when visiting greenhouses in which transgenic plants are grown. Particularly pollen may attach to persons and be transmitted out of the greenhouse when attached to body parts or clothing. Because of the small size of many pollen grains (ranging from 4 – 250 µm, according Jones and Jones 2001)), it will be hard to exclude transmission of pollen grains even when following strict sanitation and containment measures. However we assume that the spread of pollen due to presence of humans in the greenhouse does not change when using biocontrol instead of chemical control systems.

The fate of pollen when transported by arthropods or humans from the contained GM crops is outside the scope of the study. But in general the chance that such an event will lead to pollination of a conspecific or taxonomically closely related plant seems to be very low when it concerns species that is not an obligate or frequent flower visiting species. But real flower visitors such as bees and hoverflies and pollen feeding species that have a good dispersal power, may theoretically be able to cause occasional outcrossing. The relationship of insect pests and natural enemies have to pollen and the potential of those species to escape and disperse is therefore crucial when it comes to estimate risks in any pest management system.

4 The biocontrol system: crops, pests and natural enemies

As discussed above, we assume that attachment of pollen to insects and mites is the main route for potential dissemination of transgenes. All types of pollen may in principle attach to all types of natural enemies, as shown by the study of Pu et al. (2014). However, the risk of dissemination may strongly depend on the species of natural enemy used, as this may depend on ability to fly, the flower visiting behaviour and the ability to consume pollen and the type of pollen.

Obviously, natural enemies that have the ability to fly increase the potential of pollen dissemination when these are attached to their body. Several species of natural enemies have been observed to visit flowers and feed on pollen or nectar, which is summarized in Table 4.1. Some of them are considered as good pollinators, such as the syrphid *Episyrphus balteatus* (Jones and Jones 2001). Other species might only occasionally visit flowers, such as ladybird beetles or lacewings. Predators that also consume pollen may easily spread pollen as they move migrate between flowers and other plant parts, see e.g. (Hansen *et al*. 2003); (Messelink and Janssen 2014); (Weintraub *et al*. 2007).

There are also natural enemies that cannot fly, but that do visit flowers for pollen consumption, such as generalist predatory mites (McMurtry and Croft 1997). However, although they cannot fly, these small mites might easily be transported by humans after visiting the greenhouse crop. Also their small size and low visibility help them to escape without notice.

Some species of natural enemies have rarely or never been observed in flowers, but do consume pollen that has fall on the leaves, such as the predatory bug *Orius majusculus*. So these predators may still easily disseminate attached pollen attached to their bodies, in particular when they are good flyers such as the *Orius* bugs.

Apart from the behaviour of natural enemies and their ability to fly, also the morphology of natural enemies may play a role in pollen transport. Large hairy species may carry many more pollen grains than small-sized natural enemies with less hair (Table 3.1.) Some species of natural enemies are very small, for example phytoseiid predatory mites are only 380-450 µm large. These mites may still carry pollen grains, but some larger pollen grains may not attach as easily as the small pollen grains.

Although the number of pollen grains carried per individual may be largely different among species, eventually it is about the total carrying capacity of a population that matters. A study with thrips showed that individuals carried on average only 15 pollen grains, but it was estimated that thrips populations, when abundant, could deliver more than 5500 pollen grains to a single flower ovule (Mound and Terry 2001). So small-sized species of natural enemies may still be important for pollen transfer when their population numbers are high.

Ladybirds are notorious aphids feeders, but in particular the larvae also feed substantially on pollen (Seagraves 2009). It is not known to which extend adult beetles actively visit pollen rich flowers or contribute to pollination.

Based on flight ability, flower visiting behaviour (which in most cases means pollen attachment) and pollen consumption, we may categorize species of natural enemies in three groups with low, moderate and high risks of pollen dispersal:

1. High risk: ability to fly, flower visiting behaviour and/or consumption of pollen
2. Moderate risk: flower visiting behaviour and/or pollen consumption but not able to fly or able to fly but no flower visiting behaviour and/or pollen consumption
3. Low risk: not able to fly, no flower visiting behaviour and/or no pollen consumption

Based on these risk criteria, we categorized the natural enemies that are currently used in greenhouse crops for biological pest control. These data are presented in Table 4.1.

Table 4.1

Flight ability, flower visiting behaviour, pollen feeding and potential risk of pollen dissemination by arthropod natural enemies as they are currenty used in biocontrol.

Type	family	species	Flight ability	flower visits	pollen feeding	potential risk
predatory bugs	Anthocoridae	*Orius laevigatus*	yes	yes	yes	high
		Orius majusculus	yes	no	yes	high
	Miridae	*Macrolophus pygmaeus* *Nesidiocoris tenuis* *Dicyphus hesperus*	yes	yes	yes	high
predatory beetles	Coccinellidae	*Adalia bipunctata* *Cryptolaemus montrouzieri* *Delphastus catalinae*	yes	yes	Yes	high
predatory mites	Phytoseiidae, Type III and IV*	many e.g. *Amblyseius swirskii* *Neoseiulus cucumeris*	no	yes	yes	moderate
	Phytoseiidae, Type II*	*Neoseiulus californicus*	no	no	yes	moderate
	Phytoseiidae Type 1*	*Phytoseiulus persimilis*	no	no	no	low
	Laelapidae	*Stratiolaelaps scimitus* *Hypoaspis aculeifer*	no	no	?	low
	Macrochelidae	*Macrocheles robustulus*	no	no	?	low
predatory midges	Cecidomyiidae	*Aphidoletes aphidimyza* *Feltiella acarisuga*	yes	yes	no	high
lacewings	Chrysopidae	*Chrysoperla* spp.	yes	yes	yes	high
hoverflies	Syrphidae	*Episyrphus balteatus*	yes	yes	yes	high
parasitoids	Aphelinidae	*Aphelinus abdominalus* *Encarsia formosa* *Eretmocerus eremicus* *Eretmocerus mundus*	yes	probably not	no	moderate
	Braconidae	*Aphidius* spp *Aphelinus* spp *Praon volucre* *Ephedrus* spp.	yes	yes	no	high
	Encyrtidae	*Anagyrus pseudococci* *Leptomastix dactylopii*	yes	yes	no	high
	Eulophidae	*Diglyphus isaea* *Dacnusa sibirica*	yes	yes	no	high
	Trichogrammatidae	*Trichogramma brassica* *Trichogramma evescens*	yes	yes	no	high

* According to classification based on life-style (McMurtry and Croft 1997).

The consideration to use natural enemies in GM crops for biological pest control not only depends on the species of natural enemy used, but also on the efficacy of the natural enemies in that particular crop (Table 4.2). Many natural enemies maintain a close relationship with specific plants because of their plant feeding habits or requirements for oviposition tissue (Messelink *et a*l. 2014). This explains why some pests, such as western flower thrips, can be controlled very well in sweet pepper, but not in ornamental plants. Some other pests, such as aphids, are hard to control with natural enemies in all cropping systems. In contrast, spider mites are in general controlled very well by natural enemies. The alternative of chemical control might in some cases be less effective than biological control, particularly in those cases where pests have become resistant to pesticides. This has been recorded for spider mites and thrips (Van Leeuwen *et al*. 2010), (Puinean *et al.* 2013) and is a common observation in practice (Messelink, personal observations). In fact, this resistance to pesticides has been the reason for many growers to switch to integrated pest management systems and the application of natural enemies. In some cases pesticide applications may make plants more vulnerable for other non-target pest species (Szczepaniec *et al*. 2013). No systematic overview or data is currently published to compare the presence of pests in quantitative way for conventional or biological control systems. When it is expected that pest abundance is higher under biocontrol systems this should be taken into account when judging the safety of experimental systems. In such case additional pollen spread by the pest species should be incorporated in the risk analysis.

Table 4.2

Efficacy of biological control of the most important pest species for some cropping systems. Green refers to good control, orange to moderate results and red to poor results. An 'x' means that the pest is not a problem in that crop. Details of these cropping systems can be found in the appendix (Table A-G).

Crop	**Aphids**	**Leafminers**	**Spider mites**	**Western flower thrips**	**Whiteflies**
tomato	🟧	🟩	🟧	x	🟩
sweet pepper	🟥	x	🟩	🟩	🟩
cucumber	🟧	x	🟩	🟩	🟩
strawberry	🟩	x	🟩	🟧	🟧
rose	🟧	🟩	🟩	🟧	🟧
gerbera	🟧	🟩	🟩	🟧	🟧
chrysanthemum	🟧	🟩	🟩	🟥	x

Summarizing, when there is a wish or need to apply biological pest control with natural enemies in GM crops in greenhouses the following criteria should be considered:

1. The presence and abundance of pollen in the GM crop.
2. The efficacy of chemical control measures compared to that of natural enemies in the crop and its effect on pest abundance.
3. The species of natural enemies to be used and their capacity to disseminate pollen based on the ability to fly, flower visiting behaviour and ability to feed on pollen.
4. The combined effect of pests and natural enemies on potential pollen spread.

5 Case studies for modified crops

Potentially all crops can be subjected to genetic modification for scientific explorations or to develop commercially interesting crops. The interaction between crops, natural enemies and the way biological control agents are used is likely to be case specific. Yet some general principles can be found by analysing a number of cases that differ in plant characteristics, the pest complex present and the natural enemies used.

Many fundamental scientific studies using genetic modification are performed with a limited number of 'model' crops of which the genetic properties are well studied and experimental conditions are highly standardized. This crop category includes *Arabidopsis*, Tobacco and *Medicago* but also more commercial crops such as Potato are Tomato have a status as model crop. Commercial crops that are currently under study to improve production properties or to make them market-ready (risk free), including commodity crops such as grasses, cereals, rice, maize, semi-tropical world crops (cotton, soybean, cassave, banana), *Solanum* crops (tomato, potato), Cruciferous crops (cabbage, canola, oil seed rape, broccoli), production trees (pine, eucalyptus, poplar), fruits (grape, apple) and ornamental plants.

In addition to these main economic crops many other plant species have been used in research for genetic modification. A complete list of the species for which the COGEM (2014) has provided containment measures to prevent spread of seeds and pollen is available at www.cogem.net. This list also shows that for many plant species spread of transgenic pollen by insects is relevant. Spread of seeds is assumed to be mainly caused by wind or via the soil/medium in which plants are grown.

Worldwide, however, the diversity of crops that is genetically modified by research is quickly expanding. Plants are modified for a wider range of purposes including experiment for genetic mechanisms, development of resistance, chemical properties, growing characteristics, nutritious value and pharmaceuticals. Though transgenic -crop studies sometimes remain in almost sterile conditions, many will be grown in less clean (greenhouse) conditions and hence will be attacked sooner or later by pests and diseases that should be chemically or biologically controlled. Most widespread polyphagous insects such as aphids and thrips or plant feeding mites are most likely to occur in such situations. These pest species typically include species that are small-sized, attacking different crops, being tolerant for chemical treatments and highly reproductive. Biocontrol has typically developed for such wide-spread pests and hence is likely to be applicable for many of those GM crops. But still in most cases it should be adapted to the specific situation.

To select case studies that cover a wide range of crops and are relevant for the short term risk assessment we selected 4 crop systems to analyse the risk of spreading GM material when pests would be biologically controlled. The focus is on flowering and pollen producing crops as pollen is likely to be the most risky material to be spread from experimental conditions by pest insects or their natural enemies. The case studies also typically try to cover different combinations of pests and natural enemies. The natural enemies may vary with respect to their potential uptake of pollen and the dispersal capacity. When introducing biocontrol agents always a cost-benefit analysis should be made with regard to the control results and the spreading potential of transgenic plant material.

5.1 Arabidopsis

Arabidopsis is one of the most frequently used model plants for genetic studies. As it belongs to the Brassicaceae, knowledge from this plant is of general importance for breeding programs in the many varieties of *Brassica* that are major crops such cabbage and oil seed rape. All cruciferous crops are attacked by a wide variety of insect pests under field conditions but only a few of these play a role in greenhouse studies (Bush *et al.* 2006).

Procedures for growing *Arabidopsis* (Anderson 1997) mostly include guidelines to prevent spread of thrips, fungus gnats (Sciaridae) and aphids. Other species that may occur at lower frequency are spider mites and whiteflies. Though *Arabidopsis* is considered as a self-pollinating species, outcrossing can potentially occur by a variety of insects that visit flowering *Arabidopsis* including wild bees, syrphid flies and thrips species (Hoffmann *et al.* 2003). So there are surely insects – both pests and natural enemies- that are able to carry Arabidopsis pollen.

Fungus gnats can be successfully biologically controlled by the *Israelensis* strain of *Bacillus thuringiensis* or by the nematode *Steinernema feltiae*. Both biocontrol agents are not able to transmit any transgenic plant material themselves, while the fungus gnats are able to transport pollen. However no information was found about dispersal and pollination capacity. Some fungus gnats species –other than those in greenhouses - have been shown to play a key role in pollination of some Orchids, Araceae and Saxifragacaea (Vogel 2000, Okuyama 2004) So it is expected the typical greenhouse species are likely to be also able to transport pollen.

Thrips species such as *Frankliniella occidentalis*, which is a frequent pest in Arabidopsis experiments, is controlled on a regular base by the predatory mites such as *Neoseiulus cucumeris* and *Stratiolaelaps scimitus* (Bush *et al.* 2006). As both thrips and predatory mites can transport pollen and are vulnerable to unintentional transport by humans (clothing) the cost/benefit of biocontrol versus chemical control in terms of pollen spreading should be taken into account. Preventive measures and monitoring of spread of both the pest and its predators are useful tools for reducing the risk. Some protocols can be found at:
http://bioscigreenhouse.osu.edu/files/research-arabidopsis-protocols.pdf
http://bti.cornell.edu/wp-content/uploads/2014/04/BTI_GH_POLICIES_Sec_6.pdf

5.2 Sweet Pepper

Sweet pepper has a long tradition of a successful application of several natural enemies for a variety of pests (Ramakers 2004). The use of predatory *Orius* bugs against thrips species and predatory mites against both thrips and plant feeding mites (Annex 1, Table B) form the core of the biocontrol system in sweet pepper. Sweet pepper produces abundant pollen that does not only occur in flowers but also drops in considerable amounts on the leaves where it is consumed by natural enemies. For this crop the likelihood that pollen attaches to all insects present in the crop seems to be high.

For sweet pepper the application of predatory *Orius* bugs (also called flower bugs) is most illustrative as these bugs are strong flyers that also feed on pollen and thrive on pollen as well as on the thrips for which they are applied. Their association with flowers and pollen is strong (Van den Meiracker and Ramakers 1991), which increases the chance that substantial pollen is attached to them. When there is food shortage (low prey or pollen densities) or under high temperature condition they tend to move a lot through the crop. Both larvae and adults can easily escape through small openings and react to light (Booij 2014). When they effectively control thrips, the associated potential pollen spread by the pests may outweigh the pollen dissemination risk of the natural enemies. From half open tunnel systems with sweet pepper crops it is known that *Orius* bugs frequently move between natural vegetation and sweet pepper switching between various plant species with abundant prey and pollen sources (Bosco *et al.* 2008), (Bosco and Tavella 2013). In this way the sweet pepper case is a typical example of a system where more quantitative information is needed for a proper risk analysis. As long as such information is lacking, containment measures should be strict.

5.3 Tomato

Widespread tomato pests that have to be controlled in many cases are whiteflies, spider mites, leaf miners and aphids (Table A, Annex 1). Tobacco whiteflies (*Bemisia tabaci)* are also able to spread virus diseases, so effective control is needed when this pest is present. In contrast to sweet pepper tomato plant produce less pollen which is confined in the flowers with little pollen fall-down.

The major pest species mites and whiteflies are associated with leaves rather than the flowers so the contact between pests and pollen-sources is limited. Whiteflies can be effectively controlled by mirid predatory bugs, mainly *Macrolophus pygmaeus*, or parasitoids *(Encarsia, Eretmocerus)*. The predatory bugs can reach high densities, as the also feed and reproduce on the plants in absence prey. Moreover, they are relatively large and good flyers, so the risk of pollen spread by the predators is rather high, whereas the risk for the small whitefly parasitoids is assumed to be rather low. However, this hypothesis needs to be tested as no experimental data are available to support this hypothesis.

Effectiveness of predatory mites *Phytoseiulus persimilis* to control spider mites is in general reliable, but efficacy is hampered by the glandular trichomes of tomato plant. Particularly the high glandular trichome densities on the tomato stems hamper the mobility of predatory mites. Both the pest and predator stay on the tomato leaves and are not very mobile, thus the potential risk of pollen spread is probably low.

As in the other crops the balance between pests and predators and the respective pollen spreading power is a key factor in risk estimates. For the tomato case biocontrol of whiteflies seems to be more risky than that of spider mites as whiteflies are likely to transport pollen and are active fliers.

5.4 Strawberry

Major pests of strawberry are spider mites, thrips and aphids. For aphids, spider mites, whiteflies and thrips control, the options are almost similar to those given for sweet pepper where predatory mites and bugs are the major control agents (Table D, Annex 1). Pollen in strawberry flowers is abundant but (depending on the growing system) is not very likely to fall substantially on the leaves. But as the pests and the natural enemies are likely to occur both on flowers and leafs and also feed on pollen potential pollen transport is likely. In terms of the additional contribution of pollen spread by natural enemies in this case, everything depends on the effectiveness of control.

Strawberry aphids under greenhouse conditions is quite a different story. Effective control systems have been reported using ladybird beetles, lacewings and Aphidius parasitoids.
Of those three agents, the parasitoid seem to be the least risky for pollen attachment but they are not always effective. *Adalia bipuncatata* has potential to control aphids but both adults (flying) and larvae (adhering to clothing) are able to transport pollen. Both adult and larvae of coccinellid species consume substantial amounts of pollen (Lundgren 2009), but no information was found in their role in transport. At least they are not regarded as flower visitors so their role in potential cross pollination problems is judged to be negligible.

6 Risk driven containment measures in different pest control settings

When using biocontrol against pests on genetically modified crops, the same criteria for risk prevention could be applied as in experiments where arthropod pests are chemically controlled. This means that the biocontrol agent should preferably be as effective as the alternative chemical treatments in terms of pest levels that do not harm the crop in an unacceptable way and does not increase the risk of gene-escape from the experimental climate room or greenhouse.

Taking the baseline that strict guidelines for containment of transgenic plants are meant to avoid the spread of transgenic plant material into the environment the management of organisms that often infest such plants should focus on the risk of different management options fir the spread of that material. For the current study we conclude that spread of pollen is the key issue to prevent which depends on the numbers and properties of the organisms present rather than the chemical or biological control systems to be used. It is the spreading capacity of transgenic material of the pest species and –in case of biocontrol- the natural enemies that counts for the potential risk and the additional containment measures to be taken.

There seems no additional risk involved with biocontrol in crops where pollen are absent or infertile. In that case pest control can be chemical or biological according to the effectivity required and the presence or even escape of arthropods in the facility do not create any risk for spread of transgenes. This means that standard containment measures can be applied for working protocols and handling of plants and soil (which are the primary source of transgenic material).

Where pollen do play a role, care should be taken in several ways to avoid spread of transgenes by the pest species as well as the natural enemies in case biocontrol is applied.
In this case the least risky situation is where the pest species as well as the natural enemies are not associated with flowers and when their densities, their mobility as well as their tendency to escape is low. A typical example is when caterpillars are controlled by parasitoids. In the case of caterpillars the application microbial control with Bacillus thuringiensis can be considered as an alternative for chemical control.

When under biocontrol or regimes densities of pests and natural enemies are expected to be higher, the potential risk of escaping organisms is assumed to be higher but still the risk can be low when both the pest as well as the natural enemies are not associated with flowers and the potential transport of pollen is low. This is the case in control of spider mites with not-pollen feeding predatory mites or mirid bugs.

Also the risk of biological white fly control is likely to be low in pollen-poor plants as the control often is very good and the parasitoids are not assumed to carry much pollen. However as both the pest and the parasitoids are good flyers and easily visit other plant when coming into the environment, containment measures in the facilities should be more strict

In particular care should be taken when the main pest is a flower-visiting species such as thrips, as they tend to carry higher pollen loads and are able to fly and easily attach to clothing. An additional problem might occur when predatory mites are used that do like pollen. Potentilally potential pollen spread might be even higher when a highly mobile pollen feeding natural enemy such as *Orius* is used.

In that case high control effectivity is needed and pest levels should be monitored during the experiment. For such situations containment restrictions should be followed closely, even though the real risk of outcrossing mediated by escaping thrips or *Orius* bugs in the natural environment is unknown. For thrips control predatory mites might be preferred over predatory bugs when effective, as bugs are likely to be more risky in pollen spread due to their high mobility and flying capacity

In almost all cropping systems very hard to control aphids effectively with natural enemies, so control should preferably be done with pesticides. Ineffective control may result in high aphid densities, which induces winged morphs that can more easily spread pollen than un-winged morphs. Also the potential uses of predators such as lady birds or hoover flies is not without risk as they are good fliers and both larvae and adults consume pollen and are able to transport them.

In all cases where pollen play a role, the choice of natural enemies such as parasitoids, predatory mites or predatory bugs could be based on their pollen spreading capacity. For the most risky systems where biocontrol is moderately effective and the pest as well as the natural enemy can transport pollen biocontrol with microbials (if available) or chemical control might be a better option.

But in case biocontrol is more effective or requiered for other reasons, more strict containment options should be advised for the facilities. In case pollen transport should be prevented with high certainty the risk of escape of insects and mites can be minimized using the guidelines for containment of transgenic insects as described by Booij (2013).

In particular when the risk of outcrossing is considerable, e.g. when the same or related plants are grown within the dispersal range of the pests and biocontrol agents, such strict containment may be deemed necessary. In such cases chemical control may be preferred or the use of microbial control with entomopathogenic bacteria (Bt), fungi or viruses can be considered.

7 Discussion and recommendations

The risk assessment for application of biological pest control in experimental and other pre-market test facilities with GM crops is most easy for those crops that do not produce (fertile) pollen, as pollen seems to be the major route for gene-transfer outside the facilities. The fate of genetic material that escapes from GM facilities is out of the scope of this report but the focus of GM plant containment tends to be on seeds and in particular pollen as this may lead to genetic ‘contamination’ of natural populations of plants. Neither the biocontrol agents used for the control of insects and mites nor the pests themselves are known to transport seeds, but many species of both categories can potentially carry pollen. In case pests and natural enemies are associated with flowers (or consume pollen) the application of biological control can lead to risk for pollen spread in particular when they are mobile and occur in higher densities.

In case of higher risk combinations for crops, pests and natural enemies the applicability of biocontrol depends on the efficacy of the pest control strategy in relation to the pollen-spreading capacity of both the prey and the biocontrol agent. In such cases where there is a positive trade-off between the advantage of applying biocontrol and the potential risks involved, containment measures need extra attention. Another option is to choose for microbial control with entomopathogenic bacteria or fungi when available and effective.

Current knowledge on the pollen spreading capacity of most natural enemies used in biocontrol (as well as their prey) is very limited, even more so when it comes to their relative role in cross-fertilization of plants, which is the major concern for escape of transgenes. Pollen attachment very much depends on their exposure towards pollen, morphological properties of the insects and mites as well as pollen properties. Pollen biomass and insect biomass present in the system will finally affect the amount of pollen potentially transported.

Pest species such as fungus gnats, thrips and mites, and natural enemies such as predatory mites and bugs are all able to feed and carry pollen to some extent. For a proper analysis of the risks more research in this field it is needed to quantify potential pollen spread in a more quantitative way. With the current knowledge only qualitative estimates expert guesses can be made on the risk of pollen spread by pests and natural enemies. The step from pollen spread risk to outcrossing-pollination risk was outside the scope of this stud. It is good to realize that in pollination ecology the current opinion is that, apart from bees, hoover flies and some beetles, other insects play an insignificant role in pollination in most cases. For transgenic risk research, however also rare events may be relevant depending on the impact of such events.

When transport of pollen by flower visiting species that may escape from GM-crop facilities is the main point of concern, the question about applicability of biocontrol in such facilities in this study focused on the risk of escaping pollen carrying pests and natural enemies. For most combinations of pests and natural enemies applied, this risk is regarded to be low as long as the pest control is sufficient effective and the usual containment measures are applied. It is assumed that pollen spread and pollination potential in those cases is probably insignificant and should not be an obstacle for biocontrol application. In the case effectivity is variable and the species involved substantially transport pollen and visit flowers (hence pollen spread is much more likely) additional containment measures should be applied in a way that is similar to those formulated for genetically modified insects. This could be the case for example in a pollen rich crop such as sweet pepper in combination with flower thrips and *Orius* bugs, that do both carry pollen and are highly mobile.
However, to reduce the uncertainty about the extent that pests and biocontrol species transport pollen and are able to cause cross pollination after occasional escape, much more knowledge is needed.

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Annex 1 Efficacy of biological control in different cropping systems

Table A

*Most important pests in **tomato crops** and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
whiteflies	*Trialeurodes vaporariorum* *Bemisia tabaci*	*Macrolophus pygmaeus* (predator) *Encarsia formosa* *Eretmocerus eremicus*(parasitoids)	combination of generalist predator + parasitoids is very effective. Predators need inoculative releases and can be boosted with alternative food: Artemia/Ephestia
leafminers	*Liriomyza bryoniae, L. trifolii and L. huidobrensis*	*Diglyphus isaea* *Dacnusa sibirica* (parasitoids)	not always effective (reasons not clear)
Aphids	several species, e.g. *Myzus persicae* *Aulacorthum solani* *Macrosiphum euphorbiae*	Aphidius parasitoids (*Aphidius matricariae, A. colemani, A. ervi*)	Not always effective
caterpillars	several species, e.g *Chrysodeixis chalcites* *Lacanobia oleracea*	Trichogramma spp. (egg parasitoids)	natural enemies not always effective
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	not always effective, overkill needed
tomato russet mite	*Aculops lycopersici*	generalist predatory mites, e.g. *Amblyseius swirskii*	poor establishment because of glandular trichomes
western flower thrips (only on young plants)	*Frankliniella occidentalis*	predatory mites, predatory bugs	no establishment natural enemies because of glandular trichomes
mealybugs (not common)	*Pseudococcus viburni*	parasitoids and *Cryptolaemus montrouzieri*	efficacy in tomato not known, in ornamentals not always effective
tomato psyllid*	*Bactericera cockerelli*	not available	-

***** not present in Europe

Table B

Most important pests in ***sweet pepper*** *crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
Aphids	several species, e.g. *Myzus persicae* *Aulacorthum solani* *Aphis gossypii* *Macrosiphum euphorbiae* etc.	*Macrolophus pygmaeus* (generalist predatory bug) *Aphidoletes aphidimyza* (predatory midge) *Episyrphus balteatus* (syrphid) *Adalia bipuctata* (ladybird beetle) several species of parasitoids (Aphelinus, Aphidius, Ephedrus, Praon)	often not effective enough
caterpillars	several species, e.g *Chrysodeixis chalcites* *Lacanobia oleracea*	Trichogramma spp. (egg parasitoids)	natural enemies not always effective
western flower thrips	*Frankliniella occidentalis*	*Orius laevigatus* (predatory bug) *Amblyseius swirskii* (predatory mite) several other species of predatory mites	very successful and stable system
Echinothrips	*Echinothrips americanus*	*Orius majusculus* (predatory bug) *Amblydromalus limonicus* (predatory mite)	establishment of *O. majusculus* can be a problem, but is general very effective
tarnished plant bug the common nettle bug the common green capsid	*Lygus rugulipennis* *Liocoris tripustulatus* *Lygocoris pabulinus*	not available	-
whiteflies (not common)	*Bemisia tabaci*	*Amblyseius swirskii* (predatory mite) *Eretmocerus eremicus* (parasitoid)	very successful and stable system
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	very effective
broad mites	*Polyphagotarsonemus latus*	*Amblyseius swirskii* (predatory mite)	very effective
pepper weevil* (q-organism)	*Anthonomus eugenii*	not available	-
green leafhopper	*Empoasca vitis*	not available	-

***** not present in Europe

Table C

Most important pests in ***cucumber*** *crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
western flower thrips	*Frankliniella occidentalis*	*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* (predatory mites)	in general very successful and stable system, predatory mites need support (rearing sachets/pollen)
Aphids	several species, but mainly *Aphis gossypii*	*Aphidoletes aphidimyza* (predatory midge) *Aphidius colemani* (parasitoid)	not always effective
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	in general very effective
whiteflies	*Trialeurodes vaporariorum* *Bemisia tabaci*	*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* (predatory mites) *Encarsia formosa* *Eretmocerus eremicus*(parasitoids)	in general very successful and stable system
caterpillars	several species, e.g *Chrysodeixis chalcites* *Lacanobia oleracea*	Trichogramma spp. (egg parasitoids)	natural enemies not always effective
tarnished plant bug the common nettle bug the common green capsid	*Lygus rugulipennis* *Liocoris tripustulatus* *Lygocoris pabulinus*	not available	-

Table D

Most important pests in greenhouse ***strawberry*** *crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
western flower thrips	*Frankliniella occidentalis*	*Amblyseius swirskii* *Amblydromalus limonicus* (predatory mites) *Orius laevigatus* (predatory bug)	Not always effective due to poor establishment of predators
Aphids	several species	*Aphidoletes aphidimyza* (predatory midge) *Episyrphus balteatus* (syrphid) *Adalia bipuctata* (ladybird beetle) Chrysoperla carnea (lacewings) several species of parasitoids (Aphelinus, Aphidius, Ephedrus, Praon)	not always effective
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* *Neoseiulus californicus* (predatory mites)	in general very effective
whiteflies	*Trialeurodes vaporariorum*	*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* (predatory mites) *Encarsia formosa* *Eretmocerus eremicus*(parasitoids)	can be a successful and stable system
caterpillars	several species	Trichogramma spp. (egg parasitoids)	natural enemies not always effective

Table E

*Most important pests in **rose** crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
western flower thrips	*Frankliniella occidentalis*	several species of leaf-dwelling predatory mites (*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* *Amblyseius cucumeris,* *Euseius gallicus*) *Macrocheles robustulus* (soil-dwelling predatory mite)	limited effects
Echinothrips	*Echinothrips americanus*	*Amblydromalus limonicus* (predatory mite)	limited effect
Aphids	several species, e.g. *Myzus persicae* *Aulacorthum solani* *Aphis gossypii* *Macrosiphum euphorbiae* etc.	*Aphidoletes aphidimyza* (predatory midge) *Episyrphus balteatus* (syrphid) *Adalia bipuctata* (ladybird beetle) several species of parasitoids (Aphelinus, Aphidius, Ephedrus, Praon) *Aphidoletes aphidimyza*	often not effective enough
caterpillars	several species, e.g *Chrysodeixis chalcites* *Lacanobia oleracea*	Trichogramma spp. (egg parasitoids)	natural enemies not always effective
whiteflies	*Trialeurodes vaporariorum* *Bemisia tabaci*	*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* (predatory mites) *Encarsia formosa* *Eretmocerus eremicus*(parasitoids)	in general successful and stable system
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	very effective
mealybugs (not common)	*Planococcus citri* *Pseudococcus longispinus* *Pseudococcus viburni*	parasitoids and *Cryptolaemus montrouzieri*	not always effective
armoured scales	*Aulacaspis rosae*	*Rhyzobius lophanthae*	not always effective
rose leafhopper	*Edwardsiana rosae*	not available	-

Table F

*Most important pests in **gerbera** crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
whiteflies	*Trialeurodes vaporariorum* *Bemisia tabaci*	*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* (predatory mites) *Encarsia formosa* *Eretmocerus eremicus*(parasitoids)	successful in summer, not in winter at low temperatures
Echinothrips	*Echinothrips americanus*	*Amblydromalus limonicus* (predatory mite)	limited effect
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	very effective
Tarsonemid mites (including broad mites)	*Polyphagotarsonemus latus* *Tarsonemus violae*	*Amblyseius swirskii* (predatory mite)	not always effective
Aphids	several species, e.g. *Myzus persicae* *Aulacorthum solani* *Macrosiphum euphorbiae* etc.	*Aphidoletes aphidimyza* (predatory midge) *Episyrphus balteatus* (syrphid) *Adalia bipuctata* (ladybird beetle) several species of parasitoids (Aphelinus, Aphidius, Ephedrus, Praon)	often not effective enough
caterpillars	several species, e.g *Chrysodeixis chalcites* *Lacanobia oleracea* *Duponchelia fovealis* *Clepsis spectrana*	Trichogramma spp. (egg parasitoids) *Stratiolaelaps scimitus*(soil-dwelling predatory mite against Duponchelia)	natural enemies not always effective
western flower thrips	*Frankliniella occidentalis*	several species of leaf-dwelling predatory mites (*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* *Amblyseius cucumeris,* *Euseius gallicus*) *Macrocheles robustulus* (soil-dwelling predatory mite)	not always effective
leafminers	*Liriomyza trifolii*	*Diglyphus isaea* *Dacnusa sibirica* (parasitoids)	in general effective and stable system
mealybugs	*Planococcus citri* *Pseudococcus longispinus* *Pseudococcus viburni*	parasitoids and *Cryptolaemus montrouzieri*	not always effective

Table G

Most important pests in ***chrysanthemum*** *crops and options for biological control.*

common name pest	scientific name pest	available natural enemies	efficacy biological control
western flower thrips	*Frankliniella occidentalis*	several species of leaf-dwelling predatory mites (*Amblyseius swirskii* *Amblydromalus limonicus* *Amblyseius montdorensis* *Amblyseius cucumeris* *Macrocheles robustulus* (soil-dwelling predatory mite) *Orius laevigatus* *Orius majusculus* (predatory bugs)	limited effects, unstable system because of short growing cycles and poor establishment of predators
spider mites	*Tetranychus urticae*	*Phytoseiulus persimilis* (predatory mite)	very effective
leafminers	*Liriomyza trifolii*	*Diglyphus isaea* *Dacnusa sibirica* (parasitoids)	in general effective and stable system
Aphids	several species, but mainly *Aphis gossypii*	*Aphidoletes aphidimyza* (predatory midge) *Aphidius colemani* (parasitoid)	not always effective
tarnished plant bug the common nettle bug the common green capsid	*Lygus rugulipennis* *Liocoris tripustulatus* *Lygocoris pabulinus*	not available	-

Corresponding address for this report:
P.O. Box 16
6700 AA Wageningen
T 0317 48 06 70
The Netherlands
www.wageningenUR.nl/en

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