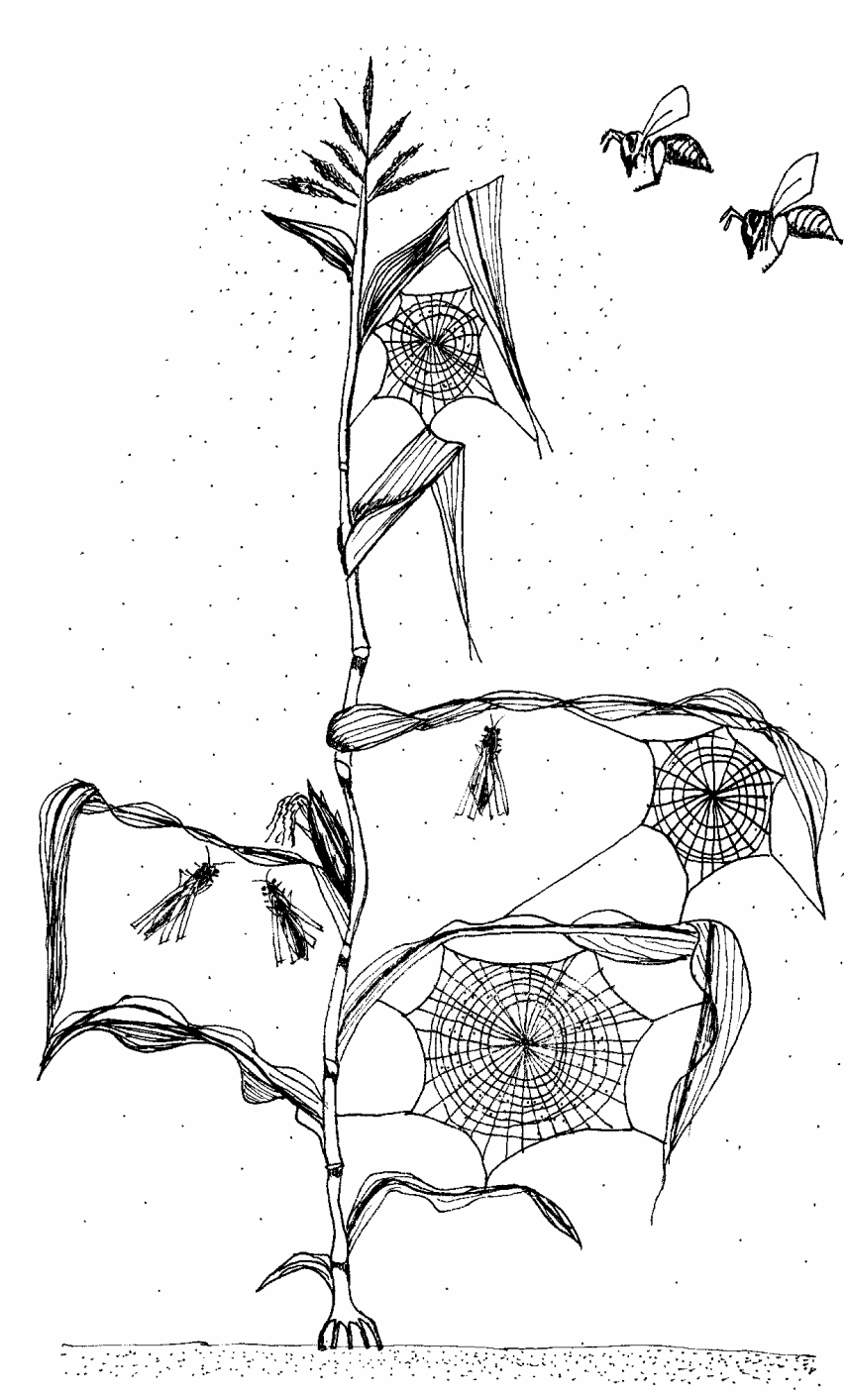
A risk assessment of genetically modified organisms:

Potential effects of *Bt* maize on spiders



As our circle of knowledge expands,
so does the circumference of darkness surrounding it.

Attributed to Albert Einstein

A risk assessment of genetically modified organisms:

Potential effects of *Bt* maize on spiders

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Summary

Concerns have been raised that genetically modified *Bt* maize expressing the Cry1Ab protein of the soil bacterium *Bacillus thuringiensis* (*Bt* toxin) may harm non-target organisms, and there is a general call and need for a risk assessment of *Bt* maize. Spiders are important pest predators in agroecosystems and in maize, and can be exposed to the *Bt* toxin by active *Bt* maize pollen feeding, by ingesting their pollen-dusted webs and by preying on herbivorous or pollen-collecting prey. In this thesis, the risk potentially arising from *Bt* maize for foliage-dwelling spiders was assessed by (i) recording base line data of spiders in maize fields and adjacent margins, (ii) by an evaluation of adequate sampling methods for foliage-dwelling spiders, (iii) by the investigation of exposure pathways of *Bt* maize to spiders and (iv) by assessing the actual *Bt* effects on foliage-dwelling spiders on laboratory and field scale.

For recording base line data as well as for the evaluation of different sampling methods of the foliage-dwelling fauna in maize fields and field margins, foliage-dwelling spiders were collected in maize fields and on adjacent margins in Bavaria, South Germany. Two different sampling methods were evaluated: drop cloth sampling and suction sampling. Maize fields and adjacent field margins were colonized by a typical spider assemblage, consisting of juvenile web-building spiders e.g. Theridiidae (cob-web spiders), Linyphiidae (sheet-web spiders), Tetragnathidae (long-jawed spiders) and Araneidae (orb-web spiders, sensu stricto) in decreasing order with a dominance of space-web spiders (Theridiidae and Linyphiidae). Generally, field margins harboured more spider individuals and spider species than maize fields. Suction sampling with a small suction device proved to be a more efficient and consistent sampling method for foliage-dwelling spiders than drop cloth sampling. Abundance and species richness of foliage-dwelling spiders in maize was shown to be fairly high, implying that spiders of higher strata may play a more important role in biological control than suspected up to now.

To gain information on the potential exposure of spiders to *Bt* maize pollen, data on the temporal occurrence, spatial range and dispersion of maize pollen, and thus potentially *Bt* maize pollen densities in field margins in Bavaria, South Germany was acquired. The *Bt* maize event 176 (variety “Navares” by Syngenta) shed pollen generally during July. Pollen numbers deposited in field margins were highly variable and decreased with distance to field edge. The main factors determining pollen densities on field margins were various climate parameters, growth stage of the maize and distance to field edge. Cry1Ab amounts in *Bt*

maize event 176 pollen was with 2.5 µg/g pollen found to be in the range reported so far. Furthermore, *Bt* maize pollen was quantified in orb-webs in maize fields and on adjacent field margins by counting pollen in spider webs which were exposed in maize fields and on field margins during maize anthesis. In both habitat types, web-building spiders may be exposed to high amounts of *Bt* maize pollen. An enzyme-linked immunosorbent assay (ELISA) proved an uptake of the Cry1Ab protein by spiders via the consumption of *Bt* maize pollen dusted webs. The exposure of spiders to potential *Bt*–contaminated prey was investigated by the assessment of the actual prey spectra and the selectivity of webs as well as individuals of two orb-web spider species, the garden spider *Araneus diadematus* (Clerck) and the wasp-like spider *Argiope bruennichi* (Scopoli) (both species Araneae: Araneidae) on two different structured field margins. In general, prey spectra of both spider species on field margins consisted of a few, in arable land frequent, taxa (e.g. Diptera, Sternorrhyncha, Heteroptera, Coleoptera) and were dominated by Diptera. In spider webs, small-sized, broad-winged prey items such as Sternorrhyncha were caught easily, whereas mobile prey with good optical skills such as Diptera and Hymenoptera could probably avoid spider webs. Armed prey with strong mandibles or stings such as Coleoptera and Hymenoptera were avoided by individuals of both spider species. However, both spider species differed in their selectivity to Apidae. Whereas Apidae were avoided by individuals of *A. diadematus*, *A. bruennichi* showed no avoidance towards this prey. In conclusion, the investigation of possible exposure pathways of *Bt* maize to spiders showed that spiders are potentially exposed to *Bt* maize pollen in spider webs in maize fields and on field margins as well as to *Bt*-contaminated prey on field margins. However, the wasp-like spider may be more exposed to *Bt* pollen-collecting prey than the garden spider.

The potential effect of *Bt* maize on spiders was investigated in a laboratory assay and a 3-year monitoring of *Bt* maize on field scale in Bavaria, South Germany. The laboratory experiment was conducted to evaluate possible effects of the consumption of *Bt* maize pollen on juvenile *A. diadematus*. This study showed no effects of *Bt* maize pollen on weight increase, survival, moult frequency, reaction time towards prey and various web variables of *A. diadematus*. However, a pyrethroid insecticide (Baythroid) affected weight increase, survival and reaction time of spiders negatively. A 3-year field-scale monitoring on the foliage-dwelling spider fauna of *Bt* maize fields and adjacent margins showed no negative effect on individual numbers, species richness and guild structure of foliage-dwelling spiders in maize fields and adjacent field margin strips.

Concerning the risk assessment of genetically modified plants, ecological risk is defined as a function of exposure and effect, i.e. the product of the likelihood of exposure and the magnitude of an adverse effect. Including the fact that no lethal effect of *Bt* maize on spiders on laboratory as well as on field scale was found, a high risk of *Bt* maize event 176 to spiders can not be confirmed with this thesis. However, as this is the first study on this topic and high variation as well as small effect sizes may have masked existing effects, further studies on different temporal and spatial scales are required to allow general statements. Furthermore, studies on potential sublethal or longterm effects should be conducted to assess a possible effect on spider populations and the concurrent biocontrol efficacy of spider assemblages in agroecosystems.

Zusammenfassung

Genetisch veränderter *Bt* Mais exprimiert das Protein Cry1Ab (*Bt* Toxin) des Bodenbakteriums *Bacillus thuringiensis*. Da Hinweise auf mögliche Effekte des *Bt* Toxins auf Nichtzielorganismen existieren, wird eine Risikoabschätzung von *Bt* Mais notwendig. Spinnen sind wichtige Gegenspieler von Schädlingen in Agrarökosystemen und können aufgrund einer beabsichtigten Aufnahme von *Bt* Maispollen, durch das regelmäßige Fressen ihrer Netze mit anheftenden *Bt* Maispollen und über das Fressen von *Bt*-kontaminierter herbivorer oder pollensammelnder Beute mit dem *Bt* Toxin in *Bt* Mais in Berührung kommen. In dieser Arbeit wurde das Risiko von *Bt* Mais gegenüber pflanzenbewohnenden Spinnen durch (i) die Aufnahme der Spinnenfauna in Maisfeldern und angrenzenden Feldrändern, (ii) der Evaluierung von adäquaten Sammelmethoden für pflanzenbewohnende Spinnen, (iii) der Untersuchung von verschiedenen Expositionswegen von *Bt* Mais für Spinnen und (iv) der Untersuchung eines tatsächlichen Effekts von *Bt* Mais auf Spinnen in Labor und Freiland abgeschätzt.

Für die Aufnahme der Spinnenfauna und für die Evaluierung von verschiedenen Sammelmethoden wurden pflanzenbewohnende Spinnen in Maisfeldern und an angrenzenden Feldrändern in Bayern, Süddeutschland, gesammelt. Dazu wurden zwei verschiedene Sammelmethoden angewendet, das Abklopfen der Pflanzen mit einem Klopfschirm und das Absaugen der Pflanzen mit einem tragbaren Akkusauger. Maisfelder und angrenzende Feldränder wurden von einer typischen Spinnengemeinschaft bewohnt, die sich aus juvenilen netzbauenden Spinnen der Familien Theridiidae (Kugelspinnen), Linyphiidae (Baldachinspinnen), Tetragnathidae (Streckerspinnen) und Araneidae (Radnetzspinnen) zusammensetze und vor allem von raumnetzbauenden Spinnen (Theridiidae und Linyphiidae) dominiert wurde. Im Allgemeinen wurden Feldränder von mehr Spinnenindividuen und –arten besiedelt als Maisfelder. Das Saugen mit einem kleinen tragbaren Akkusauger stellt sich als die effektivere und genauere Methode zum Erfassen von pflanzenbewohnenden Spinnen im Vergleich zum Klopfen mit einem Klopfschirm heraus. Es zeigte sich, dass die Abundanz und der Artenreichum von pflanzenbewohnenden Spinnen in Maisfeldern relativ hoch war. Dies deutet auf eine wichtigere Rolle von Spinnen der höheren Vegetationsstraten hin, als zuvor angenommen wurde.

Um eine potenzielle Exposition von Spinnen an Feldrändern gegenüber *Bt* Mais zu untersuchen, wurden Informationen zur zeitlichen und räumlichen Verteilung sowie zur Ausbreitung von *Bt* Maispollen gewonnen. Event 176 *Bt* Mais (Sorte „Navares“ von

Syngenta) blühte generell im Juli. Die Anzahl der Pollen, der sich an Feldrändern ablagerte, unterlag großen Schwankungen und nahm mit zunehmendem Abstand vom blühenden Maisfeld ab. Die Pollendeposition an Feldrändern wurde durch verschiedene klimatische Faktoren, durch das Wachstumsstadium des Mais und den Abstand zum Maisfeld beeinflusst. Der Gehalt an Cry1Ab in Event 176 *Bt* Mais war mit 2.5 µg/g innerhalb des Wertebereichs, der in der Literatur angegeben wird. Des Weiteren wurde in Maisfelder und Feldrändern die Menge der *Bt* Maispollen in Spinnennetzen quantifiziert, indem der Pollen, der sich in Spinnennetzen während der Maisblüte verfangen hatte, ausgezählt wurde. In beiden Habitattypen waren Spinnen hohen Mengen von *Bt* Maispollen ausgesetzt. Ein ELISA-Test erbrachte den Nachweis der Aufnahme des Proteins Cry1Ab durch Spinnen über das Fressen von Spinnennetzen, die *Bt* Maispollen enthielten. Die Exposition von Spinnen gegenüber potenziell *Bt*-kontaminierter Beute wurde anhand einer Erfassung des tatsächlichen Beutespektrums und der Selektivität von Spinnennetzen und Spinnenindividuen von zwei Radnetzspinnenarten, der Gartenkreuzspinne *Araneus diadematus* (Clerck) und der Wespenspinne *Argiope bruennichi* (Scopoli) (beide Arten Araneae: Araneidae) an zwei unterschiedlich strukturierten Feldrändern untersucht. Im Allgemeinen setzte sich das tatsächliche Beutespektrum von Radnetzspinnen an Feldrändern aus wenigen, in Ackerland häufigen Tiergruppen zusammen (Diptera, Sternorrhyncha, Heteroptera, Coleoptera) und wurde von Dipteren dominiert. Sternorrhyncha, Beutetiere von meist geringer Größe und breiten Flügeln wurden leicht in Spinnennetzen gefangen, während mobilere Beutegruppen mit gutem Sehvermögen wie Dipteren und Hymenopteren wahrscheinlich Spinnennetze meiden konnten. Wehrhafte Beutegruppen mit kräftigen Mandibeln und/oder Stacheln wurden von beiden Spinnenarten gemieden. Jedoch unterschieden sich die Spinnenarten hinsichtlich ihrer Selektivität gegenüber Apidae. Während Apidae von den Individuen der Gartenkreuzspinnen gemieden wurde, zeigt die Wespenspinne keine Aversion gegenüber dieser Beutegruppe. Zusammenfassend zeigte die Untersuchung, dass Spinnen sowohl gegenüber *Bt* Maispollen in Spinnennetzen in Maisfeldern und an angrenzenden Feldrändern als auch gegenüber potentiell *Bt*-kontaminierter Beute an Feldrändern ausgesetzt sind. Insbesondere die Wespenspinne kann mit *Bt*-Pollen sammelnden Blütenbesuchern in Kontakt kommen.

Ein möglicher Effekt von *Bt* Mais auf Spinnen wurde anhand eines Laborversuchs und eines dreijährigen Freilandmonitorings untersucht. Das Laborexperiment wurde durchgeführt, um einen möglichen Effekt einer Aufnahme von *Bt* Maispollen auf juvenile *A. diadematus* zu erforschen. Diese Studie zeigte keinen Einfluss von *Bt* Maispollen auf die Gewichtszunahme,

Überlebenszeit, Häutungsfrequenz und Reaktionszeit gegenüber Beute sowie auf verschiedene Netzbauparameter von *A. diadematus*. Ein Insektizid, das Pyrethroid Baythroid, beeinflusste jedoch die Gewichtszunahme, die Überlebenszeit und die Reaktionszeit negativ. Ein dreijähriges Monitoring in Maisfeldern und angrenzenden Feldrändern zeigte keine negativen Effekte auf die Anzahl der Spinnenindividuen, den Artenreichtum und die Gildenzusammensetzung von pflanzenbewohnenden Spinnen in Maisfeldern und angrenzenden Feldrändern.

In Hinblick auf die Risiko-Abschätzung von genetisch veränderten Pflanzen wird das ökologische Risiko als eine Funktion von Exposition und Effekt, d.h. als Produkt der Eintrittswahrscheinlichkeit und der Höhe des Effekts eines möglichen Störfaktors definiert. In Anbetracht dessen, dass sowohl auf Labor- als auch auf Feldmaßstabsebene kein negativer Effekt von *Bt* Mais auf Spinnen gefunden wurde, kann ein hohes Risiko ausgehend von Event 176 *Bt* Mais auf Spinnen mit dieser Arbeit nicht bestätigt werden. Um jedoch eine allgemeine Aussage hinsichtlich eines Effekts von *Bt* Mais auf Spinnen machen zu können, sind weitere Untersuchungen auf unterschiedlichen, zeitlich und räumlich skalierten Ebenen nötig, da eine hohe Varianz und kleine Effektgrößen einen vorhandenen Effekt überdeckt haben könnten. Außerdem sollten Studien zu potenziell subletalen Effekten und Langzeit-Effekten durchgeführt werden, um einen möglichen Einfluss von *Bt* Mais auf Spinnenpopulationen und damit auf deren Effizienz in der biologischen Schädlingsbekämpfung in Agrarökosystemen abschätzen zu können.

1 Introduction

Bt pesticides based on the bacterium *Bacillus thuringiensis* which produces insecticidal proteins during sporulation, the so-called Cry proteins, are used in agriculture, because they are described as effective and specific against pest organisms (Van Frankenhuyzen 1993). However, *Bt* pesticides have the disadvantage to be active against pests at best only few weeks under field conditions, and mining insects which are protected inside plant stems, are not affected by a surface pesticide application. Therefore, transgenic plants (*Bt* plants) were developed which express Cry proteins of *B. thuringiensis* in plant tissue providing resistance against (mining) pests (Whalon & Wingerd 2003).

Predators and parasitoids are natural enemies of pest organisms in agroecosystems and constitute an important element of biological control for sustainable agriculture. Therefore, these beneficial organisms should not be harmed by plant production measures such as pesticides (Croft 1990). Despite the reported selectivity, there is evidence that Cry proteins may harm non-target organisms such as predators and parasitoids (Groot & Dicke 2002). Furthermore, the Cry protein expressed in *Bt* plants is modified and may be further processed in the plant metabolism which could affect the selectivity and toxicity of Cry toxins to non-targets (Fearing 1997, Perlak et al. 1991). As the area of planted transgenic insect resistant plants increase worldwide (James 2003), a risk assessment of transgenic insect resistant plants is needed and demanded (e.g. European Parliament and Council 2002, Andow & Hilbeck 2004).

In this thesis, the potential risk of *Bt* maize event 176, expressing the Cry1Ab toxin, to foliage-dwelling spiders, as general predators, was assessed on different temporal scales as well as on laboratory and field scale.

The following chapters provide information about the mode of action of Cry1 toxins known so far. Furthermore, differences between *Bt* pesticides and transgenic *Bt* plants are discussed and information on known non-target effects of Cry toxins is provided. Finally, methods of risk assessment of genetically modified plants (GMPs) are introduced and the choice of spiders as test organism for a GMP risk assessment is justified. In the subsequent conceptual approach, the studies of this thesis are shortly described and references to publications on which this thesis is based are given.

1.1 *Bacillus thuringiensis* toxins and its mode of action

The gram-positive, spore-forming soil bacterium *Bacillus thuringiensis* (Berliner) produces protein crystals during sporulation. Besides several other virulence factors, these crystals consist of entomopathogenic proteins, called "Cry proteins" (De Maagd 2001). Different Cry proteins exist, derived from different *B. thuringiensis* strains which were classified according their toxicity to insect taxa. So Cry1 proteins are described to be toxic to Lepidoptera, Cry2 proteins to Lepidoptera and Diptera, Cry3 proteins to Coleoptera and Cry4 to Diptera (Höfte & Whiteley 1989). Due to the insecticidal effectiveness and the specificity to pest targets of Cry proteins, commercially available pesticides based on *B. thuringiensis* were used in agriculture since the 1930s (Van Frankenhuyzen 1993). The specificity of *Bt* toxins is due to the mode of action which includes several steps involved in specificity. In the following, an overview of the mode of action of Cry1 toxins from *B. thuringiensis* var. *kurstaki* on lepidopteran larvae is given. However, the mode of action and determinants of the specificity of *Bt* toxins have not been completely understood so far (see reviews in Gill et al. 1992, Schnepf et al. 1998, De Maagd et al. 2001, Bravo et al. 2004).

After the ingestion of *B. thuringiensis* var. *kurstaki* spores by a lepidopteran larva, the protein crystals dissolve in the alkaline environment of the insect midgut. Consequently, a Cry1 protein of 130 kDa (Cry protoxin) is released in the midgut and subsequently, a fragment of the Cry protoxin is cleaved from the C-terminal of the protein leading to an activated toxin of 60 kDa. This cleavage of *Bt* protoxins in the insect gut, a proteolysis, is mediated by bacterial proteases and trypsin as well as by specific lepidopteran midgut proteases (Rukmini et al. 2000, Miranda et al. 2001). An activated Cry1 toxin consists of three main protein domains (I-III) (Grochulski et al. 1995). The hydrophobic domain I on the N-terminal of the protein is a bundle of seven amino acid α-helices. Domains II and III are located on the C-terminal of the protein and consist of amino acid β- sheets. Domain I inserts into the midgut epithelium and is involved in the formation of transmembran spanning channels. Whereas domain II is responsible for the binding on a specific midgut cell receptor, domain III is probably involved in both functions of domain I and II (Dean et al. 1996).

After the activation of the Cry1 toxin, it binds to specific receptors on the brush border membrane of midgut epithelium cells (Van Rie et al. 1990). Several receptors participate in the mode of action such as the receptor BT-R_1, a cadherin (Vadlamudi et al. 1995) and the aminopeptidase N (Luo et al. 1997). The binding on receptors leads to an irreversible insertion of α-helices of domain I into the midgut membrane (Bravo et al. 2004) which

contributes to a formation of ion channels (English et al. 1995). Ion channels mediate an influx of ions into the midgut membrane cells which is followed by an osmotic water influx and results in the burst of midgut epithelium cells (Knowles & Ellar 1987). Organisms susceptible to Cry1 toxins die from gut lysis and septicemia as well as from paralysis and starvation after the ingestion of Cry proteins (Heimpel & Angus 1959, Gringorten 2001). To summarise, an alkaline midgut environment for the solution of protein crystals, midgut proteases for the activation of the protoxin as well as midgut ephithelium receptors for the catalysis of transmembran channel forming are steps responsible for the specificity of Cry1 toxins.

The mechanism of action of Cry1 toxins in other organisms than lepidopteran larvae is not investigated so far. Nevertheless, Cry toxins bind not only to midgut epithelium cells, but also to several other epithelium cells (Gill et al. 1992) and show even toxicity to insect brain cells (Cerstiaens et al. 2001). In addition, *Bacillus thuringiensis* var. *kurstaki* producing Cry1 toxins may also cause chromosome aberrations in Orthoptera (Ren et al. 2002) which suggests another mode of action of Cry1 proteins than the described one.

1.2 Genetically modified *Bt* plants

As conventional *Bt* pesticides on the base of the bacterium *B. thuringiensis* have a low persistence under field conditions necessitating costly multiple spraying and do not reach boring pests (Whalon & Wingerd 2003), genetically modified crops were developed which produce Cry toxins themselves (De Maagd et al. 1999). To date, genetically modified insect resistant cotton, tomatoes, corn and potatoes expressing Cry1Ac, Cry1Ab, Cry1F, Cry3A and some other Cry proteins are commercially available (Nap et al. 2003, AgBiosafety 2005).

Generally, the biosynthesis of proteins from genes occurs in two steps, the transcription from desoxyribonucleic acid (DNA) in genes to ribonucleic acid (RNA) and the translation from RNA to proteins (Knippers 1995). The transcription in plants is mainly controlled by two DNA sequences adjacent a coding gene, the promoter and the terminator. The promoter is located before the coding gene and determines the start and the terminator is located behind the coding gene and determines the end of the transcription (Brandt 2004). Thus, to develop Cry toxin expressing plant cells and plants, the target Cry gene of *B. thuringiensis* and a marker gene to identify transformed cells, as well as promoters and terminators for each of those genes were integrated in the plant genome (Fearing et al. 1997, AgBiosafety 2005).

As the expression of wild-type *B. thuringiensis* genes in plants is suboptimal due to differences between bacterial and plant genomes (De Maagd et al. 1999), wild-type *B. thuringiensis* genes were modified in order to enhance the quantity and toxicity of Cry toxins in plant tissue (Perlak et al. 1991). However, several factors determine the location and quantity of Cry1 expression in *Bt* plants. In the plant genome integrated promoters determine, in which plant tissue the Cry toxin is expressed (Bernal et al. 2002). Often wild-type or modified cauliflower mosaic promoters (CaMV 35S) were integrated in the plant genome which is expressed in green plant tissue, but not or only marginally in pollen (Wilkinson et al. 1997, Hilbeck & Andow 2002). *Bt* maize event 176, however, carries a phosphoenolpyruvate carboxylase promoter (PEPC) which is expressed in green plant tissue and additionally a maize-specific promoter which is expressed only in maize pollen (Fearing et al. 1997). So the mean Cry1Ab content in pollen of event 176 *Bt* maize is about 2 µg per g fresh weight, whereas other *Bt* maize varieties show only low Cry1Ab contents in pollen (Hilbeck & Andow 2002). Also with the same promoters, the Cry1Ab content may differ between maize varieties. Mature leaves e.g. may have a Cry1Ab concentration from approximately 0.4 to 80 µg per g fresh weight (Hilbeck & Andow 2002). Even in the same *Bt* maize variety, the Cry1Ab toxin may decrease during the vegetation period, probably dependent on an altering chlorophyll content in leaves (Fearing et al. 1997, Abel & Adamczyk 2004, for *Bt* cotton see Greenplate 1999).

As *Bt* maize event 176 produces the truncated active Cry1Ab toxin, the midgut pH as well as specific proteases of an organism as specificity factors are no longer involved in the action mechanism of the Cry1Ab toxin. Furthermore, the activated protein expressed in plants differs in size from the protein expressed in *B. thuringiensis* and truncated in the lepidopteran gut. Additionally, there is evidence that the plant-produced Cry1Ab protein is further truncated or modified in the plant metabolism (Fearing et al. 1997). There is no data available so far, how these modifications of the Cry1Ab in *Bt* plants may affect the mechanisms of toxin action of Cry1Ab. Nevertheless, there is evidence, that even small changes in Cry domains binding on receptors may cause differences in the selectivity and toxicity of Cry proteins (Haider & Ellar 1989).

In addition to a possible lower specificity of *Bt* plants due to an expression of active Cry toxins, transgenic *Bt* plants pose a higher exposure to non-targets as compared to *Bt* pesticides. This is because Cry toxins in *Bt* plant tissue are at least present for several months over the whole vegetation period of *Bt* plants (Fearing et al. 1997), whereas Cry proteins from conventional *Bt* pesticides generally remain active only for several weeks under field

conditions (Meadows 1993). Furthermore, Cry toxin-containing pollen of *Bt* plants is spread by wind or pollinators in adjacent habitats and could harm non-targets there (Jarosz et al. 2003).

1.3 Non-target effects of *Bacillus thuringiensis* and transgenic *Bt* plants

Despite the reported specificity of Cry1 proteins for lepodopteran pests, there is evidence for adverse effects of these toxins on non-target organisms. Direct negative effects of *B. thuringiensis* var. *kurstaki* and related pesticides, containing at least one of the proteins Cry1Aa, Cry1Ab and Cry1Ac were reported for Neuroptera (e.g., *Chrysoperla carnea*, Haverty 1982, Jayanthi & Padmavathamma 1996), Coleoptera (e.g., *Hippodamia convergens*, Haverty 1982), hymenopteran parasitoid species (e.g., Schuster 1994), as well as mites (e.g., *Argas persicus*, Hassanain et al. 1997) (see Glare & O´Callaghan 2000 and Groot & Dicke 2002 for review).

Bt plants expressing Cry proteins may affect non-target organisms directly by consuming *Bt* plant tissue as herbivores as well as *Bt* toxin-contaminated prey or hosts as predators and parasitoids (Hilbeck & Andow 2002). Herbivores not sensitive to *Bt* toxins may pass the toxin to its predators where *Bt* toxins may cause adverse effects (Chapman & Hoy 1991). This could be due to a specific procession of the Cry protein during digestion in herbivores which may bias or determine susceptibility to Cry proteins in predators (Miranda et al. 2001).

Out of 21 laboratory studies published in peer-reviewed journals on potential effects of Cry1 toxins on predators and parasitoids (Tab. 1), 13 (62 %) studies showed negative and 8 (38 %) studies showed no effect. In none of these laboratory assays, positive effects were demonstrated. In detail, negative effects of activated Cry1 toxins derived from transgenic plants (i.e., *Bt* maize, *Bt* cotton, *Bt* rape, *Bt* rice) or offered as pure activated toxins were reported on development and mortality of the green lacewing *Chrysoperla carnea* as well as of various species of hymenopteran parasitoids (Tab. 1). Furthermore, negative effects of *Bt* plant tissue were recorded on the development of the carabid beetle *Poecilus cupreus*, the survival of the coccinellid beetle *Propylea japonica* as well as on the survival of the heteropterans *Orius tristicolor* and *Geocoris punctipes* (Tab. 1). However, some laboratory assays on possible effects of GMPs on non-target organisms showed contradicting results. Hilbeck et al. 1998a, e.g., demonstrated an increased mortality in *Chrysoperla carnea* when an artificial diet with the pure activated Cry1Ab *Bt* toxin was offered, whereas Romeis et al.

2004 found no different mortality between Cry1Ab and control treatment for this predator. Furthermore, Schuler et al. 2004 showed a negative effect on survival of the braconid *Cotesia plutellae* when fed with *Bt*-reared *Bt*-susceptible prey, but not when fed with *Bt*-reared *Bt*-resistant prey (Tab. 1). Due to these inconsistent results, it is discussed whether the observed tritrophic effects are direct Cry1Ab effects due to a contamination of prey by Cry1Ab or if these effects are dependent on a suboptimal nutrient quality of susceptible *Bt* plant-tissue fed prey (Pilcher et al. 1997, Dutton et al. 2003a, Andow & Hilbeck 2004, Prütz & Dettner 2004, Romeis et al. 2004). However, existing laboratory studies to elucidate adverse effects of genetically modified plants are limited and mainly cover the green lacewing *Chrysoperla carnea* and several hymenopteran parasitoid species, whereas only one study addressed a carabid species and no laboratory study was conducted on spiders as dominant predatory groups in arable land (see also Lövei & Arpaia 2005, Tab. 1).

As laboratory assays under controlled environmental conditions may not minic field conditions sufficiently, also large-scale field investigations are needed for a risk assessment of GMPs (Birch & Wheatley 2005). Out of 21 field investigations published in peer-reviewed journals on potential effects of *Bt* plants expressing Cry1 proteins on predators and parasitoids, 4 (19 %) studies showed negative, 3 studies showed positive (14 %) and 16 (71 %) studies showed no or inconsistent effects of *Bt* plants on single predator or parasitoid taxa (Tab. 2). Negative field effects of *Bt* maize were described for heteropterans of the genus Nabis and the hymenopteran parasitoid *Macrocentrus cingulum* in the USA, as well as on the dipteran parasitoid family Tachnidae in France (Tab. 2). Positive effects of *Bt* maize were reported for the predatory bug *Orius insidosus*, as well as the coccinellid beetles *Coleomegilla maculata* and *Cycloneta munda* in the USA and on Neuroptera in France (Tab. 2). In Australia, *Bt* cotton showed negative effects on the spider family Salticidae and the heteropteran predators *Geocoris spec.* and *Nabis spec.* (Tab. 2).

Comparing results of laboratory assays with results of field investigations, it is conspicuous, that possible sublethal and lethal effects of *Bt* plants recorded in the laboratory, e.g., on the green lacewing *Chrysoperla carnea,* were not recorded under field conditions. This may be due to a lower exposure to Cry1 toxin under field conditions (Birch & Whitely 2005), but also due to drawbacks of existing field studies which may have prevented the detection of existing effects. So, e.g., plot sizes of existing field studies were often smaller than the common commercial field size (< 0.5 ha) which may have reduced differences between treatment plots in the abundance of mobile taxa (Duffield & Aebischer 1994, Prasifka et al. 2005, Tab. 2).

Table 1: Laboratory assays on potential effects of Cry1 toxins on parasitoids and predators published in peer-reviewed journals.

Test organism	*Bt* toxin	*Bt* plant	Event	Form offered	Parameter	Result	Reference
Heteroptera							
Anthocoridae							
Orius majusculus	Cry1Ab	Maize	Bt 11	*Bt* plant fed prey	Development	0	Zwahlen et al. 2000
	Cry1Ab	Maize	Bt 11	*Bt* plant fed prey	Mortality	0	Zwahlen et al. 2000
Orius insidiosus	Cry1Ab	Maize	176	*Bt* plant pollen	Development	0	Pilcher et al. 1997
	Cry1Ab	Maize	176	*Bt* plant pollen	Survival	0	Pilcher et al. 1997
	Cry1Ab	Maize	Mon 810	*Bt* plant silk	Development	0	Al-Deeb et al. 2001
	Cry1Ab	Maize	Mon 810	*Bt* plant silk	Mortality	0	Al-Deeb et al. 2001
Orius tristicolor	Cry1Ac	Cotton	531	*Bt* plant fed prey	Survival	-	Ponsard et al. 2002
Lygaeidae							
Geocoris punctipes	Cry1Ac	Cotton	531	*Bt* plant fed prey	Survival	-	Ponsard et al. 2002
Miridae							
Cyrtorhinus lividipennis	Cry1Ab	Rice	n.s.	*Bt* plant fed prey	Development	0	Bernal et al. 2002
	Cry1Ab	Rice	n.s.	*Bt* plant fed prey	Survival	0	Bernal et al. 2002
	Cry1Ab/Cry1Ac	Rice	n.s.	*Bt* plant fed prey	Development	0	Bernal et al. 2002
	Cry1Ab/Cry1Ac	Rice	n.s.	*Bt* plant fed prey	Survival	0	Bernal et al. 2002
Nabidae							
Nabis spec.	Cry1Ac	Cotton	531	*Bt* plant fed prey	Survival	0	Ponsard et al. 2002
Reduviidae							
Zelus renardii	Cry1Ac	Cotton	531	*Bt* plant fed prey	Survival	0	Ponsard et al. 2002
Neuroptera							
Chrysopidae							
Chrysoperla carnea	Cry1Ab	Maize	176	*Bt* plant pollen	Development	0	Pilcher et al. 1997
	Cry1Ab	Maize	176	*Bt* plant pollen	Survival	0	Pilcher et al. 1997
	Cry1Ab	Maize	176	*Bt* plant fed prey	Development	-	Hilbeck et al. 1998b
	Cry1Ab	Maize	176	*Bt* plant fed prey	Mortality	-	Hilbeck et al. 1998b
	Cry1Ab	Maize	Bt 11	*Bt* plant fed prey	Development	-[b]	Dutton et al. 2002
	Cry1Ab	Maize	Bt 11	*Bt* plant fed prey	Mortality	-[b]	Dutton et al. 2002
	Cry1Ab			*Bt* toxin in diet	Development	0	Romeis et al. 2004
	Cry1Ab			*Bt* toxin in diet	Longevity	0	Romeis et al. 2004
	Cry1Ab			*Bt* toxin in diet	Mortality	0	Romeis et al. 2004
	Cry1Ab			*Bt* toxin in diet	Development	0	Hilbeck et al. 1998a
	Cry1Ab			*Bt* toxin in diet	Mortality	-	Hilbeck et al. 1998a
	Cry1Ab			*Bt* toxin in diet of prey	Development	-	Hilbeck et al. 1999
	Cry1Ab			*Bt* toxin in diet of prey	Mortality	-	Hilbeck et al. 1999

Table 1: Laboratory assays on potential effects of Cry1 toxins on parasitoids and predators published in peer-reviewed journals (continued)

Test organism	*Bt* toxin	*Bt* plant	Event	Form offered	Parameter	Result	Reference
Chrysoperla carnea	Cry1Ac			*Bt* toxin in diet	Mortality	0	Sims 1995
Coleoptera							
Carabidae							
Poecilus cupreus	Cry1Ab	Maize	Mon 810	*Bt* plant fed prey	Survival	-	Meissle et al. 2005
Coccinellidae							
Coleomegilla maculata	Cry1Ab	Maize	176	*Bt* plant pollen	Development	0	Pilcher et al. 1997
	Cry1Ab	Maize	176	*Bt* plant pollen	Survival	0	Pilcher et al. 1997
Hippodamia convergens	Cry1Ac			*Bt* toxin in diet	Mortality	0	Sims 1995
Propylea japonica	Cry1Ab	Rice	n.s.	*Bt* plant pollen	Development	0	Bai et al. 2005
	Cry1Ab	Rice	n.s.	*Bt* plant pollen	Survival	-	Bai et al. 2005
Hymenoptera							
Braconidae							
Cotesia flavipes	Cry1Ab	Maize	176	*Bt* plant fed hosts	Development	-	Prütz & Dettner 2004
	Cry1Ab	Maize	176	*Bt* plant fed hosts	Development	-	Prütz et al. 2004
Cotesia marginiventris	Cry1Ab	Maize	176	*Bt* plant fed hosts	Development	-	Vojtech et al. 2005
	Cry1Ab	Maize	176	*Bt* plant fed hosts	Survival	-	Vojtech et al. 2005
	Cry1Ac	Cotton	531	*Bt* plant fed hosts	Development	-	Baur & Boethel 2002
	Cry1Ac	Cotton	531	*Bt* plant fed hosts	Longevity	-	Baur & Boethel 2002
	Cry1Ac	Cotton	531	*Bt* plant fed hosts	Fecundity		Baur & Boethel 2002
Cotesia plutellae	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts[a]	Parasitism rate	0	Schuler et al. 2003
	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts[a]	Development	0	Schuler et al. 2003
	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts	Survival	-	Schuler et al. 2004
	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts[a]	Development	0	Schuler et al. 2004
	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts[a]	Survival	0	Schuler et al. 2004
Diaeretiella rapae	Cry1Ac	Rape	n.s.	*Bt* plant fed hosts	Emergence rate	0	Schuler et al. 2001
Microplitis mediator	Cry1Ac			*Bt* toxin in diet	Development	0	Liu et al. 2005
	Cry1Ac			*Bt* toxin in diet	Survival	0	Liu et al. 2005
	Cry1Ac			*Bt* toxin in diet of prey	Development	-	Liu et al. 2005
	Cry1Ac			*Bt* toxin in diet of prey	Survival	-	Liu et al. 2005
Encyrtidae							
Copidosoma floridanum	Cry1Ac	Cotton	531	*Bt* plant fed hosts	Development	-	Baur & Boethel 2002
	Cry1Ac	Cotton	531	*Bt* plant fed hosts	Survival	-	Baur & Boethel 2002
Pteromalidae							
Nasonia vitripennis	Cry1Ac			*Bt* toxin in diet	Mortality	0	Sims 1995

-: negative effect, +: positive effect, 0: no effect, [a]: *Bt*-resistant hosts, [b] : negative effect when fed with *Bt*-reared lepidopetran larvae, no effect when fed with *Bt*-reared mites or aphids

Table 2: Field studies of potential effects of *Bt* plants expressing Cry1 toxins on parasitoids and predators published in peer-reviewed journals.

Test organism	*Bt* toxin	*Bt* plant	Event	Field/Plot size	Duration (years)	Country	Parameter	Result	Reference
Araneae									
Oxyopidae									
Oxyopidae	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Salticidae									
Salticidae	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	-	Whitehouse et al. 2005
Araneae	Cry1Ab	Maize	176	7-29 ha	2	Germany	Diversity	0	Volkmar & Freier 2003
	Cry1Ab	Maize	176	1.2-1.7 ha	1	France	No. individuals	0	Candolfi et al. 2004
	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	incon.	Poza et al. 2005
	Cry1Ab	Maize	176	1500 m²	1	Germany	No. individuals	0	Meissle & Lang 2005
	Cry1Ab	Maize	Mon 810	29 m²	1	USA	No. individuals	0	Hassell & Shepard 2002
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
	Cry1Ac	Cotton	531	212-1058 m²	3	USA	No. individuals	0	Hagerty et al. 2005
	Cry1Ac/Cry2Ab	Cotton	15985	212-1058 m²	3	USA	No. individuals	0	Hagerty et al. 2005
Heteroptera									
Anthocoridae									
Orius insidosus	Cry1Ab	Maize	176	0.4 ha	1	USA	No. individuals	0	Orr & Landis 1997
	Cry1Ab	Maize	176	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
	Cry1Ab	Maize	Mon 810	900 m²	1	France	No. individuals	0	Bourguet et al. 2002
	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	incon.	Wold et al. 2001
	Cry1Ab	Maize	Mon 810	32 m²	2	USA	No. individuals	+	Musser & Shelton 2003
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
	Cry1Ab	Maize	Bt11	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
Orius spec.	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Anthocoridae	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	incon.	Poza et al. 2005
	Cry1Ab	Maize	Mon 810	29 m²	1	USA	No. individuals	0	Hassell & Shepard 2002
Lygaeidae									
Geocoris spec.	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	incon.	Daly & Buntin 2005
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	-	Whitehouse et al. 2005
Nabidae									
Nabis americoferus	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
Nabis spec.	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	-	Daly & Buntin 2005
Nabidae	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	0	Poza et al. 2005
	Cry1Ab	Maize	Mon 810	29 m²	1	USA	No. individuals	0	Hassell & Shepard 2002

Table 2: Field studies of potential effects of *Bt* plants expressing Cry1 toxins on parasitoids and predators published in peer-reviewed journals (continued).

Test organism	*Bt* toxin	*Bt* plant	Event	Field/Plot Size	Duration (years)	Country	Parameter	Result	Reference
Nabidae	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
	Cry1Ac	Cotton	531	212-1058 m²	3	USA	No. individuals	incon.	Hagerty et al. 2005
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	-	Whitehouse et al. 2005
Neuroptera									
Chrysopidae									
Chrysoperla carnea	Cry1Ab	Maize	176	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
	Cry1Ab	Maize	Mon 810	900 m²	1	France	No. individuals	0	Bourguet et al. 2002
	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
	Cry1Ab	Maize	Bt11	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
Chrysopidae	Cry1Ab	Maize	176	0.4 ha	1	USA	No. individuals	0	Orr & Landis 1997
	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	incon.	Poza et al. 2005
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Hemerobiidae									
Hemerobiidae	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Neuroptera	Cry1Ab	Maize	176	1.2-1.7 ha	1	France	No. Individuals	+	Candolfi et al. 2004
Coleoptera									
Cantharidae									
Chauliognathus spec.	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Carabidae									
Harpalus pennsylvanicus	Cry1Ab	Maize	Mon 810	0.39 ha	2	USA	No. individuals	0	Lopez et al. 2005
Microlestis linearis	Cry1Ab	Maize	Mon 810	0.39 ha	2	USA	No. individuals	0	Lopez et al. 2005
Poecilus lucublandus	Cry1Ab	Maize	Mon 810	0.39 ha	2	USA	No. individuals	0	Lopez et al. 2005
Carabidae	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Cicindellidae									
Cicindellidae	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	incon.	Daly & Buntin 2005
Coccinellidae									
Adalia bipunctata	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
Coccinella septempunctata	Cry1Ab	Maize	176	0.4 ha	1	USA	No. individuals	incon.	Orr & Landis 1997
	Cry1Ab	Maize	Mon 810	900 m²	1	France	No. individuals	0	Bourguet et al. 2002
	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	incon.	Wold et al. 2001

Table 2: Field studies of potential effects of *Bt* plants expressing Cry1 toxins on parasitoids and predators published in peer-reviewed journals (continued).

Test organism	*Bt* toxin	*Bt* plant	Event	Field/Plot size	Duration (years)	Country	Parameter	Result	Reference
Coccinella septempunctata	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
Coleomegilla maculata	Cry1Ab	Maize	176	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
	Cry1Ab	Maize	Mon 810	32 m²	1	USA	No. individuals	+	Musser & Shelton 2003
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
	Cry1Ab	Maize	Bt11	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Cycloneda munda	Cry1Ab	Maize	176	0.12-0.77 ha	3	USA	No. individuals	+	Pilcher et al. 2005
	Cry1Ab	Maize	Bt11	0.12-0.77 ha	3	USA	No. individuals	0	Pilcher et al. 2005
	Cry1Ab	Maize	n.s.	0.4 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Diomus spec.	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Harmonia axyridis	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
	Cry1Ab	Maize	Mon 810	32 m²	1	USA	No. individuals	0	Musser & Shelton 2003
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Hippodamia convergens	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
Hippodamia tredecimpunctata	Cry1Ab	Maize	Mon 810	21-543 m²	2	USA	No. individuals	0	Wold et al. 2001
Scymnus spec.	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
Coccinellidae	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	0	Poza et al. 2005
	Cry1Ab	Maize	Mon 810	29 m²	1	USA	No. individuals	0	Hassell & Shepard 2002
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	0	Daly & Buntin 2005
	Cry1Ac	Cotton	531	212-1058 m²	3	USA	No. individuals	incon.	Hagerty et al. 2005
	Cry1Ac/Cry2Ab	Cotton	15985	212-1058 m²	3	USA	No. individuals	incon.	Hagerty et al. 2005
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Staphylinidae									
Staphylinidae	Cry1Ab	Maize	176	0.4-0.7 ha	3	Spain	No. individuals	incon.	Poza et al. 2005
	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	incon.	Daly & Buntin 2005
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Hymenoptera									
Braconidae									
Cardiochiles nigriceps	Cry1Ab	Tobacco	n.s.	60-70 plants	2	USA	Parasitism rate	incon.	Johnson & Gould 1992
	Cry1Ab	Tobacco	n.s.	6-28 plants	2	USA	Parasitism rate	0	Johnson et al. 1997
Eriborus terebrans	Cry1Ab	Maize	176	0.4 ha	1	USA	No. individuals	0	Orr & Landis 1997
Macrocentrus cingulum	Cry1Ab	Maize	176	0.12-0.77 ha	3	USA	No. individuals	-	Pilcher et al. 2005
	Cry1Ab	Maize	Bt11	0.12-0.77 ha	3	USA	No. individuals	-	Pilcher et al. 2005

Table 2: Field studies of potential effects of *Bt* plants expressing Cry1 toxins on parasitoids and predators published in peer-reviewed journals (continued).

Test organism	*Bt* toxin	*Bt* plant	Event	Field/Plot size	Duration (years)	Country	Parameter	Result	Reference
Braconidae	Cry1Ab	Maize	176	n.s.	1	Spain	No. species	0	Pons & Starý 2003
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Ichneumonidae									
Campoletis sonorensis	Cry1Ab	Tobacco	n.s.	60-70 plants	2	USA	Parasitism rate	incon.	Johnson & Gould 1992
	Cry1Ab	Tobacco	n.s.	6-28 plants	2	USA	Parasitism rate	incon.	Johnson et al. 1997
Mymaridae									
Mymaridae	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	0	Whitehouse et al. 2005
Hymenopteran parasitoids	Cry1Ab	Maize	176	0.4 ha	1	USA	No. individuals	0	Orr & Landis 1997
	Cry1Ab	Maize	Mon 810	900 m²	1	France	No. individuals	0	Bourguet et al. 2002
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Formicidae									
Solenopsis invicta	Cry1Ac	Cotton	531	212-1058 m²	3	USA	No. individuals	0	Hagerty et al. 2005
	Cry1Ac/Cry2Ab	Cotton	15985	212-1058 m²	3	USA	No. individuals	incon.	Hagerty et al. 2005
Formicidae	Cry1Ab	Maize	Mon 810	525 m²	2	USA	No. individuals	incon.	Daly & Buntin 2005
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	No. individuals	incon.	Whitehouse et al. 2005
Diptera									
Syrphidae									
Syrphus corollae	Cry1Ab	Maize	Mon 810	900 m²	1	France	No. individuals	0	Bourguet et al. 2002
Syrphidae	Cry1Ab	Maize	176	1.2-1.7 ha	1	France	No. individuals	0	Candolfi et al. 2004
	Cry1Ab	Maize	n.s.	4-20 ha	1	USA	No. individuals	0	Jasinski et al. 2003
Tachinidae									
Tachinidae	Cry1Ab	Maize	176	200 m²-1 ha	1	France	Parasitism rate	-	Bourguet et al. 2002
Natual enemies (in general)	Cry1Ab	Maize	176	1.2-1.7 ha	1	France	No. individuals	0	Candolfi et al. 2004
	Cry1Ab	Maize	Mon 810	22-45 m²	2	USA	No. individuals	incon.	Pilcher et al. 1997
	Cry1Ac	Cotton	531	0.4 ha	3	China	No. species	incon.	Men et al. 2003
	Cry1Ac	Cotton	531	0.4 ha	3	China	Diversity	incon.	Men et al. 2003
	Cry1Ac	Cotton	531	0.6-1.62 ha	2	USA	No. individuals	0	Sisterson et al. 2004
	Cry1Ac	Cotton	531	0.6-1.62 ha	2	USA	Diversity	0	Sisterson et al. 2004
	Cry1Ac	Cotton	531	1-80 ha	3	Australia	Diversity	incon.	Whitehouse et al. 2005
	Cry1Ac/Cry2Ab	Cotton	15985	1-80 ha	3	Australia	Diversity	0	Whitehouse et al. 2005

n.s.: not specified, - : negative effect, + : positive effect, 0 : no effect, incon. : inconsistent effect

In conclusion, due to limitations in numbers, scope and probably due to a low statistical power of both existing laboratory and field studies (Lövei & Arpaia 2005, Birch & Wheately 2005), generalisations for an effect of Cry1 toxins to predators and parasitoids on the base of existing laboratory and field studies are difficult. Therefore, to clarify a direct or indirect effect of Cry1 toxins on predators and parasitoids, further investigations, e.g., laboratory studies on natural enemies neglected so far and further long-term and large-scale field studies are needed.

1.4 Risk assessment of genetically modified plants

As genetically modified plants (GMPs) may have negative environmental effects i.e. non-target organisms may be harmed by insect resistant crops, and the area of GMPs increases worldwide (James 2003), public and scientific concerns have been expressed against the approval of GMP varieties. So, several countries developed regulatory systems for the deliberate release of transgenic crops (Conner 2003). The European Community e.g. developed the EU Directive 2001/18/EC (European Parliament and Council 2001) which demands a pre-release risk assessment to investigate the potential toxicity of GMPs as well as a post-release monitoring of GMPs in order to assess possible long-term or unexpected effects. However, standardised models for assessing potential risks of GMP do not exist so far and several approaches are discussed. Some authors proposed quantitative risk or safety assessments (Wolt et al. 2003, Howard & Donnelly 2004) as well as systems derived from ecotoxicological models (Jepson et al. 1994, Dutton et al. 2003b). Andow & Hilbeck (2004) suggested an ecological model based on a synopsis of an ecotoxicological model as well as an nonindigenous species model (see also Andow & Zwahlen in press).

For a risk assessment of GMPs, test organisms should be selected according to their ecological and economic significance, the likelihood of exposure to GMPs as well as a possible sensitivity to products of transgenic plants (Jepson et al. 1994, Dutton et al. 2003b, Hilbeck & Andow 2002). However, as complex agroecosystems are often difficult to understand due to limited knowledge especially of food webs, selection of test organisms according to their ecological significance is often not trivial (Andow & Hilbeck 2004).

Generally, ecological risk can be defined as a function of exposure and effect i.e. the product of the likelihood of exposure and the magnitude of the effect (Breckling & Müller 2000), so both terms should be investigated in GMP risk assessment models (Sears et al. 2001, Dutton et al. 2003b, Cowgill & Atkinson 2003, Andow & Hilbeck 2004).

An exposure assessment of GMPs to predators should include the investigation of GMP characteristics i.e. toxin expression in different plant tissues and dispersal of toxin containing pollen in adjacent habitats, the investigation of characteristics of potential prey i.e. availability to predators, feeding habits, potential processing of ingested toxin, as well as characteristics of the predators i.e. occurrence, abundance, prey spectrum and prey selectivity under field conditions (Dutton et al. 2003b, Hilbeck & Andow 2002).

To investigate potential lethal or sublethal effects of GMPs on a test organism, laboratory assays should be conducted, in which GMP material or pure integrants of GMPs are offered to test organisms. Furthermore, the applied GMP product in the laboratory assay should be tested in a bioassay with a known susceptible organism for biological activity (Andow & Hilbeck 2004). However, laboratory assays may not mimic field conditions sufficiently which may lead to a unrealistic high exposure of Cry1 toxins to test organisms or to a non-detection of possible unintended effects present under field conditions (Birch & Wheatley 2005). Therefore, in addition to laboratory assays, semi-field trials and a long-term field monitoring under realistic farming systems under consideration of different temporal and spatial scales should be conducted (Jepson et al. 1994, Hilbeck & Andow 2002, Züghart & Breckling 2003, Graef et al. 2004, Birch & Wheatley 2005).

Mathematically, either exposure or effect must be zero to disprove a risk. However, nonsignificant statistical results do not necessarily imply an absence of an effect as an existing effect may not have been detected with the applied test methodology or experimental design (Marvier 2002), i.e. the absence of proof is no proof for absence. Thus, for a risk assessment, the type II error, the false acceptation of a null hypothesis, is especially of interest. Therefore, some risk assessment studies provide retrospective power analyses for nonsignificant results (e.g. Romeis et al. 2004). However, the usefulness of these power analyses for interpreting nonsignificant results is discussed due to logical flaws e.g. the statistical power is dependent on the p-value of nonsignificant results (Nakagawa & Forster 2004). Alternatively, effect sizes and corresponding confidence intervals of nonsignificant results were recommended to interpret nonsignificant results (Colegrave & Ruxton 2003, Nakagawa & Forster 2004). Furthermore, a sufficient sample size should be chosen for risk assessment investigations to gain an acceptable statistical power (Lang 2004, Lövei & Arpaia 2005).

1.5 Spiders as test organisms for a risk assessment of genetically modified plants

Spiders as test organisms for a GMP risk assessment fulfil several criteria described above:

Spiders are an abundant and species rich predator group in arable land (Samu & Szinetár 2002, Nyffeler & Sunderland 2003). Spiders have an ecological function in agroecosystems, because they are a diverse order showing various life styles and feeding on prey from different trophic levels (Nyffeler et al. 1994) which may stabilise a biocoenosis (Fagan 1997). Furthermore, spiders feed largely on pest organism such as Diptera, Sternorrhyncha and Auchenorhychna in agroecosystems (Kajak 1965, Nyffeler & Benz 1979, Nyffeler 1982, Lang et al. 1999), and spider assemblages can limit pest populations in arable land resulting in a reduction of herbivory (Riechert & Lawrence 1997). Thus, spiders have economic value in agroecosystems (Riechert & Bishop 1990). Especially web-building spiders are important pest predators as they feed not only on pests directly, but even abandoned webs keep on catching and killing pests (Sunderland 1999). Additionally, spiders are harmed by many pesticides sublethally or lethally (Sterk et al. 1995), and parameters of spiders and spider webs e.g. may reflect sublethal effects, as drugs and pesticides bias the spiders` web-building behaviour (Witt 1971, Samu & Vollrath 1992). Therefore, spiders are described as good indicators of ecological and environmental risks (Marc et al. 1999).

Spiders are exposed to GMP products e.g. *Bt* maize by several pathways (Fig. 1): On the one hand, spiders may be exposed to Cry toxin-contaminated *Bt* maize pollen by intentional pollen feeding (Vogelei & Greissl 1989). Furthermore, spiders are exposed to *Bt* maize pollen dusting spider webs (Appendix: Fig. 1 and Fig. 2). This is because spiders may ingest pollen in spider webs due to the eating of their webs including adhering particles in order to "recycle" spider silk (Smith & Mommson 1984). On the other hand, spiders may be exposed to Cry toxins via preying on herbivores eating *Bt* maize-tissue and *Bt* pollen-loaded pollinators (Gregory 1989, Nyffeler & Breene 1991), and pollinators may transport considerable pollen amounts including maize pollen (Hirschfelder 1950, Vaissière & Vinson 1994, Odoux 2004) (Appendix: Fig. 3 and Fig. 4). Therefore, due to their ecological and economic significance, their potential exposure the products of transgenic plants as well as their sensitivity to pesticides, especially foliage-dwelling web-building spiders are appropriate test organisms for a risk assessment of GMPs.

However, spider guilds with different biologies are probably not exposed to products of GMPs equally. So not all spider taxa build webs, in which pollen may be caught. As hunting spiders catch their prey directly without a web (Nyffeler et al. 1994) and not all web-

building spiders eat their web incl. adhering pollen (e.g., Linyphiidae, Carrel et al. 1999), a potential exposure to *Bt* maize pollen via web recycling in these spider groups is unlikely. On the other hand, foliage-dwelling orb-web spiders (Araneidae) do recycle their webs incl. pollen (Smith & Mommson 1984). Furthermore, orb-web spiders scrunch their prey to a mash from which the nutrients were imbibed including proteins (e.g., Cry1Ab) of pollen during extra intestinal digestion (Appendix: Fig. 4). Thus, orb-web spiders may be especially exposed to *Bt* maize pollen due to higher pollen concentration in their habitat and additionally to *Bt* pollen adhering on pollinator prey.

Despite their ecological significance and potential exposure to Cry1Ab toxin of *Bt* maize, not one laboratory study on potential lethal or sublethal effects of products of *Bt* maize on spiders exists (Lövei & Arpaia 2005, Tab. 1). Most field assays showed no effect of *Bt* maize on spiders (Hassell & Shepard 2002, Jasinski et al. 2003, Poza et al. 2005, Candolfi et al. 2005, Meissle & Lang 2005, Daly & Buntin 2005, Tab. 2). However, a recent study of Whitehouse et al. 2005 recorded a negative effect of *Bt* cotton expressing the Cry1Ac protein on jumping spiders (Salticidae).

Existing field studies of *Bt* plants mainly cover the taxon Araneae in general and not spider guilds or spider species (Tab. 2) which may be more appropriate due to potential differences in the exposure to products of GMPs to spider guilds, as described above. Furthermore, only one European study exists which includes spiders and which was conducted with plots in realistic field sizes during more than 2 years (Poza et al. 2005). Therefore, further investigations are needed including laboratory studies as well as long-term and field-scale studies on potential effects of GMPs on spider species and spider guilds.

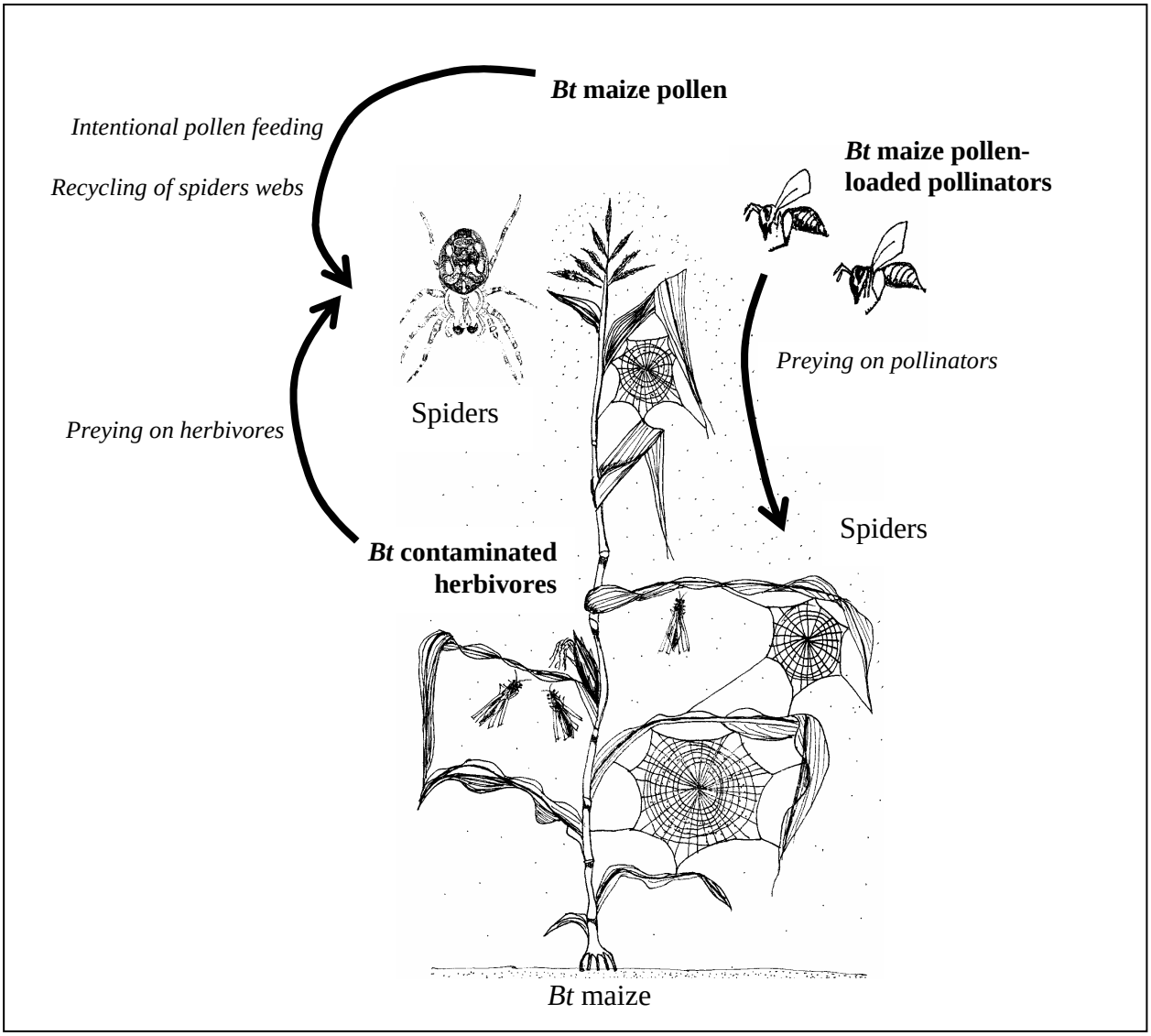

Figure 1: Potential exposure pathways of *Bt* maize to spiders.

Spiders may be exposed to products of *Bt* maize via intentional feeding of *Bt* maize pollen, recycling of pollen-dusted spider webs as well as via preying on *Bt*-contaminated herbivores and pollinators.

2 Conceptual and methodological approach

2.1 Conceptual approach

In this thesis, the risk potentially arising from genetically modified *Bt* maize for foliage-dwelling spiders was assessed by (i) recording base line data of spiders in maize fields and adjacent margins, (ii) by an evaluation of adequate sampling methods for foliage-dwelling spiders, (iii) by the investigation of exposure pathways of *Bt* maize to spiders and (iv) by assessing the actual *Bt* effects on foliage-dwelling spiders.

The thesis is based on the following publications listed below:

I Ludy, C. & Lang, A., 2004: How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods. – *Journal of Applied Entomology* 128, 501-509.

II Lang, A., Ludy, C. & Vojtech, E., 2004: Dispersion and deposition of *Bt* maize pollen in field margins. – *Journal of Plant Diseases and Protection* 111, 417-428.

III Ludy, C. & Lang, A.: *Bt* maize pollen exposure and impact on the garden spider *Araneus diadematus.* – Accepted, *Entomologia Experimentalis et Applicata*.

IV Ludy, C.: Prey spectra and prey selection of orb-web spiders (Araneae: Araneidae) on field margins: significance for an exposure assessment of *Bt*-contaminated prey. – Submitted to *Agriculture, Ecosystems & Environment*.

V Ludy, C., 2004: Intentional pollen feeding in the garden spider *Araneus diadematus*. – *Newsletter of the British Arachnological Society* 101, 4-5.

VI Ludy, C. & Lang, A.: A 3-year field-scale field monitoring of foliage-dwelling spiders (Araneae) in transgenic *Bt* maize fields and adjacent field margins. – Under revision *Biological Control.*

2.2 Methodological approach

In the following, an outline of the aims and methods of the investigations of the thesis with reference to the corresponding publications, shown on page 25, is given. An overview of the aspects of a risk assessment included in this thesis is shown in Fig. 2.

Spiders are important predators in agroecosystems and useful bioindicators. However, concerning risk assessment of GMPs, spiders have been neglected so far (Lövei & Arpaia 2005). Also, base line data of spiders is poor, especially of foliage-dwelling spiders in maize fields, a dominant crop in Mid-Europe (Nyffeler & Sunderland 2003). Furthermore, an assessment is lacking of adequate sampling methods for recording the abundance of foliage-dwelling spiders in maize fields and their margins for a monitoring of GMPs. Therefore, the abundance and spider species composition in maize fields and adjacent field margin was described (**I**, **VI**) and sampling methods were evaluated for recording foliage-dwelling spiders (**I**). Six different maize fields and adjacent artificial field margins were established at three locations. Maize plants in fields as well as stinging nettle shrubs on field margins were sampled during the vegetation season by a drop cloth (Appendix: Fig. 5) as well as by a hand-held suction sampler (Appendix: Fig. 6). Both sampling methods and habitat types were evaluated according to their reliability and effectiveness and with regard to the proportion of spider guilds obtained.

To assess the ecological risk of GMPs, information about the exposure as well as about a potential effect of a possible adverse factor must be available. An exposure analysis of *Bt* maize to foliage-dwelling spiders requires information about characteristics of *Bt* maize, spider prey and spiders themselves (Hilbeck & Andow 2002). *Bt* maize characteristics i.e. time of maize anthesis, Cry1Ab toxin content in *Bt* maize pollen, pollen distribution and wind dispersal (**II**) as well as *Bt* maize pollen amount in spider webs in maize fields and on adjacent field margins (**III**) was provided. The latter was done by keeping adult orb-web spiders (Araneae: Araneidae) in the laboratory where they built webs in wooden frames. After spiders had built webs in the frames, frames with webs but without spiders were exposed in maize fields and in different distances on adjacent margins during maize anthesis. After an exposure of 24 hours, exposed webs were photographed and the adhering pollen were counted from enlarged photographs. Characteristics of spider prey i.e. the occurrence and abundance of potential prey was recorded in a field experiment (**IV**). On two field margins differing in flower density, a malaise trap (Appendix: Fig. 7) as well as sticky traps (Appendix: Fig. 8)

were installed. Caught potential prey items were collected after exposure and were identified to order or family level in the laboratory. Spider characteristics which bias their exposure to *Bt* maize i.e. the uptake of the Cry1Ab toxin via *Bt* maize pollen as well as the prey spectrum and the prey selectivity of spiders was investigated in the laboratory or in the field, respectively. The ingestion of the Cry1Ab toxin by spiders via *Bt* maize pollen contamined webs was investigated by dusting webs of juvenile garden spiders with *Bt* maize pollen (**V**). Just after eating their webs, the spiders were killed by freezing. After defrosting, the spiders` gastrointestinal systems were dissected and analysed with an enzyme-linked immunosorbent assay (ELISA) for Cry1Ab content. Furthermore, juvenile and adult garden spiders were observed to record a possible deliberate feeding on *Bt* maize pollen. Prey spectrum and prey selectivity of two orb-web spiders, the garden spider *Araneus diadematus* (Appendix: Fig. 9) and the wasp-like spider *Argiope bruennichi* (Appendix: Fig. 10) (both Araneae: Araneidae) were investigated by exposing wooden frames with spiders and spider webs on two field margin types, a flower-poor and a flower-rich field margin (Appendix: Fig. 11 and Fig. 12) (**IV**). Prey caught in the spider web as well as actual prey eaten by the spider was directly observed. This observation took place for individuals of each spider species during three days on each field margin type. Prey selectivity of spider webs and spiders was determined by a comparison with the data of the potential prey caught with malaise traps and sticky traps simultaneously.

Bt maize may affect spiders directly via pollen feeding and indirectly via herbivorous and pollen-colleting prey. In a laboratory assay, the potential impact of *Bt* maize pollen on juvenile garden spiders was investigated (**III**). Juvenile garden spiders were kept in the laboratory in wooden frames to allow web building. Webs were treated with *Bt* maize pollen (variatey “Navares” by Syngenta) as well as with conventional maize pollen (near-isogenic variety “Antares” by Syngenta) as a negative control and with the insecticidal pyrethroid Baythroid® as a positive control. After treatment, webs were photographed and lethal and sublethal parameters such as development, reaction toward prey and web building were recorded. Additionally, the biological activity of the Cry1Ab toxin of *Bt* maize pollen was investigated by a bioassay with a target organism of *Bt* maize, the European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae). In addition to the laboratory assay, a 3-year monitoring of foliage-dwelling spiders in *Bt* maize and adjacent margins under a realistic farming practice was conducted (**VI**). Three *Bt* maize fields (“Navares”, Sygneta) and three conventional maize fields (“Antares” by Sygenta) as controls as well as adjacent field margins were established on three locations in Bavaria, South Germany. The spider abundance,

species richness and composition of foliage-dwelling spiders were recorded in maize fields and field margins during the vegetation seasons of three years.

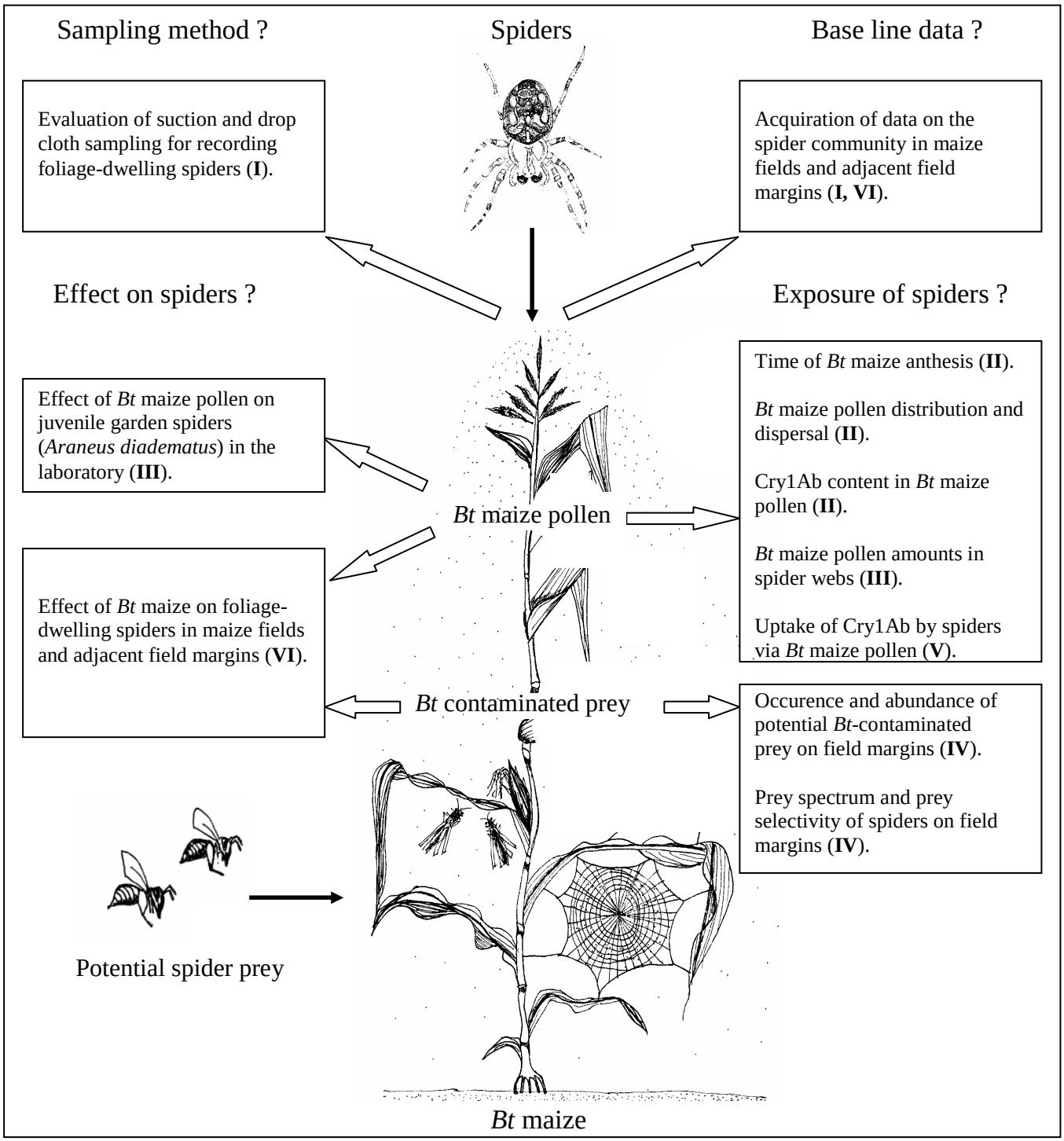

Figure 2: Overview of the aspects of a risk assessment of *Bt* maize concerning foliage-dwelling spiders included in this thesis.

Spider communities in maize fields and field margin were examined to acquire base line data and sampling methods were evaluated to record foliage-dwelling spiders (upper boxes). For the assessment of the exposure of spiders to *Bt* maize via *Bt* pollen and *Bt* contaminated prey, characterisitics of *Bt* maize, of potential spider prey and of spiders were investigated (lower right boxes). The potenial effect of *Bt* maize on spiders was assessed on laboratory as well as on field scale (lower left boxes). Bold roman letters in brackets refer to the corresponding publications given on page 25.

3 Main results and general discussion

In the following, the main results of this thesis are given and discussed. In Fig. 3 on overview of the results achieved in this thesis are shown.

3.1 Base line data of foliage-dwelling spiders in maize fields and neighbouring field margins

For the assessment of the potential exposure of test organisms to GMPs, field data on abundance and species composition of test organisms in agroecosystems must be available. Therefore, the spider abundance and species richness in maize fields and on adjacent field margins were investigated (**I**,**VI**).

The spider communities in maize ecosystems and adjacent margins showed a high abundance as well as a high species richness and mainly consisted of juvenile individuals of the web-building spider families Theridiidae (cob-web spiders), Linyphiidae (sheet-web spiders), Tetragnathidae (long-jawed spiders) and Araneidae (orb-web spiders, sensu stricto) with a dominance of space-web spiders (Theridiidae and Linyphiidae). Typical of agroecosystems, a few spider species dominated the spider communities in maize fields as well as in field margins, e.g. the theridiid species *Theridion impressum* L. Koch, the linyphiid species *Oedothorax apicatus* (Blackwall) and *Meioneta rurestris* (C.L. Koch) in maize fields as well as the araneid *Aculepeira ceropegia* (Walckenear) on field margins (Appendix: Table 1). The spider abundance in both maize fields and field margins increased during the vegetation season and peaked in July-August. These findings correspond to the results of several other studies on foliage-dwelling spider communities in arable land (Luczak 1979, Nyffeler 1982, Barthel 1997, Samu & Szinetár 2002, Nyffeler & Sunderland 2003). Whereas the relative spider family composition in maize fields and field margins was more or less constant throughout the years, the absolute numbers of spider individuals and species differed considerably. This high variation in spider abundance and species richness in agroecosystems was also found by Nyffeler & Sunderland (2003) and Samu & Szinetár (2002).

The dominance of juvenile web-building spiders in agroecosystems can be explained with a good dispersal ability of these spiders through “ballooning” behaviour i.e. the aerial dispersal by means of releasing silk threats (Decae 1987). A good dispersal ability is an important feature of field-inhabiting spiders as managed fields are disturbed regularly by agricultural practices (ploughing, insecticide applications, harvesting), and so spiders have to

re-immigrate from reproduction and hibernation habitats into field habitats regularly (Bishop & Riechert 1990). An immigration of spiders may also be necessary for field margins which may develop anew each year under realistic agricultural management conditions. However, even these ephemeral field margins are found to be an important habitat especially for free hunting spiders as well as orb-web building spiders due to a complex vegetation structure which provides e.g. shelter and web attachment (Uetz 1991), resulting in a generally higher spider species richness and abundance on field margins than in maize fields.

3.2 Catching foliage-dwelling spiders in maize: An evaluation of sampling methods

As the sampling method bias the gained individual number of spiders as well as the spider community composition in a sample (Green 1999, Meissle & Lang 2005), the choice of an appropriate sampling method is important to investigate a possible effect of GMPs on foliage-dwelling spiders. Therefore, two methods for sampling foliage-dwelling spiders in maize fields and adjacent margins were evaluated which were shown to be efficient sampling methods for foliage-dwelling spiders: suction sampling and drop cloth sampling (Meissle & Lang 2005) (**I**).

It was demonstrated that with a small hand-held suction sampler, more reliable results were achieved than with a drop cloth due to a lower variation in suction than in drop cloth samples. A higher variation in drop cloth samples can be explained with the accidentally shaking of neighbouring plants at the same time while sampling one target plant in the dense vegetation of a maize field. This may lead to an additional abseiling of spiders from non-target plants which then land on the drop cloth, leading to an overestimation of spider individuals in drop cloth samples. So, suction samples can be assigned to one plant more accurately, because with this method only spiders which are located directly on the plant, are collected (see also Nuessly & Sterling 1984). Moreover, a higher proportion of Linyphiidae, often small-sized spiders which hide between plant structures (Alderweireldt 1994), but which make up a large proportion of the spider community in maize fields and margins, was acquired by the suction sampler (see also Meissle & Lang 2005, Costello & Daane 1997). Furthermore, working with a small hand-held suction sampler turned out to be more convenient and time-saving in dense maize fields than drop cloth sampling with a bulky drop cloth and the corresponding beating stick.

Due to its reliability, efficacy and convenience, the usage of a hand-held suction sampler was applied for a subsequent monitoring of foliage-dwelling spiders in this study and is recommended as an appropriate tool for sampling foliage dwelling spiders, e.g., for a monitoring of GMPs.

3.3 Potential exposure of spiders to products of *Bt* maize

3.3.1 Characteristics of *Bt* maize event 176

Characteristics of *Bt* maize varieties determine the Cry1Ab concentration in the environment and so the potential exposure of spiders in maize fields and adjacent field margins (Hilbeck & Andow 2002, Dutton et al. 2003b). Therefore, information on the Cry1Ab content in *Bt* maize pollen as well as the time of *Bt* maize pollen shedding, dispersal of *Bt* maize pollen and the pollen amount in spider webs in maize fields and on field margins was acquired (**II**, **III**).

The Cry1Ab content in *Bt* maize pollen event 176 was approximately 2.5 µg/g pollen which corresponds to data found in literature (Hilbeck & Andow 2002) (**II**). It was shown that the anthesis and pollen shedding of *Bt* maize event 176 generally occurs in July (**II**) which corresponds to the phenology of maize given in Zscheischler et al. (1990) for Mid-Europe. Pollen deposition on micro slides in maize fields and adjacent field margins was with 66 ± 70 pollen per cm² (mean ± 1 SD) very variable in this study. Pollen deposition on field margins declined rapidly with an increasing distance to the flowering maize field. In a distance of 10 m from the maize field, six times less pollen was deposited compared to pollen deposition inside maize fields. Besides the distance to an flowering maize field, several other factors influenced the deposition of pollen i.e. the BBCH stage of maize and various climatic factors, such as air temperature, wind speed and precipitation (**II**).

Also the pollen amount in spider webs in maize fields and on field margins was very variable. The average amount of *Bt* maize pollen in spider webs was in maize fields 1044 ± 1193 pollen per spider web (mean ± 1 SD) and on field margins 381 ± 205 (mean ± 1 SD) during maize anthesis. The maize pollen concentration in spider orb webs increased in maize fields in dependence to the height position. In a height of 170 cm in a maize field, the average pollen amount in spider orb webs was six times higher than on the ground. On the other hand, the mean number of maize pollen in spider webs decreased on adjacent field margins in dependence of the distance to maize fields. However, in a distance of 10 m from maize fields,

more than half of the pollen amount found in spider webs inside maize fields was recorded in this study (**III**).

3.3.2 Characteristics of potential spider prey

The potential prey spectrum of spiders on field margins was investigated as spiders in maize fields and on field margins may be exposed not only to *Bt* maize pollen in spider webs but also to *Bt*-contaminated herbivorous and pollen-collecting prey (**IV**). Potential prey of orb-web spiders was defined as all flying insects available in an investigated flower-rich or flower-poor field margin habitat, respectively. Diptera dominated the potential prey spectrum by approximately 50 % on both field margin types. Other frequent potential prey types were Hymenoptera, Coleoptera and Heteroptera which could reach together a proportion over 30 % on the potential prey spectrum.

However, many potential prey taxa can minimise the likelihood of predation by spiders due to morphological properties and defence mechanisms. For example, less Diptera and Hymenoptera were caught by spider webs in this study which means a smaller proportion of these taxa were captured in spider webs as compared to the proportion potentially available in the habitat. This underrepresentation of Diptera and some Hymenoptera in spider webs may be due to their good visual power which allows a detection and avoidance of spider webs.

Potential prey taxa may also defend spider attacks after got stuck in the web. Coleoptera and some Hymenoptera possess powerful mandibles or stings to ward off the spider, and some species are able to struggle free from a spider web due to smooth surface structures (Nentwig 1982). These characteristics of potential prey types may also be responsible for the underrepresentation of these prey types on the actual prey spectrum of spiders as shown in this study. Several studies on the prey selectivity of spider webs and spiders confirmed these results (Kajak 1965, Uetz 1990, Nentwig 1985).

Differences between the potential prey spectrum and the actual prey spectrum of spiders could not only be due to web or spider avoidance mechanisms of potential prey, but also be due to features of potential prey taxa which facilitates a predation by spiders. Hence, light and broad-winged prey types such as Sternorrhyncha may get stuck in spiders webs easily (Nentwig 1982) which is shown in a positive selectivity of spider webs i.e. a overrepresentation of Sternorrhyncha in spider webs as compared to the potential availability.

3.3.3 Characteristics of spiders

Characteristics of potential prey influence the possibility to get stuck in spider webs, while the actual prey spectrum refers to the prey species being killed or eaten by the spiders eventually. The actual prey spectrum of spiders on field margins is of interest to assess the final potential exposure to *Bt*-contaminated prey (**IV**). Orb-web spiders generally consumed a mean number of nine prey items per seven hours on field margins, and their actual prey spectrum generally consisted of Diptera, Sternorrhyncha, Heteroptera and some Coleoptera with a strong dominance of Diptera by over 50 % on a flower-poor field margin. On a flower-rich field margin, however, pollen-collecting Apidae were caught by spiders frequently and may contribute up to 37 % to the spider´s actual prey spectrum which corresponds to a mean number of one bee eaten, e.g., by the wasp-like spider *A. bruennichi*. Similar prey spectra for orb-web spiders as in this study were described by several authors e.g. Kajak (1965), Nyffeler (1982) and Nentwig (1985).

Besides potential prey characteristics, also morphological and behavioural features of different spider species may influence the actual prey spectrum. So, the wasp-like spider *A. bruennichi* fed on more Apidae which got stuck in a web than the garden spider *A. diadematus*. This more efficient predation on Apidae by the wasp-like spider can be explained by longer legs as well as behavioural adoptions on defensive prey such as massive silk wrapping of prey (Eisner & Dean 1976, Olive 1980).

To get exposed to the Cry1Ab via pollen-collecting Apidae or *Bt* maize pollen in spider webs, spiders have to ingest the Cry1Ab from *Bt* maize pollen in the field. This was investigated by ELISA and by the observation of spiders, whose webs were dusted with *Bt* maize pollen or were hit by pollen-carrying pollinators, respectively (**V**). Intentional (*Bt* maize) pollen feeding was documented in garden spiders in both juvenile and adult developmental stages. Furthermore, ELISA proved an uptake of Cry1Ab from *Bt* maize pollen applied in spider webs at least by 65 % of juvenile spiders, whose webs were treated with *Bt* maize pollen. However, an uptake of whole *Bt* maize pollen grains is not likely as maize pollen have a size of 90 µm (Aylor 2002) and spiders can only ingest particles ≤ 1 µm (Foelix 1992). So spiders probably dissolve nutrients incl. proteins of pollen by extra-intestinal digestion and absorb the digestive juice including nutrients afterwards.

3.4 Effect of *Bt* maize on foliage-dwelling spiders

The potential effect of *Bt* maize on foliage-dwelling spiders was assessed on laboratory as well as on field scale. In a laboratory assay, a possible effect of *Bt* maize pollen on juvenile garden spiders (*A. diadematus*) was conducted (**III**) and in a 3-year field assay, the potential effect of *Bt* maize on foliage-dwelling spiders was assessed under realistic farming conditions (**VI**).

3.4.1 Laboratory assay on the potential effect of *Bt* maize pollen on the garden spider *Araneus diadematus*

No effects of *Bt* maize pollen-treated spider webs on juvenile garden spiders were detected concerning various development and web-building parameters compared to webs treated with conventional maize pollen. A correct exposure of garden spiders to *Bt* maize pollen was guaranteed by the application of a *Bt* maize pollen amount which corresponded to pollen densities in spider webs during maize anthesis in the field and by a test of the biological activity of the Cry1Ab toxin in the used *Bt* maize pollen on the target organism *Ostrinia nubilalis*. In conclusion, *Bt* maize pollen adhering to spider webs had no lethal or sublethal effect on juvenile garden spiders.

However, a general statement concerning a presence or an absence of a possible adverse effect of *Bt* maize pollen on spiders would require further laboratory investigations, as small effect sizes and high variation may have prevented the detection of a possible effect. On the other hand, spraying spider webs with the conventional insecticidal pyrethroid Baythroid® caused clear adverse effects in juvenile garden spiders resulting e.g. in a lower weight increase, a longer reaction time towards prey and a reduced survival as compared to pollen-treated spiders. Sublethal and lethal effects of pyrethroids on spiders are well known (Samu & Vollrath 1992, Lengwiler & Benz 1994, Sterk 1995). An absence or a missed detection of effects of Baythroid on web-building parameters could be due to high variations in the web-building behaviour of juvenile spiders (Heiling & Herberstein 2000) which were chosen as test organisms in this study. However, flowering maize fields harbour mainly juvenile spiders, which requires the choice of juvenile developmental stages for bioassays with *Bt* maize pollen.

3.4.2 Long-term field-scale monitoring of foliage-dwelling spiders in *Bt* maize field and adjacent field margins

A 3-year field monitoring of foliage-dwelling spiders in *Bt* maize fields showed in the years 2001-2002 no effect and 2003 a positive effect on spider abundances. No negative impact of *Bt* maize on spiders was found. Furthermore, no effect was detected on the species richness and the guild structure of foliage-dwelling spiders in *Bt* maize fields. Even during maize anthesis in July, where the highest exposure of *Bt* maize via pollen to spiders during the vegetation season can be assumed, no significant negative effect of *Bt* maize on spiders was detected. Consequently, a huge lethal effect of *Bt* maize on spider communities can be excluded. An observed increase of the spider abundance in *Bt* maize fields as compared to conventional maize fields in one year may be explained with possible pleiotropic effects of *Bt* maize as the transmission of the Cry1Ab gene from *B. thuringiensis* into the maize plant genome may be linked with physiological alterations (Saxena & Stotzky 2001). Therefore, differences in the phenology as well as a possible higher abundance of potential spider prey in *Bt* maize as compared to conventional maize may have caused an increase in the spiders' abundance. This study is the first long-term study on field scale covering different spider guilds with a possible differing exposure to products of GMPs. However, as in the laboratory study, small effect sizes and a high variation may have masked *Bt* effects which makes general statements to possible effects of *Bt* maize on spiders difficult.

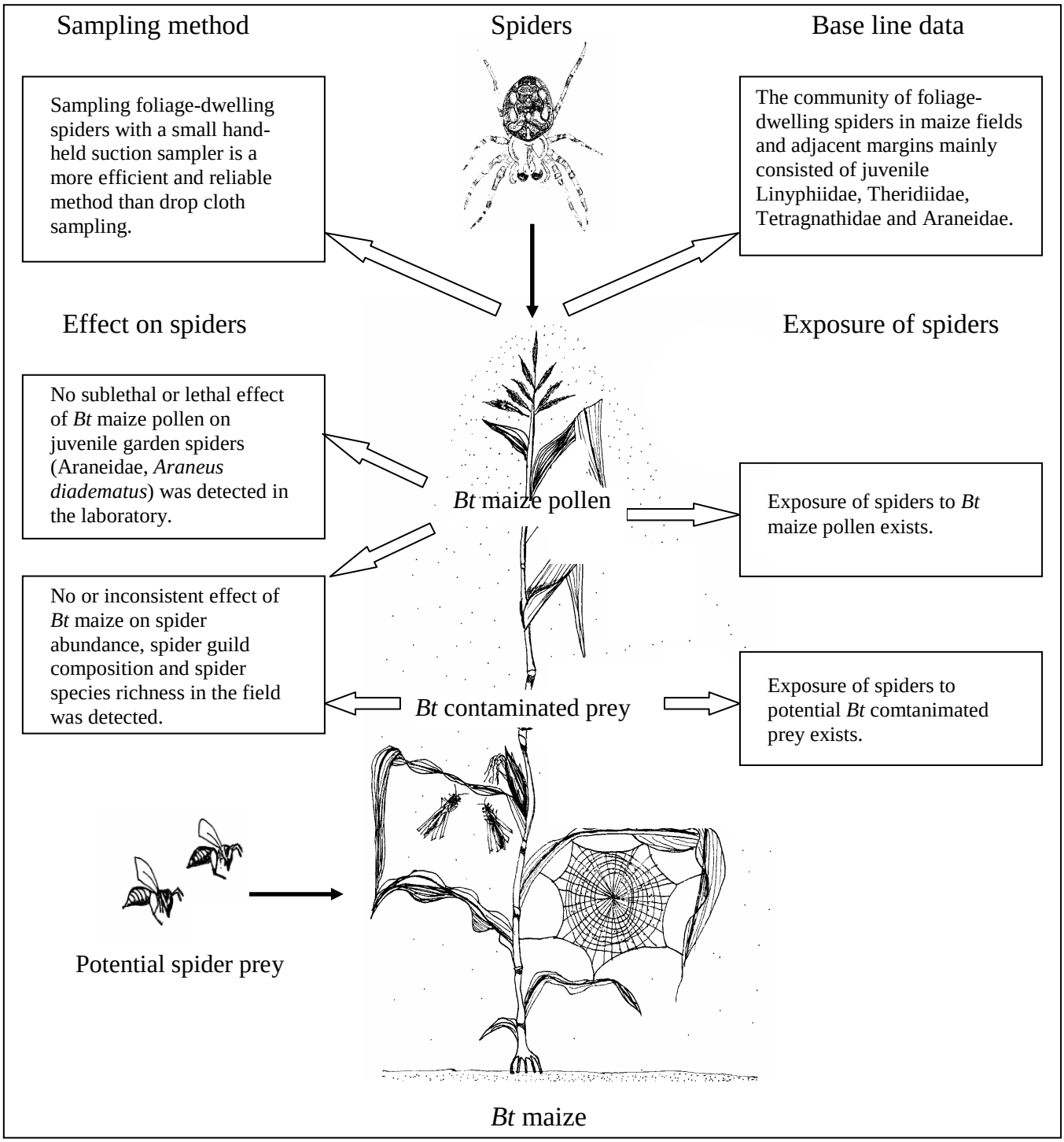

Figure 3: Overview of the results of the investigations of this thesis covering different aspects of a risk assessemnt of *Bt* maize concerning foliage-dwelling spiders.

A small suction sampler is an appropriate sampling method for recording the spider community in maize fields and field margins which consisted mainly of juvenile web-building spiders (upper boxes). Despite there is an exposure of spiders to *Bt* maize pollen and potential *Bt*-contaminated prey (lower right boxes), laboratory and field studies showed no direct lethal effect of *Bt* maize on foliage-dwelling spiders (lower left boxes).

4 Synopsis

Spiders are potentially exposed to products of *Bt* maize via different routes. Beside the feeding on *Bt* maize pollen which adhere to spider webs, intentional pollen feeding or recycling of webs incl. containing pollen, spiders may be exposed by preying on *Bt* contaminated herbivores and *Bt* maize pollen-carrying pollinators. In this thesis, foliage-dwelling web-building spiders in maize fields were potentially more exposed to *Bt* maize pollen in spider webs than ground-dwelling spiders. Off *Bt* maize fields, *Bt* maize pollen were dispersed and deposited. Also in spider webs on field margins, considerable amounts of *Bt* were found. Due to "recycling" of *Bt* maize pollen dusted webs, it was proven that spiders may ingest the Cry1Ab protein from *Bt* maize pollen.

Besides a potential exposure to *Bt* contaminated herbivorous prey which may occur during the whole vegetation season of *Bt* maize, a potential exposure to *Bt* maize pollen and *Bt* maize pollen-carrying pollinators is restricted to *Bt* maize anthesis which generally takes place in July. At this time, juveniles of many web-building spider species inhabiting field habitats, e.g., *Theridion impressum* and *Aculepeira ceropegia*, hatch from cocoons in maize fields or in reproduction habitats of spiders (Schäfer 1976, Barthel 1997) and immigrate into fields or adjacent margins (Bishop & Riechert 1990). This results in an increase in the spider abundance in July and August as found in this study, and these juvenile spiders are exposed to *Bt* maize pollen dusting their webs.

Field margins harboured a higher spider abundance and species richness than adjacent fields and were appropriate habitats for orb-web spiders in this study. Common orb-web spider species on field margins are the wasp-like spider *Argiope bruennichi* and the garden spider *Araneus diadematus* (Barthel 1997). Whereas the wasp-like spider has an annual life cycle, the garden spider shows probably a biannual life cycle, but both spider species generally mature in July-August (Dahl 1931). Due to a biannual life cycle, juveniles as well as adults of the garden spider may occur simultaneously in July during maize anthesis. Therefore adult wasp-like spiders as well as several developmental stages of the garden spider are potentially exposed to *Bt* maize pollen in spider webs as well as *Bt* pollen-contaminated pollinators. Pollinators, e.g., the honey bee *Apis mellifera,* are able to transport relatively high maize pollen amounts (Vaissière & Vinson 1994), but at the same time, may defend spider attacks effectively. Therefore, an exposure to *Bt* maize pollen-contaminated pollinator may be more likely for adult wasp-like spiders as this spider species has morphological and behavioural adoptions to subdue large-sized and armed prey (Eisner & Dean 1976),

demonstrated in a higher consumption of Apidae caught in the spider web by the wasp-like spider as compared to the garden spider in this study. A potential exposure via *Bt*-contaminated herbivorous prey may be possible due to a passage of the Cry1Ab toxin from prey to spiders. Additionally, an insufficient nutrient quality of *Bt*-affected prey may also influence predators negatively (Harwood et al. 2005, Dutton et al. 2002). This is because prey taxa which dominated the actual prey spectrum of orb-web spiders on field margins such as Diptera, Heteroptera and Coleoptera, may ingest the Cry1Ab toxin via green plant tissue or pollen (Dutton et al. 2003b, Harwood et al. 2005), and thus may be harmed by the *Bt* toxin (e.g. Indrasith et al. 1992, Ponsard et al. 2002).

In conclusion, the exposure analysis, as it was conducted in this study, showed that there is a potential exposure of foliage-dwelling spiders in maize fields and on field margins via *Bt* maize pollen in spider webs as well as via prey i.e. herbivores and pollinators.

A laboratory assay investigating the potential effect of *Bt* maize pollen on the garden spider as well as a 3-year field-scale monitoring did not give any evidence for an adverse effect of *Bt* maize in spiders. Despite a low sample size and high variations, a direct lethal effect of *Bt* maize on spiders can most likely be excluded. However, possible sublethal effects of *Bt* maize of spiders need to be clarified.

Considering the absence of a clear direct lethal effect of *Bt* maize event 176 on both laboratory and field scale, a high risk of *Bt* maize event 176 on spiders cannot be confirmed on the base of the results of this study.

5 Conclusions and outlook

The aim of this thesis was to assess the potential risk of *Bt* maize event 176 to foliage-dwelling spiders. On the base of several investigations on occurrence and sampling of foliage-dwelling spiders in maize fields and on adjacent field margins as well as on the potential exposure and effect of products of *Bt* maize on spiders, the following conclusions were drawn:

(i) Maize fields and adjacent field margins harbour an individual- and species-rich spider community which refers to an important biocontrol function of foliage-dwelling spiders in agroecosystems. Therefore, foliage-dwelling spiders are valuable test organisms for a risk assessment of GMPs and should also be included in further ecological studies.

(ii) Suction sampling with a small hand-held suction sampler is an appropriate method to record foliage-dwelling spiders in maize fields and field margins and is recommended for future risk assessment studies or a post-release monitoring of GMPs.

(iii) Foliage-dwelling spiders in maize field as well as in adjacent field margins are potentially exposed to products of *Bt* maize via pollen and *Bt*-contaminated prey.

(iv) A direct lethal effect of *Bt* maize event 176 on spiders was not found.

(v) A high risk of *Bt* maize event 176 to spiders cannot be confirmed in this study.

This thesis includes several important aspects of a risk assessment of *Bt* maize event 176 expressing the *Bt* protein Cry1Ab on foliage-dwellling spiders. The applied methodology of this thesis is an appropriate tool for investigating potential effects of other *Bt* events of *Bt* maize expressing Cry1Ab or other Cry proteins on biological control agents.

However, making generalisations concerning a risk of *Bt* maize event 176 on spiders on the base of this thesis is difficult, because high variations and small effect sizes may have masked existing effects. Therefore, to clarify a potential adverse impact of the cultivation of *Bt* maize event 176 on spiders, a monitoring on longer temporal and larger spatial scales should be extended with more replicates than in this study to enhance statistical power to detect also small effects (Perry et al. 2003, Lang 2004), e.g., as is was conducted in a British study on potential ecological side effects of hercicide-tolerant crops (“farm scale evaluations”, e.g., Haughton et al. 2003). Furthermore, long-term studies on laboratory and field scale are

needed to assess possible chronic and sublethal effects of products of *Bt* maize event 176 on spiders and spider populations, e.g., effects on fecundity and longevity of spiders which may bias the biocontrol efficacy of spider assemblages in agroecosystems.

In addition to the aspects of an exposure assessment of *Bt* maize pollen for spiders included in this study, information on an uptake of Cry1Ab via *Bt* maize pollen by different spider guilds with different biologies could be useful to assess the exposure of spider communities to *Bt* maize pollen.

Furthermore, the risk assessment of spiders to products of *Bt* maize should be more extended concerning tritrophic interactions of spiders with *Bt*-contaminated prey, as direct *Bt* effects as well as indirect effects via insufficient nutrition quality of *Bt*-contaminated prey may exist (Strohmeyer et al. 1998).

Therefore, data on the actual exposure of spiders to *Bt*-contaminated prey should be available, e.g., data on prey spectra of different spider guilds in maize fields and on field margins, data on the uptake and content of Cry1Ab in actual spider prey (e.g., herbivores and pollinators with pollen loads), data on the uptake of Cry1Ab via pollen and *Bt*-contaminated prey by spiders under field conditions in maize fields and on field margins (only one recent study exists: Harwood et al. 2005). In addition, the biological activity of *Bt* toxins ingested by spiders (e.g., with bioassays on *Bt*-suspectible organisms) should be conducted. This test is useful, because common methods to quantify *Bt* proteins, such as the enzyme-linked immunosorbend assays (ELISA) give no information on the biological activity of proteins which is importat for a exposure assessement on non-targets.

Indirect effects of *Bt*-contaminated spider prey are possible, as there is evidence that several taxa of actual spider prey may be harmed by *Bt* toxins, e.g., Diptera (Indrasith et al 1992), Sternorrhyncha (Ashouri et al. 2001) and Heteroptera (Ponsard et al. 2002). To clarify such potential indirect effects which may harm spiders sublethally or lethally, laboratory studies with *Bt*-contaminated spider prey should be conducted.

In conclusion, more information on chronic and sublethal effects as well as on indirect effect of *Bt* maize event 176 on spiders are needed to exclude an adverse effect of *Bt* maize on spiders and spider populations. An overview of further investigations needed to assess a potential risk of *Bt* maize event 176 is given in Fig. 4.

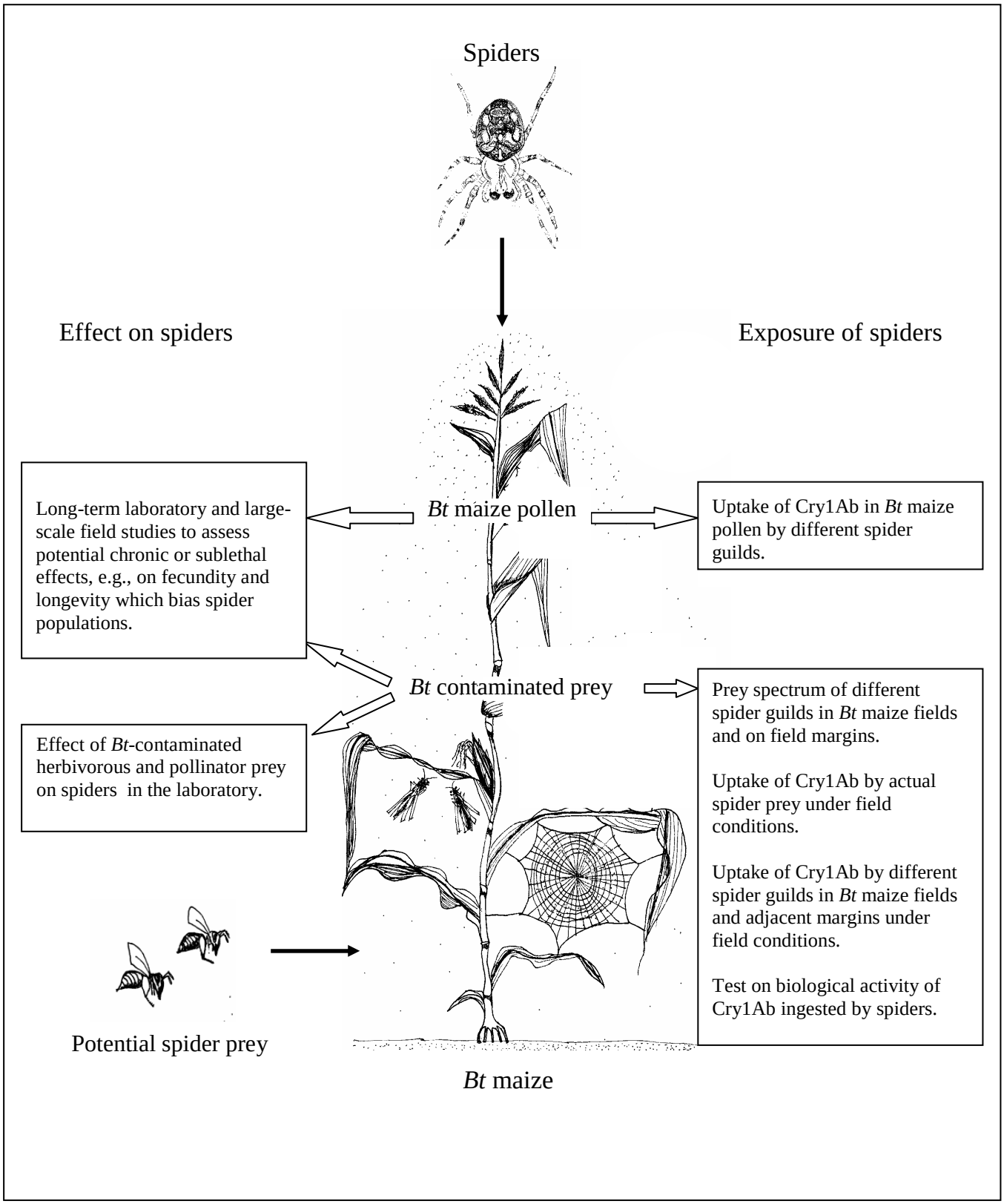

Figure 4: Overview of aspects of a risk assessment of *Bt* maize concerning foliage-dwelling spiders which should be investigated in future studies.

For the further assessment of the exposure of spiders to *Bt* maize via *Bt* pollen and *Bt* contaminated prey, further characteristics of spiders and potential spider prey should be investigated (right boxes). To clarify the potential effect of *Bt* maize on spiders, laboratory and field studies should be extended (left boxes).

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List of publications

Ludy, C. & Lang, A., 2004: How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods. – *Journal of Applied Entomology* 128, 501-509.

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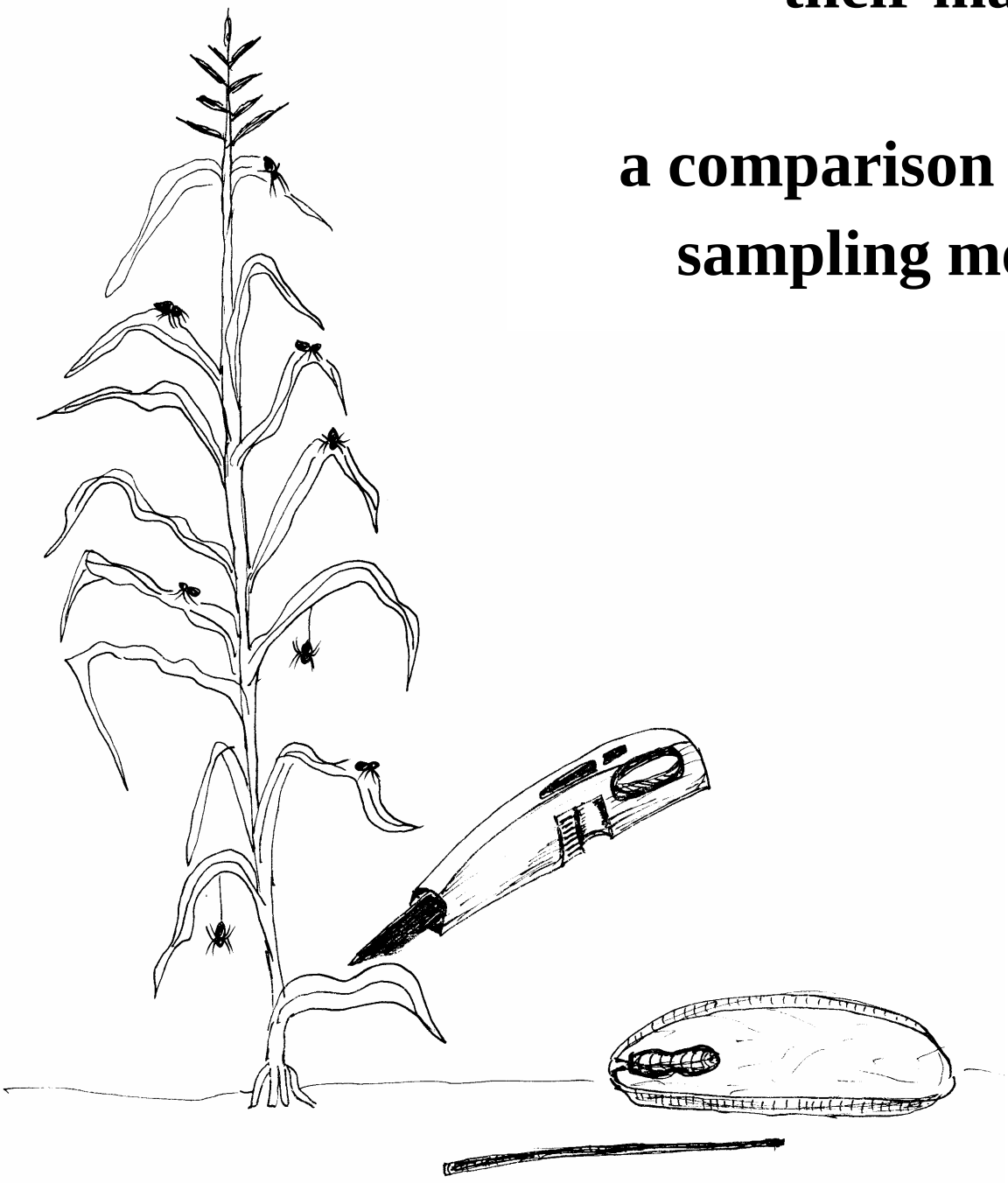
Ludy, C. & Lang, A.: *Bt* maize pollen exposure and impact on the garden spider *Araneus diadematus.* – Accepted, *Entomologia Experimentalis et Applicata*.

Ludy, C.: Prey spectra and prey selection of orb-web spiders (Araneae: Araneidae) on field margins: significance for an exposure assessment of *Bt*-contaminated prey. – Submitted to *Agriculture, Ecosystems & Environment*.

How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins:

a comparison of two sampling methods



How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods.

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Abstract

The foliage-dwelling spider fauna was collected in maize fields and on stinging nettles in adjacent margins in Bavaria, South Germany. Two different sampling methods were evaluated: drop cloth sampling and suction sampling. The overall catch was dominated by juvenile spiders, web-building spiders, and spiders of the families Theridiidae, Linyphiidae, Tetragnathidae and Araneidae (in decreasing order). Field margins harbored more species than maize fields, whereas the total spider abundance was higher in the maize crop. Web-building spiders such as Theridiidae and Linyphiidae were prominent in maize by individual numbers. Suction sampling with a small suction device proved to be a more efficient and consistent sampling method for foliage-dwelling spiders than drop cloth sampling. Density and species richness of foliage-dwelling spiders in maize was shown to be fairly high, implying that spiders of higher strata may play a more important role in biological control than suspected up to now.

Key words: Spider abundance, species richness, seasonal dynamics, suction sampling, drop cloth sampling, *Zea mays*, field margins

1 Introduction

Spiders belong to the most numerous invertebrate predators of arable land in Europe (Nyffeler & Sunderland 2003). Although they are generalists, a diverse spider fauna can contribute to the limitation of on various pest species (Marc & Canard 1997). Therefore spiders have an important function in pest control (e.g. Lang 2003). However, in studies in arable land spiders are often sampled with pitfalls which provide only information about ground-dwelling active spiders (e.g. Downie et al. 1996, Lang 2000). In contrast, there is only scarce information about foliage-dwelling spiders occurring in arable fields, despite the fact that the community of foliage-dwelling spiders can be as abundant and as relevant for pest control as ground-dwelling spiders (e.g. Carter & Rypstra 1995). Therefore, there is a need of more data concerning foliage-dwelling spiders in agricultural habitats. Although maize belongs to the prominent crops in Central Europe, there hardly exists any data on the spider fauna in maize fields. Moreover, the few papers dealing with spiders in maize only cover ground-dwelling spiders (e.g. Alderweireldt & Desender 1990). In agricultural landscapes, field margins have a great importance, because they increase structural complexity of arable landscapes, thereby promoting populations of natural enemies (Thies & Tscharnke 1999). Established field margin strips (sensu Marshall & Moonen 2002) can serve as overwintering sites for spiders (Lemke & Poehling 2002). These margins can also accommodate reproducing spider populations and act as an important "source" for spiders, immigrating into fields (Kajak & Lukasiewicz 1994). Common sampling methods for foliage-dwelling spiders are visual search including quadrate sampling (Nyffeler & Benz 1979, Asselin 1988, Barthel 1997), sweep net sampling (Samu et. al. 1996a), suction sampling (Topping & Sunderland 1994) and drop cloth sampling (Culin & Yeargan 1983). Although visual search is an effective method for recording foliage-dwelling spiders (Amalin et al. 2001), this sampling method is time consuming, and small spiders are likely to be overlooked (Nobre et al. 2000). Sweeping is an ineffective method to catch foliage-dwelling spiders, because only 2-10 % of the absolute population numbers of arthropods are captured with this sampling method (Haas 1980). Here, suction samples and drop cloth samples were used to collect the foliage-dwelling spider fauna as these methods are considered to be the most efficient ones (Green 1999, Nobre et. al. 2000). The objectives of the study were: (1) to obtain baseline data on spider abundance and species richness in maize fields and adjacent field margin strips, and (2) to compare and to evaluate the two sampling methods for their efficiency in monitoring foliage-dwelling spiders in agricultural habitats.

2 Material and methods

2.1 Location and study sites

This study was carried out on three research farms of the Bavarian State Research Center for Agriculture located in Swabia, Frankonia and Upper Bavaria (South-Germany). At each location the spider fauna was recorded in two maize fields (varieties "Antares" and "Navares" from Syngenta) and in two margin strips adjacent to these fields, resulting in a total number of six fields and six margin strips. Each maize field was two hectares large, and no insecticides, but herbicides were applied once in May ("Lentagran" and "Zintan Pack" from Syngenta; "Mikado" from Bayer). On the northern edge of each maize field, a margin strip (50 x 6 m) was established in the end of April. These margin strips were planted with stinging-nettles (*Urtica dioica*) and various others herbs (*Sinapis arvensis*, *Lotus corniculatus*, *Daucus carota*). Each plant species covered a plot of 10 x 6 m per margin strip. Only stinging nettle plots were sampled in this study. Nettles were chosen as the focus plants as they are very abundant in agricultural land, and were also the dominant plant species occurring along other maize field edges on the research farms. Stinging nettle shrubs were obtained from local field populations nearby, and were then planted in the concerning plots of each field margin strip. Approximately 36 - 40 nettle shrubs (diameter circa 20 cm at top of shrub) were planted per field margin strip (i.e. about 0.6 shrubs per m^2), and fertilized regularly with nitrogen to secure proper growth.

2.2 Sampling dates and methods

The study was carried out from the 4th of July to the 25th of September in 2001. Spiders were recorded twice in a month (but only once in September), resulting in five sampling dates per site. On each sampling date, 20 maize plants per maize field, and 20 stinging nettle shrubs per margin strip were randomly selected. The spider fauna was then recorded by sampling half of the plants by suction sampling and half of the plants by drop cloth. This resulted in an overall number of 60 sampled plants per sampling period, per sampling method, and per maize plant or nettle shrub, respectively (3 locations * 2 fields per location * 10 plants). In maize fields, the selected plants were located in the middle of the maize field and had at least a distance of 20 m to the edge of the maize field. On average, ten maize plants covered one square meter

(distance between maize rows was 75 cm and 15 cm between single maize plants within a row). The phenology of maize plants (after Meier 1997) is shown in Tab. 1. In margin strips, the spider density was not referred to the area of one square meter, because stinging nettles showed a different performance in the different plots during the season, and, therefore, they were distributed quite heterogeneously. In consequence, the number of plants did not correspond uniformly to a standardized area of one square meter, and spider abundance is presented only as numbers per plant. The used suction sampler was a modified small vacuum cleaner with a suction hole opening area of 3,0 x 0,6 cm (producer: "Quelle"). The plants were sampled from the top to the bottom by holding the suction sampler directly onto the plant. Each maize plant was sampled for 33.68 ± 11.12 s, and nettle shrubs for 36.78 ± 17.95 s (means ± 1 SD). For drop cloth sampling, each plant was beaten 20 times with a plastic stick from the top to the bottom of the plant. High plants (> 180 cm) were bent over a drop cloth (diameter of 74 cm), and then knocked with a plastic stick. Spiders knocked down on the drop cloth were then collected by hand. Knocking took 27.98 ± 8.5 s per maize plant, and 14.55 ± 4.36 s per nettle shrub (means ± 1 SD).

Table 1: Height, principal growth stages and BBCH-identification keys of maize plants with regard to the different sampling periods of the three sampling sites.

Date	Activity	Mean height (cm)	Principal growth stage
17.04.-04.05.	Sowing	0	0: Germination
04.07.-06.07.	1st Sampling	120	5: Inflorescence emergence, heading
17.07.-24.07.	2nd Sampling	220	6: Flowering, anthesis
08.08.-09.08.	3rd Sampling	245	7: Development of fruit
22.08.-24.08.	4th Sampling	245	8: Ripening
19.09.-25.09.	5th Sampling	225	9: Senescence
28.09.-25.10.	Harvest	225	9: Senescence

2.3 Identification

Sampled spiders were fixed in 70 % ethanol, brought to the laboratory and identified according to Heimer & Nentwig (1991) and Roberts (1985, 1987, 1995). Species were classified according to Platnick (2003). Juvenile spiders were identified to genus or family level, if possible. The recorded spiders were divided in two main guilds: stationary web building spiders (Theridiidae, Linyphiidae, Tetragnathidae, Araneidae, Dictynidae), and mobile hunting spider (Lycosidae, Pisauridae, Miturgidae, Anyphaenidae, Clubionidae, Philodromidae, Thomisidae, Salticidae).

2.4 Statistical analyses

Analysis of variance (ANOVA) was used to analyze the effects of the main factors “habitat type” (i.e. “maize field” and “margin strip”) and “sampling method” (i.e. “suction sampling” and “drop cloth sampling”) on the dependent variables “total number of species” (1) and “number of individuals” (2). The effects of the main factors “habitat type” and “sampling method” were also analyzed on the dependent variables “proportion of guilds” (3), “proportion of stages of development” (4) and “proportion of families” (5). Additional factors tested were “spider guild” (i.e. “web building spiders” and “hunting spiders”), “stage of development” (i.e. “juveniles” and “adults”), and “spider family” (various spider families). A repeated-measurement ANOVA was performed to analyze the effects of “habitat type”, “sampling method”, “family” and “time” (5 sampling dates) on the dependent variable “number of spider individuals” (6). Kolmogorov-Smirnov one-sample test was used for testing the normal distribution of data. To create normal distribution and/or heterogeneity of variances of the data, proportional data were arcsin-transformed and other data were log-transformed. Sen and Puri`s nonparametric test was conducted to test for homogeneity of variances. Post hoc comparisons were conducted with the Tukey honest significant differences test (HSD). All statistical analyses were carried out with the software Statistica 5.0 (StatSoft inc. 1995). The variation of data was described by the coefficient of variance (cv). A modified *F*-test ($F = cv_1^2/cv_2^2$) was used to analyze differences in the variation of data from suction and drop cloth samples with regard to the number of individuals and the number of spider species captured in both habitat types (field and margin). All average values are presented as arithmetic means with 1 SD.

3 Results

3.1 Spider fauna of margin strips and maize fields

A total of 647 individuals and 40 species were recorded in maize fields and field margins (Tab. 2). The overall mean proportion of juvenile spiders (80 ± 15 %) was clearly higher than the proportion of adult spiders (20 ± 17 %) in both habitat types (main factor "stage of development": ANOVA, $F_{1.40} = 132.72$, $p < 0.001$). Also, the overall average proportion of web building spiders (87 ± 10 %) was higher than the proportion of hunting spiders (13 ± 12 %, main factor "spider guild": ANOVA, $F_{1.40} = 390.87$, $p < 0.001$). The mean spider density in maize fields was 5.9 (minimum-maximum: 3-12) spiders per 10 plants which matches to 5.9 spiders per m². The mean spider number in margin strips was 4.9 (minimum-maximum: 2-7) spiders per 10 stinging nettle shrubs (Tab. 3a).

Table 2: Summary of captured spiders in margin strips and maize fields, 5 sampling dates * 3 locations * 2 habitat types * 10 plants (maize or stinging nettle shrubs, respectively).

Species	Maize field	Margin strip	Total
Araneae			
unidentified	1	1	2
Theridiidae			
Achaearanea sp.		1	1
Achaearanea riparia (Blackwall, 1834)		1	1
Enoplognatha latimana Hippa & Oksala, 1982		2	2
Enoplognatha ovata (Clerck, 1757)		1	1
Episinus sp.		1	1
Episinus angulatus (Blackwall, 1836)		1	1
Paidiscura pallens (Blackwall, 1834)	1		1
Theridion sp.	2	6	8
Theridion impressum L. Koch, 1881	7	14	21
unidentified	117	68	185
Linyphiidae			
Bathyphantes gracilis (Blackwall, 1841)	1		1
Erigone atra Blackwall, 1833	9		9
Erigone dentipalpis (Wider, 1834)	2	2	4
Tenuiphantes tenuis (Blackwall, 1852)		5	5
Linyphia triangularis (Clerck, 1757)	1		1
Meioneta rurestris (C. L. Koch, 1836)	5	8	13
Meioneta fuscipalpa (C. L. Koch, 1836)		1	1
Microlinyphia sp.	1	2	3
Microlinyphia pusilla (Sundevall, 1830)	1	3	4

Species	Maize field	Margin strip	Total
Oedothorax apicatus (Blackwall, 1850)	15	7	22
Porrhomma microphthalmum (O. P.-Cambridge, 1871)		2	2
unidentified	47	18	65
Tetragnathidae			
Metellina sp.	2		2
Pachygnatha degeeri Sundevall, 1830	1	1	2
Tetragnatha sp.	89	48	137
Tetragnatha montana Simon, 1874		1	1
Tetragnatha extensa (Linnaeus, 1758)		1	1
Araneidae			
Agalenatea redii (Scopoli, 1763)		1	1
Aculepeira ceropegia (Walckenaer, 1802)	2	14	16
Araneus diadematus Clerck, 1757		1	1
Araneus quadratus Clerck, 1757		1	1
Araniella sp.	4	6	10
Araniella cucurbitina (Clerck, 1757)		1	1
Larinioides sp.	1	2	3
Singa sp.		1	1
unidentified	22	14	36
Lycosidae			
Pardosa sp.	5	5	10
Pardosa lugubris (Walckenaer, 1802)	1		1
Pisauridae			
Pisaura mirabilis (Clerck, 1757)		7	7
Dictynidae			
Nigma sp.	1		1
Dictyna sp.	1	1	2
Miturgidae			
Cheiracanthium sp.		1	1
Cheiracanthium erraticum (Walckenaer, 1802)		1	1
Anyphaenidae			
Anyphaena accentuata (Walckenaer, 1802)		1	1
Clubionidae			
Clubiona sp.		2	2
Philodromidae			
Philodromus sp.	4	6	10
Thomisidae			
Misumenops tricuspitatus (Fabricius, 1775)	1	7	8
Xysticus sp.	6	16	22
Xysticus audax (Schrank, 1803)	1		1
Unidentified	4	3	7
Salticidae			
Evarcha arcuata (Clerck, 1757)		1	1
Euophrys sp.		1	1
Unidentified	1		1
Total no. of individuals	356	291	647
Total no. of species	21	34	40

Overall, Theridiidae were more abundant than Tetragnathidae, Araneidae and other families, while Linyphiidae were more abundant than Araneidae and other families (main factor "habitat type": ANOVA, $F_{4.100}$ =11.45, $p < 0.001$, Tukey HSD test: $p < 0.05$ in all cases). *Oedothorax apicatus*, *Meioneta rurestris* and *Theridion impressum* were the dominant species in both habitat types (Tab. 2).

Table 3: Number of species, number of individuals and proportions (%) of stages of development and guilds (a) as well as the coefficients of variation (cv, %) of the total number of species and individuals (b) in margin strips and maize fields provided by suction and drop cloth sampling (n = 12 each).

	Habitat type		**Sampling method**	
	Maize field	**Margin strip**	**Suction sampling**	**Drop cloth sampling**
(a)				
Total no. of species	**6.66 ± 2.19 #**	**8.75 ± 2.86**	8.00 ± 2.37	7.42 ± 3.09
No. of individuals	5.93 ± 2.71	4.87 ± 2.74	4.50 ± 1.60	6.30 ± 3.34
Stages of development				
Juveniles	83 ± 15 a	76 ± 16 a	76 ± 15 a	85 ± 15 a
Adults	17 ± 16 b	24 ± 18 b	24 ± 15 b	15 ± 16 b
Guilds				
Web building spiders	93 ± 4 a	82 ± 12 b	87 ± 8 a	87 ± 13 a
Hunting spiders	7 ± 4 c	18 ± 15 c	13 ± 8 b	13 ± 16 b
(b)				
Total no. of species (cv)	33.02	32.73	29.68	41.64
No. of individuals (cv)	45.75	56.30	**35.53 #**	**53.06**

Differences between treatments of a main factor are in bold letters and marked with a # ($0.05 < p < 0.10$) (ANOVA or T-Test, respectively). Significant differences within groups of the main factors "Stages of development" and "Guilds" are marked with different letters ($p < 0.05$, Tukey HSD Test). Means ± 1 SD.

In maize fields 356 individuals and 21 species were caught, and in margin strips a total of 291 individuals and 34 species (Tab. 2). *Erigone atra* was often collected in maize fields, whereas *Aculepeira ceropegia*, *Pisaura mirabilis* and *Misumenops tricuspitatus* were among the abundant species in margin strips (Tab. 2). The higher number of species in margin strips resulted mainly from the occurrence of more species of Araneidae (7 in strips vs. 3 in maize) and hunting spiders (10 in strips vs. 5 in maize, Tab. 2). This difference in species number between the two habitat types was nearly significant (main factor "spider species": ANOVA, $F_{1.20} = 3.42$, $p = 0.08$, Tab. 3a). An interaction of "habitat type" and "time" indicated a higher abundance of spider individuals in maize fields in the end of August (repeated-measurement ANOVA, $F_{4.400} = 2.48$, $p < 0.05$, Fig. 1a; not affirmed by Tukey HSD test). Other significant

differences between the habitats were a higher percentage of web-builders in maize fields (interaction "habitat type" and "spider guild": ANOVA, $F_{1.40} = 12.40$, $p < 0.01$, Tukey HSD test: $p < 0.05$, Tab. 3a), and a different proportion of families (interaction "habitat type" and "spider family": ANOVA, $F_{4.100} = 2.91$, $p < 0.05$). The latter was presumably due to the higher dominance of Theridiidae and Linyphiidae in maize fields (Fig. 2a). There was no difference between maize fields and margin strips in the percentages of stages of development (interaction "habitat type" and "stages of development": ANOVA, $p > 0.05$), and in the coefficients of variances of mean species numbers and mean individual numbers (F-test: $p > 0.05$ in both cases, Tab. 3b).

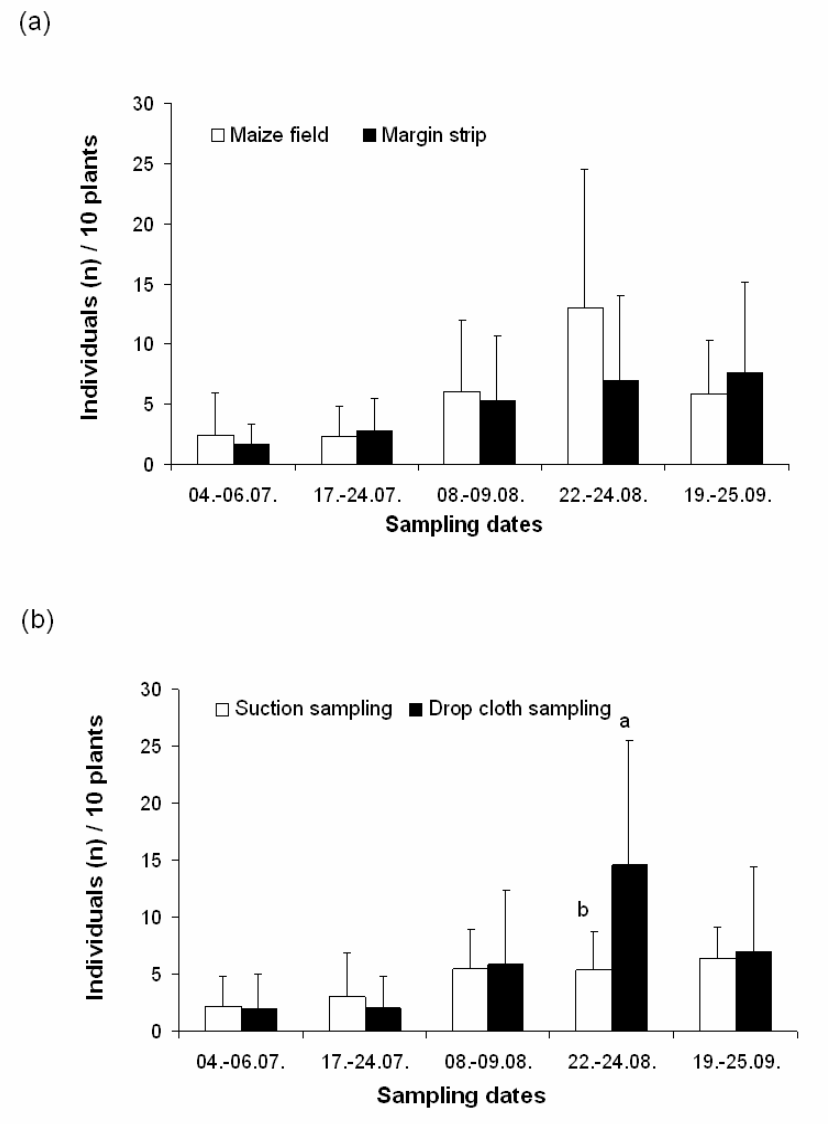

Figure 1: Mean number (+ 1 SD) of spider individuals in maize fields and margin strips during different sampling periods, both sampling methods pooled (a); mean number (+ 1 SD) of spider individuals caught with different sampling methods during different sampling periods, both habitat types pooled (b). Significant differences (Tukey HSD test: $p < 0.05$) are marked with different letters, n = 12 (each habitat type or sampling method, respectively).

3.2 Seasonal dynamics

The number of spider individuals changed during the sampling time (main factor "time": repeated-measurement ANOVA, $F_{4.400} = 29.80$, $p < 0.001$). In August, more spiders were caught than in July (Fig. 3a). Different families showed different seasonal dynamics (interaction "spider family" and "time": repeated-measurement ANOVA, $F_{16.400} = 3.24$, $p < 0.001$): Theridiidae, Tetragnathidae and Araneidae had a peak in August and September (Fig. 3c-e), while Linyphiidae showed no differences during the sampling time (Fig. 3b).

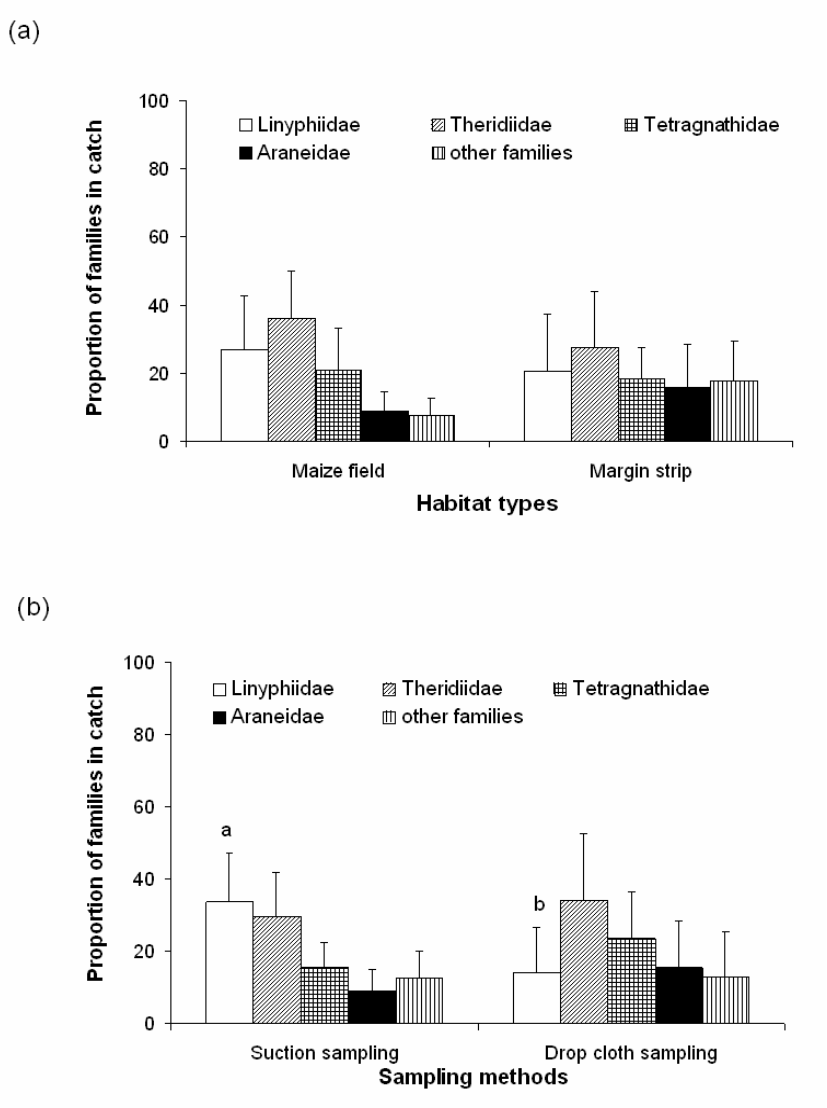

Figure 2: Proportions (%) of families in maize fields and margin strips (seasonal means + 1 SD), both sampling methods pooled (a); proportions (%) of families caught with suction and drop cloth sampling (seasonal means + 1 SD), both habitat types pooled (b). Significant differences (Tukey HSD test: $p < 0.05$) are marked with different letters, n = 12 (each habitat type or sampling method, respectively.

3.3 Effect of sampling methods

Pooled over all sampling occasions, there was no difference in the total number of species and mean number of spider individuals between suction samples and drop cloth samples (main factor “sampling method”: ANOVA, $p > 0.05$ in both cases, Tab. 3). Only in August, drop cloth sampling recovered more spider individuals than suction sampling (interaction “sampling method” and “time”: ANOVA, $F_{4.400} = 4.85$, $p < 0.001$; Tukey HSD test, $p < 0.001$, Fig. 1b). The proportions of stages of development were different between suction samples and drop cloth samples (interaction “sampling method” and “stage of development”: ANOVA, $F_{1.40} = 4.92$, $p < 0.05$), and the data suggested that this was due to the higher number of juvenile spiders in drop cloth samples (Tab. 3). Also, the coefficient of variance (cv) of the individual spider number tended to be higher in drop cloth samples (F-test, $p = 0.09$, Tab. 3b), while the cv of the species numbers did not differ between the sampling methods ($p > 0.05$, Tab. 3b). The proportion of Linyphiidae was higher in suction samples than in drop-cloth samples (interaction “sampling method” and “spider family”: ANOVA, $F_{4.100} = 5.51$, $p < 0.001$; Tukey HSD test, $p < 0.01$, Fig. 2b), while the numbers of the other families were similar in both sampling methods. The proportions of guilds did not differ between suction samples and drop cloth samples (interaction “sampling method” and “spider guild”: ANOVA: $F_{1.40} = 0.26$, $p > 0.05$, Tab. 3a).

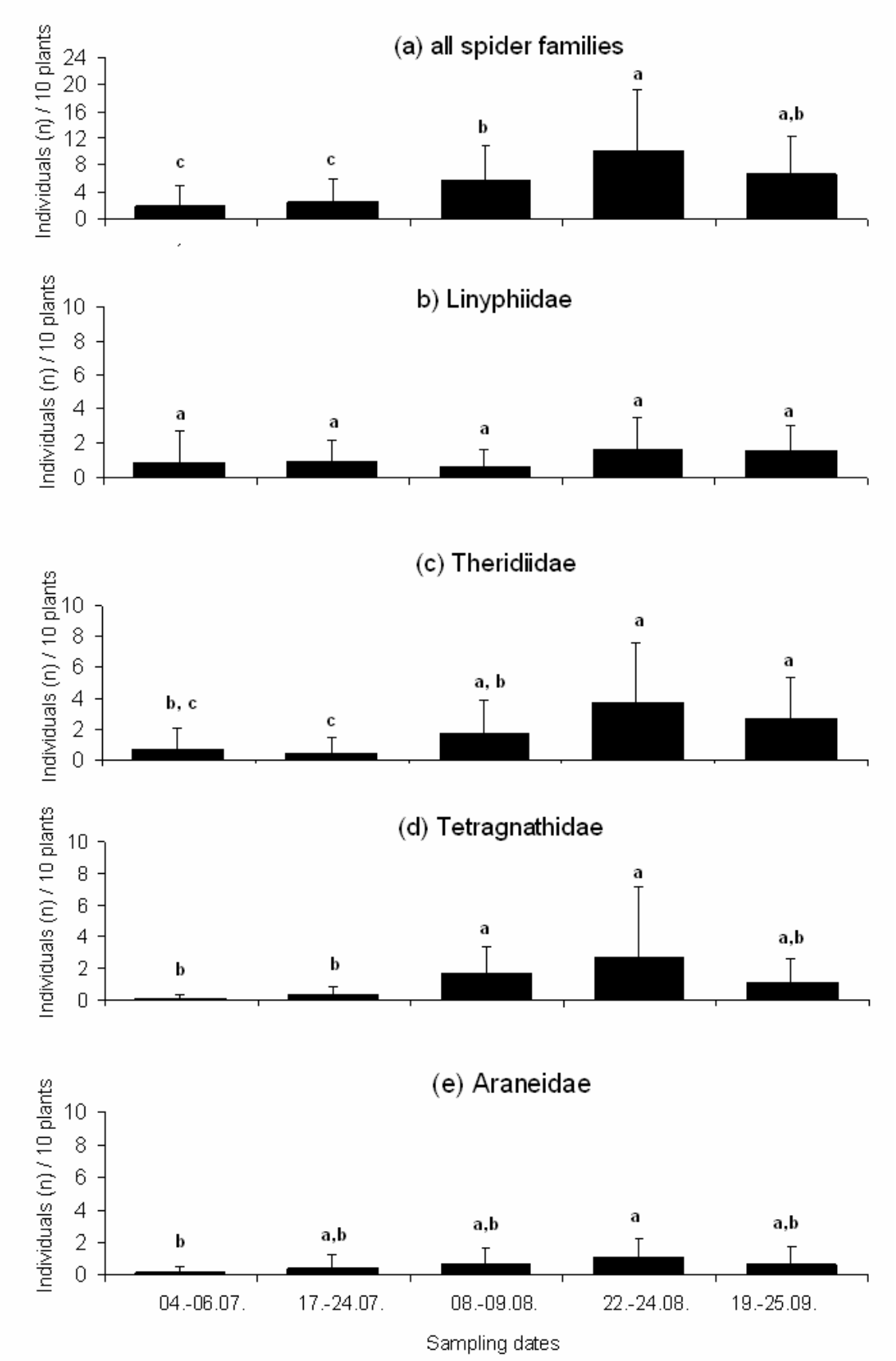

Figure 3: Seasonal dynamics of individuals of all spiders (a), and selected families (b - e), habitat types and sampling methods pooled. Significant differences are marked with different letters (Tukey HSD test: $p < 0.05$), n = 24 each sampling period. Means + 1 SD.

4 Discussion

4.1 Spider fauna of maize fields and adjacent margin strips

The foliage-dwelling spider fauna in maize fields and margin strips mainly consisted of web-building spiders belonging to Linyphiidae, Theridiidae, Tetragnathidae and Araneidae (in decreasing dominance). The high proportion of juveniles and web-building spiders (mostly Linyphiidae, Theridiidae and Tetragnathidae) may indicate a recent colonization by ballooning in both habitat types (Suter 1999) as especially juveniles and these spider families migrate into arable habitats by aerial dispersal (Barthel 1997). Krause (1987) found a similar amount of about 70 percent of juvenile spiders in higher vegetation layers of field margins and fields. Barthel (1997) as well as Nyffeler & Benz (1979) found a much lower density of 0.01 respectively 0.1 foliage-dwelling spiders per m^2 in maize fields as opposed to an average of 2 to 13 spiders in the present study. However, the density of foliage-dwelling spiders was probably underestimated in those studies, because small and hidden spiders are likely to be overlooked by visual search which was the method applied. A mean density of five spiders per square meter in this study may appear too low to have a significance for biological control. However, spider densities within this range may already have an effect on aphid numbers (Lang 2003). Moreover, the efficiency in killing prey is not only dependent on the abundance and biomass of spiders. Efficiency in killing prey also depends on the number and size of the spider webs, because small pests may die in spider webs independent from predation by a spider. Besides, spiders often capture and kill more prey than they consume ("wasteful killing"), which contributes to their biocontrol potential independent from saturation level of the spiders (Sunderland 1999). For example, up to 1000 insects may be present in a spider web at a given moment, and not all are eaten by the spider (Nyffeler et al. 1994). Moreover, the spiders that are most efficient at capturing pest organisms are those that forage on the plant itself, because this is the location preferred by the pests (Maloney et al. 2003). Therefore, the biocontrol role of foliage-dwelling spiders in maize fields may be more important than suspected (Nyffeler & Benz 1987). The variance of abundance and species richness was high in both habitat types. High variations of spider species richness in agroecosystems are well known (Samu & Szinetár 2002). This may be due to the influence of spider biocoenoses of the habitats and landscape around margins and fields (Wolters et al. 1999).

In maize fields, the families of Linyphiidae and Theridiidae dominated by number. In consequence, members of these families were also very prominent in maize fields (i.e. *O. apicatus*, *M. rurestris*, *E. atra*, *T. impressum*). This dominance of only a few species is typical for agroecosystems (Samu & Szinetár 2002, Nyffeler & Sunderland, 2003). In maize fields, the proportion of web-building spiders was higher than in margin strips. As web-building spiders such as Linyphiidae frequently disperse by ballooning, this may indicate a higher importance of ballooning as immigration mechanism in maize fields as compared to margin strips. In small margin strips, hunting spiders, which often immigrate by ground dispersal, could reach this new habitat from adjacent "sources", whereas these spiders hardly get to the center of fields by ground dispersal (Bishop & Riechert 1990, Frank & Nentwig 1995). The higher species richness of margin strips was mainly due to more species of Araneidae and hunting spiders. This result may be attributed to the complex and denser vegetation structures found in margins, which provides the necessary microhabitats and web attachment features (e.g. Hatley & Macmahon 1980). On the other hand, the linyphiid spider *E. atra* prefers open habitats and avoids places with dense vegetation (Downie et al. 2000), and consequently was more often collected in maize fields. Rare or threatened species were not very abundant and only one red list species was found. *Meioneta fuscipalpa* (1 individual on the margin strip at Schwarzenau, Tab. 2) is classified in the category "R" of the Red Data Book of Bavaria (Blick & Scheidler 2004; R = very rare or geographically restricted species).

4.2 Seasonal dynamics

The spider families Theridiidae, Tetragnathidae and Araneidae had an abundance peak in the end of August, whereas the individual numbers of Linyphiidae stayed on the same level without a clear peak during the season. This may be due to the univoltine phenology of most Theridiidae, Tetragnathidae and Araneidae, and the hatching of juveniles of abundant field-inhabiting species of these families during August, e.g. the theridiid *T. impressum*, species of the genus *Tetragnatha*, and the araneids *A. ceropegia* and *Mangora acalypha* (Schäfer 1976; Barthel 1997). In contrast, Linyphiidae have a multivoltine phenology with different development stages occurring at the same time (Topping & Sunderland 1998). Also, these spiders show ballooning behavior in all development stages over the whole season (Weyman et al. 1995). This can lead to more or less constant individual number during the vegetation season (Samu & Szinetár 2002).

In general, the increase in height of maize plants and stinging nettle shrubs (C. Ludy, pers. obs.) during the season is correlated with vegetation complexity and a concurrent increase in spider abundance as more spiders find appropriate microhabitats in higher or denser plants (Hartley & Macmahon, 1980). A spider peak at the end of the season was also found in other agricultural habitats such as garden plots (Bishop & Riechert 1990), alfalfa, soybean (Culin & Yeargan 1983), and wheat (Topping & Sunderland 1994).

4.3 Evaluation of sampling methods

Drop cloth sampling and various kinds of suction sampling are reported to be efficient methods for collecting foliage-dwelling spiders, e.g. in vineyards (Costello & Daane 1997; Nobre et al. 2000), in heath (Canard 1981), and in orchards (Amalin et al. 2001). In this study drop cloth as well as suction sampling yielded good results in terms of species number and individuals obtained, especially when compared to similar studies in maize crops which applied visual searching (Nyffeler & Benz 1979, Barthel 1997). Drop cloth and suction sampling recorded the foliage-dwelling spider assemblage similarly in terms of species number, individual abundance and proportion of spider guilds in both habitat types. Therefore, the results appear reliable as they were obtained by two different methods in a quite comparable pattern. A disadvantage of drop cloth sampling may be the inconvenience of the equipment in dense vegetation. The cloth and the stick may tap plants other than the selected one quite easily, and the additional catch from surrounding plants may lead to an overestimation of spider densities. This may be especially the case at high spider densities, for instance at times of high ballooning activity of juvenile spiders. Possibly this happened in our August sample. In August more individuals and more juvenile spiders were found with drop cloth sampling. As especially the juveniles balloon at the end of the season (Bishop & Riechert 1990), and as the spider densities were highest in August, drop cloth sampling possibly overestimated abundances on this sampling occasion. The more time needed for drop cloth sampling in maize field compared to margin strips could be due to the inconvenience of the drop cloth in high vegetation, because high maize plants have to be bent over the drop cloth, which is time consuming.

However, suction sampling recovered more Linyphiidae as compared with the drop cloth, which was also found by Costello & Daane (1997) in vineyards, and by Meissle & Lang (unpublished data) in other maize fields. Linyphiid spiders often prefer moist microhabitats as found in the center of nettle shrubs (Samu et al. 1996b), hide in maize axils, or build their web near the ground beneath the maize roots (Alderweireldt 1994). These places can be directly sampled by suction devices but not adequately by drop cloth sampling.

In comparing the two sampling methods suction sampling appears to be more efficient and consistent than drop cloth sampling, which was shown in a higher proportion of Linyphiidae and a lower variance in captured individual numbers. Suction devices collect hidden specimens, the spiders are sucked in immediately (and cannot get lost in an additional sorting step), the catch can be attributed to one plant, and small suction devices as the one used can be handled in the field quite conveniently. A disadvantage of suction sampling is that sucked in spiders may be damaged to a extent that species identification is no longer possible. However, there are more disadvantages to drop cloth sampling: hidden specimens are not fully collected, the catch cannot be attributed to one plant unequivocally, after knocking down spiders must be sorted from the cloth by hand (which is time consuming and may also result in the loss of escaping individuals), the sampling devices are unhandy in dense and high vegetation such as maize, and all these drawbacks may lead to inconsistent catches as demonstrated by the higher variation of the drop cloth samples.

In conclusion, we would recommend the use of small suction samplers for collecting foliage-dwelling spiders, especially in maize crops, as suction devices are more likely to record the spider densities correctly, which was also found by other authors (e.g. Nuessly & Sterling 1984).

Conclusions

The foliage-dwelling spider fauna in maize fields and field margin strip was dominated by web-building spiders. In margin strips, more species occurred than in maize fields, which illustrated the importance of this habitat to enhance pest predator numbers and richness in agricultural landscapes.

In maize fields, foliage-dwelling spiders are more abundant than suggested so far, and may have important impact on pest populations. Suction sampling (with a small suction sampler) is a more reliable sampling method for recording foliage dwelling spiders than drop cloth sampling, especially in maize fields. This study provided evidence that foliage-dwelling spiders are abundant and diverse predators of agroecosystems and are worth to be included in further ecological field studies.

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Dispersion and deposition of *Bt* maize pollen in field margins

Dispersion and deposition of *Bt* maize pollen in field margins.

Pollenflug von *Bt*-Mais in angrenzende Feldränder.

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Abstract

The purpose of the study was to gain information on the temporal occurrence, spatial range and dispersion of maize pollen, and thus potentially *Bt* maize pollen densities in field margins in Bavaria, South Germany. This information is an important part of the risk assessment of *Bt* maize as it characterises the potential environmental exposure of non-target organisms to *Bt* pollen. The majority (91%) of maize fields shed pollen in July, but the beginning of anthesis could be recorded as early as June 27 and as late as August 8. The Bt176 event "Navares" shed pollen about one week earlier than the average anthesis date of other maize varieties. Pollen numbers deposited in field margins were highly variable, and decreased with distance to field edge. The main factors determining pollen densities were relative humidity, growth stage of the maize, and distance to field edge explaining together 48% of the variance of pollen amounts. The more pollen were deposited in maize field margin, the more pollen was found on leaves of wild carrots located at maize field edge. Cry1A(b) amounts in pollen of the events Bt176 and Mon810 were found to be roughly in the range reported so far. The results provide helpful and effective information with regard to the evaluation of the exposure of butterflies to *Bt* maize pollen.

Key words: Cry1A(b), *Bacillus thuringiensis*, *Zea mays*, corn, field edge, maize pollen dispersal, pollen shedding, *Daucus carota*, ELISA, toxin content, environmental exposure, risk assessment, transgenic plants

Zusammenfassung

Zur ökologischen Risikoabschätzung von Bt-Mais ist es notwendig, Informationen über das zeitliche Auftreten von Maispollen sowie Menge und Reichweite des Polleneintrages in Feldränder zu berücksichtigen, da diese Faktoren die Exposition betroffener Nichtziel-Organismen mitbestimmen. Der Großteil der untersuchten Maisfelder (91%) blühte im Juli, der Beginn der Pollenschüttung konnte aber auch frühestens Ende Juni oder spätestens Anfang August liegen. Der Blühbeginn der Bt176-Sorte „Navares“ lag circa eine Woche früher als der Durchschnittswert der anderen Maissorten. Die Maispollendichte in Feldrändern war sehr variabel und nahm mit steigender Entfernung zum Maisfeld ab. Relative Feuchte, Blühstadium des Mais’ und Entfernung zum Maisfeld waren die Faktoren, welche die Pollendichte am stärksten beeinflussten (R2 = 48%). Je mehr Maispollen im Maisfeldrand eingetragen wurden desto mehr Pollen fanden sich auch auf Blättern von Wilder Möhre. Die Konzentration an nachgewiesenem Cry1A(b)-Toxin in Bt176- und Mon810-Mais lag im wesentlichen im bekannten Bereich. Die Ergebnisse tragen zu einer verbesserten Einschätzung der Exposition durch Bt-Maispollen von Schmetterlingslarven am Feldrand bei.

Stichwörter: Cry1A(b), *Bacillus thuringiensis*, *Zea mays*, Mais, Feldrand, Pollenverbreitung, Pollenschüttung, *Daucus carota*, Wilde Möhre, ELISA, Toxin-Konzentration, Exposition, Risikoabschätzung, transgene Pflanzen

1 Introduction

Bt maize is a transgenic crop which has been engineered with genes of the soil bacterium *Bacillus thuringiensis* (*Bt*). All current commercial *Bt* maize events in Europe were transformed with genes which express a truncated form of the insecticidal delta-endotoxin (Cry1Ab) specific against lepidopteran species. The toxin is produced in most tissues of the maize plants, though in different concentrations. The target organism of *Bt* maize is the european corn borer (Lepidoptera, Pyralidae: *Ostrinia nubilalis*). Due to its specificity against Lepidoptera the *Bt* toxin has been considered as relatively safe for non-target organisms (Glare & O'Callaghan 2000). However, *Bt* maize pollen may be deposited by wind on host plants of non-target butterfly larvae occurring along field edges (Pleasants et al. 2001), and leaves containing surface-deposited pollen may be consumed by the larvae (Hansen Jesse & Obrycki 2000, Wraight et al. 2000). Adverse effects of *Bt* maize pollen consumption have been reported for larvae of several non-target butterfly species both in the laboratory and the field (e.g. Losey et al. 1999, Hellmich et al. 2001, Stanley-Horn et al. 2001, Zangerl et al. 2001, Felke et al. 2002, Felke & Langenbruch 2003). The actual risk for butterfly populations in the field can be described as a function of the adverse effect (toxicological hazard) and exposure (environmental dose) (Wolt et al. 2003). Therefore, knowledge of the environmental exposure of the butterflies to *Bt* pollen is needed in order to assess the risks associated with cultivation of *Bt* maize. This environmental exposure is determined by various components, for example by characteristics of the *Bt* maize and its pollen such as toxin concentrations and dispersion parameters of the pollen of *Bt* maize (Sears et al. 2001). Particularly important are the time of pollen shed (because of the temporal overlap with the occurrence of butterfly species), and the distance of pollen dispersal and amount of pollen deposition (because of the extent and the spatial range of the exposure) (Wolt et al. 2003). Information on toxin contents of *Bt* maize pollen has increased recently (e.g. Fearing et al. 1997, Hansen Jesse & Obrycki 2000, Sears et al. 2001). However, published data on pollen dispersion are still limited and mainly restricted to studies from the U.S. (Raynor et al. 1972, Oberhauser et al. 2001, Pleasants et al. 2001), and often refer to outcrossing rates which are difficult to convert into actual pollen densities deposited in field margins (e.g. Emberlin et al. 1999, Treu & Emberlin 2000). At the Bavarian State Research Centre for Agriculture potential environmental impacts of *Bt* maize are studied since several years (bioSicherheit.de 2003, Lang et al. in press). Within the framework of this extensive research programme data were obtained about pollen

characteristics of two *Bt* maize events, Bt176 from Syngenta and Mon810 from Monsanto, and of conventional non-transgenic maize varieties. Here, we present results about the time of maize pollen shedding, the amount of pollen deposition in dependence of the distance to maize fields, surface-deposited pollen on a host plant of butterflies, and of the toxin content in the pollen of the above *Bt* maize events.

2 Material and methods

2.1 Time of pollen shed

The examination and evaluation of maize varieties belongs to the official tasks of the Institute of Crop Production and Plant Breeding at the Bavarian Research Centre for Agriculture, and the registered data are stored in databases. Maize varieties to be assessed were planted on state research and contract farms throughout Bavaria, South Germany. Then, basic parameters of the plants were analysed during the season until harvest, among others the date of the onset of pollen shedding. The temporal range of the onset of maize pollen shedding in Bavaria was analysed for the years 1995 to 2000 (after 2000 the onset of pollen shedding was no longer part of the obligatory data recording scheme, therefore we could not analyse more recent data). Overall, 307 different maize varieties were analysed on a total number of 1680 fields at 22 different localities. Additionally, the Bt176 variety “Navares” was cropped on 21 fields (each 2-ha large) between 2000 and 2003, and the date was recorded of the start of pollen shedding.

2.2 Pollen deposition and dispersion

Analysis of pollen deposition and dispersion was performed during three years (in July, 2000 – 2002) on two state research farms, in Grub near Munich (11°46’49’’ east, 48°10’09’’ north) and in Neuhof near Donauwörth (10°47’10’’ east, 48°47’09’’ north). At each location a 2-hectare large field was cropped with Bt176 maize (variety “Navares”, Syngenta). Pollen deposition was measured using microscope slides (76 * 26 mm) covered with a thin coat of Vaseline®. These slides were fixed on top of wooden stakes ranging in height from 6 to 20 cm (Tab. 1). The slides were exposed during maize anthesis, either within the maize field (-5 m from field edge), directly at the maize field edge (0 m), or outside the maize field in various distances to the field edge (1 m – 10 m, Tab. 1, Fig. 2). The slides were placed around

noontime, exposed for 24 hours, and then taken to the laboratory where the pollen were counted. Deposited pollen grains on slides were scanned and automatically counted with an image analyser (Leica Qwin), or counted manually with a microscope (when pollen densities were very high or when slides were soiled). In total, 300 slides were placed in and along maize fields during the study (70% in Neuhof, and 30% in Grub; 12% in 2000, 42% in 2001, and 46% in 2002; 12% along the western edge of the field, 25% along the eastern edge, and 63% along the northern edge). Stage of maize anthesis was recorded according to the principal growth stages of maize (BBCH code) following Meier (1997). Weather conditions were registered by stations located on the research farms (air temperature at 200 cm height, relative humidity, precipitation, wind speed at 250 cm height).

A multiple linear regression analysis with stepwise selection was applied to the data to identify the key variables determining pollen deposition. When necessary, data were transformed to meet assumptions of multiple regression analysis (see transformations in parentheses).The focus variable was “pollen per cm2” (Ln(x+1)), and the independent variables “air temperature” (Ln(x+1)), “precipitation” (Ln(x+1)), “humidity” (arcsine(x)), “wind speed” (x), “distance to maize edge” (square root of Ln(x+10)), “height of slide” (1/x), “field edge orientation” (x, dummy variable), BBCH code of maize anthesis (arcsine(x/67)), and “site” (x, dummy variable). The analysis was carried out using SPSS, version 11.

2.3 Surface-deposited pollen on *Daucus carota*

Deposition of maize pollen on leaves of *Daucus carota* was analysed during full anthesis (BBCH stage = 65) on 22, 23 and 26 July 2002 at the northern edge of a 2-hectare large field on the research farm Neuhof, which was cropped with Bt176 (“Navares”). *D. carota* was chosen as this is an important food plant for larvae of the common swallowtail (*Papilio machaon*), a butterfly occurring regularly in agricultural land and along maize field margins (Ebert & Rennwald 1991a, Lang 2004). Carrot leaves were obtained from greenhouse cultures of *D. carota*. One leaf was put in each flask filled with water and closed with a foamed plastic cap. These flasks were buried at the maize field edge (in a distance of 20 cm) in such a way that the cap fitted even with the soil surface. The leaves were 15 cm long and were all taken from young plants, because common swallowtails prefer young and smaller carrot plants (Ebert & Rennwald 1991a). The described approach assured a standardised exposure of the carrot leaves to pollen shedding. The carrot leaves were placed in the field for 24 hours. After that the tip of each leaf was collected by carefully pressing a transparent adhesive tape on the

leaf, cutting the tip with scissors, and transferring the sample onto a plain white cardboard. Maize pollen on the surface was counted under a microscope, and the carrot leaf was scanned and its area measured by an image analyser (Leica Qwin). Pollen on the underside of the leaves was not counted because these are considered to be negligible (Pleasants et al. 2001). For each leaf, pollen density was calculated as the pollen count divided by the leaf area. On each sampling date 12 carrot leaves were exposed giving a total of 36 samples. At the same time maize pollen deposition on microscope slides (see above) was also measured in order to calculate the proportion of the pollen shed that accumulates on the surface of carrot leaves (i.e. pollen numbers per cm^2 on carrot leaves divided by pollen numbers per cm^2 on slides). Slides were placed on the field edge at same height (9.00 ± 1.59 cm, mean ± 1SD) as the tips of the carrot leaves in such a way that each slide was flanked by two leaves.

2.4 Toxin content of *Bt* maize pollen

Pollen were collected from *Bt* maize fields established on the research farms at Grub, Neuhof, Puch near Fürstenfeldbruck (11°13'00'' east, 48°11'11'' north), and Schwarzenau near Kitzingen (10°12'40'' east, 49°48'19'' north). Pollen was collected and tested from event Mon810 ("Novelis", Monsanto), event Bt176 ("Navares", Syngenta), and isogenic non-*Bt* maize varieties as control ("Nobilis", Monsanto, and "Antares", Syngenta,). After collection pollen was sieved through a 1mm-mesh and stored at -20°C for up to 14 months (on average for 4 months). Levels of Cry1Ab toxin in pollen were determined by ELISA using the EnviroLogix QuantiPlate™ Kit for Cry1Ab/Cry1Ac (Adgen®). The limit of quantification of the kit was 250ppb. Two to three sets of aliquots from each pollen sample were analysed, and the mean was calculated from that. Prior to toxin analysis the pollen was dried at 28°C for 12 hours, sieved through a 0.1-mm mesh, and was then either ground or sonicated in order to release the *Bt* toxin. The weight of pollen grains of Bt176 maize "Navares" was determined in order to calculate the Cry1Ab amount per single pollen. Pollen samples were dried and treated as described, their mass determined, and then suspended in 10 ml water. An aliquot of 15 µl was taken, applied onto a plain surface, and the number of pollen counted under a binocular. From that count the number of pollen in the initial sample and the individual mass of single pollen grains was calculated.

3 Results

In a six-year survey of various maize varieties (1995-2000) the earliest start of maize pollen shedding was June 27, and the latest date August 8 (Fig. 1a). The majority (91%) of maize anthesis took place in July, and the average value was 18 July ± 9 days (mean ± 1SD). The Bt-variety "Navares" started pollen shedding on average on 12 July ± 7 days (mean ± 1SD) with the start of anthesis ranging from June 30 to July 26 (Fig. 1b).

The amount of pollen deposited on slides was highly variable, and ranged from 0 to 429 grains per cm^2 (Fig. 2, Tab. 1). There was a negative relationship between pollen number deposited and distance to the maize field, but the maximum amount found in 10 m distance was still 93 pollen per cm^2 (Fig. 2). Pollen amount was also influenced by numerous factors other than distance to field edge (Fig. 3a-d). Therefore, a multiple regression analysis was conducted with the independent variables distance to field edge, height of slide, air temperature, relative humidity, wind speed, precipitation, orientation of field edge, growth stage of maize, and site (see Tab. 1 for descriptive data). All independent variables together explained a variance of 64% ($p < 0.001$). However, the most influential variables determining deposited pollen grains per cm2 were humidity, distance to field edge and growth stage according to the equation (variables in sequential order as incorporated by the stepwise model):

pollen density = 17.11 – 3.37*humidity – 4.00*distance to field – 3.26*growth stage ($R2 = 0.48$, $p < 0.001$; for the transformations of the variables see "material and methods" section).

The more pollen were deposited in the maize field margin the more pollen were recorded on leaves of *D. carota* (Fig. 4). The average number (mean ± 1SD) of pollen per cm2 leaf was 33.46 ± 24.86 (minimum: 7.14; maximum: 93.00; 95% confidence interval of mean: 25.04 – 41.87). Of the slide-deposited pollen a proportion of 31.64 ± 14.10% was found on the surface of carrot leaves (minimum: 12.44; maximum: 63.53; 95% confidence interval of mean: 26.87 – 36.41).

The pollen of the event Bt176 "Navares" contained 2.59 ± 0.40 µg Cry1Ab protein / g dry weight pollen (n = 10, mean ± 1SD; 95% confidence interval of mean: 1.68 – 3.51). Of the 6 pollen samples tested of Mon810 "Novelis", 3 samples contained too little *Bt* to be quantified. When the latter 3 samples were assigned a value of 250ppb (i.e. the threshold level of quantification), the average of Mon810 pollen was 0.25 ± 0.09 µg Cry1Ab protein / g dry weight pollen; when those samples were attributed a value of nil, the resulting average value

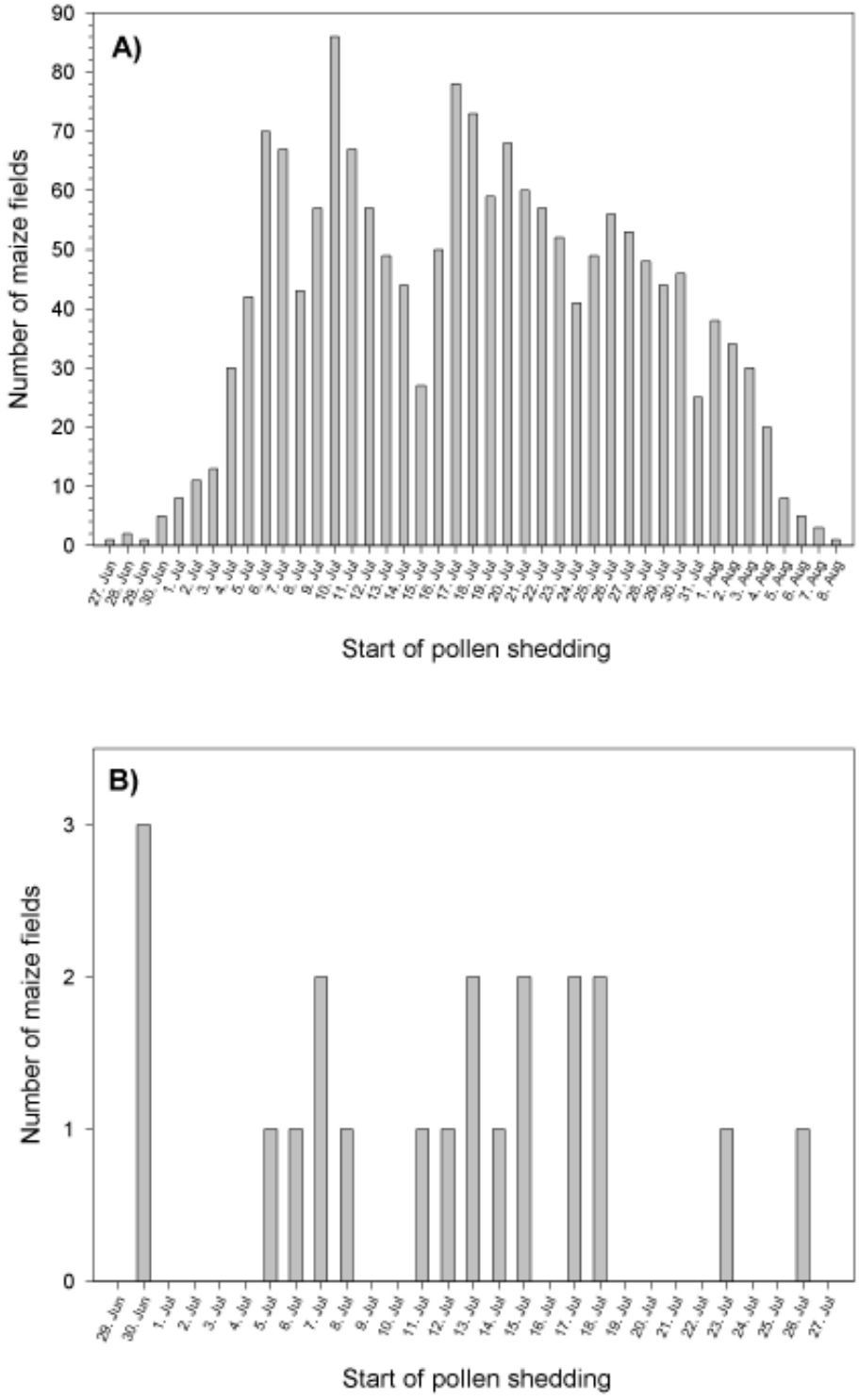

Figure 1: (A) Start dates of pollen shedding of maize fields in Bavaria (n = 1680 fields established at 22 locations during the years 1995 – 2000); (B) Start dates of pollen shedding of Bt176 maize fields in Bavaria (n = 21 fields cropped with variety "Navares" at 7 locations between 2000 and 2003).

was 0.13 ± 0.16 µg *Bt* protein / g dry weight pollen (n = 6, means ± 1SD). Both values are significantly different from the Bt176 pollen (Mann-Whitney U-test, $p < 0.001$). Of the 14 pollen samples tested of non-Bt varieties (10 "Antares", 4 "Nobilis") only one "Antares" sample gave a positive signal, which was probably due to contamination.

Bt176 "Navares" pollen contained on average 2602 ± 384 grains per 1 mg dried pollen (n = 29 samples), i.e. calculated weight of a single pollen grain was 0.392 ± 0.055 µg (means ± 1SD). Hence, a single pollen grain contained 1.015 ± 0.141 pg Cry1Ab protein (mean ± 1SD) assuming an average Cry1Ab amount of 2.59 ppm.

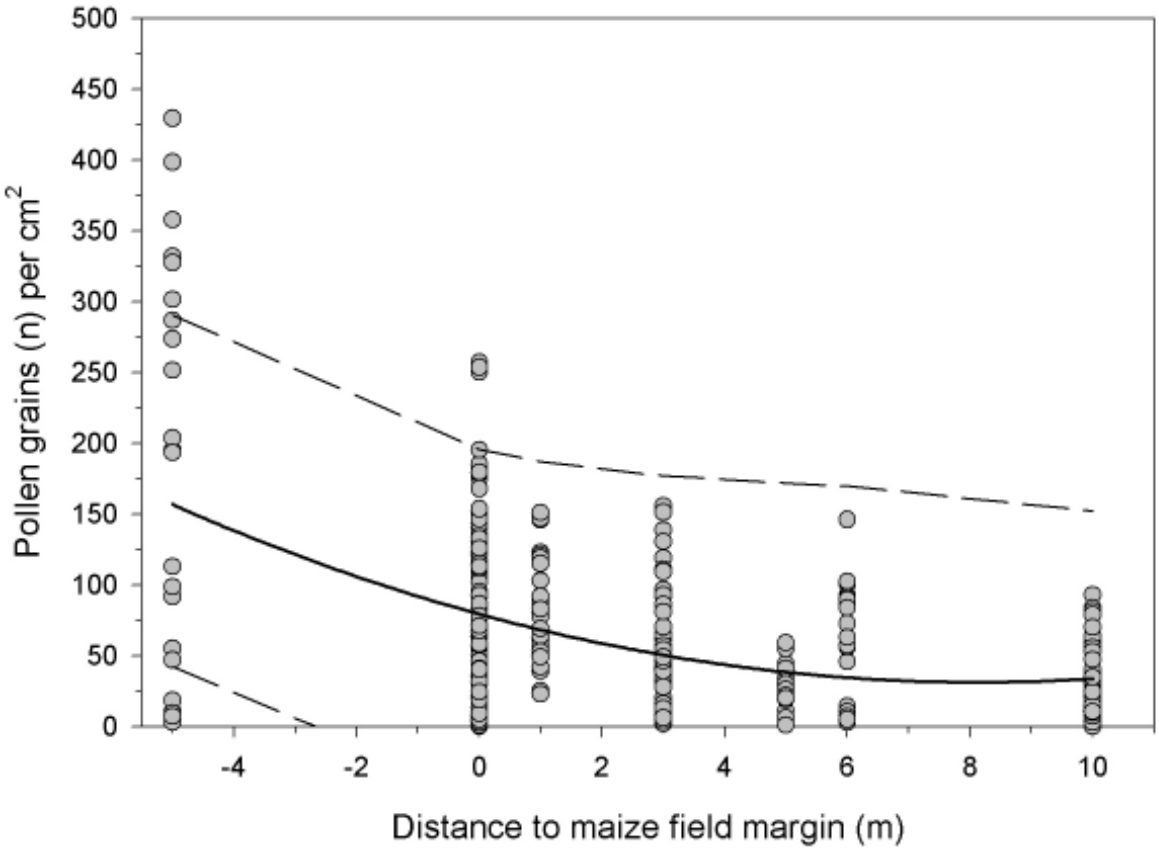

Figure 2. Densities of maize pollen deposited along maize fields in dependence of the distance to field (number of pollen grains on microscope slides coated with Vaseline®). The regression equation is $y = 74.75 – 9.71x + 1.32x^2 – 0.08x^3$, $R^2 = 0.23$, $p < 0.001$, n = 300; the regression line is given with the upper and lower limit of the 95% confidence interval.

Table 1: Descriptive data of pollen deposition and pollen dispersion experiment. N = 300 each variable. The 95% confidence interval is given for the number of pollen grains recorded on microscope slides coated with Vaseline® (24-hours counts).

Variable	**Mean**	**1SD**	**Minimum**	**Maximum**	**95% CI**
Pollen grains per cm^2 slide	65.92	70.00	0	429	57.97 – 73.87
Distance to field edge (m)	2.69	4.26	-5	10	
Height of slide (cm)	17.37	2.70	6	20	
Maize growth stage (BBCH)	64.99	1.05	63	67	
*Air temperature (°C)**	17.38	3.59	11.6	24.3	
*Relative humidity (%)**	73.29	12.21	52.6	97.4	
*Wind speed (m/s)**	1.11	0.74	0.1	3.1	
*Precipitation (mm)***	3.47	6.49	0	20.5	

* Based on daily average
** Based on daily sum

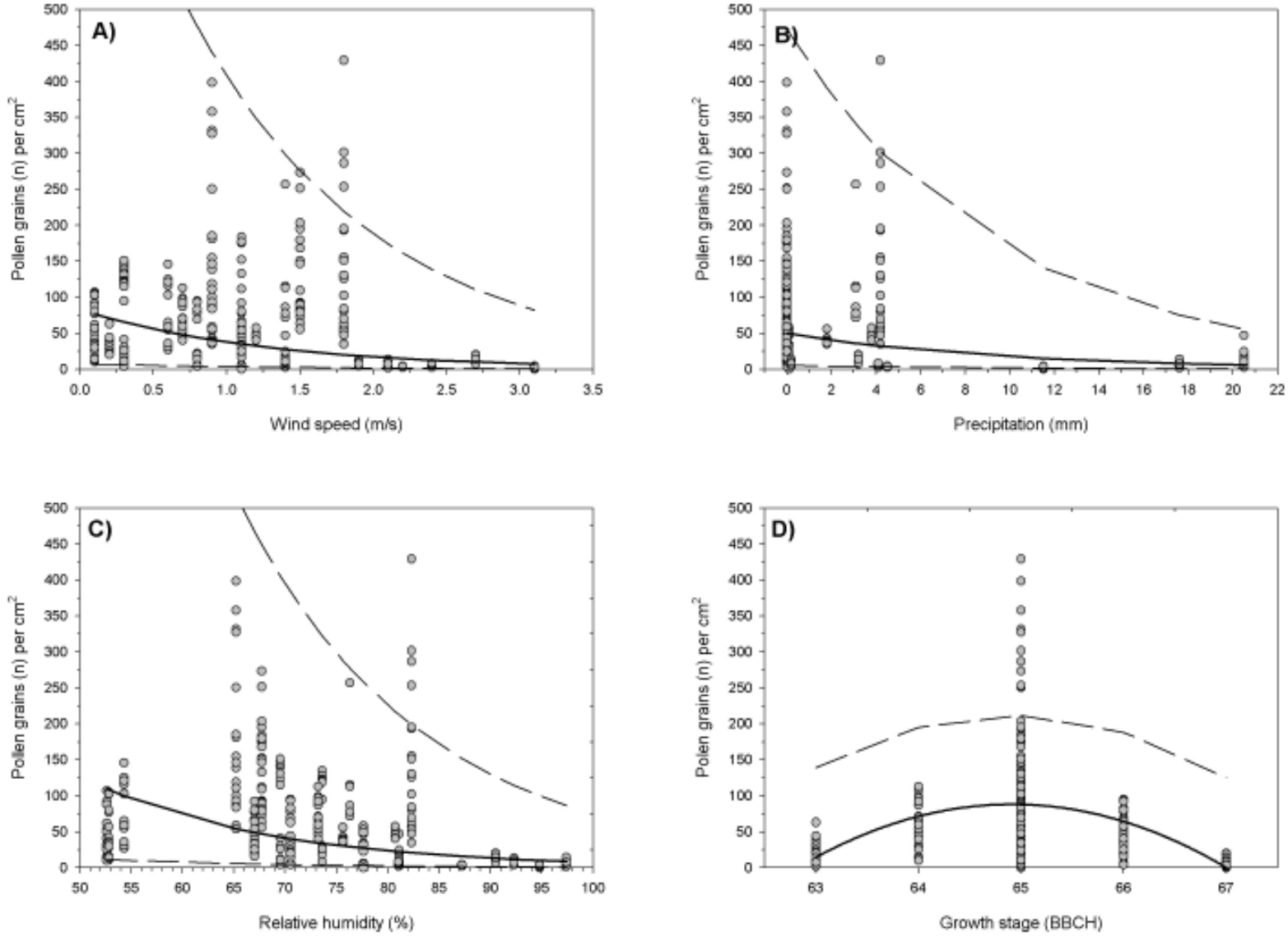

Figure 3: Densities of maize pollen deposited along maize fields in dependence of (A) wind speed, (B) precipitation, (C) relative humidity, and (D) growth stage of maize. The regression lines (with lower and upper limit of 95% confidence interval) follow the equations: (A) wind speed, $y = 82.49\ e^{-0.78x}$, $R^2 = 0.19$, $p < 0.001$; (B) precipitation, $y = 50.37\ e^{-0.11x}$, $R^2 = 0.27$, $p < 0.001$; (C) relative humidity, $y = 2182.09\ e^{-0.06x}$, $R^2 = 0.27$, $p < 0.001$; (D) growth stage, $y = -86634 + 2671x - 20.58x^2$, $R^2 = 0.21$, $p < 0.001$ (n = 300 each graph).

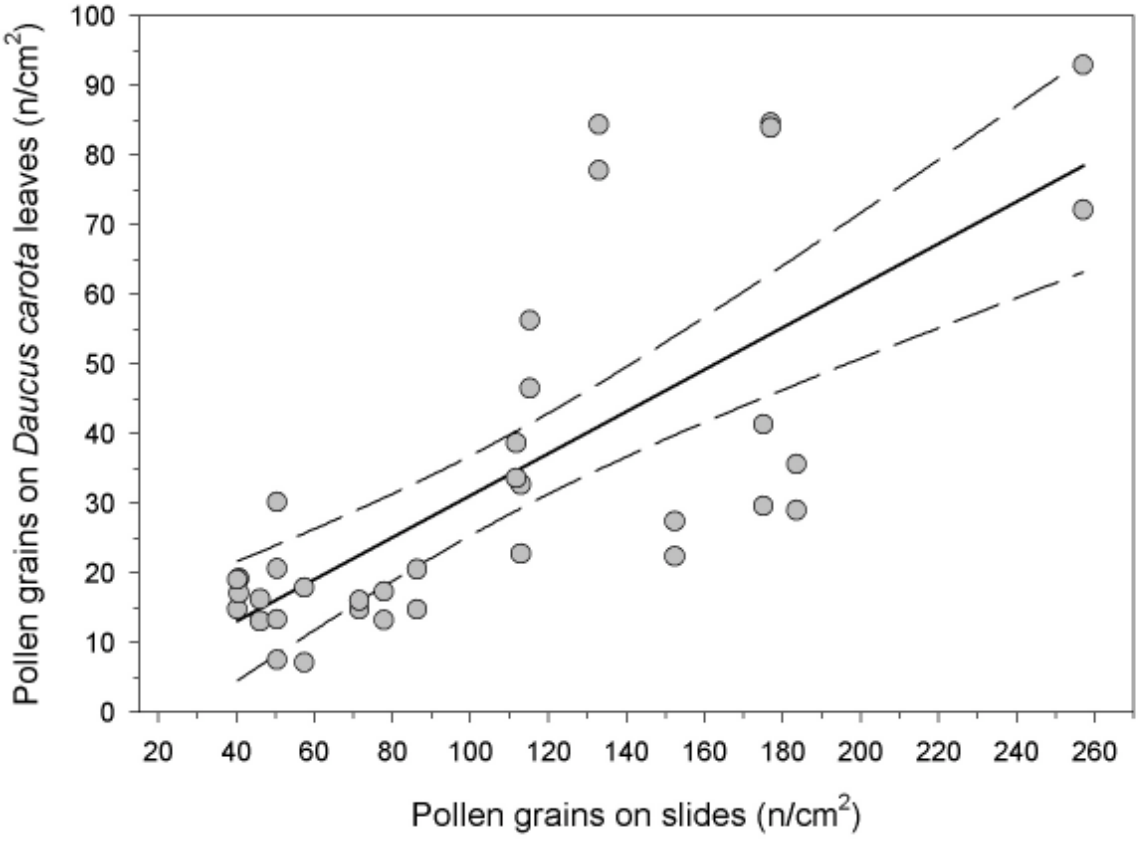

Figure 4: Densities of maize pollen on leaves of *Daucus carota* in dependence of pollen deposited on maize field edge (measured as pollen grains on microscope slides coated with Vaseline®). The linear regression (with 95% confidence interval) follows the equation: $y = 0.99 + 0.30x$, $R^2 = 0.54$, $p < 0.001$, $n = 36$.

4 Discussion

The majority of the maize fields shed pollen during July, which is typically in mid-Europe (Zscheischler et al. 1990). The surveyed maize varieties included early as well as late cultivars resulting in a representative data set and covering the entire span of possible starting dates of anthesis. The tested *Bt* maize "Navares" is an early variety, and consequently shed pollen about one week earlier than the average, nevertheless shedding *Bt* fields could be observed until the end of July. Usually pollen anthesis continues for 5 - 8 days, but under favourable conditions the vast majority of pollen shedding may occur within a 2-day period (Treu & Emberlin 2000, Wolt et al. 2003). But as maize fields can shed pollen up to 10 - 14 days after the onset of anthesis (Treu & Emberlin 2000, Oberhauser et al. 2001) we conclude that pollen shedding maize fields may be found until the third week of August in Bavaria.

Maize pollen may be drifted by wind as far as 800 m (Salamov 1940, cited in Treu & Emberlin 2000) or even 2000 m (Fleischmann 1942), but due to their large size and weight

only a very small proportion of maize pollen grains are deposited further than 60 m away from the "source" field (Raynor et al. 1972). Here, we considered maize pollen deposition within a 10 m distance to the fields as over 90% of maize pollen is deposited within this range (Eastham & Sweet 2002), and the typical field margins of the considered maize fields were 10 m broad on average (Lang 2004). The pollen densities deposited on the maize field margins were extremely variable. They could be considerable high even at a distance of 10 m, but at any time and any distance pollen numbers could also be very low. Despite this high variance distance to field margins was identified as one of the most influential variables determining pollen densities in field margins. Growth stage of maize was also an important variable affecting pollen numbers as during the BBCH stage 65 ("upper and lower parts of tassel in flower") (Meier 1997) the vast majority of maize pollen were shed. Relative humidity was a strong factor as it integrates over several other related factors, e.g. precipitation and temperature. Pollen is no longer dispersed by wind during rain, and the rain may also remove pollen grains from the microscope slides. Moreover, less pollen will be shed if it is too humid or too wet (Zscheischler et al. 1990). All variables analysed explained 64% of the variance of pollen densities, and the remaining variance may be explained by factors such as wind direction, frictional turbulences, or thermal convections (Emberlin et. al. 1999, Treu & Emberlin 2000, Feil & Schmid 2001). The average wind direction at our sites is from west, and it is known that wind direction plays a large role in the deposition level of maize pollen (Pleasants et al. 2001). We suppose that small-scale local wind conditions and the fact that northern field edges were over-represented in our sample masked a stronger influence of the field edge orientation. Numbers of deposited pollen on slides in field margins are difficult to compare with similar studies, mainly because the exposure time of the microscope slides differs among the studies, often other distances to maize field edges were tested, or no data are available on the height of the exposed slides or of the anthesis stage of the maize. Despite this uncertainty, it seems that the maize pollen densities recorded in our study are fairly higher than pollen depositions outside maize fields reported so far (e.g. Raynor et al. 1972; Wraight et al. 2000). However, the pattern of pollen deposition is equivalent to other studies, i.e. a considerable decline of pollen numbers within the first few meters off the maize field (Raynor et al. 1972, Hansen Jesse & Obrycki 2000, Wraight et al. 2000, Stanley-Horn et al. 2001, Zangerl at al. 2001).

On average, one third of the pollen which drifted into field margins was found on the surfaces of wild carrot leaves which is in the range recorded by other authors (Pleasants et al. 2001). The surface-deposited pollen increased linearly with increase of pollen deposition, and

reached maximum values up to 93 pollen per cm² leaf area. Pollen densities within the range recorded are known to cause adverse effects on butterfly larvae feeding on food plants dusted with Bt176-pollen (Hansen Jesse & Obrycki 2000, Stanley-Horn et al. 2001, Zangerl et al. 2001, Felke & Langenbruch 2003). Moreover, our values are based on 24-hours counts, and surface-deposited pollen numbers may accumulate on the leaves during the pollen shedding period provided there is no rain or strong wind. Rain does not only reduce maize pollen drift but also washes off pollen deposited on plant leaves. Rain events may remove as much as 54 - 86% of the maize pollen on plant leaves (Pleasants et al. 2001, Stanley-Horn et al. 2001; Zangerl et al. 2001). Other studies about maize pollen deposited on plants growing along field margins exist about milkweed plants (*Asclepias syriaca*), food plant of the monarch butterfly (*Danaus plexippus*), and of wild parsnip (*Pastinaca sativa*), a food plant of the black swallowtail (*Papilio polyxenes*), but also a food plant of the European common swallowtail (Ebert & Rennwald 1991a, Hansen Jesse & Obrycki 2000, Pleasants et al. 2001, Zangerl et al. 2001). In these studies, the densities of surface-deposited maize pollen on milkweed and parsnip leaves were much higher than pollen numbers on wild carrot leaves. The main factor responsible for these higher pollen densities was the recording of pollen which have accumulated on the plants over several days. Milkweed plants were much higher than the young carrot plants, and leaves at a higher position can receive more pollen (Pleasants et al. 2001, Zangerl et al. 2001). Leaf structure will probably affect number of pollen grains per leaf area, e.g. carrot leaves are fine and pinnate, while milkweed and parsnip leaves have a cohesive plane structure. Pollen may possibly adhere better on leaves with a high number of hairs on their surface, such as nettles, *Urtica dioica* (Felke & Langenbruch 2003). Leaf orientation, i.e. the angle of the leaf, may also play a role (Pleasants et al. 2001).

The *Bt* maize event 176 has a high pollen expression typically ranging in concentration between 1.1 to 2.9 µg / g of Cry1Ab protein in pollen (Pilcher et al. 1997, Hansen Jesse & Obrycki 2000, Sears et al. 2001, Hansen Jesse & Obrycki 2002, Wolt 2003). With an average of 2.59 µg Bt protein / g pollen the results of this study lie within the upper range of the reported *Bt* contents in pollen. Among others, differences in the *Bt* concentration in maize tissues may also be attributable to the variety, for example the variety "Valmont", which is based on event 176, seems to express a *Bt* concentration in pollen about four times lower than the one detected in this study for "Navares" (Nguyen Thu & Jehle, personal communication). Our upper estimate of *Bt* concentration in pollen of Mon810 was definitely overestimated as we arbitrarily set the samples below the detection threshold to a value of 250ppb (i.e. 0.25 µg Bt protein / g pollen).

However, our lower estimation of 0.13 µg Bt protein / g pollen is still among the highest reported values, which range typically between 0.002 and 0.09 µg / g (Wraight et al. 2000, EPA 2001, Sears et al. 2001, Nguyen Thu & Jehle, personal communication).

One purpose of the study was to gain information of the range and distribution of maize pollen densities and thus potentially *Bt* corn pollen densities, to which butterfly larvae on field margins may be exposed. Apart from the toxic effect of *Bt* maize pollen consumption, this information is crucial for an evaluation of the impact of *Bt* maize on butterflies (Sears et al. 2001). The results showed that maize fields may shed pollen from end of June until end of August with a peak in July. Thus, many butterfly species occurring during the summer months may be exposed to wind drifted maize pollen (Ebert & Rennwald 1991a,b). Our data integrated a large number of different maize varieties from early to late cultivars. *Bt* maize events may differ in time of pollen shedding which should be accounted for when assessing temporal overlap of butterfly occurrence with maize anthesis. Nevertheless, any conventional maize variety can possibly be used to produce future transgenic *Bt* maize hybrids, thus our data demonstrate the potential range. Further, the spatial overlap between area cultivated with *Bt* maize, occurrence of concerned butterfly species, and their host plants must be taken into account (Oberhauser et al. 2001), which may vary on a regional scale. We could show that maize pollen deposition along field edges and in margins can be considerably high, and is reflected in concurrent pollen densities on a butterfly food plant. The established regression relation allows the estimation of pollen on leaves of wild carrots in dependence on pollen numbers recorded with microscope slides (within the studied range of 0 m - 10 m distance to maize field), a method much more convenient than recording pollen densities on the plant leaves themselves. Knowledge of naturally occurring maize pollen densities on food plants is indispensable for assessing the expected effect of *Bt* maize on butterfly larvae along field edges (together with the toxin amount of the *Bt* maize pollen, and its toxic effect on butterfly larvae). For example, larvae of the common swallowtail, *P. machaon*, do not only feed on wild carrots but also on quite a number of other plants, e.g. on wild parsnip, which seems to exhibit higher maize pollen numbers per leaf area than wild carrots (Zangerl et al. 2001). But knowledge of maize pollen densities on potential food plants of butterfly larvae is still very deficient, and there is clearly an urgent need for more studies of this kind.

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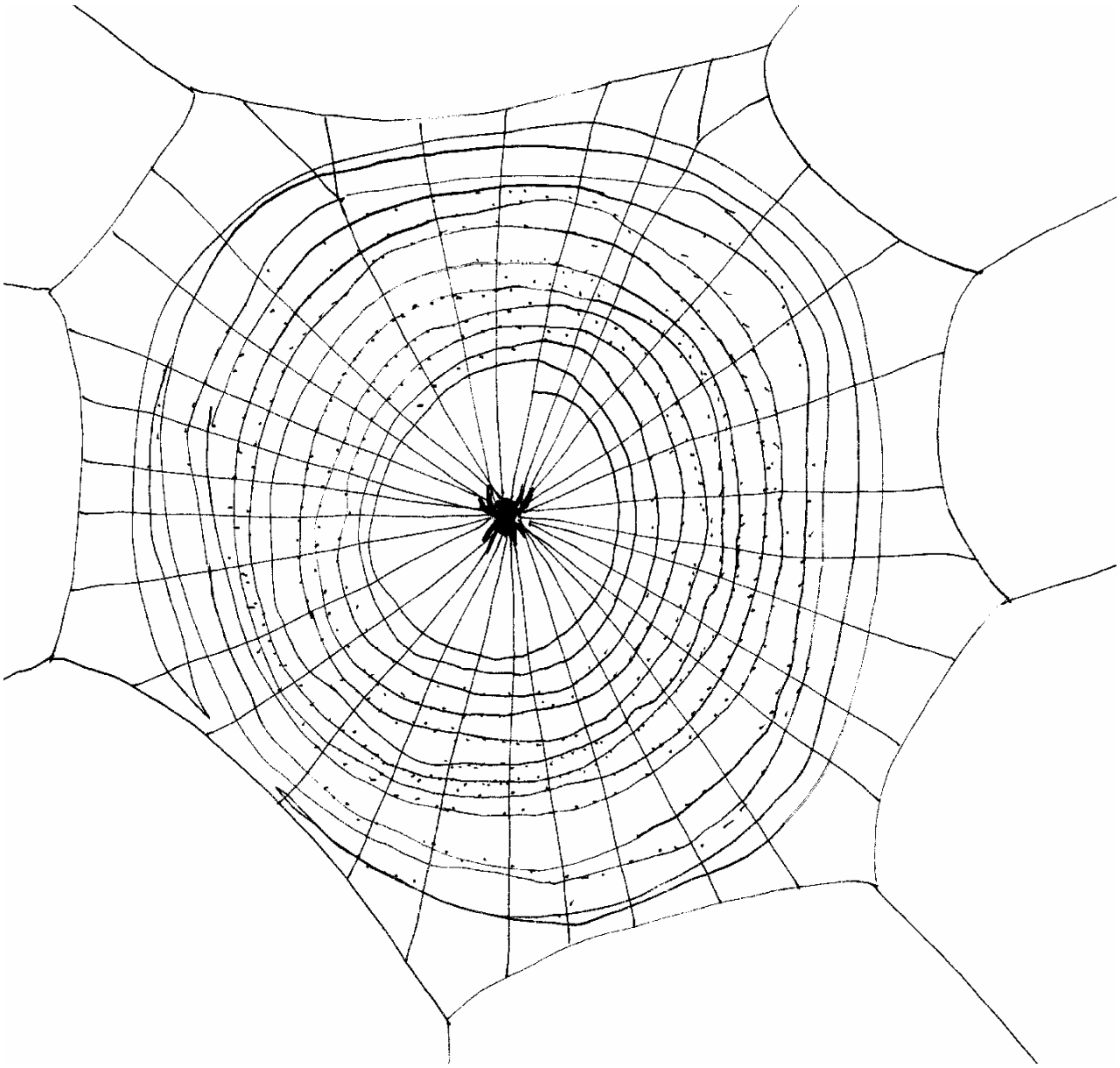
Bt maize pollen exposure and impact on the garden spider, *Araneus diadematus* (Araneae: Araneidae)



Bt maize pollen exposure and impact on the garden spider, *Araneus diadematus* (Araneae: Araneidae).

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Abstract

Concerns have been raised that *Bt* maize pollen may have adverse effects on non-target organisms; consequently, there is a general call for *Bt* maize risk assessment evaluating lethal and sublethal side effects. Spiders play an important economic and ecological role as pest predators in various crops including maize. Especially web-building spiders may be exposed to the Cry1Ab toxin of *Bt* maize by the ingestion of pollen via "recycling" of pollen dusted webs and intentional pollen feeding. In this study, the potential *Bt* maize pollen exposure of orb-web spiders was quantified in maize fields and adjacent field margins, and laboratory experiments were conducted, to evaluate possible effects of *Bt* maize pollen consumption on juvenile garden spiders, *Araneus diadematus* (Clerck) (Araneae: Araneidae). In maize fields and neighbouring field margins, web-building spiders were exposed to high amounts of *Bt* maize pollen. However, a laboratory bioassay showed no effects of *Bt* maize pollen on weight increase, survival, moult frequency, reaction time, and various web variables of *A. diadematus*. A pyrethroid insecticide (Baythroid) application affected weight increase, survival, and reaction time of spiders negatively. In conclusion, the insecticide tested showed adverse effects on the garden spider while the consumption of *Bt* maize pollen did not. This study is the first one on *Bt* maize effects on orb-web spiders, and additional research is recommended in order to account for further spider species, relative fitness parameters, prey-mediated effects, and of possible long-term chronic consequences of *Bt* exposure.

Key words: GMO, risk assessment, non-target organisms, Cry1Ab toxin, *Bacillus thuringiensis, Zea mays,* Araneae, Araneidae

1 Introduction

Genetically modified *Bt* maize commercially grown in Europe expresses the activated and truncated protein Cry1Ab of the soil bacterium *Bacillus thuringiensis* var. *kurstaki* (Berliner) in plant tissues including pollen (Fearing et al. 1996). The Cry1Ab protein is described as specifically toxic against Lepidoptera, which include economically relevant pests in maize stands (Gill et al. 1992, Burkness et al. 2001). Due to this specifity, *Bt* maize is considered to be harmless to non-target organisms outside the order Lepidoptera (Glare & O'Callaghan 2000, O'Callaghan et al. 2005), but there are indications that invertebrate predators consuming *Bt* toxins or consuming prey that had fed on *Bt* toxins may be affected adversely (Hilbeck et al. 1998a, b, 1999, Dutton et al. 2002). However, the latter results conflict with other studies reporting no negative impact, and there is an ongoing debate as to whether recorded negative effects of Cry1Ab are through indirect bitrophic and tritrophic pathways rather than direct effects of the *Bt* toxin itself (Pilcher et al. 1997, Andow & Hilbeck 2004, Romeis et al. 2004). In general, laboratory data on the impact of *Bt* maize toxin on invertebrate natural enemies is limited (Lövei & Arpaia 2005), and there is a common need and call for *Bt* maize risk assessment (e.g., European Parliament and Council 2001, Zühart & Breckling 2003). Criteria for the choice of indicator species as test organisms for a *Bt* maize risk assessment should include ecological and/or economic significance of the organisms in (agro)ecosystems, existing exposure pathways, the degree of the exposure of the organisms to the GMO, as well as acute and chronic toxicity of the transgenic product (Jepson et al. 1994, Dutton et al. 2003, Hilbeck & Andow 2002, Andow & Hilbeck 2004).

Spiders are abundant invertebrate predators in arable land, belong to the dominant predators in maize fields, and play an important economic and ecological role as natural enemies in various crops including maize (Lang et al. 1999, Sunderland 1999, Albajes et al. 2003, Nyffeler & Sunderland 2003, Lang 2003, Candolfi et al. 2004, Ludy & Lang 2004). Spiders, especially web-building spiders, may be exposed to the Cry1Ab toxin of *Bt* maize through different exposure routes. *Bt* maize pollen is dispersed by wind (Lang et al. 2004) and may be collected by spider webs inside and outside maize fields (Bera et al. 2002, Ludy 2004). By recycling their webs, orb-web spiders consume pollen adhering to the sticky web spiral, which may even be an essential additional food source for juveniles (Smith & Mommsen 1984, Ludy 2004), while hunting spiders without a web may actively forage for the pollen (Vogelei & Greissl 1989).

Furthermore, spiders may take up toxin by preying on herbivorous prey that has fed on *Bt* maize tissue (cf. Dutton et al. 2002), or catch maize pollen-collecting insects. Accordingly, the presence of Cry1Ab endotoxins was recorded in over 7% of spiders collected in a transgenic *Bt* maize field, indicating a long-term exposure to insecticidal *Bt* toxins (Harwood et al. 2005).

Surprisingly, there has not been one laboratory experiment testing the impact of *Bt* maize on spiders, nor are there studies quantifying the field exposure of spiders to the toxin of transgenic *Bt* maize apart from the recently published analysis of Harwood et al. (2005), despite the ecological and economic significance of spiders and their potential exposure to *Bt* toxin (Lövei & Arpaia 2005). Behavioural parameters of indicator species may be more sensitive markers of a toxic effect than sheer mortality. Orb webs of spiders are true and direct reflections of the spiders' complex web-building behaviour (e.g., Vollrath 1986, 1992), and orb-web geometry is affected by and does indicate the exposure to pesticides (Samu & Vollrath 1992, Lengwiler & Benz 1994, Hesselberg & Vollrath 2004). Alteration of web structure patterns may even have an ecological effect as prey capture efficacy is influenced by web geometry (Eberhard 1986).

The garden spider, *Araneus diadematus* (Clerck) (Araneae: Araneidae), is a common (pest) predator in various habitats neighbouring arable fields (Hänggi et al. 1995); it occurs also in maize fields (Nyffeler 1982) and can be easily kept in the laboratory (Zschokke & Herberstein in press), all of which makes it an ideal "model spider" for studying *Bt* maize effects. In this study, we quantified potential *Bt* maize pollen exposure of orb-web spiders in the field by recording the number of maize pollen grains found in orb webs located in maize fields and adjacent margins. The effect of *Bt* maize pollen consumption on lethal and sublethal parameters including web-building behaviour was studied in the laboratory by applying *Bt* pollen to the webs of juvenile *A. diadematus*.

2 Material and methods

2.1 Potential field exposure of orb-web spiders to *Bt* maize pollen

For the study of potential *Bt* maize pollen exposure, orb-web building spiders were kept in the laboratory. Here, they could build their webs within wooden frames (Fig. 1A). These webs were then exposed for a standardised time period within and at the edge of pollen shedding *Bt*

maize fields (Fig. 1B), and subsequently numbers of maize pollen grains deposited in the webs were analysed. In general, by using the term 'exposure' we refer to potential exposure of spiders to pollen and it is not implied that 'potential exposure' necessarily equals 'effective exposure', the latter implying the intake of a substance (Moriarty 1988).

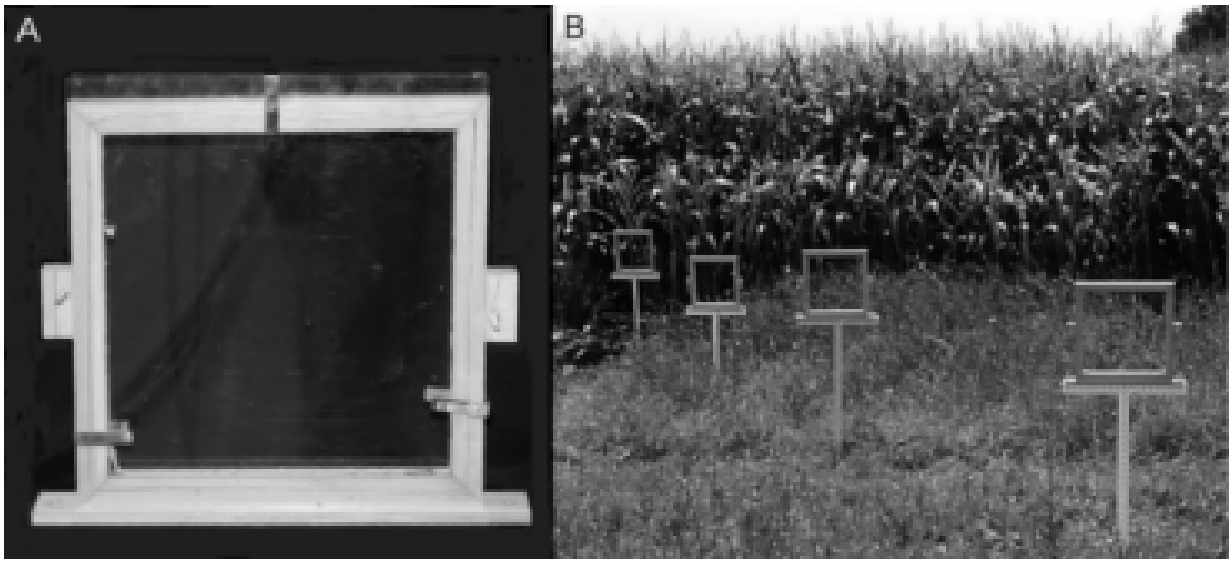

Figure 1: Wooden frame covered with two plastic plates for keeping orb-web spiders in the laboratory (A), and the exposure of spider webs in different distances from a maize field to determine pollen amounts in webs (B).

2.1.1 Obtaining spider webs

Field-collected adult females of various orb-web spider species of the family Araneidae [*Araneus diadematus* (Clerck), *Argiope bruennichi* (Scopoli), *Aculepeira ceropegia* (Walckenaer), and *Larinioides* spec. (Caporiacco)] were used for the investigation of horizontal distribution and vertical dispersion of *Bt* maize pollen in orbwebs. Gross web parameters of araneid species are similar, and species-specificy is uncommon (Eberhard 1990). Spiders were allowed to build their webs within wooden frames of 30 x 30 cm (Fig. 1A). Two transparent plastic plates enclosed both open sides of the frames to prevent spiders from escaping, the plates being covered on the inside with Vaseline® to prevent spiders from attaching silk threads to the plate (Hesselberg & Vollrath 2004, Zschokke & Herberstein in press). The spiders were supplied with *Drosophila* flies and water every day. Prior to the exposure of spider webs to maize pollen in the field, spiders were carefully removed from their webs with a small brush, because the webs were exposed in the field without the spiders.

This was done to prevent a destruction of the web by the spider, and to prevent the loss of escaping spiders, which were needed and used to build new webs in the laboratory.

2.1.2 Exposure of spider webs in the field

Bt maize pollen numbers deposited in spider webs were investigated in July 2002 during maize anthesis on two state research farms in Upper Bavaria and Swabia (South Germany). In Upper Bavaria, a *Bt* maize field of 30 x 50 m, and in Swabia a *Bt* maize field of 2 ha was planted (at both locations maize event Bt176, cultivar "Navares" from Syngenta, Basel, Switzerland). The event Bt176 is one of the two events being currently registered for the cultivation of *Bt* maize in Europe, the other one being MON810. In the smaller field (30 x 50 m), the vertical distribution of maize pollen was studied within the field (i.e., different heights of spider webs). At the larger field (2 ha), the horizontal distribution of maize pollen was studied on the northern field margin (i.e., different distances of webs to field edge).

For the investigation of the vertical distribution of *Bt* maize pollen, frames with spider webs (but without the spider) were mounted in different heights of 20, 80 cm, and 170 cm in the middle of the pollen shedding *Bt* maize field (BBCH growth stadium 6.5, i.e., "upper and lower parts of tassel in flower", Meier 1997). Each height was replicated three times on four sampling occasions between 28 July and 31 July, 2002, resulting in a total of 36 webs (3 heights*3 replicates*4 dates). Each web was exposed for 24 h, which was considered to be a realistic exposure time as spiders were reported to recycle their webs once a day (Breed et al. 1964). During this investigation, the weather conditions were 23.2 ± 2.2 °C for air temperature, 0.83 ± 0.43 m/s for wind velocity (arithmetic mean of daily means ± SD), and 5.95 ± 11.83 mm for precipitation (arithmetic mean of daily sums ± SD).

For the analysis of the horizontal dispersion of *Bt* maize pollen, spider webs were exposed at a height of 80 cm and 5 m within the field (- 5 m) as well as at distances of 0 m (maize field edge), 3, 6, and 10 m to the maize field in the adjacent field margin (Fig. 1B). On this field margin, natural succession of the vegetation community was allowed to grow (Fig. 1B). The *Bt* maize field was in full anthesis (BBCH growth stadium 6.5, Meier, 1997). The experiment took place on five sampling occasions between 18 July and 24 July, 2002. Each distance was replicated four times per day, resulting in a total of 100 webs (5 distances*4 replicates*5 dates). As the exposed webs were frequently destructed by wind or moving plant material, only 53 webs out of 100 could be analysed. Again, webs were exposed for a 24-h period per sampling date. This field assay was conducted under the following weather

conditions (arithmetic means of daily means ± 1 SD): air temperature of 15.5 ± 2.4 °C, wind velocity of 1.40 ± 0.59 m/s, and precipitation (arithmetic mean of daily sums ± SD) of 4.36 ± 7.62 mm.

After field exposure, frames with spider webs were sealed on both sides with plastic plates for transportation. In the laboratory, pollen loaded webs were photographed with a digital camera against a black background. Pollen grain numbers in spider webs were counted manually from enlarged paper copies of the digital pictures. The reliability of the pollen counting approach was evaluated by comparing pollen numbers counted directly from spider webs by eye with pollen counted from enlarged paper copies of the referring webs. The regression equation was: "Pollen counts from paper copies" (n) = 0.434 + 0.971* "Directly counted pollen" (n) (R^2 = 0.93, P<0.001, n = 53).

2.1.3 Statistical analysis

Linear regression analyses were calculated to analyse the amount of maize pollen in spider webs in dependence of "height in maize field" (cm) and of "distance from maize field edge" (m), respectively. In addition, a multiple regression with backward selection was applied to estimate the influence of the independent variables "area of spider webs (cm^2)", "air temperature (°C)"and "wind velocity (m/s)" (both daily means), "precipitation (mm)" (daily sums), on number of pollen in webs. The probability of F-to-remove was 0.10. When necessary, data were log or log(x+1) transformed to meet the assumptions of linear regression.

2.2 Uptake of the Cry1Ab protein by spiders

Spiders ingest *Bt* maize pollen and take up Cry1Ab protein (Ludy 2004). To quantify the amount of Cry1Ab that was ingested by spiders while pollen feeding, an enzyme-linked immunosorbent assay (ELISA) was conducted. For this purpose, 20 juvenile garden spiders (*A. diadematus*) were kept under standardised conditions (temperature 20 °C, L10:D14 regime) in wooden frames (10 x 10 cm) in the laboratory, and were fed with fruit flies daily. *Bt* maize pollen (cultivar "Navares" from Syngenta) was collected in July 2002 from fields in Frankonia and Swabia, South Germany, located on experimental farms of the Bavarian State Research Center for Agriculture (LfL 2005). Obtained pollen was frozen and stored at -18 °C for 10 months prior to the tests. Before use in the laboratory tests, pollen was defrosted and

desiccated in a drying chamber at 30 °C for 2 days, and was then sieved through a 0.1-mm mesh. After the spiders had built webs regularly, webs were dusted with *Bt* maize pollen (cultivar "Navares", Syngenta). Right after the spiders had recycled their webs, the spiders were frozen at –18 °C and stored for 6 months. Then spiders were defrosted at 5 °C, and washed with water to remove pollen possibly adhering to the spider. Additionally, spiders were checked carefully for adhering pollen under a binocular microscope. Subsequently, the spiders' gastrointestinal systems were dissected and taken up in cyclo-hexylaminopropane sulfonic acid buffer (CAPS buffer: 50 mM, pH 10.5). The dissected tissue was analysed for Cry1Ab content with a commercial ELISA kit (EnviroLogix QuantiPlate™ kit for Cry1Ab/Cry1Ac from Neogen Europe Ltd., Auchincruive, UK. Also, the Cry1Ab content of the applied *Bt* maize pollen was quantified with the ELISA kit. The limit of detection of the kit was 0.14 ppb, however, the limit of quantification of Cry1Ab was 0.50 ppb.

2.3 Test of biological activity of the Cry1Ab protein

The biological activity of the Cry1Ab in *Bt* maize pollen used in the spider laboratory assay was tested on a target organism of *Bt* maize, the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). Pollen was obtained and processed as described in "Uptake of the Cry1Ab protein by spiders" (see above). L1-L2 larvae of *O. nubilalis* were kept in 24-well tissue culture plates with one larva per well. Wells were filled with 0.5 ml of an artificial diet with 1% conventional maize pollen (n = 18), 1% *Bt* maize pollen (n = 15), or without pollen (n = 17), respectively. Due to a shortage of pollen we applied an amount of *Bt* pollen which was below the LC_{50} for *O. nubilalis* (Meise 2003). Mortality of the larvae was registered each day after the start of the bioassay for 7 days. ANOVA was used to analyse the effect of the factor "diet" (i.e., diet with *Bt* maize pollen, with conventional maize pollen, and without pollen) on the dependent variable "survival" (days). Larvae, which survived the whole observation time of the experiment (7 days), were set to the survival time of 7 days. Least significant differences (LSD) test was conducted for multiple comparisons between diets.

2.4 Effects of *Bt* maize pollen on *Araneus diadematus*

2.4.1 Spiders of the bioassay

The laboratory assays to study the effect of *Bt* maize pollen consumption on orb-web spiders were carried out with juvenile garden spiders (*A. diadematus*). The spiders were obtained from cocoons made by field-collected female garden spiders. The spiderlings were kept in wooden frames (10 x 10 cm) and provided with *Drosophila* flies and water every 2nd day. About 5 weeks after hatching, a sufficient number of spiders built small webs in the frames regulary; these were then used for the laboratory assay.

2.4.2 Experimental procedure

Spiders were fed with *Bt* maize pollen; one control group was given conventional maize pollen, and a second control was treated with an insecticide. *Bt* maize pollen (cultivar "Navares" from Syngenta) and conventional maize pollen (near-isogenic cultivar "Antares", Syngenta) were obtained and processed as described in "Uptake of the Cry1Ab protein by spiders" (see above). The insecticide used was the pyrethroid Baythroid50® from Bayer AG, Leverkusen, Germany (active ingredient Cyfluthrin), and was applied in a concentration of 2 µl/l. As we intended to study sublethal effects of *Bt* maize pollen and Baythroid50® on web parameters (see below) it was crucial that enough spiders would survive. A relevant field dose of a pyrethroid insecticide (Samu et al. 1992), however, would have been lethal for juvenile *A. diadematus* (Samu & Vollrath 1992). Considering that we needed enough spiders alive for recording web building parameters, we calculated and applied a dose referring to 0.8 µg Cyfluthrin per g spider, which is below the LD_{50} of 2.2 µg/g spider for Cypermethrin, the active ingredient of another pyrethroid (Samu & Vollrath 1992).

Out of the total of 60 spiders that built webs in wooden frames, spiders were assigned randomly to the three different treatments: conventional maize pollen, *Bt maize* pollen, and the insecticide Baythroid. Prior to the assay, all spiders were weighed ("initial weight"), and were fed 1-2 *Drosphila* flies daily during the experiment. For pollen application to a spider web, the wooden frame was carefully rotated by 90° together with the web and the spider. Then, pollen was applied over the capture spiral of the web from above with the help of a small pollen-loaded brush. Webs of spiders in the pollen treatments were additionally sprayed daily with water using a fine sprayer. Webs of insecticide-treated spiders were sprayed with a

water-based Baythroid solution (2 µl/l) instead of pure water. Webs were sprayed until they were satiated with fluid, and the applied Baythroid amount was calculated from the area and the density of the sticky spiral of the treated webs according to a general water absorbance estimation of spider webs (Samu & Vollrath 1992).

The construction of new webs was controlled daily, and all treatments (pollen and insecticide) were applied to four webs built by each spider in order to standardise pollen and insecticide amount. This approach meant that the treatment duration differed among spiders depending on the web building frequency of individual spiders.

2.4.3 Data recording and processing

After each pollen application, the spider web was photographed with a digital camera to determine the amount of pollen in the web. The webs were photographed using an illuminated and velvet lined box (described in Zschokke 2002). Pollen grains in spider webs were counted manually from enlarged paper copies of the digital pictures. During the experiment, every feeding of the spiders was recorded by a digital video camera to record the reaction time of the spider to prey. The reaction time was defined as the time the spider needed to respond to a fruit fly, i.e., the time span between the time the fly was caught in the web and the first movement of the spider towards the fly. As flies sometimes rested motionless when get stuck in the spider web, reaction times of the spider were prolonged by such a fly behaviour. Therefore, only cases with reaction times < 1 s were included into the analysis to prevent a bias due to fly behaviour. Reaction time analysing was carried out with the program CyberLink PowerDirector™ 1.1, CyberLink Corp., Taipei, Taiwan. Mortality and moulting of the spider (if occurring) was recorded daily until the last spider died. The first web that spiders built after the last pollen or insecticide application was photographed with a digital camera and the spider was weighed at this date ("end weight"). After treatment, the length of the tibia of the first right leg was recorded by measuring exuviae or dead spiders with a stereo microscope and an ocular micrometer. Possible "web building inhibition" was recorded and defined as the time span that the spider took to build a new web after the last treatment web. Various web parameters of the first web after treatment were analysed with the image analysing program Scion image for Windows 4.0 (Scion Corp., Frederick, USA): length of radii (cm), length (cm) and area (cm²) of the sticky spiral, as well as density of the sticky spiral (length of sticky spiral divided by the area of sticky spiral (cm/cm²) (see Zschokke 1999 for web nomenclature).

2.4.4 Statistical analysis

Analysis of covariance (ANCOVA) was used to analyse the effect of the main factor "treatment" (i.e., conventional maize pollen, *Bt* maize pollen, and Baythroid) on the recorded dependent variables: spider weight increase ("end weight" minus "initial weight", mg), survival (days), moult frequency (numbers of moults in 20 days), "web building inhibition" (days), and reaction time (ms, means of measures during treatment). For all analyses, except for the dependent variable "reaction time", the covariate "treatment duration" (time between the first and the last treatment application) was included, because treatment duration differed among spiders (see above). Likewise, treatment effects on web geometry were tested with ANCOVA: length of radii (cm), length of sticky spiral (cm), area of sticky spiral (cm^2), and the density of the sticky spiral (cm per cm^2 spider web). In all analyses of web geometry variables, the covariates "length of tibia" and "treatment duration" were included. The covariate "length of tibia" was considered, because the size of a spider web is influenced by the leg length of spiders (Vollrath 1987).

All variables and covariates were log(x+1)-transformed to create normal distribution and/or homogeneity of variances. Least significant differences (LSD) test was applied for pairwise comparisons. Furthermore, observed and standardised effect sizes (Cohen`s d) and the corresponding 95% confidence intervals were calculated from estimated means for pairwise comparisons of each dependent variable (Nakagawa & Foster 2004) following a SPSS script described in Smithson (2001), which also gives an indication of the statistical power to detect differences between treatments. The SPSS script can be downloaded from the internet at http://www.anu.edu.au/psychology/people/smithson/details/CIstuff/CI.html. All statistical analyses were carried out using SPSS, version 11 (SPSS inc., Chicago, USA). All average values presented are arithmetic means ± SD and all tests are two-sided.

3 Results

3.1 *Bt* maize pollen loads in spider webs

In the maize field, the average pollen number per spider web was 1044 ± 1193 (95% CI: 698–1391). Exposed spider webs had a mean area of the sticky spiral of 197.35 ± 198.58 cm^2 resulting in an average of 6.87 ± 6.05 pollen grains per cm^2 web area.

There was a positive relationship between the pollen load in spider webs and the height of their position in the maize field (R^2 = 0.29, $p < 0.01$; Fig. 2). Other variables were contributing to the variance in pollen numbers according to the regression equation: Pollen amount in spider web (n) = 3080.02 + 1241.96*Log_{10} (height in maize field) + 294*sticky spiral area – 202.85*air temperature (R^2 = 0.46, $p < 0.01$).

In the field margin neighbouring the *Bt* maize field, the mean pollen number found per spider web was 381 ± 205 (95% CI: 313–450). Exposed spider webs had a mean area of the sticky spiral of 187.49 ± 97.64 cm^2 resulting in an average number of 2.55 ± 2.07 pollen grains per cm^2 web area. There was a negative relationship between the pollen amount in spider webs and the distance to the maize field (R^2 = 0.12, $p = 0.01$; Fig. 3). Other variables were contributing to the variance in pollen numbers according to the regression equation: Pollen amount in spider web (n) = 1927.06 – 14.88*distance – 63.51*air temperature – 370.14*wind speed + 9.26*precipitation (R^2 = 0.38, $p < 0.001$).

3.2 Uptake of the Cry1Ab protein by spiders

The Cry1Ab protein was detected in the gastrointestinal system of 13 out of 20 garden spiders (65%), whose webs were treated with *Bt* maize pollen. The applied *Bt* maize pollen had a mean Cry1Ab concentration of 2657.81 ± 537.34 ppb. The Cry1Ab amount in the gastrointestinal system of spiders was <0.5 ppb.

3.3 Test of biological activity of the Cry1Ab protein

Diet had an effect on the survival time of *O. nubilalis* larvae ($F_{2,47}$ = 8.67, $p = 0.001$, ANOVA), and the *Bt* pollen was biological active. Mean survival times of *O. nubilalis* larvae fed with conventional maize pollen was 6.94 ± 0.24 days (n = 18), 5.00 ± 2.27 days (n = 15) when fed with *Bt* maize pollen, and 6.71 ± 1.21 days (n = 17) in the group on a diet without pollen. Survival time was significantly lower for *Bt* maize pollen fed larvae as compared to the conventional maize pollen treatment ($p = 0.002$), or larvae without any pollen ($P<0.001$, LSD test in both cases). After 7 days, 53% of the larvae fed a diet with *Bt* maize pollen had died, whereas mortality of larvae on a diet without *Bt* maize pollen was only 6%.

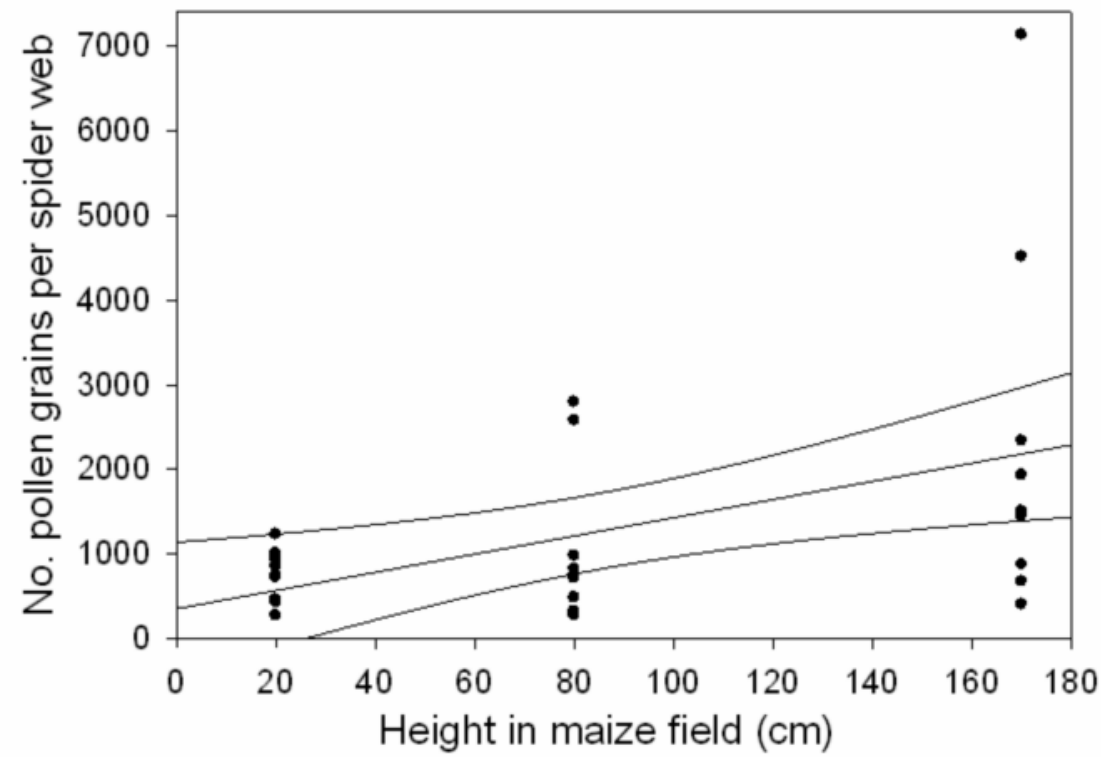

Figure 2: Number of *Bt* maize pollen recorded in webs of Araneidae in dependence of the vertical position of the spider webs (height) in the maize field. The regression equation is: $y = 361.11 + 10.72x$, $R^2 = 0.29$, $p = 0.002$, $n = 32$ (linear regression is given with the upper and lower limit of the 95% confidence interval).

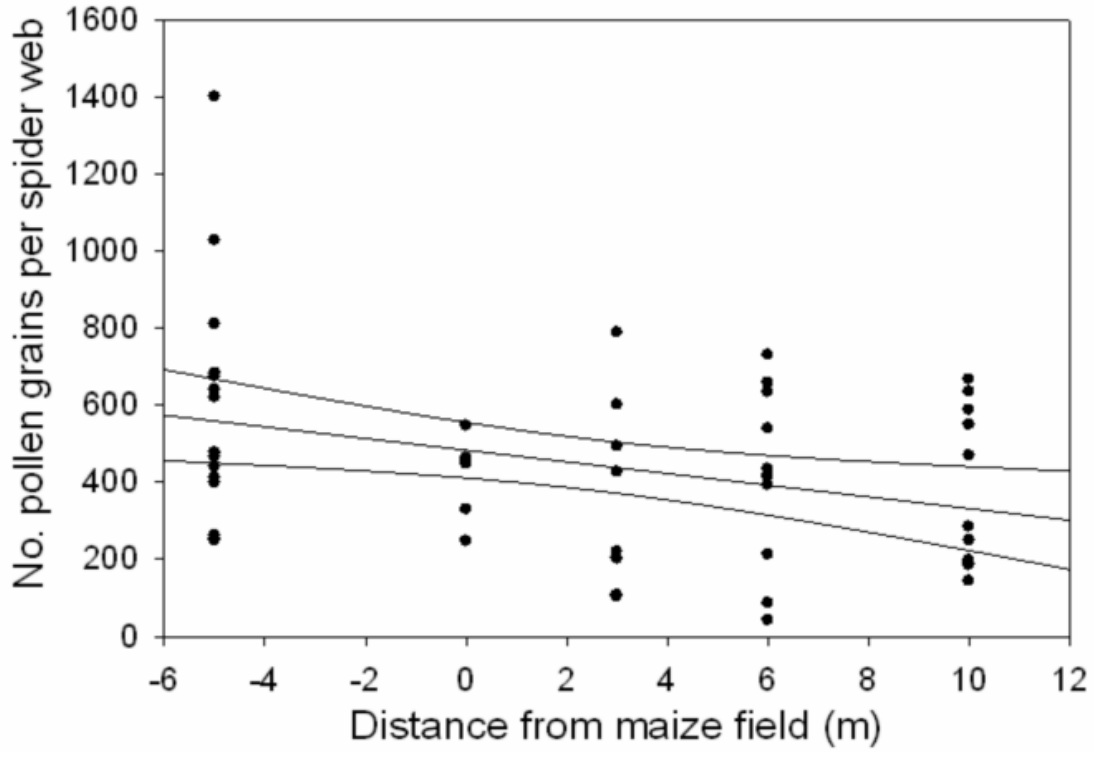

Figure 3: Number of *Bt* maize pollen recorded in webs of Araneidae in dependence of the distance to the maize field. The regression equation is: $y = 483.81 - 15.16x$, $R^2 = 0.12$, $p = 0.01$, $n = 53$ (linear regression is given with the upper and lower limit of the 95% confidence interval).

3.4 Effect of *Bt* maize pollen and insecticide on *A. diadematus*

Treatment conditions and characteristics of test spiders can be obtained from Tab. 1. Treatment showed an effect on spider variables "weight increase" ($F_{2,39}$ = 5.03, p = 0.01), survival ($F_{2,36}$ = 7.74, p = 0.002), and reaction time ($F_{2,37}$ = 2.90, p = 0.07), but not on moult frequency ($F_{2,35}$ = 0.38, p = 0.69) (ANCOVA in all cases, Fig. 4). The covariate "treatment duration" had a positive effect on "weight increase" ($F_{1,39}$ = 12.29, p = 0.001), but no relationship with the variables "survival time" ($F_{1,36}$ = 2.34, p = 0.13) and "moult frequency" ($F_{1,35}$ = 0.23, p = 0.64).

Table 1: Treatment conditions and characteristics of spiders treated with conventional maize pollen, *Bt* maize pollen and the insecticide Baythroid (arithmetic means ± 1SD).

	Conventional maize pollen	***Bt* maize pollen**	**Insecticide**
Applied pollen numbers per web (number)	871.01 ± 536.14	1177.56 ± 902.16	no pollen
Applied Baythroid 50 amount per web (nl)	no insecticide	no insecticide	0.05 ± 0.01[a]
Treatment duration (days)	10.00 ± 3.59	10.36 ± 3.72	10.38 ± 3.64
Tibia length (mm)	1.61 ± 0.26	1.48 ± 0.38	1.49 ± 0.61
Initial spider weight (mg)	3.04 ± 2.01	2.79 ± 1.88	3.58 ± 2.23
Final spider weight (mg)	6.31 ± 2.40	5.79 ± 2.43	5.97 ± 3.43
Age of spiders (days)	42.56 ± 1.85	42.43 ± 2.06	42.14 ± 1.79

[a] Baythroid amount was estimated on base of the area and the density of the sticky spiral of the treated webs and the estimated water absorbance after Samu & Vollrath (1992).

There was no difference between conventional and *Bt* maize pollen treated spiders concerning spider weight increase (Fig. 4A), survival (Fig. 4B), moult frequency (Fig. 4C), and reaction time (Fig. 4D) (LSD test in all cases, Tab. 2). However, Baythroid treated spiders had a reduced weight increase, a lower survival, and a longer reaction time (Fig. 4, Tab. 2).

Treatment did not cause web building inhibition ($F_{2,35}$ = 0.28, p = 0.76): After termination of the treatment, spiders treated with conventional maize pollen built their first web after 2.00 ± 2.24 days, *Bt* maize treated spiders after 2.09 ± 1.92 days, and Baythroid treated spiders after 1.46 ± 0.66 days. The covariate "treatment duration" had no influence on "web inhibition" ($F_{1,35}$ = 0.37, p = 0.55).

There was no treatment effect recorded on any of the web variables (Fig. 5, ANCOVA in all cases): "length of radii" ($F_{2,34}$ = 0.38, p = 0.86), "length of sticky spiral" ($F_{2,34}$ = 0.14, p = 0.87), "area of sticky spiral" ($F_{2,34}$ = 0.12, p = 0.88), and "density of sticky spiral" ($F_{2,34}$ = 1.67, p = 0.20). None of the covariates "treatment duration" and "length of tibia" had an influence on web variables (variables: "length of radii": $F_{1,34}$ = 0.05, p = 0.83; $F_{1,34}$ = 1.19, p = 0.28; "length of sticky spiral": $F_{1,34}$ = 0.67, p = 0.42; $F_{1,34}$ = 1.47, p = 0.23; "area of sticky spiral": $F_{1,34}$ = 0.36, p = 0.56; $F_{1,34}$ = 0.32, p = 0.57; "density of sticky spiral" $F_{1,34}$ = 0.43, p = 0.51; $F_{1,34}$ = 2.54, p = 0.12). Observed and standardised effect sizes (Cohen`s d) and corresponding 95% confidence intervals for pairwise comparisons of each dependent variable are shown in Tab. 2.

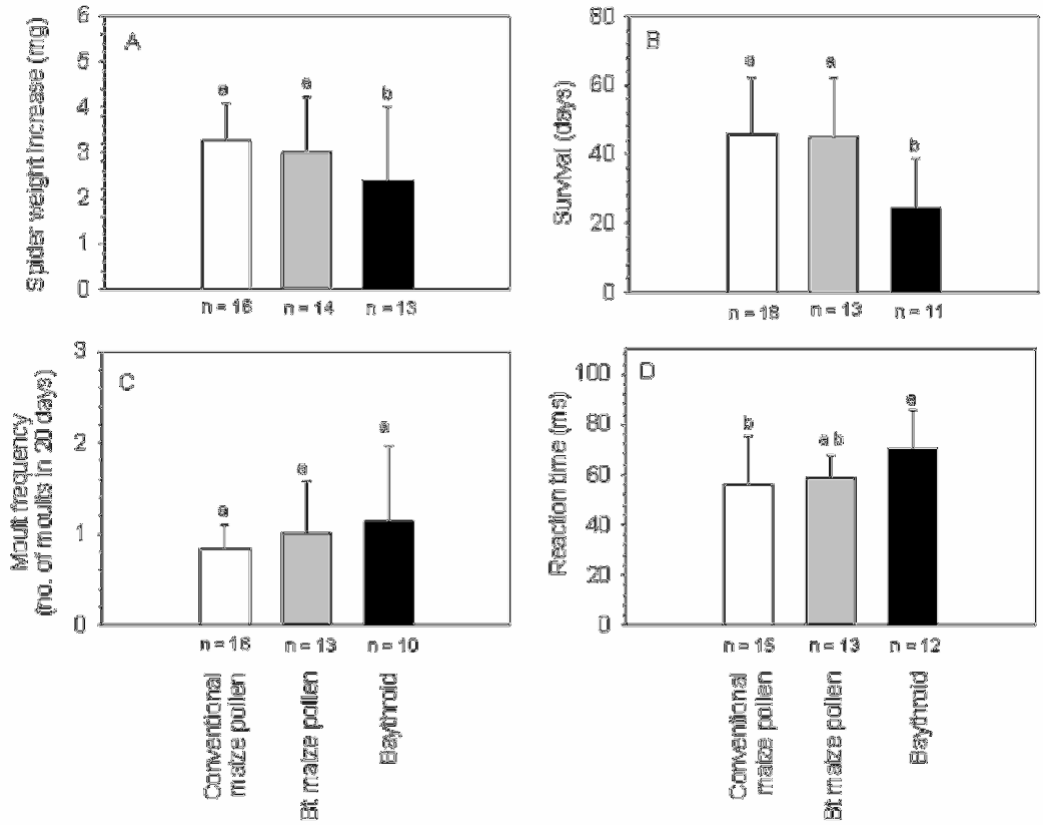

Figure 4: (A)Weight increase, (B) survival,(C) moult frequency, and (D) reaction time towards prey of *Araneus diadematus* spiders in the laboratory treated with conventional maize pollen, Bt maize pollen and the insecticide Baythroid (arithmetic means + SD). Columns capped with the same letter do not differ significantly (LSD test, $p > 0.05$).

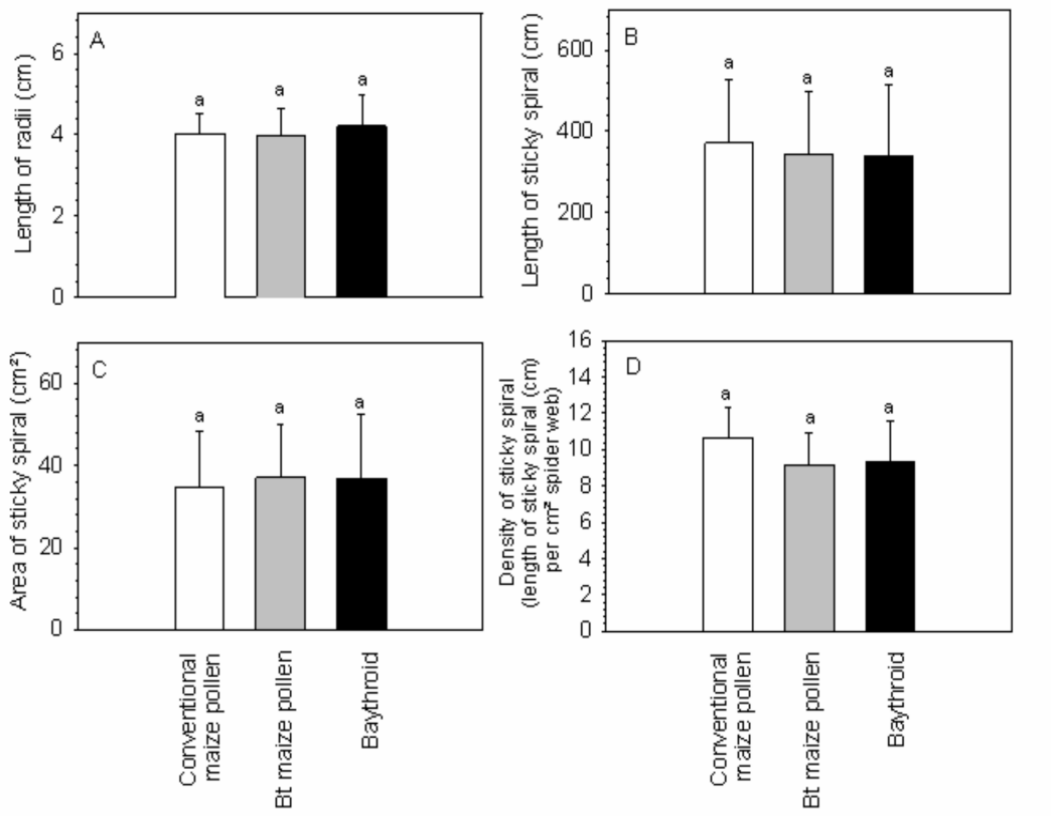

Figure 5: (A) Length of radii, (B) length of sticky spiral, (C) area of sticky spiral, and (D) density of sticky spiral of *Araneus diadematus* spiders in the laboratory treated with conventional maize pollen, Bt maize pollen, and the insecticide Baythroid (arithmetic means + SD). Sample sizes are 15 spiders for conventional pollen, 11 spiders for Bt maize pollen, and 13 spiders for Baythroid treatment. Columns capped with the same letter do not differ significantly (LSD test, $p > 0.05$).

Table 2: P-values, observed (d obs.) and standardised effect sizes (Cohen`s d) with the corresponding 95% confidence intervals for Cohen's d (95% CI of d) for pairwise comparisons (LSD test) of various dependent variables.

Comparison	*p*-value	d obs.	Cohen`s d	95% CI of d
Weight increase (mg)				
Conv – Bt	0.44	0.25	0.14	-0.44 - 1.00
Conv – Insecticide	< 0.01	0.98	0.58	0.37 - 1.96
Bt – Insecticide	0.03	0.73	0.45	0.08 - 1.68
Survival (days)				
Conv – Bt	0.99	0.55	< 0.01	-0.19 - 0.19
Conv – Insecticide	< 0.01	21.25	0.72	0.58 - 2.32
Bt – Insecticide	< 0.01	20.70	0.72	0.52 - 2.33
Moult frequency (number / 20 days)				
Conv – Bt	0.42	-0.04	0.15	-0.43 - 1.04
Conv – Insecticide	0.56	-0.03	0.12	-0.56 - 1.03
Bt – Insecticide	0.87	0.01	0.03	-0.72 - 0.86
Reaction time (ms)				
Conv – Bt	0.41	-2.44	0.16	-0.43 - 1.06
Conv – Insecticide	0.02	-13.90	0.48	0.15 - 1.76
Bt – Insecticide	0.14	-11.46	0.31	-0.19 - 1.36
Length of radii (cm)				
Conv – Bt	0.96	-0.15	0.01	-0.76 - 0.80
Conv – Insecticide	0.46	-0.23	0.14	-0.49 - 1.07
Bt – Insecticide	0.45	-0.22	0.16	-0.47 - 1.12
Length of sticky spiral (cm)				
Conv – Bt	0.86	10.43	0.04	-0.71 - 0.85
Conv – Insecticide	0.60	16.49	0.10	-0.55 - 0.94
Bt – Insecticide	0.75	6.06	0.07	-0.67 - 0.93
Area of sticky spiral (cm^2)				
Conv – Bt	0.63	-3.38	0.10	-0.59 - 0.97
Conv – Insecticide	0.87	-3.02	0.03	-0.68 - 0.81
Bt – Insecticide	0.74	0.36	0.07	-0.67 - 0.94
Density of sticky spiral (cm / cm^2)				
Conv – Bt	0.13	1.35	0.31	-0.18 - 1.41
Conv – Insecticide	0.12	1.23	0.30	-0.16 - 1.36
Bt – Insecticide	0.98	-0.12	0.01	-0.42 - 0.43

4 Discussion

4.1 Potential exposure of spiders to *Bt* maize pollen in the field

This study demonstrated that orb-web spiders are potentially exposed to maize pollen, both in the maize field and on its margin. Potential exposure to *Bt* maize pollen could be very high, but at the same time very variable. However, it is unclear how much of the pollen spiders will take up. Spider webs positioned higher in the maize field received a larger pollen amount which is in accordance with higher concentrations of airborne maize pollen in 2 m as compared to 20 cm above ground (Jarosz et al. 2003). Spider webs on field margins contained less pollen than webs within maize fields. Following maize pollen dispersion on maize field margins (Lang et al. 2004), webs at a distance of 10 m to the maize field edge had only half as much pollen than webs located directly in or at the maize field. Web spider species and individuals building their webs at a higher position and/or closer to a *Bt* maize field would, therefore, be clearly more exposed to *Bt* maize pollen. Web position, distance of the web to the maize field, and pollen deposited by shedding maize could explain only a small proportion of the pollen load in webs. Other (meteorological) factors may contribute to the variance of pollen amounts in spider webs such as temperature, turbulences, thermal convections, or wind speed, wind direction, and precipitation (e.g., Feil & Schmidt 2001, Jarosz et al. 2003, Lang et al. 2004). Features of the spider webs themselves possibly contribute to the variance of pollen load. For instance, the stickiness of spider webs is influenced by temperature, probably due to desiccation of the sticky spiral glue (Edmonds & Vollrath 1992). Furthermore, strong wind or insects caught in the web may cause single silk threads to merge thus decreasing the web's overall catching area (C. Ludy, personal observation). Preying on and catching pollen collecting bees may result in even higher *Bt* maize pollen exposure of orb-web spiders (Hirschfelder 1950, Ibrahim & Selim 1972), and would warrant further investigation.

Orb-web spiders are not only exposed to maize pollen, they also feed on it. As maize pollen has a width of 90 µm (Aylor, 2002), it is unlikely that spiders, especially juveniles, consume pollen grains as a whole, because spiders are generally only able to ingest particles ≤1 µm (Foelix 1992). Therefore, spiders would consume maize pollen by external digestion through various digestive enzymes, and suck up the dissolved nutrients. Nevertheless, the Cry1Ab protein (or derivatives) could be detected in garden spiders that had recycled their *Bt*-maize pollen loaded webs. Spiders not only consume pollen accidentally when recycling their

webs (Smith & Mommsen 1984), but also utilise and feed on pollen intentionally, and may use it as an additional food source (Vogelei & Greissl 1989, Ludy 2004). The fact that only in 65% of the spiders Cry1Ab was detected may be due to *Bt* concentrations below the limits of detection, or may indicate that spiders do not always consume the pollen in the web.

4.2 Effect of *Bt* maize pollen on spiders

Bt maize pollen consumption had no detectable effect on juvenile garden spiders (*A. diadematus*) as compared to the consumption of conventional maize pollen. The lethal and sublethal parameters of spiders studied were unaffected, as were the recorded web variables. It has to be noted that we used pollen from the *Bt* maize event Bt176, which has a toxin content several times higher than pollen from the event MON810 (e.g., Lang et al. 2004), the other *Bt* maize event registered in Europe, therefore exposure by MON810 would even be lower. This would also correspond to field studies that have not found adverse effects of *Bt* maize on the abundance of spiders (Hassell & Shepard 2002, Jasinski et al. 2003). In this study, applied *Bt* maize pollen densities corresponded to the actual field situation, spiders took up the *Bt* toxin, and the *Bt* toxin of the pollen was still biologically active. It is acknowledged, however, that high variation and small effect sizes of the data of the *Bt* comparison resulted in low power to detect effects, and that an existing *Bt* effect may have been missed due to limited sample size. In particular, web designs of individual spiders are highly variable (Hesselberg & Vollrath 2004), which may be true especially for juvenile spiders (Heiling & Herberstein 2000). Therefore, more mature or adult spiders have been suggested as indicator organisms (Witt & Reed 1965). However, we chose, and still recommend taking juvenile spiders, because the majority of the spider community in the maize fields during anthesis are juvenile stages (Ludy & Lang 2004, Meissle & Lang 2005).

The insecticide Baythroid caused clear adverse effects on spiders, proving that the experimental design was appropriate to detect effects. In the case of significant results, effect sizes were much larger than for the comparisons *Bt* vs. conventional pollen, resulting in a much higher power to statistically prove differences with the given sample size (cf. Meissle & Lang 2005). Survival and weight increase of insecticide-treated spiders were reduced. Furthermore, pyrethroids can lead to a reduced mobility of spiders (Jagers op Akkerhuis et al. 1997), which may possibly be responsible for the recorded longer reaction times of Baythroid treated garden spiders. This negative impact of a pyrethroid insecticide confirms several other laboratory and field studies (e.g., Pékar 2002, Meissle & Lang 2005).

In addition to the negative effect of the insecticide itself, the pollen treatments received an additional protein supply through the maize pollen, which may have contributed to differences in survival and weight increase. In contrast to the present study, several other studies found an impact of insecticides on web geometry (Samu & Vollrath 1992, Lengwiler & Benz 1994). In this study, the applied Baythroid concentration was relatively low in order to prevent an early loss of test spiders. As mentioned above, the high variability of web parameters possibly obstructed the detection of an effect. Moreover, in studies demonstrating a negative pesticide effect on web-geometry parameters, pesticides were mostly applied orally or topically (e.g., Samu & Vollrath 1992, Lengwiler & Benz 1994), which probably results in higher doses compared to insecticide sprayed webs.

Conclusions

Web-building spiders are exposed to *Bt* maize pollen in maize fields and adjacent field margins. Most likely, orb-web spiders will feed on the *Bt* maize pollen caught in their webs, but the laboratory assay showed no adverse effects of *Bt* maize pollen consumption on the garden spider, *A. diadematus*, while an insecticide had a clear negative impact. Spiders are common and abundant predators in arable land important for pest control, and this study together with the so far published field results suggests that cultivation of *Bt* maize is more compliant with biological control provided by spiders than the application of a pyrethroid insecticide. However, this is the first study investigating potential exposure and effects of *Bt* maize on an orb-web spider in the laboratory. Therefore, the database is still too small to allow for generalisations. Considering the important ecological and economic role of spiders, we recommend to study further species including tests of sublethal parameters, of relative fitness variables, and of possible long-term chronic effects. So far, in field studies about the effect of *Bt* maize on spiders, web-building spiders were neglected. Considering the high potential exposure of web spiders to *Bt* maize pollen, more attention should be paid to (web-building) spiders of higher strata in *Bt* maize risk assessment.

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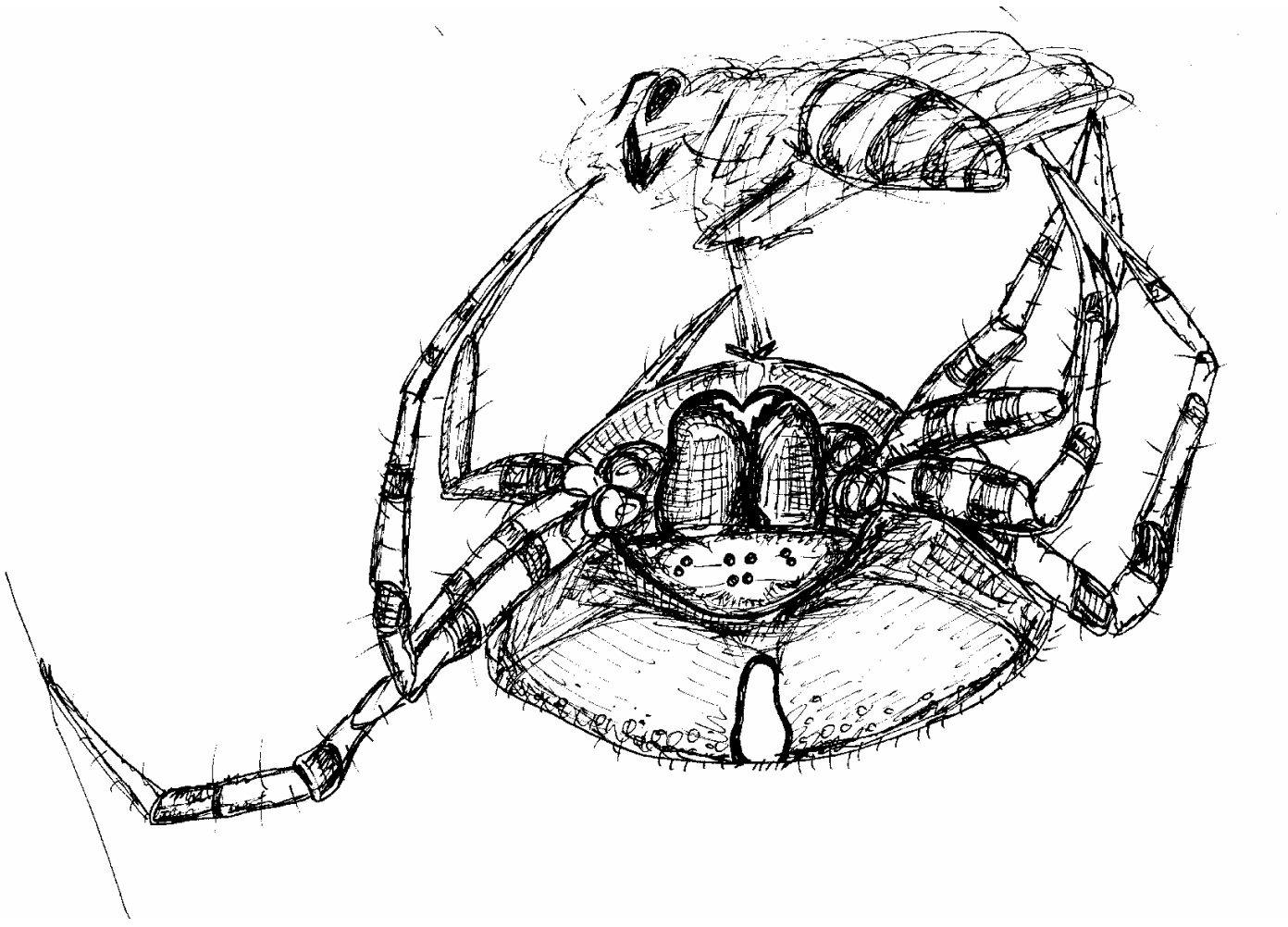
Prey spectra and prey selection of orb-web spiders (Araneae: Araneidae) on field margins



Prey spectra and prey selection of orb-web spiders (Araneae: Araneidae) on field margins: significance for an exposure assessment of *Bt*-contaminated prey.

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Abstract

Genetically modified *Bt* plants may have negative effects on non-target organisms and therefore a risk assessment including an exposure analysis of *Bt* plants is needed and demanded. Beside a possible direct adverse effect of *Bt* plants e.g. via pollen consumption, predators could be exposed indirectly to *Bt* toxins via herbivorous or pollen-collecting prey. As the *Bt* toxin content in different prey taxa varies due to different feeding habits of prey, data on the prey spectrum and prey selection of predators in agroecosystems are needed for an exposure assessment of *Bt* plants to predators. In this study, prey spectra and the selectivity of webs as well as individuals of two orb-web spider species, the garden spider *Araneus diadematus* and the wasp-like spider *Argiope bruennichi*, were recorded on two different structured field margins in Bavaria, South Germany. In general, prey spectra of both spider species consisted of a few, in arable land frequent, prey taxa and were dominated by Diptera. In spider webs, small, broad-winged prey items such as Sternorrhyncha were caught easily, whereas mobile prey with good optical skills such as Diptera and Hymenoptera could probably avoid spider webs. Prey armed with strong mandibles or stings, such as Coleoptera and Hymenoptera, were avoided by individuals of both spider species. However, both spider species differed in their selectivity to Apidae. Whereas Apidae were avoided by individuals of *A. diadematus*, *A. bruennichi* showed no avoidance or preference towards this prey. Taken into account both web and spider selectivities, orb-web spider on field margins preferred Sternorrhyncha and Apidae, but avoided other Hymenptera in this study. Thus, orb-web spiders on field margins were potentially exposed to *Bt*-contaminated herbivorous prey and *Bt*-pollen collecting prey. However, further field and laboratory studies are needed to quantify an uptake of *Bt* toxins by orb-web spiders via different prey items in the field and to assess a possible effect of these *Bt* toxins on orb-web spiders.

Key words: Prey composition; prey selectivity; Araneidae; field margins; risk assessment of genetically modified plants

1 Introduction

Genetically modified *Bt* plants (e.g. *Bt* corn, *Bt* potato, *Bt* cotton, *Bt* tomato), which are cropped increasingly worldwide (James 2003), express entomopathogenic Cry proteins (Cry1Ab, Cry1Ac, Cry3A, Cry9F and some others) derived from the soil bacterium *Bacillus thuringiensis* (*Bt* toxin) in plant tissue including pollen. Despite the fact that *Bt* proteins are described as specific toxins against lepidopteran, dipteran and coleopteran pests (Gill et al. 1992), there is evidence, that *Bt* toxins could harm non-target predators and parasitoids sublethally or lethally (e.g. Hilbeck et al. 1998, Ponsard et al. 2002; see Groot & Dicke 2002 for review). Therefore, an ecological risk assessment of *Bt* plants is needed and demanded (e.g. European Parliament and Council 2001). A risk assessment of *Bt* plants should include an exposure analysis of predators as ecological and economical important organisms (Dutton et al. 2003, Andow & Hilbeck 2004). Beside a direct exposure of predators to *Bt* proteins by the intentional consumption of *Bt* plant materials including pollen (Rijn et al. 1997), predators also could be indirectly exposed by the consumption of *Bt* contaminated prey which may pass the *Bt* protein to predators (Hilbeck & Andow 2002, Harwood et al. 2005). However, the *Bt* protein content in herbivores having fed on *Bt* plants depends on feeding habits of different prey taxa (Head et al. 2001, Raps et al. 2001, Dutton et al. 2002, Dutton et al. 2003). Therefore, qualitative and quantitative data on prey consumption and prey selection of test organisms in agroecosystems should be available due to an exposure analysis of predators to *Bt* plants.

Spiders are a species-rich and abundant predator group in agroecosystems and have there ecological and economical significance due to a pest control function (Sunderland 1999, Nyffeler & Sunderland 2003, Lang 2003). Orb-web spiders (Araneidae) occur in arable land frequently (Nyffeler 1982, Barthel 1997, Ludy & Lang 2004) and field margins provide an appropriate vegetation structure for web-building and thus are valuable habitats for orb-web spiders (McNett and Rypstra, 2000).

Spiders are potentially exposed to products of *Bt* plants by *Bt* pollen feeding (Ludy, 2004) or by feeding on *Bt*-contaminated herbivorous or pollen-collecting prey (Harwood et al. 2005, Gregory 1989). Several studies analysed prey consumption and prey spectra of orb-web spiders in grassland (Kajak 1965, Pasquet 1984, Nyffeler & Breene 1991) and arable land (Nyffeler 1982, Nyffeler & Benz 1979), but no such work was conducted on field margins so far.

Furthermore, most of the existing studies on the prey of spiders did not address the potential prey (prey available in a habitat), which biases the actual prey of predators in a habitat (prey eaten) (Sih & Moore 1990). Beside prey availability, prey selection of predators occurs according handling efforts and palatability of prey (Sih & Moore 1990, Lang & Gsödl 2001), which is also true for spiders (Riechert & Luczak 1982, Nentwig 1987). For web-building spiders, prey selection occurs on two levels: the selection biased by characteristics of the orb-web e.g. microhabitat or visibility to potential prey ("web selectivity") and the selection biased by characteristics of the spider ("spider selectivity") e.g. specific prey capture behaviour (Riechert & Luczak 1982, Uetz 1990).

In this study, the prey spectrum as well as the prey selectivity of webs and individuals of two orb-web spider species on two different structured field margin types was investigated. The aim of the study was describe prey composition and prey selection of orb-web spiders on field margins and to elucidate their potential exposure of *Bt* contaminated prey.

2. Material and methods

2.1 Exposure of spiders in the field

Adult females of the garden spider *Araneus diadematus* (Clerck) and the wasp spider *Argiope bruennichi* (Scopoli), which occur on field margins frequently (Barthel 1997) were collected on field margins and were then kept in the laboratory in wooden frames (size 30 x 30 cm²) to allow web building ("spider frames", see Ludy & Lang (submitted) for description). Spiders were fed with *Drosophila* flies and supplied with water every day in the laboratory for several weeks before they were exposed on field margins to potential flying prey. This was done to generate a comparable nutrient level in the spiders, as nutrient conditions of spiders could bias their selection for prey (De Crespigny et al. 2001).

Two different field margins adjacent to maize fields in Swabia and Frankonia (South Germany) were chosen for prey analysis. In Swabia, the field margin was covered with stinging nettles (*Urtica dioica*), birdsfoot trefoil (*Lotus corniculatus*), wild mustard (*Sinapis arvensis*) and wild carrot (*Daucus carota*), but without flowering plants (flower-poor margin). The field margin in Frankonia was dominated by blooming common tansy (*Tanacetum vulgare*) (flower-rich margin). Both field margins had a size of approximately 50 x 7 m. "Spider frames" were installed on metal posts in different heights according the natural vertical stratification of the spider species (Nyffeler 1982): Frames with *A. diadematus* were

exposed in a height of 80 cm, frames with *A. bruennichi* in a height of 20 cm. The investigation was conducted during three days for each spider species and field margin type, respectively, from July 7 to August 11, 2003. Each day, 3-4 "spider frames" inclusive spiders were exposed on field margins. Observation periods and climatic conditions are shown in Tab. 1.

Table 1: Climatic conditions during the observation periods of actual prey of two orb web spider species on two field margin types (means ± 1SD, n = 3 each).

Date	Spider species	Field margin	Air temperature (°C, daily means)	Wind velocity (m/s, daily means)	Precipitation (mm, daily sums)
07.07-10.07.2003	*Araneus diadematus*	flower-poor	18.63 ± 0.74	1.13 ± 0.35	0.00 ± 0.00
23.07.-25.07.2003	*Araneus diadematus*	flower-rich	20.63 ± 1.45	1.23 ± 0.38	1.57 ± 2.45
05.08.-07.08.2003	*Argiope bruennichi*	flower-poor	26.50 ± 0.79	0.80 ± 0.10	0.00 ± 0.00
09.08.-11.08.2003	*Argiope bruennichi*	flower-rich	26.73 ± 0.23	1.36 ± 0.49	3.46 ± 3.31

2.2 Prey consumption and prey preference analysis

Exposed "spider frames" with spider webs and spiders were observed seven hours a day from 11:00 to 18:00, giving a total of 154 web hours (3-4 spiders x 7 hours x 3 days x 2 spider species, see Uetz 1990). Spider webs were observed from a distance of 1.5 to 2 meters to minimize the disturbance of potential prey and spiders. All prey items, which were caught by the spider web and which were eaten by the spider were counted and classified into taxonomic groups at order or family level. In the following, the taxonomic group "Hymenoptera" does not contain "Apidae" as this family was treated separately as potential pollen-carrying prey. As spiders may ingest small prey by web "recycling" without attacking (Nentwig 1985), prey items, which were present in the web after the exposure duration, were assigned to actually eaten prey.

The assessment of potential prey was conducted by means of sampling flying prey items on the field margins simultaneously with the exposure of the "spider frames". Therefore, a malaise trap (Townes 1962) with a capture area of 1.8 m² was installed on each field margin, parallel oriented to the exposed spider webs, and the collecting head was filled with 5% acetic acid. Additionally, sticky traps in the same size of the "spider frames" (30 x 30 cm i.e. 0.09 m²) were set up next to each exposed "spider frame". During the observation of *A. diadematus* on the flower-poor field margin, only the malaise trap was installed.

Altogether 12 malaise trap samples were taken for assessing the potential prey spectrum of both spider species (1 malaise trap sample x 3 days x 2 field margin types x 2 spider species).

Sticky traps consisted of transparent plastic plates, which were covered with a clear film spread with Aurum® sticky non-drying glue. After the exposure duration, the films covered with non-drying glue and adhering items were collected. Potential prey items caught with malaise traps and sticky traps were taxonomically classified in the laboratory to order or family level. Altogether, 11 sticky trap samples were taken for assessing the potential prey of *A. diadematus* (3-4 sticky trap samples x 3 days x 1 field margin type), and 24 sticky trap samples were taken for the assessing the potential prey of *A. bruennichi* (4 sticky trap samples x 3 days x 2 field margin types).

2.3 Statistical analysis

2.3.1 Actual prey

Analyses of variance (ANOVA) was conducted with the independent variables “prey” (six different taxonomic groups) and “field margin” (“flower-poor margin” and “flower-rich margin”) and the dependant variable “numbers of eaten prey” for both spider species separately. To test the homogeneity of variances, Sen and Puris nonparametric test was conducted. Kolmogorov-Smirnov one-sample test was used for testing the normal distribution of data. For statistical analysis, means over the exposure duration of the dependant variable was calculated. As spiders did not always build webs every day during the experimental dates, the same spider individuals could not be used on all three days. So, the sample size of the spiders was with 5-7 spiders higher than the exposed 3-4 spiders per day. Furthermore, the dependant variable was log x +1-transformed to create normal distribution and homogeneity of variance of the data set. Post hoc comparisons were conducted with the least significance differences (LSD) test. The ANOVA was calculated using the software Statistika 0.5. All average values presented are arithmetic means ± 1SD and the tests are two-sided.

2.3.1 Prey preference

The selection of prey was analysed with the forage ratio (Savage 1931, cited in Manly et al. 2002):

$$w_i = o_i/\pi_i \qquad (1)$$

The forage ratio ranges from 0 (maximum negative selection) to infinity (maximum positive selection). A forage ratio with a value of 1 indicates no selection. The foraging ratio was calculated to determine the "web selectivity" with $o_{i\ (web\ selectivity)}$ as the frequency of prey of the taxonomic group i caught in spider webs and $\pi_{i\ (web\ selectivity)}$ as the frequency of available prey of the taxonomic group i collected in the habitat by malaise traps and sticky traps (potential prey). Furthermore, the foraging ratio was calculated for the "spider selectivity" with $o_{i\ (spider\ selectivity)}$ as the frequency of prey of the taxonomic group i eaten by the spider (actual prey) and $\pi_{i\ (spider\ selectivity)}$ as the frequency of prey of the taxonomic group i caught in the spider web. Frequencies of prey caught in spider webs as well as frequencies of actual prey were calculated on the basis of summed data of spider individuals and observation dates. Malaise trap data were converted to the area of sticky traps and then arithmetic means of malaise trap data and sticky trap data were calculated. With these means, frequencies of potential prey were determined. The standard error of the forage ratio for the selectivity of webs or spiders, respectively, was calculated by means of the following formula:

$$se_{(wi)} = [(1- \pi_i)/(u_+ * \pi_i)]^{1/2} \qquad (2)$$

with $u_{+\ (web\ selectivity)}$ as total number of prey caught in webs or $u_{+\ (spider\ selectivity)}$ as the prey eaten by spiders, respectively (Manly et al. 2002). The statistical significance of the forage ratio was determined with the chi-square statistic:

$$X^2_{(selectivity)} = (w_i - 1)^2/se_{(wi)}^2 \qquad (3)$$

with one degree of freedom to test the null hypothesis $H_{0\ (web\ selectivity)}$, that spider webs catch prey in frequencies to prey availability or, respectively, the null hypothesis $H_{0\ (spider\ selectivity)}$, that spiders feed on prey in frequencies to prey caught in the spider web. The significance level of 5% was adjusted with the Bonferroni correction to 0.7 % (Rice 1989). The chi-square statistic for web and spider selectivity was calculated with the software MS Excel by using the formulas 1-3.

3 Results

3.1 Potential prey

A total of 353 potential prey items were recorded with a malaise trap and 2059 items with 3-4 sticky traps during three days as potential prey for *A. diadematus*. A mean number of 44.54 ± 48.17 potential prey per web area (i.e. 0.09 m²) and seven hours was caught for *A. diadematus*. As potential prey for *A. bruennichi*, a total of 237 potential prey items were recorded by a malaise trap and 6849 potential prey items by four sticky traps during three days. A mean number of 143.67 ± 133.35 potential prey items were caught per web area (i.e. 0.09 m²) and seven hours. The potential prey specta of both spiders species and both field margin types were dominated by Diptera, followed by Hymenoptera, Coleoptera and Heteroptera by numbers and proportions (Tab. 2).

Table 2: Mean number and proportions of potential prey items for two orb-web spider species on two different field margin types. [a]: only malaise trap data available, all other values are based on malaise and sticky trap data. The mean number is presented as arithmetic means ± 1 SD. The taxonomic group Hymenoptera does not include Apidae.

	Araneus diadematus			
	Flower-poor margin		**Flower-rich margin**	
	Prey numbers/web area[a]	%	Prey numbers/web area	%
Heteroptera	0.08 ± 0.06	11.4	2.33 ± 0.95	2.6
Sternorrhyncha	0.00 ± 0.00	0.0	0.78 ± 0.82	0.9
Coleoptera	0.15 ± 0.00	21.4	4.87 ± 0.41	5.5
Hymenoptera	0.05 ± 0.00	7.1	21.74 ± 10.50	24.6
Apidae	0.00 ± 0.00	0.0	0.08 ± 0.05	0.1
Diptera	0.34 ± 0.10	48.6	43.38 ± 4.98	49.1
other taxa	0.08 ± 0.03	11.4	15.19 ± 1.78	17.2
all groups	**0.70 ± 0.03**	**100.0**	**88.38 ± 6.07**	**100.0**
	Argiope bruennichi			
	Flower-poor margin		**Flower-rich margin**	
	Prey numbers/web area	%	Prey numbers/web area	%
Heteroptera	5.66 ± 0.43	2.2	1.87 ± 0.61	7.2
Sternorrhyncha	0.25 ± 0.33	0.1	0.00 ± 0.00	0.0
Coleoptera	5.43 ± 0.82	2.1	0.55 ± 0.19	2.1
Hymenoptera	17.6 ± 7.01	6.7	5.96 ± 1.88	22.9
Apidae	0.00 ± 0.00	0.0	0.03 ± 0.01	0.1
Diptera	151.99 ± 47.71	58.2	16.12 ± 4.19	61.9
other taxa	80.36 ± 4.52	30.8	1.51 ± 0.39	5.8
all groups	**261.29 ± 54.11**	**100.0**	**26.06 ± 5.31**	**100.0**

3.2 Actual prey

Overall, the garden spider *A. diadematus* fed on 292 prey items on both field margin types and consumed a mean number of 13.50 ± 7.46 (3 to 26, minimum to maximum) prey items per web and seven hours a day. The prey of *A. diadematus* generally consisted of 1.30 ± 2.74 (0 to 9) Heteroptera, 2.7 ± 2.68 (0 to 8) Sternorrhyncha, 1.07 ± 1.62 (0 to 5) Coleoptera, 0.58 ± 0.90 (0 to 3) Hymenoptera without Apidae, 0.28 ± 0.79 (0 to 3) Apidae, 7.10 ± 5.49 (0 to 15) Diptera and 0.47 ± 0.48 (0 to 1) other taxa.

The wasp spider *A. bruennichi* consumed a total prey number of 106 in both field margin types and fed on a mean number of 4.87 ± 4.63 (1 to 17) prey items per web and seven hours consisting of 0.37 ± 0.46 (0 to 1) Heteroptera, 0.64 ± 0.97 (0 to 3) Sternorrhyncha, 0.29 ± 0.38 (0 to 1) Coleoptera, 0.14 ± 0.30 (0 to 1) Hymenoptera without Apidae, 0.55 ± 0.65 (0 to 2) Apidae, 2.85 ± 3.40 (0 to 10) Diptera, and 0.03 ± 0.09 (0 to 1) other taxa.

Table 3: Results of an analysis of variance (ANOVA) on the number of eaten prey groups of two orb web spider species on two different field margins

	Araneus diadematus				***Argiope bruennichi***			
Source of variation	**df**	**MS**	***F***	***P***	**df**	**MS**	***F***	***P***
Prey	6	0.58	10.14	0.01	6	0.27	13.31	< 0.01
Field margin	1	0.38	6.70	< 0.01	1	0.32	15.63	< 0.01
Prey x field margin	6	0.23	4.08	< 0.01	6	0.22	10.53	< 0.01
Error	56	0.06			77	0.02		

In general, Diptera dominated the actual prey spectra of both spider species by numbers and proportions (Tab. 3, main factor “prey”, LSD test: $p < 0.01$ each comparison, Fig. 1), followed by Sternorrhyncha, which were more consumed than all other prey groups except for Diptera by *A. diadematus* (LSD test: $p < 0.01$ each comparison, Fig. 1) and which were more consumed than Hymenoptera without Apidae and “other taxa” by *A. bruennichi* (LSD test: $p < 0.05$ each comparison, Fig. 1). Additionally, *A. bruennichi* fed on more Apidae than “other taxa” (LSD test: $p < 0.05$, Fig. 1).

On the flower-poor field margin, *A. diadematus* fed on a total prey number of 233 and on the flower-rich margin on a total prey number of 59. The average prey consumption in *A. diadematus* was on the flower-poor field margin 18.87 ± 6.30 (10 to 26) prey items and on the flower-rich field margin 8.13 ± 3.66 (3 to 13) prey items per web and seven hours. *A.*

bruennichi consumed on the flower-poor margin a total prey number of 81 and on the flower-rich margin a total prey number of 25. The average prey consumption in *A. bruennichi* was 8.08 ± 5.19 (3 to 17) prey items on the flower-poor field margin and 2.12 ± 1.10 (1 to 4) in the flower-rich margin per web and seven hours. Both spider species consumed less prey items on the flower-rich field margin than on the flower-poor margin (Tab. 3, main factor "field margin", Fig. 1). This was due to a lower consumption of Diptera on the flower-rich field margin than on the flower poor margin of both spider species (Tab. 3, interaction "prey x field margin", LSD test: $p < 0.001$ each comparison, Fig. 1). Additionally, *A. diadematus* consumed more Coleoptera on the flower-poor margin than on the flower-rich field margin (LSD test: $p = 0.01$). In contrast, *A. diadematus* consumed more Heteroptera (LSD test: $p = 0.04$) and *A. bruennichi* consumed more Apidae (LSD test: $p = 0.02$) on the flower-rich margin.

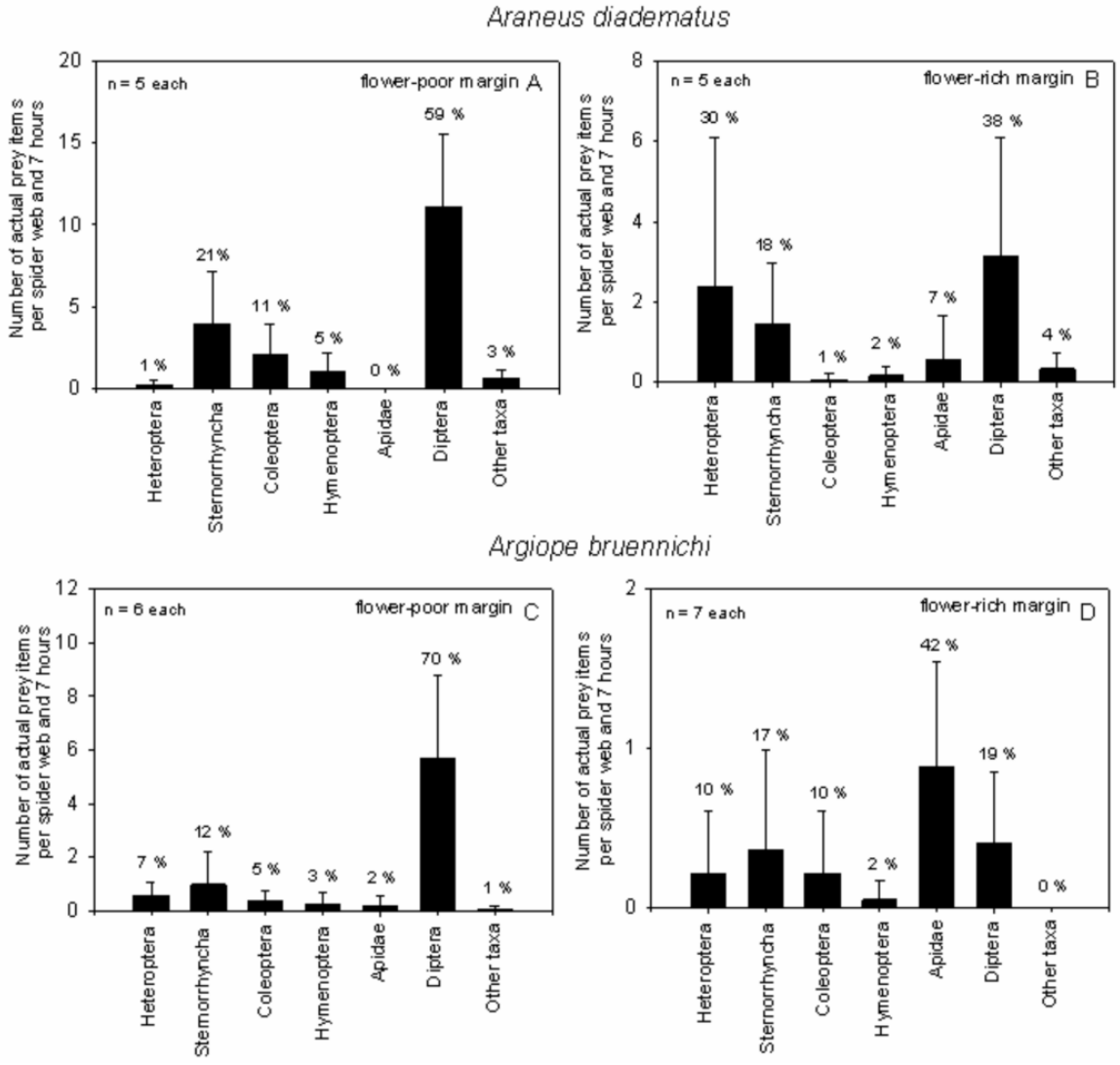

Figure 1: Numbers and frequencies of actual eaten prey of *Araneus diadematus* (A,B) and *Argiope bruennichi* (C,D) on a flower-poor (A,C) and a flower-rich (B,D) field margin (arithmetic means ± 1SD) (Note different ranges of x-axes). The taxonomic group Hymenoptera does not include Apidae.

3.3 Selectivity of webs and spiders

The selectivity of spiders webs and spider individuals (forage ratio w_i) of both spider species and the statistical significance of the selectivities are shown in Tab. 4. On both field margin types, webs of *A. diadematus* and *A. bruennichi* selected Sternorrhyncha positively, but spider individuals showed no selection towards this prey type. Taking into account both web and spider selectivities, Sternorrhyncha were positively selected by both spider species. The selection of webs for Heteroptera was inconsistent, both negative and positive, and also the spiders individuals had no consistent aversion or preference for this prey group. Altogether this resulted in a inconsistent selection of Heteroptera by both spider species. Webs caught Coleoptera without or even with a positive selection, but individuals of both spider species avoided this prey. The contradicting selectivity of webs and spider individuals to Coleoptera led to a negative selectivity by *A. diadematus* and a preference or no selectivity, respectively, in *A. bruennichi*. Spider webs of both species selected Hymenoptera without Apidae on the flower-rich margin negatively, and also spider individuals avoided this prey group, which contributed to an overall negative selection of Hymenoptera without Apidae by both spider species. Diptera were selected negatively by spider webs on the flower-rich margin, but spider individuals showed no selection towards this prey type, generally resulting in no selection of both spider species for Diptera. If available, pollen-collecting Apidae were selected positively by spider webs, but whereas this prey was avoided by individuals of *A. diadematus*, *A. bruennichi* showed no selection. However, there was an overall positive selection of Apidae by both spider species.

Table 4: Forage ratio (w_i), chi-square statistic (X^2) and significance level (p-level) for the selectivity of spider webs (a), spiders (b), and both webs and spiders (c) to various prey taxa of two orb-web spider species on two field margin types. After a Bonferroni correction, significant selectivities have a significance level $p < 0.007$. The taxonomic group Hymenoptera does not include Apidae.

	Araneus diadematus						***Argiope bruennichi***					
	Flower poor margin			**Flower rich margin**			**Flower poor margin**			**Flower rich margin**		
	w_i	X^2	p-level	w_i	X^2	p-level	w_i	X^2	p-level	w_i	X^2	p-level
Heteroptera	0.24[a]	397.98	< 0.001	13.98	41.60	< 0.001	3.58	17.60	< 0.001	1.79	4.07	0.044
	0.47[b]	46.35	< 0.001	1.11	0.41	0.522	0.80	1.60	0.206	0.93	0.10	0.749
	0.11[c]	**2363.87**	**< 0.001**	**7.57**	**31.87**	**< 0.001**	**2.85**	**10.29**	**0.001**	**1.67**	**2.90**	**0.090**
Stenorrhyncha	-	-	-	1.86	9.59	0.002	97.22	35.92	< 0.001	-	-	-
	1.34	4.86	0.027	0.98	0.01	0.911	1.19	0.83	0.361	0.99	0.001	0.971
	-	**-**	**-**	**17.25**	**33.10**	**< 0.001**	**116.13**	**30.91**	**< 0.001**	**-**	**-**	**-**
Coleoptera	0.96	0.16	0.689	0.85	0.73	0.393	5.22	25.66	< 0.001	1.53	1.44	0.230
	0.58	29.06	< 0.001	0.48	30.69	< 0.001	0.46	32.12	< 0.001	1.24	0.43	0.511
	0.55	**35.33**	**< 0.001**	**0.21**	**349.82**	**< 0.001**	**2.37**	**7.51**	**0.006**	**1.90**	**2.58**	**0.108**
Hymenoptera	0.87	1.07	0.301	0.33	110.16	< 0.001	0.69	5.55	0.018	0.14	446.17	< 0.001
	0.64	11.47	0.001	0.57	10.26	0.001	0.53	14.32	< 0.001	1.24	0.43	0.511
	0.55	**22.65**	**< 0.001**	**0.10**	**1667.50**	**< 0.001**	**0.37**	**54.74**	**< 0.001**	**0.17**	**256.81**	**< 0.001**
Apidae	-	-	-	11.69	41.21	< 0.001	-	-	-	354.07	37.62	< 0.001
	-	-	-	0.37	73.80	< 0.001	0.8	1.50	0.220	1.06	0.12	0.726
	-	**-**	**-**	**83.17**	**25.81**	**< 0.001**	**-**	**-**	**-**	**375.26**	**34.82**	**< 0.001**
Diptera	0.96	0.22	0.639	0.50	65.89	< 0.001	1.12	1.05	0.306	0.26	186.00	< 0.001
	1.12	1.33	0.249	1.40	5.38	0.020	1.12	0.87	0.351	0.99	0.001	0.971
	1.08	**0.57**	**0.450**	**1.00**	**0.00**	**0.979**	**1.25**	**3.13**	**0.080**	**0.26**	**170.05**	**< 0.001**
Other taxa	0.3	188.76	< 0.001	1.98	7.11	0.008	0.02	27743.72	< 0.001	0.56	7.59	0.006
	1.01	0.00	1.000	0.82	1.08	0.298	1.59	3.18	0.075	-	-	-
	0.31	**155.07**	**< 0.001**	**0.28**	**154.57**	**< 0.001**	**0.04**	**13102.13**	**< 0.001**	**-**	**-**	**-**

4. Discussion

4.1. Prey spectrum and prey selection of orb-web spiders on field margins

Orb-web spiders consumed a mean number of 9 prey items per web and seven hours. Other quantitative studies on the prey consumption of orb-web spiders confirmed that prey numbers ranged between 5 and 20 prey items a day (Kajak 1965, Nyffeler & Benz 1979, Nyffeler 1982). Nevertheless, most of these studies based on the number of potential prey items in spider webs in 24 hours and did not address the actual eaten prey items by the spider. Therefore, the amount of actual eaten prey in 24 hours could be higher than the prey amount of spider webs given in existing studies, as spiders may drop eaten prey remains from the web (Nyffeler 1982). A direct observation of spider webs to investigate the prey consumed by the spider, as it was conducted in this study, is time consuming. However, compared to an examination of webs, a direct observation is the only possibility to assess the actual eaten prey of a spider per time unit accurately (Olive 1980, Nyffeler 1982). Actual prey quantities consumed by orb-web spider species on different field margins were highly variable and differed between field margin types by 58–75 %. High variations in the total prey of different orb-web spider species between different locations were also given in Nentwig (1985).

A few flying insect taxa such as Diptera, Sternorrhyncha, Heteroptera and Coleoptera (in a decreasing order) provided the prey spectra of orb-web spiders on field margins with a strong dominance of Diptera. This prey composition of orb-web spiders was confirmed by several other studies in different habitat types and geographic regions (Kajak 1965, Nyffeler & Benz 1979, Olive 1980, Nyffeler 1982, Pasquet 1984, Nentwig 1985, Miyashita & Shinkai 1995).

The prey composition of orb-web spiders was biased by both web characteristics and spider features (see Stowe 1986, Uetz 1990). Prey groups, which have good visual and flight abilities such as Diptera and certain Hymenoptera may actively avoid webs (Olive 1980, Craig 1986, Uetz 1990) (especially when they were stretched between wooden frames as in the present study), which resulted in negative selectivity of spider webs to this prey group. On the other hand, small sized and slow flying items with a large wing surface i.e. Sternorrhyncha, were caught by spider webs easily (Nentwig 1982). A positive selectivity of spider webs to Sternorrhyncha and a negative web selectivity to Hymenoptera were also found by Kajak (1965) and Uetz (1990) for different orb-web spider species. Nentwig (1985) recorded a negative selectivity of webs at least to Brachycera.

Spider individuals avoided prey with defence structures i.e. robust mandibles and/or stings or a bad taste such as Coleoptera and some Hymenoptera (Nentwig 1987, Henaut et al. 2001). Spiders probably discriminate potentially dangerous prey via web-borne vibrations caused by the entangled prey (Suter 1978) and do not attack, while these prey types may leave the web due to an effective escape behaviour (Nentwig 1982). Uetz (1990) described a comparable selectivity of spiders to Hymenoptera and Coleoptera as in this study.

Generally, the garden spider *A. diadematus* consumed more prey items than the wasp-like spider *A. bruennichi* on field margins. This difference in the prey quantities between both orb-web spider species may reflect a different quantity of available prey on the observation dates of *A. diadematus* and *A. bruennichi*. However, Olive (1980) described a higher encounter rate of prey in microhabitats of *Araneus* webs than in microhabitats of *Argiope* webs. Beside different microhabitats, orb webs of various spider species may differ in their visibility to prey (Nyffeler & Breene 1991, Zschokke 2002). So the visibility of webs could also bias the selectivity of webs to potential prey. However, no consistent difference was found in the selectivity of webs to various prey groups between *A. diadematus* and *A. bruennichi* in this study.

On the other hand, Apidae may dominate the actual prey spectrum of *A. bruennichi*, if available, whereas *A. diadematus* avoided Apidae as prey. This may be explained with a good ability of the genus Argiope to cope with armed prey due to morphologic features e.g. long legs and behavioural characteristics e.g. massive silk wrapping of prey (Eisner & Dean 1976, Olive 1980). Also Nyffeler and Breene (1991) found more Apidae as prey in webs of *A. bruennichi* than in *A. diadematus* webs on abandoned grassland.

Taking into account both web and spider characteristics, generally Sternorrhyncha and Apidae were preferred, Hymenoptera without Apidae were avoided, and an inconsistent selectivity was shown towards Heteroptera and Diptera by both spider species. Coleoptera were avoided by *A. diadematus*, but no consistent selection was found in *A. bruennichi*. However, these results may not only be dependent on the selectivity of webs and spiders as described above, but also on the selectivity of sampling methods for potential prey. Despite malaise traps are described to record flying insects without systematic error (Juillet 1963), species with good visual and flight abilities may avoid malaise traps in a certain extent (Kentner & Schrade 1991). Additionally, large-sized and heavy prey items were probably not caught by sticky traps (Mühlenberg 1993). So e.g. Apidae were probably underrepresented in malaise and sticky traps, whereas in spider webs, Apidae were frequently caught, possibly due to a non-visibility of webs to bees between vegetation (Nyffeler & Breene 1991).

Furthermore, possible differences in the spatial distribution of potential prey in different field margin habitats may have biased the catching success of sampling methods on both field margin types differently. Thus, flower-visiting insects on the flower-rich margin may have been restricted to patches of flowering plants and were not caught by the adjacent malaise trap, but in spider webs inside the patches. This may be an explanation for the positive selectivity of webs of *A. diadematus* for Heteroptera on the flower-rich margin. On the other hand, webs probably selected Heteroptera negatively on the flower-poor margin as the catching success of malaise and sticky traps could have been there higher than on the flower-rich margin due to a possibly more regular spatial distribution of potential prey.

4.2. Exposure of orb-web spiders to *Bt*-contaminated prey

Pollen-collecting Apidae may dominate the prey spectrum of orb-web spiders in adequate habitats. The wasp-like spider *A. bruennichi* e.g. consumed a mean number of one bee in seven hours on a flower-rich field margin. Pollinators such as the honey bee *Apis mellifera* may collect pollen of potential *Bt* plants i.e. maize in considerable amounts during anthesis (Odoux et al. 2004) and individual honey bees may transport huge pollen loads (10 mg, Vaissière & Vinson 1994), which could then be offered to orb-web spiders by predation. Orb-web spiders may use pollen adhering on pollinator prey deliberately (Gregory 1989) or accidentally via a specific feeding behaviour: In contrast to hunting spiders, which suck their prey and leave the exoskeleton of the prey items intact, generally orb-web spiders scrunch their prey inclusive adhering pollen by means of chelicera teeth to a mash, from which all digestible integrants including pollen nutrients were imbibed after extra intestinal digestion (Homann 1985). So spiders may ingest *Bt* toxins via pollen, adhering on pollinators (Ludy 2004).

Furthermore, many field inhabiting herbivores or pollen feeders which contribute to the prey of orb-web spiders, such as Heteroptera and Coleoptera, ingest *Bt* proteins of *Bt* plants and pass the protein to spider predators (Harwood et al. 2005, Obrist et al. 2005). However, there is no information on an direct uptake of *Bt* proteins e.g. via *Bt* pollen by Diptera thus far, a prey group, which dominated the prey spectrum of orb-web spiders on field margins. There is evidence, that some Sternorrhyncha, which were also often consumed by orb-web spiders, e.g. aphids of the genus *Rhopalosiphum*, do not ingest the Cry1Ab *Bt* toxin from different *Bt* maize varieties (Head et al. 2001, Raps et al. 2001), which was explained by the absence of Cry1Ab from phloem, the feeding site of aphids.

However, the *Bt* toxin content in plant tissues may differ between *Bt* plant species as well as between varieties (Bernal et al. 2002, Hilbeck & Andow, 2002) and an uptake of *Bt* proteins from other *Bt* plants than *Bt* maize by Sternorrhyncha needs to be investigated.

As *Bt*-contaminated spider prey may be harmed by *Bt* toxins (Diptera: Indrasith et al. 1992, Sternorrhyncha: Ashouri et al. 2001, Heteroptera: Ponsard et al. 2002), spiders could be affected negatively by an uptake of the *Bt* protein itself, but also by an insufficient nutrient quality of *Bt*-affected prey (Strohmeyer et al. 1998, c.f. Romeis et al. 2004). However, there is no evidence for a direct negative impact of *Bt* proteins to spiders from several field studies so far (Hassell & Shepard 2002, Jasinski et al. 2003). Nevertheless, laboratory assays on possible effects of genetically modified organisms to spiders via herbivorous or pollen-collecting prey are still lacking broadly (Lövei & Arpaia 2005).

Thus, for a risk-assessment of *Bt*-contaminated prey to spiders, further investigations on the actual *Bt* toxin content of spider prey under field conditions should be conducted. Furthermore, tritrophic laboratory assays with various contaminated prey types are needed to clarify the passage of *Bt* toxins from prey to orb-web spiders as well as to investigate a potential effect of *Bt* toxins on orb-web spiders.

Conclusions

The prey spectrum of orb-web spiders on field margins was dominated by Diptera. If available, pollen-collecting Apidae may dominate the prey spectrum of the wasp-like spider *A. bruennichi*. Small, broad-winged prey items such as Sternorrhyncha were overrepresented, whereas prey with a good eyesight and manoeuvrability such Diptera and Hymenoptera were underrepresented in spider webs compared to the prey potentially available in the habitat. In general, active prey and prey armed with stings and/or strong mandibles, such as Coleoptera and Hymenoptera were avoided by spider individuals. The preference towards Apidae differed between individuals of *A. diadematus* and *A. bruennichi*: Whereas *A. diadematus* avoided Apidae, *A. bruennichi* showed no selection towards or against this prey type. Taking into account both selectivities of webs and spider individuals, Sternorrhyncha and Apidae were preferred and other Hymenoptera were avoided by orb-web spiders on field margins in this study. This study showed that orb-web spiders on field margins are potentially exposed to *Bt*-contaminated pollen-collecting and herbivorous prey. To assess the exposure of orb-web spiders to *Bt* plants further field assays and tritrophic laboratory studies are necessary to assess an uptake of *Bt* toxins by orb-web spiders via prey.

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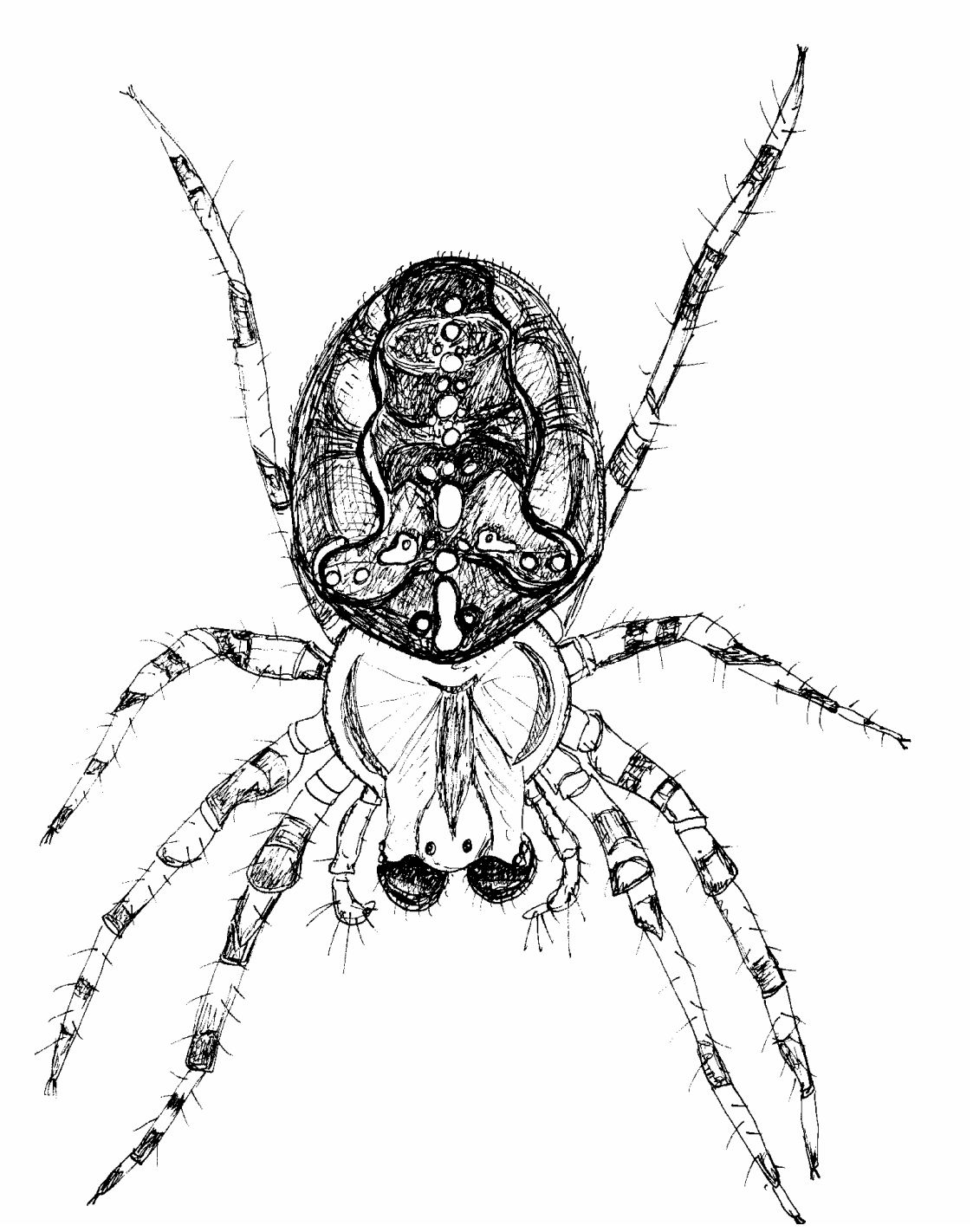
Intentional pollen feeding in the garden spider *Araneus diadematus*



Intentional pollen feeding in the garden spider *Araneus diadematus.*

Claudia Ludy

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Generally, spiders are described as carnivores. On the other hand, there are reports that spiders also ingest plant derived nutrients such as nectar (Taylor & Foster 1996, Jackson et al. 2001) and pollen (Smith & Mommsen 1984). While hunting spiders probably forage pollen on purpose in times of prey absence (Vogelei & Greissl 1989), orb-weaving spiders are believed to consume pollen accidentally by "recycling" their webs (Smith & Mommsen 1984). This unintentional pollen feeding of orb-web spiders was shown by Smith & Mommsen (1984) indirectly by demonstrating that spiders with an supply of pollen survive longer than starved spiders. So far, there is no direct proof of deliberate pollen up-take in orb-weaving spiders. In this study, intentional pollen feeding in juvenile and adult garden spiders (*Araneus diadematus*) was directly proven by visual observation, and verified with a molecular biological method.

Pollen consumption of an adult garden spider was observed in the field. The spider was kept in the laboratory under standardised conditions (temperature 20 °C, 10 h/10 h light/dark regime) for several weeks. It built its orb web in a wooden frame (30 x 30 cm), and was exposed at a height of 80 cm for seven hours on a field margin covered with flowering plants. During field exposure, a pollen-carrying wild bee was caught in the spider web. The spider wrapped up the bee with silk, but the bee could escape eventually, leaving behind the spider's silk wrapping including a mass of pollen. Later on, the spider took the silk wrapped pollen to the hub, and after a few minutes, fluid appeared on the cluster, and the pollen mass changed colour (Fig. 1).

Figure 1: Pollen eating adult female of *Araneus diadematus* (ventral view) in the field. Fluid on the pollen mass and a change of colour of the pollen (white arrow) indicated extraintestinal digestion.

Pollen feeding in a juvenile *A. diadematus* (approximately 8 weeks old with a length of about 3 mm) was visual observed in the laboratory. The spiders was kept in a wooden frame (10 x 10 cm), where it built its orb web. The spider was fed with fruit flies (*Drosophila* spec.) and was kept under the conditions described above for several weeks. Maize pollen (*Zea mays*) were applied onto the orb-web with a small brush. The spider immediately reacted to the objects in the web by pulling the radial threats. Subsequently it moved towards a pollen package, touched the pollen package with the pedipalps, and then it transported the pollen to the hub. In the following, the pollen package was held between the chelicera, and became darker in colour and coated with liquid (Fig. 2).

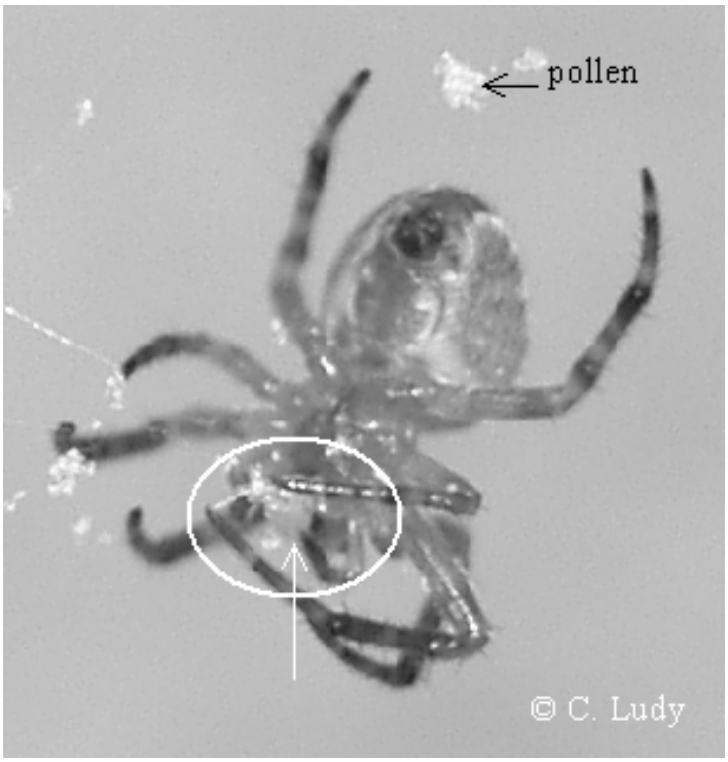

Figure 2: Maize pollen eating juvenile *Araneus diadematus* (ventral view) in the laboratory holding and consuming a pollen mass (white circle and arrow). As compared to other pollen in the spider web (black arrow), fluid on the pollen package and a change of colour of the pollen indicates extraintestinal digestion.

The following laboratory experiment was carried out to prove pollen consumption directly: Juvenile garden spiders (*A. diadematus*) were kept under the above laboratory conditions, and were fed fruit flies. The webs of 11 spiders were dusted with pollen of conventional maize (control), and to another 20 spider webs pollen of genetically modified *Bt* maize was applied (variety "Navares", event Bt176). The Bt-maize produces a protein of the entomopathogenic bacterium *Bacillus thuringiensis* (Cry1A(b) protein), which can be detected by an enzyme linked immuno sorbent assay (ELISA). After the spiders had recycled their webs, the spiders were collected and frozen at –18 ° to prevent a possible degradation of the Cry1A(b) protein and stored for six months. Subsequently, the spiders were defrosted at 5 ºC, and washed with water in order to remove any pollen possibly adhering to the spider (additionally, spiders were controlled for pollen under a binocular). Then, the gastrointestinal system of the prosoma was dissected and picked in cyclohexylaminopropane sulfonic acid buffer (CAPS buffer: 50 mM, pH 10.5). The dissected tissue was analysed for Cry1A(b) content with a commercial ELISA kit (EnviroLogix QuantiPlate™ kit for Cry1Ab/Cry1Ac from Adgen®). In 13 of the 20 spiders (65%), whose webs were dusted with Bt-pollen, but in none of the 11 spiders of the control group, the Cry1A(b) toxin was detected. The behavioural observations showed that garden

spiders can ingest pollen directly in both juvenile and adult developmental stages. Possibly, spiders recognize pollen as food by touching the pollen with taste receptors on the pedipalps. Fluid on the pollen package and concomitant colour change of pollen was likely due to the application of digestive enzymes from the spiders' midgut indicating extraintestinal digestion. Further, the results of the ELISA with spiders, whose webs were dusted with Bt-maize pollen, prove an uptake of pollen by the spider. The detection of the Cry1A(b) protein in the spiders' gastrointestinal system proved to be an effective method to verify pollen consumption. Thus, feeding Bt-maize pollen and a subsequent analysis by ELISA is a possible method to detect pollen feeding both in various spider groups and other animals.

To my knowledge, this is the first published direct evidence that orb-web spiders consume pollen, and the results suggest that both juvenile and adult spiders do so on purpose. On the other hand, spiders sometimes refused offered pollen by dislodging the pollen actively out of their webs (pers. obs.). This indicates that spiders sometimes regard pollen as a useless web load, perhaps affecting capture efficiency of the web. Spiders consuming pollen in this study were only fed with *Drosophila* flies which are of poor nutritional value for spiders (Bilde & Toft 2000), and perhaps therefore some spiders took the chance to utilise the extra protein portion of the pollen. As orb-webs can contain immense amounts of pollen in the field (Fig. 3), pollen feeding may have a substantial significance for juvenile spiders.

Figure 3: Web of an orb-weaving spider in a maize field during pollen shedding of maize containing thousands of pollen grains.

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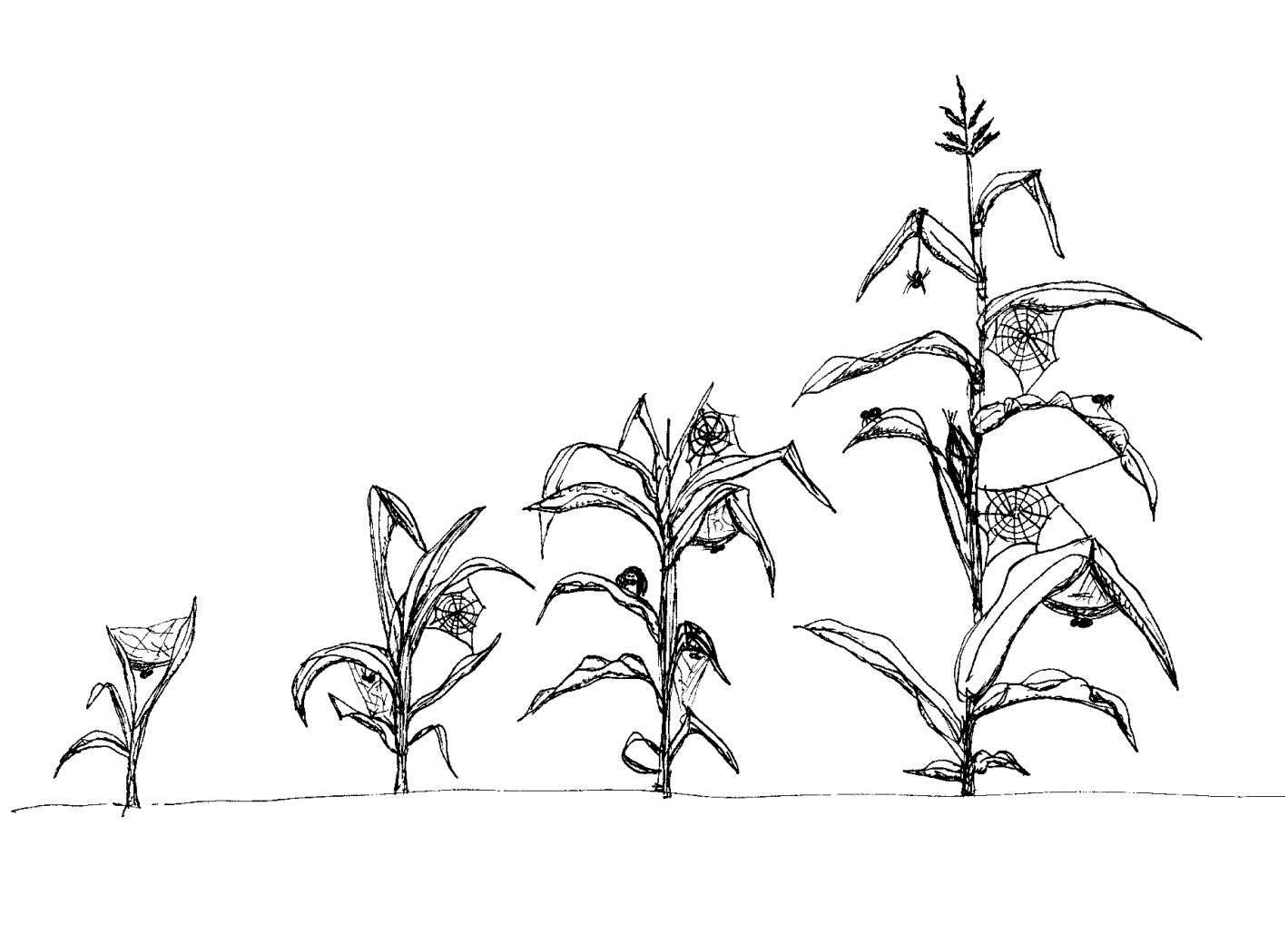
A 3-year field-scale monitoring of foliage-dwelling spiders in transgenic *Bt* maize fields and adjacent field margins



A 3-year field-scale monitoring of foliage-dwelling spiders in transgenic *Bt* maize fields and adjacent field margins.

Claudia Ludy & Andreas Lang

In revision, *Biological Control*

Abstract

Concerns have been raised that genetically modified *Bt* maize may harm non-target organisms, and there is a general call and need for a risk assessment of *Bt* maize. Spiders are important pest predators in agroecosystems and in maize, and can be exposed to the *Bt* toxin by herbivorous or pollen-collecting prey, by active *Bt* maize pollen feeding, and by ingesting their pollen-dusted webs. The foliage-dwelling spider fauna of *Bt* maize fields and adjacent margins was monitored and compared to non-transgenic maize fields. The study took place during the vegetation seasons of 2001 – 2003 in Bavaria, South Germany. Maize fields and adjacent nettle field margins were colonized by a typical spider assemblage, dominated by space-web spiders (Theridiidae and Linyphiidae). Abundance and species richness of spiders was higher in nettle margins than in maize fields. The proportion of hunting spiders tended to be higher in nettle margins, whereas space-web spiders tended to be more frequent in maize fields. *Bt* maize showed no consistent effect on individual numbers, species richness and guild structure of spiders in maize fields and adjacent nettle field margin strips. The spider abundance was higher in *Bt* treatments in 2003, whereas in 2001 and 2002 no significant differences were found. The results provide an important contribution for the implementation of case-specific and general surveillance of transgenic plants to be employed due to the regulations of the European Community**.**

Key words: *Bacillus thuringiensis*, *Zea mays*, Cry1Ab protein, genetically modified organisms, non-target effects, arthropod predators

1 Introduction

Genetically modified *Bt* maize commercially available in Europe expresses the activated and truncated protein Cry1Ab of the insect pathogen *Bacillus thuringiensis*. Cry1Ab is toxic for Lepidoptera and thus *Bt* maize is described as being protected specifically and effectively against lepidopteran pests such as the European corn borer *Ostrinia nubilalis* (Lepidoptera, Crambidae) (Gill et al. 1992, Burkness et al. 2001). Due to its specificity, the *Bt* toxin has been considered as relatively safe for non-target organisms (Glare & O'Callaghan 2000), but some adverse effects on non-targets have been reported (e.g. Hilbeck et al. 1998, Losey et al. 1999, Felke et al. 2002). As the area being cropped with *Bt* maize is increasing rapidly worldwide (James 2003), there is a general need and call for an assessment of possible environmental effects on non-target organisms associated with the commercial cultivation of transgenic crops in the field (e.g. European Parliament and Council 2001, Zügh art & Breckling 2003). Relevant indicator species to be evaluated should be selected based on the exposure of species to the transgenic product, the degree of the adverse effect of the transgenic product, the economic importance of species, the ecological and functional role of the species, and on biomass or abundance of species in the field (Jepson et al. 1994, Dutton et al. 2003, Andow & Hilbeck 2004).

In Europe, spiders are prominent invertebrate predators in agroecosystems showing high population densities and species richness in arable land in general (Samu & Szinetár 2002), and belong to the most abundant arthropod predators in maize fields in particular (e.g. Katz 1993, Lang et al. 1999, Albajes et al. 2003). Spiders are a very diverse group with different lifestyles, and feed on a wide variety of prey including most pest species (Marc et al. 1999, Nyffeler 1999). Hence, they play a vital role in agroecosystems as predators, and so are of economic value due to their pest control function in various crops including maize (e.g. Marc & Canard 1997, Lang et al. 1999). Further, spiders are among the first predators arriving in newly established crop habitats and thus provide an early season protection against pests (Bishop & Riechert 1990). Spiders are potentially exposed to the Cry1Ab toxin of *Bt* maize in various ways: Spiders may actively forage for the maize pollen (Vogelei & Greissl 1989, Ludy 2004). Spiders may consume maize pollen indirectly when recycling their pollen-dusted webs (Smith & Mommsen 1984). Spiders may ingest maize pollen when feeding on prey which has collected or consumed pollen, or is dusted with it (Gregory 1989). Spiders prey on large quantities of herbivores (Nyffeler 1999), and herbivores take up *Bt* toxin when feeding on *Bt* maize tissue and can pass it on to their predators (Dutton et al. 2002). Not only

spiders within the maize field are potentially influenced via these pathways, but also populations occurring in field margins along the maize fields may be affected, in particular by wind drifted pollen, and by herbivorous and pollen collecting prey. Despite their ecological significance and potential exposure to Cry1Ab toxin of *Bt* maize, studies considering the effect of *Bt* maize on spiders are limited in number and scope (Hassell & Shepard 2002, Jasinski et al. 2003, Volkmar & Freier 2003, Candolfi et al. 2004, Meissle & Lang 2005, Poza et al. 2005).

So far, studies published in peer-reviewed journals and evaluating the effect of *Bt* maize on arthropod non-target communities in the field were subject to the following restrictions: spiders were not included (60 % of 15 papers) or not considered on species level (further 20 %), spider populations of adjacent field margins were not studied (100 %), plot size was smaller (< 0.5 ha) than common commercial field size (60 %), the study lasted only one (47 %) or two (further 33 %) seasons, sampling dates per study season were less than three (13 %), and study site was outside Europe (40 %) (Orr & Landis 1997, Pilcher et al. 1997, Wold et al. 2001, Bourguet et al. 2002, Hassell & Shepard 2002, Musser & Shelton 2003, Jasinski et al. 2003, Pons & Starý 2003, Volkmar & Freier 2003, Candolfi et al. 2004, Lumbieres et al. 2004, Tóth et al. 2004, Meissle & Lang 2005, Pons et al. 2005, Poza et al. 2005).

The objective of this study was to provide baseline data on foliage-dwelling spiders assemblages of maize fields and adjacent margins, and to assess the potential effects of *Bt* maize on abundance, species richness and guild structure.

2 Material and methods

2.1 Study sites

The study was carried out on three research farms located in Swabia, Frankonia and Upper Bavaria (South Germany) during the seasons 2001 to 2003. At each experimental site, a pair of *Bt* maize and conventional maize field was established each two hectares large the fields being apart between 500 and 1000 m. For the *Bt* maize the event 176 “Navares” was cropped, and for control the near-isogenic variety “Antares” (both from Syngenta).

On average, ten maize plants covered one square meter (distance between maize rows was 75 cm, and 15 cm between single maize plants within a row). Herbicides but no insecticides were applied once or twice in May. Field margin strips (50 x 7 m) were established on the northern edge of each maize field. Each margin included a plot of stinging nettles (*Urtica dioica* L.) which was used for the survey of spiders in field margins. Stinging nettles were chosen, because these plants are abundant in agricultural landscapes and grow often along field margins. The nettle plot measured 10 x 6 m in 2001, and 18 x 7 m in both 2002 and 2003. In 2001, 40 nettle shrubs were planted in each plot (about 0.6 shrubs per m^2), and in 2002/03 400 shrubs per plot (about 3 shrubs per m^2). In 2001 and 2002, nettle shrubs were obtained from local field populations nearby, and were then planted in the concerning plots of each nettle field margin strip. In 2003, nettles were first sown and reared in the glasshouse and then planted in the margin strips. Nettles were regularly supplied with water and fertilized with nitrogen to secure proper growth.

2.2 Sampling dates and sampling methods

Foliage-dwelling spiders were recorded with a suction sampler which was a modified small vacuum cleaner with a suction hole opening area of 3,0 x 0,6 cm (Ludy & Lang, 2004). Both maize and nettle plants were sampled from the top to the bottom by holding and moving the suction sampler directly on the plant. In 2001, the mean suction time was 35 sec per plant (both maize plant and nettle), and in 2002/03 suction time was increased to 2 min per plant.

In maize fields, 10 maize plants were selected randomly per field and sampling occasion in 2001, and spiders on these plants were collected. This resulted in an overall number of 150 sampled maize plants per *Bt* maize field or conventional maize field, respectively (3 sites x 10 plants per field x 5 sampling dates). The sampled maize plants had at least a distance of 20 m to the field edge. In 2002 and 2003, each maize field was divided into 10 subplots each consisting of 50 maize plants (at least 20 m distance to field edge). On each sampling occasion, one maize plant per subplot was chosen randomly resulting in an overall number of 150 (2002) or 120 (2003) sampled maize plants per *Bt* maize field or conventional field, respectively (3 sites x 10 subplots x 1 maize plant per plot x 4-5 sampling dates).

In nettle field margins, 10 nettle shrubs were selected randomly per margin strip and sampling occasion in 2001, and spiders on these plants were sampled. This resulted in an

overall number of 150 sampled nettles per margin neighboring *Bt* maize fields or conventional maize fields, respectively (3 sites x 10 nettles per margin x 5 sampling dates). In 2002 and 2003, each nettle plot was divided into eight subplots (each 4.5 x 3.5 m). On each sampling occasion, one nettle shrub per subplot was chosen randomly resulting in an overall number of 120 (2002) or 96 (2003) sampled nettles per margin neighboring *Bt* maize fields or conventional fields, respectively (3 sites x 8 subplots x 1 nettle shrub per subplot x 4-5 sampling dates). For further statistical analysis average values were calculated per maize field and nettle field margin, respectively.

2.3 Identification of spiders

Sampled spiders were fixed in 70% ethanol, brought to the laboratory and identified according to Heimer & Nentwig (1991) and Roberts (1985, 1987, 1995). Species were classified according to Platnick (2005). Juvenile spiders were identified to genus or family level, if possible. Additionally, the recorded spiders were divided in three main guilds (after Nyffeler 1982): space-web spiders (Dictynidae, Theridiidae, Linyphiidae), orb-web spiders (Araneidae and Tetragnathidae), and hunting spiders (Lycosidae, Pisauridae, Miturgidae, Anyphaenidae, Clubionidae, Philodromidae, Thomisidae, Salticidae).

2.4 Statistical analyses

A repeated measures analysis of variance (ANOVA) was used to analyze the effect of the factor "*Bt*-status" (i.e. "*Bt* maize" and "conventional maize") on the dependent variable "number of individuals per plant" (means per sampling date), including the factor "sampling date" (4 sampling dates, analysis 1). The first sampling date in 2001 and 2002 was not included in the repeated measures ANOVA, because this analysis required an identical number of sampling dates in all years. For analyzing species richness, an analysis of covariance (ANCOVA) was conducted with the dependent variable "total number of species per plot" (seasonal sums; analysis 2) and the covariate "number of individuals per plot". The covariate was included in order to correct a potential effect of spider abundance on species richness. A multivariate analysis of variance (MANOVA) was conducted to analyze a possible effect of "*Bt*-status" on the composition of spider guilds (proportions of 3 different spider guilds per plot; seasonal sums of guilds; analysis 3).

Subsequently, ANOVA was conducted to specify effects on single spider guilds. Additionally, the factors "year" (2001-2003) and "habitat type" ("maize field" and "field margin") were included in all analyses to detect potential interactions with the main factor "*Bt*-status".

To test the homogeneity of variances, Sen & Puris nonparametric test was conducted. Kolmogorov-Smirnov one-sample test was used for testing the normal distribution of data. Spider abundance and species number of analyses 1 and 2 were log x+1 transformed and guild proportions of analysis 3 were arcsin-transformed to create normal distribution and/or homogeneity of variance of the data set. Post hoc comparisons were conducted with the least significance differences (LSD) test.

Standardised effect sizes, Cohen's d (Cohen 1988), were calculated together with the corresponding 95% confidence intervals of d for pair wise comparisons of dependent variables. Effect size d is a dimensionless measurement of the magnitude of an effect recorded and allows the comparison of effects among different results and studies, hence facilitating meta-analysis (Colegrave & Ruxton 2003, Nakagawa & Forster 2004). A SPSS script written by Smithson (2001) was used to calculate d and non-central confidence intervals on base of the observed value of a t-statistic of the concerned treatment comparison. The SPSS script can also be downloaded from the internet at http://www.anu.edu.au/psychology/people/smithson/details/CIstuff/CI.html (October 19, 2005). All other statistical analyses were carried out using STATISTICA for Windows, version 5.0. All average values presented are arithmetic means ± 1SD and all tests are two-sided.

3 Results

3.1 The spider community of maize fields and adjacent field margin strips

Overall, 50 foliage-dwelling spider species and 1811 individuals were recorded in three years in both maize fields and nettle field margins (Appendix: Tab. 1). Generally, space-web spiders (Linyphiidae and Theridiidae) dominated the spider community in both habitat types (Fig. 1). The most abundant species in both habitat types were *Theridion impressum* (Theridiidae, space-web spiders), *Meioneta rurestris*, and *Oedothorax apicatus* (both Linyphiidae, space-web spiders).

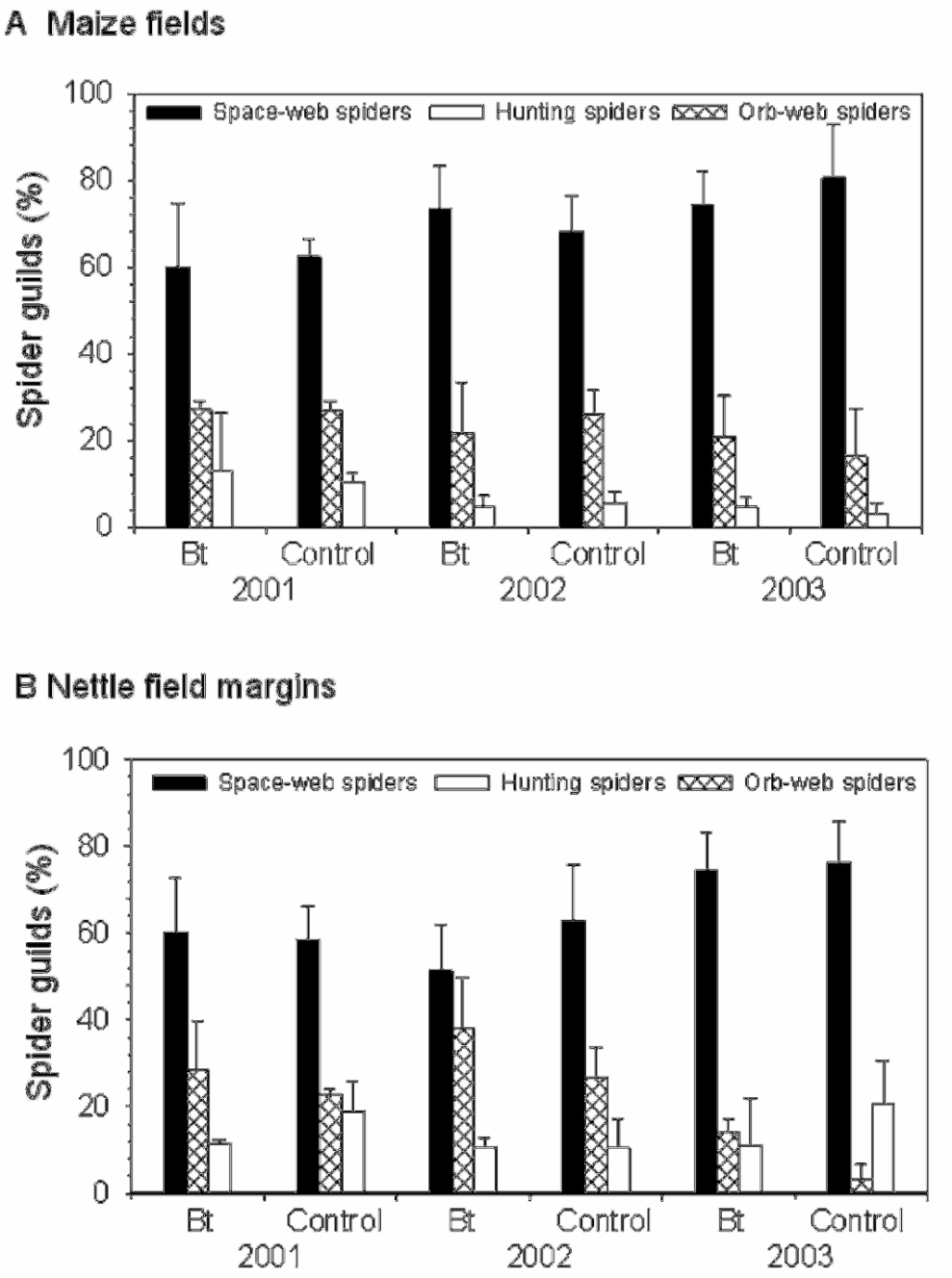


Figure 1: Proportion of spider guilds in *Bt* and control maize fields (a) and adjacent nettle field margins (b) of three years (seasonal sums + SD), n = 3 each column.

In maize fields, a total of 33 spider species and 868 individuals were found (Appendix: Tab. 1). The average spider density pooled over three years was 1.05 ± 0.65 spiders per maize plant. *Erigone atra* was often recorded in maize fields. In nettle margin strips, 44 species and 943 individuals were found (Appendix: Tab. 1). The average spider density over three years was 1.39 ± 0.93 spiders per stinging nettle shrub. *Tenuiphantes tenuis* (Linyphiidae, space-web spiders), *Aculepeira ceropegia* (Araneidae, orb-web spiders), *Pachygnatha degeeri* (Tetragnathidae, orb-web spiders) and *Misumenops tricuspitatus* (Thomisidae, hunting spiders) were often recorded in nettle margins.

The spider abundance and the species richness were higher in margins than in maize fields (abundance: Tab. 1a, factor "habitat type", Fig. 2; species richness: Tab. 1b, factor "habitat type", Fig. 3). The covariate "number of individuals" had a positive effect on the spider species richness ($F_{1,23} = 5.45$, $p = 0.03$).

The relative abundance of the different spider guilds changed from year to year and habitat type tended to affect guild proportions (Tab. 1c, factors "year" and "habitat type", Fig. 1). In 2001 and 2002, the proportion of space web spiders was lower (ANOVA: factor "Year", $F_{2,24} = 8.89$, $p < 0.01$; LSD test: $p < 0.05$ each comparison) and the proportion of orb web spiders was higher than in 2003 (ANOVA: factor "year", $F_{2,24} = 11.98$, $p < 0.001$; LSD test: $p < 0.05$ each comparison). The proportion of hunting spiders did not change over the years ($p > 0.05$) (ANOVA: factor "year", $p > 0.05$, Fig. 1). The proportions of space-web spiders tended to be higher in maize fields than in nettle field margins, whereas orb-web spiders seemed to be more frequent in maize fields in 2003 (Fig. 1).

The numbers of spider individuals changed over the season in both habitat types during all years (Tab. 1a, factor "sampling date", Fig. 4). In general, spider populations increased by the end of July or the beginning of August, and declined by the end of the season. Spider densities were also different between years, with the lowest densities in 2001 and the highest in 2002 (Tab. 1a, factor "year", Fig. 2).

3.2 Effect of *Bt* maize on foliage-dwelling spiders

A total of 24 spider species and 478 individuals were recorded in *Bt* maize fields, and in conventional maize fields 26 species and 390 individuals (Appendix: Tab. 1). The overall frequency of the different spider guilds were 69 ± 12% space-web spiders, 23 ± 8% orb-web spiders, and 8 ± 8% hunting spiders in *Bt* maize fields. The corresponding values of

Table 1. Repeated measures ANOVA on the number of spider individuals (a), ANCOVA on the number of spider species (b) and MANOVA on proportion of guilds (c) for the effect of year, habitat type and *Bt*-status.

Source of variation	**df**	**MS**	**F**	**p**
(a) Number of spider individuals				
Year	2	1.20	65.42	< 0.01
Habitat type	1	0.10	5.29	0.03
Bt-status	1	0.01	0.29	0.59
Year x habitat type	2	0.03	1.82	0.18
Year x *Bt*-status	2	0.06	3.26	0.05
Habitat type x *Bt*-status	1	0.02	1.35	0.26
Year x habitat type x *Bt*-status	2	0.02	0.82	0.45
Error	24	0.02		
Sampling date	3	0.10	10.44	< 0.01
Sampling date x year	6	0.03	3.11	0.01
Sampling date x habitat type	3	0.01	1.01	0.39
Sampling date x *Bt*-status	3	0.00	0.35	0.79
Sampling date x year x habitat type	6	0.02	1.79	0.11
Sampling date x year x *Bt*-status	6	0.01	0.63	0.70
Sampling date x habitat type x *Bt*-status	3	0.01	0.77	0.51
Sampling date x year x habitat type x *Bt*-status	6	0.01	1.10	0.37
Error	72	0.03		
(b) Number of spider species				
Year	2	0.03	4.69	0.02
Habitat type	1	0.16	24.15	< 0.01
Bt-status	1	0.01	1.43	0.24
Year x habitat type	2	0.02	2.96	0.07
Year x *Bt*-status	2	0.01	1.33	0.28
Habitat type x *Bt*-status	1	0.00	0.32	0.58
Year x habitat type x *Bt*-status	2	0.01	0.86	0.44
Error	23	0.01		
(c) Proportion of spider guilds	**df**	**Wilk's Lambda**	**F (Rao´s R)**	**p**
Year	6,44	0.39	4.36	< 0.01
Habitat type	3,22	0.71	2.92	0.06
Bt-status	3,22	0.85	1.31	0.29
Year x habitat type	6,44	0.63	1.91	0.09
Year x *Bt*-status	6,44	0.79	0.90	0.50
Habitat type x *Bt*-status	3,22	0.80	1.84	0.17
Year x habitat type x *Bt*-status	6,44	0.88	0.49	0.81

conventional maize fields were 71 ± 11% space-web spiders, 23 ± 8% orb-web spiders, and 6 ± 4% hunting spiders (Fig. 1).

A total of 36 spider species and 427 individuals were caught in nettle margin strips neighboring *Bt* maize fields, while in nettle strips neighbouring conventional maize fields 35 spider species and 516 individuals were recorded (see Appendix: Tab. 1). In nettle margins neighbouring *Bt* fields, the overall frequency of the different spider guilds were 62 ± 14% space-web spiders, 27 ± 13% orb-web spiders, and 11 ± 6% hunting spiders. The corresponding values of nettle margins along conventional maize fields were 66 ± 12% space-web spiders, 17 ± 12% orb-web spiders, and 17 ± 12% hunting spiders (Fig. 1). The proportion of guilds never differed between *Bt* maize fields and conventional maize fields or between corresponding neighbouring nettle margin strips, respectively (Tab. 1c, factor "*Bt*-status", Fig. 1).

There was a trend that the effect of *Bt* maize on spider densities was different in the successive years (Tab. 1a, interaction "year x *Bt*-status"). In 2003, spider numbers were higher in *Bt* maize fields than in conventional maize fields, whereas this difference was not recorded between *Bt* nettle margins and non-*Bt* nettle margins (LSD-test, Tab. 2a, Fig. 1). Sampling date had no significant interaction with the *Bt* treatment in both habitat types (Tab. 1a, interaction "sampling date x *Bt*-status", Fig. 4).

In all years, ANCOVA revealed no effect of *Bt*-status on species number (Tab. 1b, factor "*Bt*-status", Fig. 3). However, in 2003 species number was higher in *Bt* maize fields as compared to control maize fields (LSD-test, $P < 0.01$, Tab. 2b), but which was related to the increased abundance of spiders in *Bt* fields.

Observed standardised effect sizes (Cohen`s d) and the corresponding 95% confidence intervals for pair wise comparisons (LSD test) of each dependent variable are shown in Tab. 2. The majority of the observed differences were rather small indicating that possible effects may be of minor importance. Medium to large effect sizes (Cohen´s $d \geq 0.50$, *sensu* Cohen, 1988) were recorded for *Bt* maize fields for a decrease in abundance in 2001, and in 2003 for an increase of both abundance and species richness. In nettle margins along *Bt* maize fields, effect sizes were medium to large for an decrease in species richness in 2001, increased proportions of orb-web spiders (2002, 2003) and decreased proportions of hunters (2003). The proportions of space-web spiders showed the strongest effect for decreases in *Bt* nettle field margins in 2002 and in *Bt* maize fields in 2003 (Tab. 2).

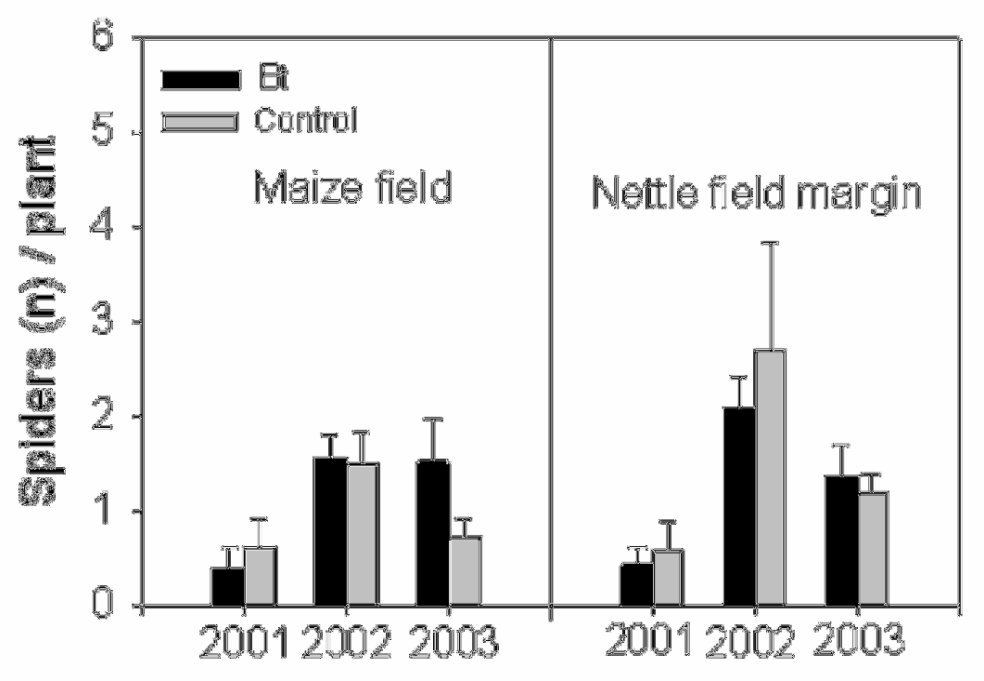


Figure 2: Number of spider individuals per plant recorded in *Bt* and control maize fields and adjacent nettle field margins of three years (seasonal means + SD), n = 3 each column.

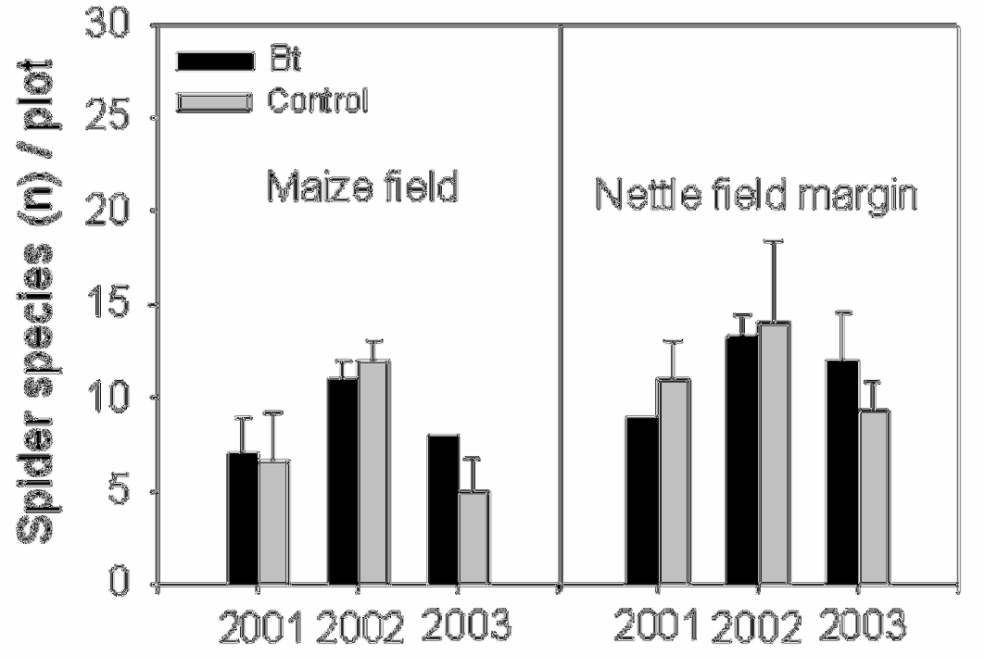


Figure 3: Number of spider species per plot recorded in *Bt* and control maize fields and adjacent nettle field margins of three years (seasonal means + SD), n = 3 each column.

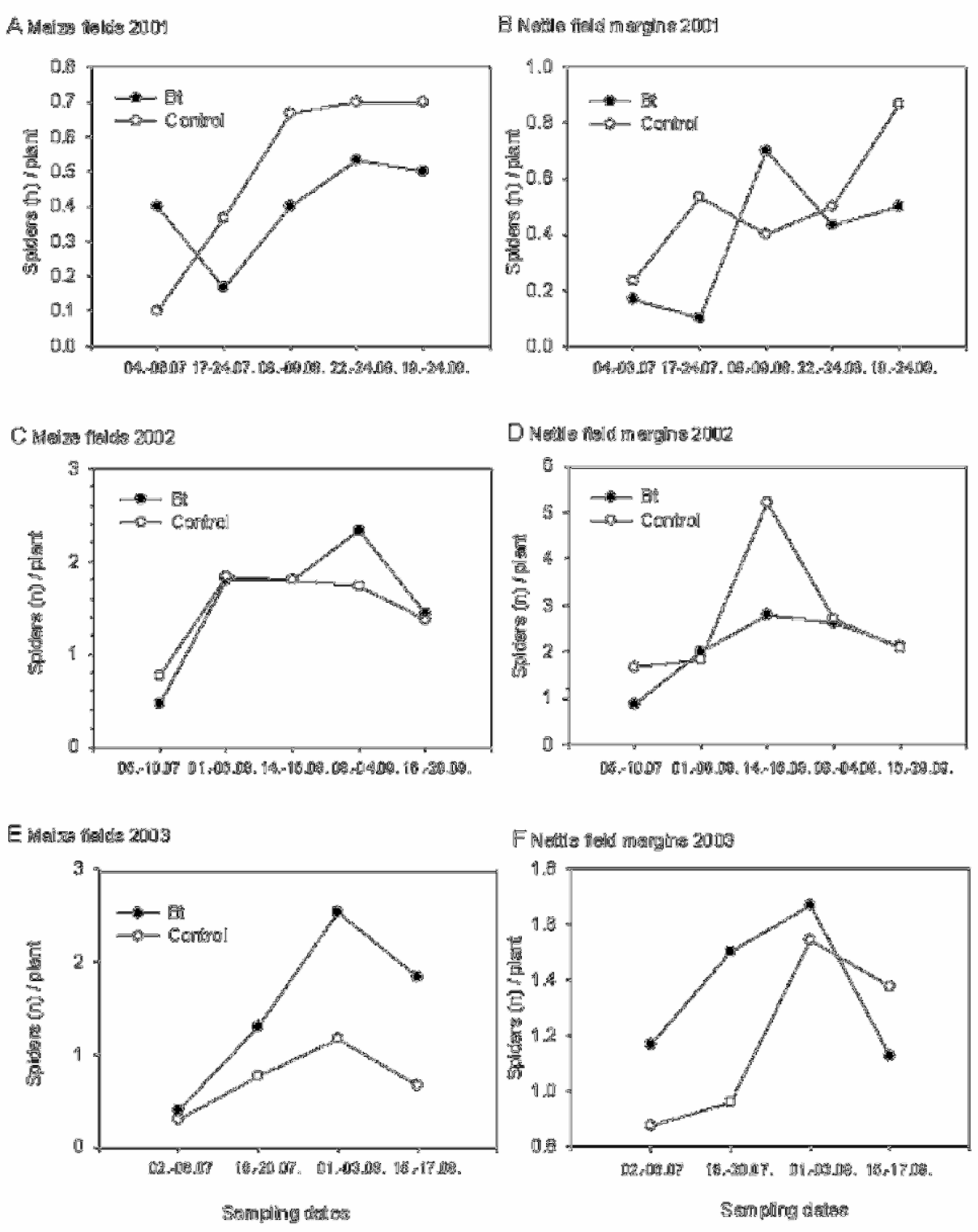


Figure 4: Seasonal dynamics of spider individuals in *Bt*- and control maize fields (A,C,E) and in adjacent nettle field margins (B,D,F) of three years (means per sampling date), n = 3 each point. Note different ranges of y-axes.

Table 2. Significances (p), observed (d obs.) and standardised effect sizes (Cohen`s d) with the corresponding 95% confidence intervals for Cohen's d (95% CI) for pair wise comparisons (LSD test) of the dependent variables "number of spider individuals" (a), "number of spider species" (b) and "proportion of spider guilds" (c) in different habitat types and during the years 2001-2003.

Dependent variable			**p**	**d obs.**	**Cohen`s d**	**95% CI**
(a) Number of spider individuals						
2001						
Conv-Bt		maize field	0.29	0.21	0.50	-0.40 – 1.34
Conv-Bt		nettle field margin	0.50	0.14	0.30	-0.54 – 1.11
2002						
Conv-Bt		maize field	0.64	-0.07	0.20	-0.62 – 1.00
Conv-Bt		nettle field margin	0.54	0.62	0.27	-0.56 – 1.08
2003						
Conv-Bt		maize field	0.01	-0.79	1.76	0.32 – 3.15
Conv-Bt		nettle field margin	0.59	-0.18	0.24	-0.59 – 1.04
(b) Number of spider species						
2001						
Conv-Bt		maize field	0.69	-0.33	0.17	-0.64 – 0.97
Conv-Bt		nettle field margin	0.26	2.00	0.53	-0.37 – 1.38
2002						
Conv-Bt		maize field	0.59	1.00	0.23	-0.60 – 1.03
Conv-Bt		nettle field margin	0.92	0.67	0.04	-0.76 – 0.84
2003						
Conv-Bt		maize field	< 0.01	-3.00	1.94	0.40 – 3.44
Conv-Bt		nettle field margin	0.15	-2.67	0.71	-0.25 – 1.61
(c) Proportion of spider guilds						
2001						
Conv-Bt	Hunters	maize field	0.76	-2.39	0.13	-0.68 – 0.93
Conv-Bt	Orb-web spiders	maize field	0.98	-0.13	0.07	-0.28 – 0.28
Conv-Bt	Space-web spiders	maize field	0.76	2.52	0.13	-0.68 – 0.93
Conv-Bt	Hunters	nettle field margin	0.35	7.58	0.43	-0.45 – 1.25
Conv-Bt	Orb-web spiders	nettle field margin	0.47	-5.66	0.33	-0.52 – 1.14
Conv-Bt	Space-web spiders	nettle field margin	0.74	-1.92	0.14	-0.67 – 0.94
2002						
Conv-Bt	Hunters	maize field	0.92	0.76	0.04	-0.76 – 0.84
Conv-Bt	Orb-web spiders	maize field	0.60	4.31	0.23	-0.60 – 1.03
Conv-Bt	Space-web spiders	maize field	0.36	-5.07	0.42	-0.45 – 1.25
Conv-Bt	Hunters	nettle field margin	0.98	-0.13	0.01	-0.24 – 0.25
Conv-Bt	Orb-web spiders	nettle field margin	0.15	-11.25	0.73	-0.24 – 1.63
Conv-Bt	Space-web spiders	nettle field margin	0.08	11.38	0.93	-0.12 – 1.90
2003						
Conv-Bt	Hunters	maize field	0.83	-1.72	0.09	-0.72 – 0.89
Conv-Bt	Orb-web spiders	maize field	0.58	-4.48	0.24	-0.59 – 1.05
Conv-Bt	Space-web spiders	maize field	0.17	6.21	0.67	-0.28 – 1.55
Conv-Bt	Hunters	nettle field margin	0.26	9.21	0.53	-0.37 – 1.38
Conv-Bt	Orb-web spiders	nettle field margin	0.19	-10.85	0.64	-0.30 – 1.52
Conv-Bt	Space-web spiders	nettle field margin	0.74	1.64	0.14	-0.67 – 0.94

4. Discussion

4.1 The spider community of maize fields and adjacent margins

Baseline data about spiders in maize field are very scarce and refer mainly to activity densities (and not population densities) of ground-dwelling species (e.g. Alderweireldt 1989, Frank & Nentwig 1995). In all years (2001–2003), the foliage-dwelling spider fauna in maize fields and adjacent nettle field margins strips mainly consisted of space-web spiders (Theridiidae and Linyphiidae). The dominance pattern of spider families and species of this three-year study was quite similar to two one-year studies of maize fields in 2001 (Ludy & Lang 2004, Meissle & Lang 2005). This indicates that a typical and relatively steady spider community exists in maize fields in terms of prevailing species and families, which is not prone to high year-to-year changes. This is further corroborated by other studies also reporting the dominance of species of Theridiidae and Linyphiidae in higher strata in maize fields and on field margins (maize fields: Nyffeler & Benz 1979, Katz 1993; margin strips: Wyss 1996, Denys & Tscharnke 2002). Species number of foliage-dwelling spiders was within the range expected for habitats in arable land (Luczak 1979, Barthel 1997). Generally, more species were found in nettle margins strips, which was mainly due to a higher species number of orb-web spiders and hunting spiders (see also Ludy & Lang 2004). The dominance of space-web spiders in maize fields and artificial sown nettle field margins could be due to the good aerial dispersal ability (ballooning) of these spiders (e.g. Plagens 1986). Both habitat types, maize fields and sown nettle margin strips, were habitats created anew each year. So, most spiders had to immigrate into these habitats each season, and spider families such as Linyphiidae and Theridiidae, which frequently disperse by ballooning, have an advantage in the colonization of newly created habitats compared to ground dispersal spiders (Bishop & Riechert 1990, Frank & Nentwig 1995). Population densities of foliage-dwelling spiders were fairly higher than detected in some other studies (Nyffeler & Benz 1979, Barthel 1997), and peaked in August.

4.2 Effect of *Bt* maize on the spider community

The three-year study showed inconsistent effects of *Bt* maize on population densities of foliage-dwelling spiders. There was no negative impact detected of *Bt* corn on spiders in the field, neither on population densities, species numbers or guild proportions. The only significant result was an increase of spider abundance in *Bt* maize fields in 2003. However, a direct positive effect of the Cry1Ab protein itself on invertebrate predators is unknown, and can probably be ruled out as an explanation. Possibly characteristics of the *Bt* maize associated with the transformation of the *Bt* gene may be responsible for effects on non target organisms. The transformation of maize with new genes can lead to pleiotropic effects i.e. may alter the physiological parameters of the transformed plants in addition to the introduced genetic construct (Saxena & Stotzky 2001). For instance, *Bt* maize is often larger in height and green for longer than the near-isogenic variety, and may also have a differential plant development rate (Hassell & Shepard 2002, Ma & Subedi 2005). Also, *Bt* maize plants are not attacked by lepidopteran pests, and therefore *Bt* maize plants stay undamaged. In consequence, *Bt* maize may harbor more non-target herbivores as spider prey later in the season, which may also lead to a higher spider abundance. Possibly, this plays a role under dry climatic conditions such as during the exceptionally hot summer in 2003, where *Bt* maize plants had less dry leaves than conventional maize plants (Lang, unpublished data). Hence, the higher spider numbers in 2003 may have been mediated by plant characteristics rather than by the *Bt* construct itself.

In 2001 spider abundance was decreased in *Bt* maize (medium effect size, *sensu* Cohen, 1988), but not significantly ($p > 0.05$). The lack of a significant result of *Bt* maize may indicate that there is no effect, or that the effect was masked by interfering factors, or could not be detected due to inadequate methods, design or statistical power (Marvier 2002). The fact that consumption of *Bt* maize pollen seems not to harm garden spiders (*Araneus diadematus*) supports the no-effect interpretation (Ludy & Lang, in press). However, it is acknowledged that with a sample size of three fields and margins resulting statistical power of the tests was relatively small in this study. Several other field studies also found no or no consistent effect of *Bt* maize on invertebrate predators, e.g. on spiders, anthocorid bugs and coccinellid beetles (Orr & Landis 1997, Pilcher et al. 1997, Wold et al. 2001, Bourguet et al. 2002, Hassell & Shepard 2002, Musser & Shelton 2003, Jasinski et al. 2003, Volkmar & Freier 2003, Meissle & Lang 2005, Poza et al. 2005).

Comparably to our study, high variation of these data sets as well as small effect sizes and/or low replication, may have been responsible for missing an existing direct or indirect effect (Bourguet et al. 2002, Perry et al. 2003, Lang 2004). Therefore, future field studies should be conducted on longer temporal and larger spatial scales and in higher replication. In addition, laboratory experiments are needed to clarify direct and indirect field effects of the Cry1Ab protein on spiders.

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Appendix

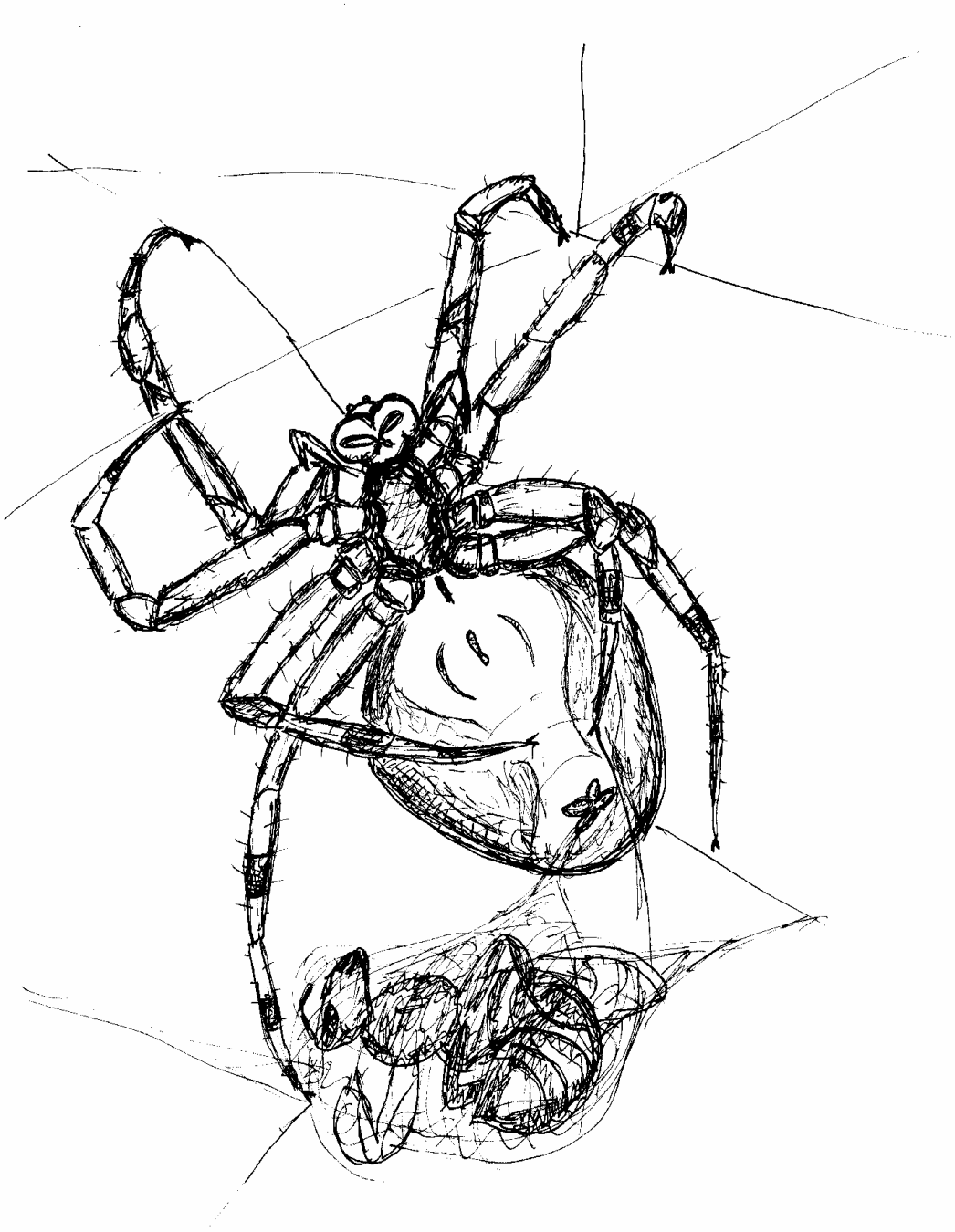

Table 1: Summary of spiders captured in *Bt* maize fields and conventional maize fields as well as in adjacent nettle margin strips (3 years * 4-5 sampling dates * 3 locations * 10 maize plants or 8 - 10 stinging nettle shrubs, respectively).

Habitat types	**Maize**		**Field margin**	
***Bt*-status**	***Bt***	**Conv**	***Bt***	**Conv**
Species				
Araneae				
unidentified	1	1		1
Theridiidae				
Achaearanea spec.	2	2	3	2
Achaearanea riparia (Blackwall, 1834)			1	1
Enoplognatha spec.	1			
Enoplognatha latimana Hippa & Oksala, 1982			1	1
Episinus spec.		1	1	2
Episinus angulatus (Blackwall, 1836)				1
Neottiura bimaculata (Linneus, 1767)		1		
Robertus neglectus (O. P.-Cambridge, 1871)		1		
Theridion spec.	1		2	4
Theridion impressum L. Koch, 1881	2	4	4	74
Juveniles	216	135	74	110
Linyphiidae				
Araeoncus humilis (Blackwall, 1841)	1			1
Bathyphantes gracilis (Blackwall, 1841)			1	
Diplocephalus cristatus (Blackwall, 1833)		1		
Diplostyla concolor (Wider, 1834)			1	
Eperigone trilobata (Emerton, 1882)			1	1
Erigone atra Blackwall, 1833	15	3	3	3
Erigone dentipalpis (Wider, 1834)	5		3	2
Tenuiphantes tenuis (Blackwall, 1852)	3	1	7	4
Linyphia triangularis (Clerck, 1757)		1		
Meioneta spec.		2	2	3
Meioneta fuscipalpa (L.C. Koch, 1836)				1
Meioneta rurestris (L.C. Koch, 1836)	4	5	14	7
Microlinyphia spec.		1	7	3
Microlinyphia pusilla (Sundevall, 1830)		1	3	
Neriene spec.	3	8	4	3
Oedothorax apicatus (Blackwall, 1850)	15	30	18	22
Oedothorax fuscus (Blackwall, 1834)			1	
Porrhomma microphthalmum (O.P.-Cambridge, 1871)			1	1
Porrhomma oblitum (O. P.-Cambridge, 1871)		1		
Juveniles	82	76	102	100
Tetragnathidae				
Pachygnatha spec.	2		5	2
Pachygnatha degeeri Sundevall, 1830	1	2	8	13
Tetragnatha spec.	61	58	78	52
Araneidae				
Aculepeira ceropegia (Walckenaer, 1802)	1	1	9	4
Araniella spec.	8	5	7	3
Araniella curcubitina (Clerck, 1757)				1
Argiope bruennichi (Scopoli, 1772)			2	

Table 1: Summary of spiders captured in *Bt* maize fields and conventional maize fields as v
adjacent nettle margin strips (3 years * 4-5 sampling dates * 3 locations * 10 maize plants o
stinging nettle shrubs, respectively) (continued).

Habitat types	**Maize**		**Field margin**	
***Bt*-status**	***Bt***	**Conv**	***Bt***	**Conv**
Cyclosa oculata (Walckenaer, 1802)	6		3	
Larinioides spec.	3	1	1	10
Larinioides c.f. *cornutus* (Clerck, 1757)				1
Nuctenea spec.		1		
Singa spec.	1		2	3
Juveniles	19	26	20	27
Lycosidae				
Pardosa spec.	5	5	8	10
Pardosa agrestris (Westring, 1861)				1
Pisauridae				
Pisaura mirabilis (Clerck, 1757)			1	6
Dictynidae				
Dictyna spec.				1
Nigma spec.	1			1
Miturgidae				
Cheiracanthium spec.			2	1
Anyphaenidae				
Anyphaena accentuata (Walckenaer, 1802)				1
Liocranidae				
Phrurolithus spec.			1	
Clubionidae				
Clubiona spec.	3	2	2	1
Clubiona terrestris Westring, 1851	1			1
Gnaphosidae				
Micaria spec.				2
Juveniles			2	2
Philodromidae				
Philodromus spec.	1	3	3	3
Tibellus spec.			1	
Tibellus oblongus (Walckenaer, 1802)				1
Thomisidae				
Misumenops tricuspitatus (Fabricius, 1775)	2	1	8	4
Ozyptila spec.				1
Xysticus spec.	11	7	7	15
Juveniles		2	1	1
Salticidae				
Euophrys spec.			1	
Heliophanus spec.				1
Phlegra spec.			1	
Juveniles		1		
Individual number	478	390	427	516
Species number	24	26	36	35

Figure 1: *Bt* maize pollen dusted web of a juvenile orb-web spider (Araneae: Araneidae) inhabiting a *Bt* maize field.

Figure 2: Web of a juvenile long-jawed spider (Araneae: Tetragnathidae) in a *Bt* maize field. The web contains considerable pollen loads.

Figure 3: In appropriate habitats, pollen-collecting bees may be consumed by orb-web spiders frequently. Here, two pollen-loaded wild bees were caught in the web of the garden spider *Araneus diadematus.*

Figure 4: The orb-web spider *Araneus diadematus* feeding on a wild bee (Apidae). Typical for orb-web spiders, the spider has scrunched its prey by means of chelicera teeth to a mash.

Figure 5: Drop cloth and beating stick used for sampling foliage-dwelling spiders.

Figure 6: Hand-held suction sampler used for sampling foliage-dwelling spiders.

Figure 7: Malaise trap for recording flying insects to assess the potential prey spectrum for orb-web spiders.

Figure 8: Sticky trap consisting of plastic plates covered with a clear film spread with non-drying sticky glue (left) for recording the potential prey spectrum beside a orb-web spider and its web built in a wooden frame (right) for recording the actual prey spectrum of the orb-web spider.

Figure 9: Adult female garden spider (*Araneus diadematus*, Araneae: Araneidae).

Figure 10: Adult female wasp-like spider (*Argiope bruennichi*, Araneae: Araneidae).

Figure 11: Flower-poor margin next to a maize field. Spiders and webs in wooden frames were exposed and observed to investigate the actual prey spectrum of orb-web spiders. The potential prey spectrum was assessed e.g. by a malaise trap as shown in the background.

Figure 12: Flower-rich margin dominated by common tansy (*Tanacetum vulgare*) next to a maize field. Spiders and webs in wooden frames were exposed and observed to investigate the actual prey spectrum of orb-web spiders. Beside a malaise trap (in the foreground), sticky traps were exposed to assess to potential prey spectrum (in the background).