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Project identification

1. Defra Project code: PS2361
2. Project title: A review of guttation and implications for pesticide exposure in non-target invertebrates and honeybees
3. Contractor organisation(s): Food and Environment Research Agency
   Sand Hutton
   York
   YO41 1LZ
4. Total Defra project costs (agreed fixed price): £ 15610
5. Project: start date ..... 01 January 2010
   end date 31/03/2010

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Guttation is the appearance of drops of xylem sap on the tips or edges of leaves of some vascular plants, such as grasses, and should not be confused with dew, which condenses from the atmosphere onto the plant surface. A recent paper on "translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees" was published by Girolami et al (2009) and this has focussed significant interest on the possible risks posed to honeybees by such a method of exposure. In response to concern from beekeepers, AFSSA recently published their opinion on the risks to bees by guttation (extra-floral secretions) and a Swiss study published by the Federal Office of Agriculture on the residues of clothianidin present in guttation fluid from treated maize and the risks to bees has also recently been published.
This project builds on those reports and summarise
1) the environmental and phenological situations where guttation may occur in crops under UK conditions and
2) the possible risks posed to bees and other non-target species.
The report also outlines gaps in knowledge which affect our ability to assess the risks posed by guttation under UK field conditions.

Guttation in a wide range of plants has been reported since at least the 1950s and is now considered a general phenomenon. It has been identified as occurring in conditions of high soil moisture and low transpiration; although there may be other factors involved such as previous levels of water stress, growth stage, root depth and soil water potential. However interactions between temperature and soil moisture are likely to vary with plant species and soil type making any predictions unreliable. Ivanoff (1963) also stated that although a number of previous authors have compiled lists of plants observed to guttate.
There are a number of factors affecting the residues of systemic pesticides in guttation fluid including formulation, metabolism within the plant, application methods, adjuvant, solubility/lipophilicity of the active ingredient, plant species.

Only one study (Girolami et al 2009) has shown a significant effect in honeybees but this should be treated with caution as the data were generated by feeding collected droplets directly to bees and in many cases sucrose was added to ensure the honeybees consumed the dose.

Guttation fluid is unlikely to be identified by honeybees as a source of sugar due to the low levels present. Bees are less subject to dessication than most terrestrial insects due to their nectar diet and high metabolic water production. Water is collected by bees to dilute thickened honey, to produce brood food from stored pollen, to maintain humidity within the hive and to maintain temperature within the brood area.

Water is not stored in combs by temperate bee colonies. The amount of water required depends on the outside air temperature and humidity, the strength of the colony and the amount of brood present.

In some circumstances dew has been reported as a source of water for insects, such as locusts, suggesting guttation fluid may be seen as a source of water more generally by arthropods. Tenebrionid beetles are reported to use dew condensate as a source of water and a pyrrhocorid (Scanthus aegyptius) and ant (Monomorium subopacum) have been reported drinking from stones and and two beetles (Carabus impressus and Coccinella septumpunctata) have been reported drinking droplets from leaves. Several butterfly and moth species are reported as drinking even though ample water is available in nectar. This is thought to be due to the need for solutes such as sodium ions.

The primary concern to date has been the consumption of guttation fluid as a source of water. Based on the data available the intake of water by drinking in non-target arthropods may range widely and up to 100% of bodyweight. The only oral toxicity data available for non-target arthropods readily available are those for the honeybee. Therefore the residues identified in guttation fluid, the intake (as a percentage of bodyweight based on a mean 100 mg/ individual) was used to calculate the percentage required to reach the LD50 for a clothianidin, thiamethoxam and imidacloprid. This shows that only a very low volume of the guttation fluid at early stages of development of the plant would be required to cause mortality. However, as studies in Switzerland showed no significant mortality in bee colonies located at the edge of treated maize fields the significance of guttation fluid as a source of water for bees is unclear. No studies have been reported to date on the effects of guttation fluid containing pesticide residues on other non-target arthroprods which have been identified above as potentially using them as a source of water, e.g. coccinelids, syrphids.

For leaves where the droplet spreads over the entire surface of the leaf, e.g. potato, an alternative exposure scenario is contact of non-target arthropods with dried residues from guttation fluid. Data on the toxicity of the three most recently studied residues in guttation fluid were extracted from the EFSA database. This comparison suggested that leaf residues may pose a risk to canopy-dwelling non-target arthropods. . For crop species where the fluid gathers as droplets at the edges or tips of leaves, e.g. monocots, a more patchwork pattern of dried residues is likely to be present and the relative proportions of uncontaminated and contaminated parts of the leaf is more likely to influence the exposure of the non-target arthropod. Therefore, where fluid gathers on the tips and edges of leafs exposure by drinking may pose a higher risk although the effects of dew redissolving pesticide residues on the leaf surface should also be considered.

Gaps in current knowledge which limit our ability to undertake an assessment of the risks posed by guttation fluid containing pesticide residues
1. Extrapolation between crops. Data currently does not allow the effect of crop type on the residues in guttation fluid to be identified. Can residues be extrapolated between crops for representative formulations with active ingredients of varying physico-chemical characteristics?
2. Effects of time since emergence on residues. Although a limited amount of data have been generated in the laboratory, what are the actual residues in a range of representative UK crops and what effects does field conditions (e.g. varying soil types) and have on the profile of residues present in guttation fluid?
3. Behaviour of non-target arthropods on plants exhibiting guttation. Do the species identified, eg parasitoid wasps, coccinellids, syrphids, honeybees, use guttation fluid as a source of water in relevant crop species?
4.Surface residues. Does the drying of guttation fluid on the surface of leaves result in significant residues of systemic pesticides and thus exposure of non-target arthropods?
4. Toxicity of surface residues to non-target arthropods. What are appropriate methodologies for assessing effects on non-target arthropods for crops, e.g. potatoes, in which guttation results in surface leaf wetting?

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
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   - the extent to which the objectives set out in the contract have been met;

- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

Introduction

Guttation is the appearance of drops of xylem sap on the tips or edges of leaves of some vascular plants, such as grasses, and should not be confused with dew, which condenses from the atmosphere onto the plant surface. A recent paper on "translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees" was published by Girolami et al (2009) and this has focussed significant interest on the possible risks posed to honeybees by such a method of exposure. In response to concern from beekeepers, AFSSA recently published their opinion on the risks to bees by guttation (extra-floral secretions) and a Swiss study published by the Federal Office of Agriculture (www.blw.admin.ch/themen/00011/00077/00590/index.html?lang=fr) on the residues of clothianidin present in guttation fluid from treated maize and the risks to bees has also recently been published. This project builds on those reports and summarise 1) the environmental and phenological situations where guttation may occur in crops under UK conditions and 2) the possible risks posed to bees and other non-target species. The report also outlines gaps in knowledge which affect our ability to assess the risks posed by guttation under UK field conditions.

Objective 1 To identify the circumstances under which guttation may occur in UK crops

Dialog online searches and literature reviews were undertaken on the crops and other plants in which guttation has been reported. Particular attention was paid to identification of UK relevant crop species reported to demonstrate guttation and the circumstances in which it has been observed, e.g. environmental conditions (such as soil moisture, air temperature), time of day, crop growth stage.

Guttation in plants has been reported since at least the 1950s, and has been identified as occurring in conditions of high soil moisture and low transpiration; although there may be other factors involved such as previous levels of water stress, growth stage, root depth and soil water potential (Takeda and Glenn (1989). Many of the reports identified by the database searches were related to the role of guttation in the development of disease through the invasion of the hydathodes of leaves by spores and bacteria, e.g. Goatley and Lewis (1966) and the ability of pesticides, particularly fungicides to act systemically to control these diseases.

Water transport in plants and the role of root pressure

Water transport in plants occurs through the xylem by two main processes, transpirational pull (water potential gradient) and root pressure (osmotic potential). Transpirational pull occurs only during hours of light when water loss occurs due to evaporation from the stomata on the surface of the leaf. Root pressure occurs, primarily during the hours of darkness, due to the accumulation of salt in the root xylem causing osmotic uptake of water and development of hydrostatic pressure in the xylem sap. Absorption of water by the surface cells in the roots increases their water potential which results in transfer of water to more deeply seated cells with lower water potential. . This water potential difference is maintained across the xylem of the stele due to the evaporation of water from the leaves in the transpiring plant during the day causing negative pressure in the xylem and the lower osmotic potential of the xylem sap. The xylem sap contains osmotically active organic and inorganic substances e.g. such as ions and sugars, catalase and peroxidase and reductase (Wilson (1923, cited by Komarnytsky 2000), at low concentrations when the plant is transpiring actively (during the day) and at high concentrations when it is not (during the night). Root pressure has been recorded to range from 3-5 atmospheres although it is apparently absent in conifers and other gymnosperms. Fisher et al (1997) demonstrated that root pressure in various climbing monocots, dicots and fern were highest (2-148 kPa) at night-time particularly around sunrise and resulted in guttation in 15 species investigated. Root presssure was absent in Passiflora, Aristolochia, Malpighiaceae and Bignoniaceae but it was widespread in Araceae, Leguminosae and Vitaceae. The metabolic activities of the root cells also have a role in root pressure as lowering oxygen levels or treatment with a respirator inhibitor, such as potassium cyanide, results in decreased root pressure. Root (osmotic) pressure is also reduced by decreasing temperature, reduction in inorganic nutrients and by metabolic inhibitors (it is increased by auxins).

Guttation

Guttation is a well described phenomena (it was first reported by Abraham Munting in 1672 (Ivanoff 1963)), not to be confused with dew or condensation, in which liquid water is released from leaves through stomata. water pores (hydathodes) situated at the edges of leaves or from vein endings in the leaf (Figure 1). Guttation is related to increased root pressure and decreased transpiration (Klepper and Kaufmann 1966) with diurnal periodicity; it occurs to a greater extent at night. Dutrochet (1867, cited by Eaton (1943)) was the first to ascribe osmosis as the cause of exudation (guttation). Wiler (1892, cited by Eaton 1943) showed the effect of temperature; warming

roots of plants resulted in exudation and Eaton (1943) demonstrated that in cotton plants exudation occurred when the osmotic potential of the xylem sap was greater than that of the culture solution but not when the reverse was true, replacing the culture solution with tap water resulted in a curvilinear relationship between the rate of exudation and the osmotic differential.

Ivanoff (1963) reviewed the conditions under which guttation occurs in plants with the summary that it occurs when conditions for absorption of water by roots are very favorable and those for transpiration are unfavorable. Cool mornings followed by warm days provide excellent conditions for guttation because in warm soils absorption is very active and at the same time, with relatively high humidity, transpiration is reduced almost to zero at night. In regions where, particularly in early spring and late autumn, the daytime temperatures are high and nights rather cool, such as mountain valleys and irrigated desert areas, plants guttate frequently and copiously. In the greenhouse situation guttation is readily induced in early morning in several plant species by keeping the soil temperature high (25-32oC), the relative humidity of the air close to 100% and the soil moisture abundant. The effects of light were reviewed by Ivanoff (1962), the guttation of maize coleoptiles increased when exposed to light; guttation reached a maximum about 2 hours after the start of illumination and decreased during dark periods. Ivanoff (1963) also stated that although a number of previous authors have compiled lists of plants observed to guttate it is now considered a general phenomenon.

Hydathodes are the structures that exude water (in guttation forced out under root pressure) together with various dissolved substances from the interior of the leaf to its surface. The structure and number of hydathodes varies from species to species but are generally modified parts of the leaf usually at leaf tips or margins, especially at the teeth (Evert 2006) and are usually a single hydathode at the leaf tip or tip of a tooth, although in some species they are distributed over the entire leaf surface, e.g. Uricaceae (nettles), Moraceae (mulberry/fig). Some hydathodes may excrete water actively, although the evidence for this is scarceand some have a dual function, e.g. the xerophytic Crassula species also absorb condensed fog or dew water and in Populus balsamifera retrieve solutes from the transpiration stream and active hydathodes or trichome hydathodes are glandular trichomes that secrete solutions of salts and other substances

In tobacco, potato and beans the entire surface of the leaf exudes the fluid. In contrast in mustard, barley and cucumber guttation drops form mainly at the edges or tips of leaves. Care must be taken in deciding whether guttation occurs as spreading over the surface of the leaf, such as lettuce, may suggest that guttation does not occur whereas in fact the surface tension of the fluid prevents formation of drops and the pools of water aggregate at the base of leaves and within the convolutions of the leaf (Ivanoff 1963). Komarnytsky et al (2000) in discussing the production of recombinant proteins through collection of guttation fluid cited species known for their high levels of guttation such as tomato and monocots particularly grasses.

Although the liquid exuded during guttation originates from the xylem sap the salt concentration of the liquid exuded is low due to its removal by the upper parts of the plant, in particular the leaves (Klepper and Kaufmann 1966). Goatley and Lewis (1966) determined the composition of guttation fluid in rye, wheat and barley seedlings. They showed that the sugar content in rye and barley was equal but in wheat it was lower. Most of the sugar in barley was glucose (38.7 mg/L) whereas in rye it comprised glucose (18.7 mg/L) , fructose (10.3 mg/L) and galactose (10.3 mg/L). In addition they detected amino acids, mostly aspartic acid (0.5-3.6 mg/L) and asparagine (1.9 -9.5 mg/L) and a range on ions including nitrate, phosphate, ammonium and other elements, e.g. iron, potassium, phosphorus. Singh et al (2008) investigated the relationship between yield in rice and guttation in cultivars. They demonstrated that those cultivars with greater panicle weights demonstrated greater guttation rates ($r^2=0.94$, $P<0.01$) and suggested that this was an evolutionary strategy for improving water balance and delivery of inorganic solutes to the panicles.

Figure1 Guttation droplets on maize and oilseed rape

Factors affecting the volume of guttation fluid
Hughes and Brimblecombe (1994) assessed the formation of guttation droplets on grass (Yorkshire fog) in rural Norfolk. They calculated the average total volume exuded per grass blade at sunrise as 1.73µl with larger droplets at sunrise and smaller droplets towards sunset. They concluded that the same conditions required for dew formation, (the dew point of air) also encourages guttation and the latter is significantly correlated with soil temperature and moisture. From this they developed an equation to calculate the guttation droplet diameter at sunrise (DIA in mm) for grasses.

DIA = 1.04+0.0643Ts - 0.021ψ
Where Ts is the mean soil temperature between sunrise and sunset at 10cm (C) and ψ is the mean soil moisture tension in the 0.25cm layer (kPa)

However such interactions between temperature and soil moisture are likely to vary with plant species and soil type and therefore are of limited value.

Hughes and Brimblecombe (1994) also reported that more than one droplet may be exuded per night; although only one would be present on the leaf tip, with others falling onto the grass beneath or rolling down the grass blade. Collection of all the exuded droplets accounted for a mean total of 9.9µl per leaf per night (including the drop present at the tip of the leaf). From this they calculated that for a grass blade density of 10464 blades per m2 the average total guttation volume was equivalent to 0.1mm of precipitation. Since the same conditions of high air humidity resulting in dew were reported as being suitable for guttation (a typical night comprised a precipitation equivalent of 0.14mm dewfall and 0.1mm guttation) it is interesting to note that the authors considered the dewfall in this study in the UK was comparable with those reported elsewhere in Europe. In a similar manner Williams et al (1998) also showed that guttation accounted for 33% of total dew (0.195mm at 0800hrs) on creeping bentgrass maintained as a golf fairway. Hughes and Brimblecombe (1994) also reported (without references) that maize, potatoes and sugarbeet also exhibit guttation with potato blight often occurring on leaf tips, which experience the greatest wetting duration. They considered that a greater wetting of the leaf area would occur on potato due to the lack of a thick waxy cuticle and therefore droplets will spread and coalesce to form a continuous film. On waxy leaves such as cabbage, sugarbeet and most grasses droplets would remain beaded. In southern England the authors calculated that 7-10% of the mean daily net radiation would be required to evaporate the combined dew and guttation derived wetness between June and August with 50% required by October.

The effects of soil moisture on guttation in barley were investigated by Zaitseva et al 1998. Using a first leaf stage plants they showed the effect of the osmotic water pressure of soil (heavy loamy with coarse silt fraction, 5% humus, neutral pH) on the level of guttation. Growth and guttation were directly correlated
G/Go = 2 (H/Ho - 0.43) where Ho maximal height and Go maximal guttation under optimal water conditions and H height and G growth is actual.
Varying the osmotic water potential of the soil so that guttation was100%, 66%, 33% or 0% of the maximal occurred at -20,-50, -300 and -1000 kPa which correspond to 32%, 25%, 21% and 19% soil moisture respectively. They also reported that another measure of soil moisture (capillary -sorptive, Pcs) could be associated with guttation with non-saline soils rich in quartz particles water is available in coarse silt at only -10 -- 60 kPa, in sand guttation stops at -20kPa and in loamy soils guttation is observed down to -400 - -500 kPa. The same authors reported that a decrease in capillary sorptive pressure of soil showed that a greater reduction in guttation than that in osmotic pressure (Zaitseva and Sudnitsyn 2001).

Takeda and Glenn (1989) reported the use of guttation in strawberries to indicate irrigation requirements. They reported that guttation occurred when the leaf water -0.01 to -0.13 MPa while onset of plant stress occurs between -0.5 and -1 MPa . Other factors affecting guttation were previous levels of water stress, root depth and soil water potential distribution within the root zone. Guttation was observed on all expanding young leaves but not consistently on the older expanded leaves thought to be due to changes in the epithem and water pores or loss of vascular function. In wheat plugging of water pores occurred in older leaves due to the condensation of the exudate. However Komarnytsky et al (2000) suggests that the change in the leaf from sink to source tissue may also be responsible for this observation. The AFSSA review of the risks to honeybees of guttation fluid gneerated in maize suggests that under gl;asshouse conditions the peak production is from the first unfolded leaf stage (BBCH 11) to the 6-7 leaf stage (BBCH 18).

Table 1. Cited species in which guttation has been reported

Common name	Ref
Potato	McCauley and Evert 1988
Wheat	Maeda and Maeda 1987, Goately and Lewis 1966
Rice	Maeda and Maeda 1988
Succulent - eg house-leek	Evert 2006
Mulberry/fig	Evert 2006
Nettle	Evert 2006
Grape vine	Tucker and Hoefert 1968
Cottonwood	Curtis and Lersten 1974
Yellow Rattle or Cockscomb	Ponzi and Pizzolongo 1992
Strawberry	Takeda et al 1991, Takeda and Glenn 1989
Barley	Bickers et al 1996, Goately and Lewis 1966
Rye	Goately and Lewis 1966
Tomato	Bickers et al 1996, Klepper and Kaufmann 1966
Sunflower	Magwa et al 1993
Cucumber	Magwa et al 1993, Biles and Abeles 1991
Japanese butterbur	Mizuno et al 2002
Japanese knotweed	Mizuno et al 2002
Tobacco	Komarnytsky et al 2000
houseplant	Pilanali 2005
Cabbage	Ruiseen and Gielink 1992
Rice	Singh et al 2008
Gametophytes of ferns	Szarek and Trebacz (1999),
Yorkshire fog, creeping bentgrass	Hughes and Brimblecombe 1994; Williams et al 1998
Sweet pepper	Klepper and Kaufmann 1966
Melons/ Squash	Ivanoff 1963
Lettuce	Curtis 1943 cited by Ivanoff 1963

Systemic pesticides in guttation fluid

Many of the reported data identified as relevant to this review were related to the distribution of systemic pesticides such as fungicides. There are a number of factors affecting the residues in guttation fluid demonstrated by the summaries below which include formulation, metabolism within the plant, application methods, adjuvant, solubility/lipophilicity of the active ingredient, plant species.

Examples include those reported by Bickers et al (1996) who assessed the effects of formulation on the biological availability of sprayed systemic fungicides by measuring concentrations of the active ingredient in guttation fluid collected from wheat barley, tomato and grapevine. The volume of guttation fluid collected from the tomato and grapevine were higher than from the monocotyledons, wheat and barley. The amounts of fungicide also varied for a single species (barley) with bitertanol below the limit of detection (<0.001 ppm), tebuconazole 0.050 ppm, triforine 0.750 ppm and triademol 0,780 ppm in guttation fluid (5.4-5.8 ul/leaf) after an application of 300ppm of the relevant formulation. Bickers et al (1996) also showed that the concentration in guttation fluid after application of triforine was positively but not linearly correlated with dose rate with a greater increase from 100 to 400ppm (<0.01 to 2.20 ppm) than from 400 to 600 ppm (2.20-3.32ppm). A slight but not significant decrease in guttation volume was detect from 4.9 µl/leaf at 100ppm to 3.9 µl/leaf at 600 ppm. A major influence on the concentration of the ai in guttation fluid was the use of additives. In the absence of an additive triflorine applied to barley at 100 ppm as the formulation Saprol Neu was not detected in guttation fluid but the addition of additives A or B (structures not identified) resulted in levels of 1.00-1.30 ppm in the guttation fluid and this was presumed to be due to enhanced penetration of the cuticle.. The time course of expression of triforine in guttation fluid after application of 300 ppm showed an increase from 0.35 ppm at 12 hrs to 0.70 ppm at 24 and 36 hrs and a decrease to 0.55ppm at 48hrs. These data showed that the amount of active ingredient at the leaf tips was negatively correlated with lipophilicity. The authors stated that differences in leaf morphology and greater leaf surface area resulted in greater volumes of guttation fluid under the conditions of the study.

Harris (1999) also used guttation to investigate the effects of formulation on systemic behaviour of fungicides (an experimental molecule Exp-F and tebuconazole) in winter wheat. He showed that the adjuvant used to apply the pesticide as well as the molecule itself affected the levels recovered in guttation fluid and this could also be correlated with the efficacy of the pesticide.

Coupland and Peabody (1981) assessed the exudation of glyphosate, fosamine and amitrole in the field horsetail (Equisetum arvense) in relation to their use in the control of this weed. Many phloem mobile herbicides (such as

glyphosate and amitrole, but not fosamine) are exuded from roots and may also be present in guttation fluid and therefore the authors were concerned whether this may limit efficacy of weed control. They showed that the levels of fosamine recovered were far lower than those of amitrole even those measured levels in xylem sap were higher for fosamine. This selectivity also occurs for other solutes such as ions. They concluded that amitrole exudation may result in significant loss under field conditions.

Coupland and Peabody (1982) also assessed the exudation of the herbicides dichlobenil and vernolate in the field horsetail. They demonstrated that following exposure to the vapour less than 0.5% of the applied dose (radioactivity) was detected in the guttation fluid with a peak 4 days after exposure. Both dichlobenil and vernolate are extensively metabolised in plants and dichlobenil has a high affinity for plant tissue. These data demonstrate the importance in understanding of plant metabolism in the prediction of the residues of pesticides in fluid exuded from leaves.

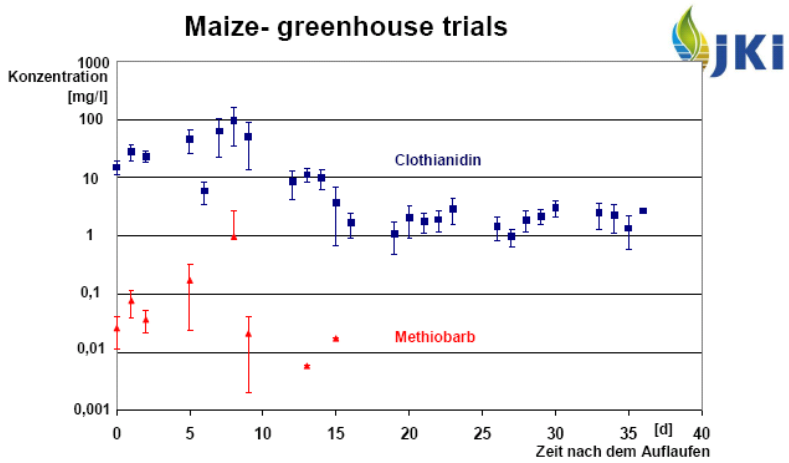
Ferreira and Seiber (1981) assessed the levels of three N-methylcarbamate insecticides (carbofuran, carbaryl and aldicarb) in guttation fluid from rice. They collected guttation fluid from the leaves of plants grown within a bell jar, i.e. high humidity. Plants were soaked in solution of each pesticide and guttation droplets collected from the leaves 1 day after treatment. The data showed high levels of all three pesticides, with levels paralleling their solubility. The authors also demonstrated that evaporation of the exuded fluid resulted in carbamate residues on the leaf surface with 8.4% (of 2mg), 12.6% (of 0.88mg) and 4.7% (of 5.1mg) of the total leaf residue of carbaryl, carbofuran and aldicarb respectively present on the outside of the leaf 1 day after root soak treatment with lower proportions on days 4 (5.6%)and 8 (2.3%).

Bickers et al (1999) assessed the transport of slow release soil applied fungicide formulations of tebuconazole and cymoxanil in barley (8 day old seedlings) and tomato (14 day old potted seedlings) by determining the levels on guttation fluid following exposure of the plants to 100% humidity and sampling at 12 hr intervals. This showed a significant difference in uptake from the slow release formulations than from the wettable powder formulations but difference between tebuconazole and cymoxanil ascribed to lipophilicity.

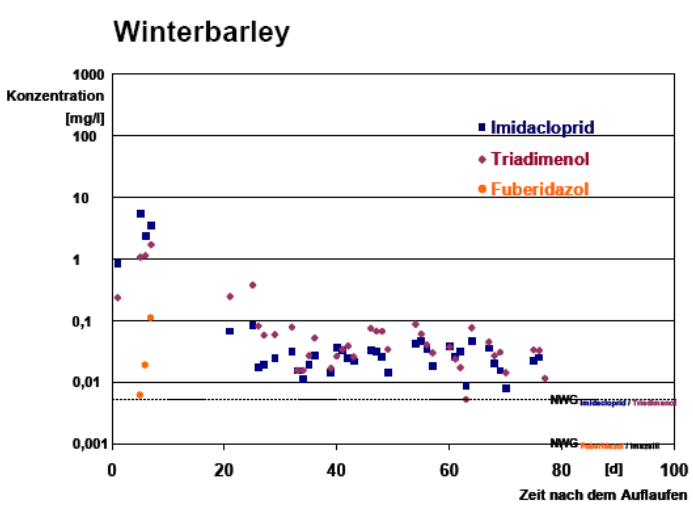
Stoller (1970) observed differences between species in the translocation of pesticides to leaves and then guttation fluid depending on the metabolism in the plant. Using Amiben (3-amino-2,5-dichlorobenzoic acid, a herbicide widely used at that time in soybean) which is more effective as a soil applied than as a foliar herbicide, he showed that 13 species (monocots and dicots) exhibited varying levels of parent compound from 1.4% (wheat) to 74% barnyard grass (Echinochloa crusgallli). He compared the amounts of the parent compound in the guttation fluid of only 2 species, 10 day old wheat and 13 day old barley plants, and showed that Amiben inhibited guttation in wheat and the levels in barley were highly variable.

Unpublished data was obtained from JKI Germany (Pistorius pers.comm) which demonstrates that a wide range of crops exhibit guttation, including maize, winter oilseed rape, winter barley, sunflower, potato and to a lesser extent sugar beet. Studies conducted by JKI have shown guttation in wheat and maize to a significant amount and frequently, in potatoes and winter oilseed rape guttation is often observed whereas in sugar beet guttation is rare. Grasses on meadows and at field edges very often showed strong guttation and obviously provide an alternative source of water in cropped areas. In terms of the residues of systemic pesticides present it appears that the residue depends on the dose and the growth stage/ development of the plants (BBCH) with high concentrations at early growth stages (See Figure 2) but further data are required to understand the relationship.

Figure 2. Presence of pesticide residues in guttation fluid in maize and winter barley grown under glasshouse conditions (Reproduced from a poster presented at a SETAC Symposium in 2009 with permission from Jens Pistorius JKI)



Means and Standard deviations of the concentration of Clothianidin and Methiocarb in guttation fluids of maize from treated seeds (n. n.- not detectable : Clothianidin. ≤ 1pg/µl, Methiocarb. ≤ 5pg/µl (µg/l))



conzentration of Imidacloprid, Triadimenol und Fuberidazol in guttation fluids of Winterbarley from treated seeds (n. n.- not detectable / LOD Fuberidazol 1 pg/µl, Imidacloprid und Triadimenol: 5 pg/µl (µg/l))

Table 2 Examples of the residues of pesticides in guttation fluid following applications to plants

Crop	Growth stage	Conditions for guttation	Volume collected (ul/leaf)	Residue ai	Ref
Wheat (cultivar Apollo)	8 day old seedling	Lab, 100% humidity 60mins	6.4	1.37ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Wheat (cultivar Avalon)	1-2 fully expanded leaves)	20C, leaf enclosed in vial	Not reported	0.15 ug (Exp) (36hrs after application of 1ug to leaf) <0.01 ug tebuconazole (48 hrs after application of 1ug to leaf)	Harris 1999
Barley (4 cultivars_ Alexis, Jana, Trixi, Corona)	8 day old seedling	Lab 100% humidity 60mins	4.8-5.6	1.36-3.95 Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Tomato	14 day old seedling	Lab 100% humidity 60mins	74.5	8.70ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Grapevine	21 day old cutting (after 3 weeks in hydroculture)	Lab 100% humidity 60mins	56.5	2.95ppm Triforine (36hrs after application of 300ppm ai to leaves)	Bickers et al 1996
Grass (Yorkshire fog)	Mature	Field conditions	1.73	N/A	Hughes and Brimblecombe 1994
Rice	125-135 days	Field- rainy season (30-32C, 80-85% RH)	62-110	N/A	Singh et al 2008
Field horsetail	4-6 shoots, 15cm	20-25C, 50-7-		Amitrole 2.88	Coupland and

Crop	Growth stage	Conditions for guttation	Volume collected (ul/leaf)	Residue ai	Ref
	high	%RH, 14/10 light cycle		ug Fosamine 0.004 ug, glyphosate 0.96 ug (36 hrs after application of 2ul 20g ai/L)	Peabody (1981)
Tobacco	2 months	95% humidity 26C light, 18C dark, 14/10h light dark cycle	1-2ml /g dry leaf per day (5ul/cm2 leaf area)	N/A	Komarnytsky et al 2000
Field bean			6ul/cm2 leaf area	N/A	Yarwood 1952 cited by Komarnytsky et al 2000
Couch grass (Agropyron repens)	3 tillers	16C light, 6C dark, 77% RH light, 95% RH dark, 14/10hr light/dark		0.11 ug glyphosate (8days after application of 50ug)	Coupland and Caseley 1979
Rice	12-18 days	12/12 hr light/dark 20C dark, 35C light, closed chamber with air flow (1L/min)		50 ug/ml Carbaryl (1 day after root soak uptake 305 ug/g plant) 254 ug/ml Carbofuran (1 day after root soak uptake 297 ug/g plant) 2152 ug/ml Aldicarb (1 day after root soak uptake 328 ug/g plant)	Ferreira and Seiber 1981

Objective 2 To identify the potential for exposure of non-target arthropods including bees to pesticides in guttation fluid

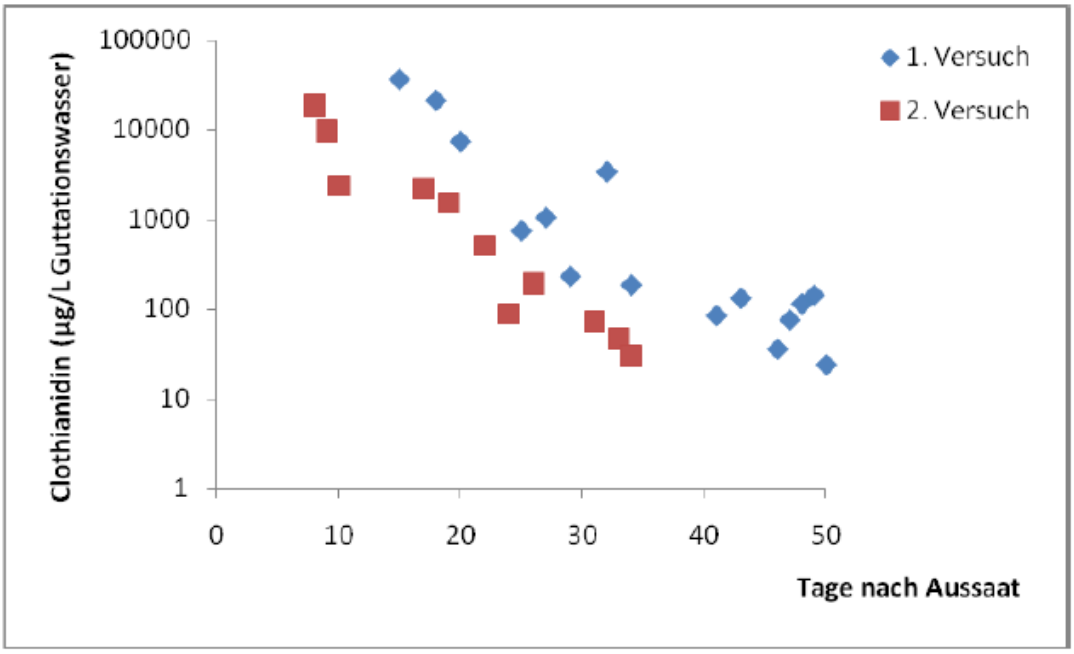
Due to the recent interest in guttation fluid as a hazard for honeybees there have only been limited data published on risks posed to non-target arthropods. Only one study (Girolami et al 2009) showed a significant effect in honeybees but as can be seen from the summary below this should be treated with caution as the data were generated by feeding collected droplets directly to bees and in many cases sucrose was added to ensure the honeybees consumed the dose. What is less clear is whether honeybees use guttation fluid on crops as a significant source of water.

Girolami et al (2009) undertook laboratory studies with honeybees in which they fed guttation fluid from treated maize to honeybees in the laboratory. The maize seeds were treated with imidacloprid (0.5mg Gaucho 350/seed), clothianidin (1.25 mg Poncho/seed), thiamethoxam (1mg Cruiser FS/seed) or fipronil (1mg Regent FS/seed) and grown in open field conditions. Guttation droplets were collected at 0800-0900 each morning for the first 3 weeks after emergence (when guttation reduced). In the field 1-3 mls of fluid could be collected from 100 plants (in the laboratory 30-150µl /plant/ day was collected) and each sample was split into two, one for chemical residue analysis and the other for bioassay. The bioassay was conducted with honeybees deprived of food and water for 2 hours before dosing and individuals dosed with guttation fluid only or guttation fluid with 15% honey. 20 minutes after fluid consumption fresh honey was provided. The time to first toxic symptoms was recorded. Field collected guttation fluid resulted in wing block within 2-9 minutes after consumption of fluid collected from plants grown from clothianidin, thiamethoxam or imidacloprid treated seed but not from control plants or plants

grown from fipronil treated seed. There was a significant delay in the consumption of guttation fluid alone and only addition of honey resulted in consumption within 5 minutes of the dose being offered. The residues in the guttation fluid from plants grown from treated seed were 47± 9.96 mg imidacloprid/ L; 23.3 ± 4.2 mg clothianidin/ L and 11.9 ± 3.32 mg thiamethoxam/ L.; no fipronil was detected. Although the authors relied on sublethal effects for their bioassay the published LD50 data are 0.0037 µg imidacloprid/bee, 0.004 µg clothianidin /bee, 0.005µg thiamethoxam /bee. Based on intake of 20µl per bee these are equivalent to test solution concentrations of 0.185 mg imidacloprid/L, 0.084 mg clothianidin/L and 0.25 mg thiamethoxam/L.. Therefore the levels in guttation fluid were 254 times the LD50 for imidacloprid, 280 times the LD50 for clothianidin and 48 times the LD50 for thiamethoxam.

The Swiss Federal Government for Agriculture commissioned a study in 2009 (www.blw.admin.ch/themen/00011/00077/00590/index.html?lang=fr) to assess the risks to honeybee colonies during sowing of maize seed treated with Poncho (25g ai/ 50,000 seeds, i.e. 0.5 mg ai/seed) through drift of dust and guttation. No effects were observed due to dust drift. Guttation fluid collected from maize after emergence (7-10 days after sowing) was reported to contain 25-37 mg clothianidin/L reducing to around 0.1 mg/L by 40 days after sowing (as above the LD50 for clothianidin is aroung 0.084 mg/L) (Figure 3). No clothianidin residues were detected in the honeybees or in honey sampled from the colonies and no increased mortality was identified at honeybee colonies placed at the edges of the treated fields and the colonies developed normally.

Figure 3 Clothianidin concentration in guttation fluid in maize in two trials in Switzerland
[Ordinate: Clothianidin (µg/L guttation fluid. Abscissa: Days after sowing. Versuch = Trial]



Shawki et al (2006) investigated the potential impact on honeybees collecting water of a spray formulation containing chlorpyrifos and cypermethrin (both non-systemic) applied to oilseed rape plants at growth stage 21-51. Guttation was encouraged by irrigation before being covered by plastic covers overnight and samples were collected before or soon after sunrise. They collected samples of guttation fluid and dew up to 10 days after spray application. Subsamples were analysed for residues. The remainder of the samples were diluted 1:1 in 50% sucrose and fed to bees to assess acute and chronic oral toxicity. Samples of the guttation fluid were also used to wet filter paper placed in cages to assess contact toxicity. The acute oral toxicity studies showed 10% or less mortality up to 7 days after treatment and the chronic toxicity studies showed no significant increase in toxicity. Analytical data showed that chlorpyrifos residues were below the limit of detection (0.8 µg/kg) and cypermethrin was also not detected in guttation fluid, chlorpyrifos residues in dew peaked at 3.7 µg/kg on day 4 after spray application.

Reguirements for water in honeybees and other non-target arthropods
Honeybees
Guttation fluid is unlikely to be identified by honeybees as a source of sugar due to the low levels present, Seeley (1986) identified 15% sugar as threshold of interest to nectar foraging bees. Bees are less subject to dessication than most terrestrial insects due to their nectar diet and high metabolic water production. Water is collected by bees to dilute thickened honey, to produce brood food from stored pollen, to maintain humidity within the hive and to maintain temperature within the brood area. Water is not stored in combs by temperate bee colonies. The amount of water required depends on the outside air temperature and humidity, the strength of the colony and the amount of brood present. Water carrier bees carry water in the honey stomach and can carry 40-50µl in this way.

Vissher et al (1996) cited by Nicolson 2009 reported mean water loads of 44mg in honeybees collecting water under desert conditions. When groups of honeybees were confined to a cage at 35-40C and provided with a 67% sugar solution they consumed about 10 µl of water per bee per day. Seeley (cited by Nicolson 2009) estimated average annual requirements of 25 litres for a single wild colony in cold temperate conditions. Shawki et al (2006) reported that bees generally collect water up to 50m from the hive suggesting local sources tend to be used when available. Usual sources of water identified for honeybees are those that are moderately mineralised and include rainwater and puddles and it has been suggested that the presence of decomposing plant material generates olfactory stimuli attractive to foragers. There have been no published reports to date of honeybees collecting guttation droplets from crops.

Other non-target arthropods

There has been much recent interest in the potential exposure of "water-carrier" honeybees to guttation droplets but there may also be other crop-dwelling non-target invertebrates that may be exposed either to the guttation fluid through ingestion or by contact following the drying of exuded droplets on the leaves. Assessment of the potential impact of pesticides in guttation fluid is dependent on a number of factors which will be assessed:

Bumble bees have been observed to collect water from a water trough in warm conditions (Ferry and Corbet cited by Nicolson 2009) and this was thought to be for the benefit of the colony rather than individual water deficit. Paper wasps and hornets have also been reported to use water for cooling their nests but highly social stingless bees do not exhibit this behaviour (Nicolson 2009).

Edney (1977) produced the most comprehensive guide the use of water by terrestrial arthropods including a review of the uptake of water by drinking. He summarised that many arthropods are known to drink water if it is available. Stored product insects are considered to consume the most water due to the nature of their dry diet with Tenebrio increasing their water content from 65% to 85% when provided with a source of water. Larvae of the tomato moth (Diataraxia oleraceae) (normal water content 90%) are reported to consume water with a 300mg insect consuming 40mg in one minute. The amount of water consumed obviously depends on the water content of the food source with phytophagous larvae consuming the least.

In adult arthropods honeybees are reported by Edney (1977) to consume up to their own weight in water in 1 minute. In some circumstances dew has been reported as a source of water for insects, such as locusts, suggesting guttation fluid may be seen as a source of water more generally by arthropods. Tenebrionid beetles are reported to use dew condensate as a source of water and a pyrrhocorid (Scanthus aegyptius) and ant (Monomorium subopacum) have been reported drinking from stones and and two beetles (Carabus impressus and Coccinella septumpunctata) have been reported drinking droplets from leaves. Several butterfly and moth species were reviewed by Edney as drinking even though ample water is available in nectar. This is thought to be due to the need for solutes such as sodium ions. A summary table produced by Edney is shown below to demonstrate the wide range of species in which drinking has been observed.

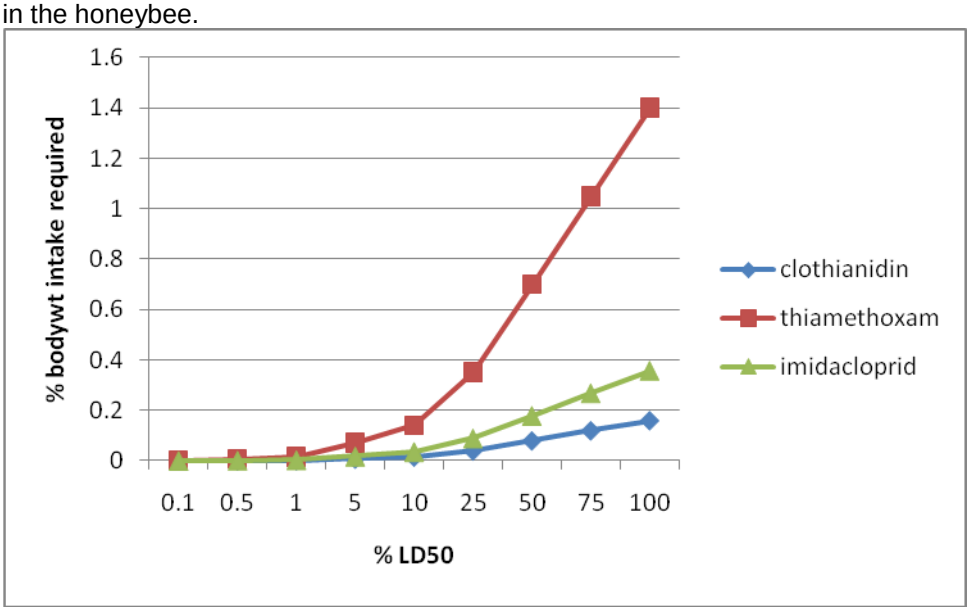
Table 3 A representative list of relevant arthropods known to drink water or absorb it from moist surfaces (from Edney 1977)

Taxon	Remarks
Insecta	
Orthoptera	
Gryllus	Drinks by mouth
Acridium	Drinks by mouth, cutaneous absorption claimed
Locusta	Drinks >100mg /g in 30min when dehydrated
Isoptera	
Hodotermes	Newly emerged alates drink and store water in modified salivary sacs
Hemiptera	
Rhinocoris, Platymeris, Reduvius	Drink water drops in the laboratory
Dysdercus	Drinks water needed for elimination of excess inorganic ions in diet.
Coleoptera	
Various adults	
Calandra, Silvanus, Geotrupes, Trichius, Clytus, Oryzaephilus, Psylloides	Drink by mouth in culture
Tenebrio larvae	Access to water affects growth rate
Agriotes larvae	Drink when dehydrated
Ptinus	Access to water affects development
Lepidoptera	
Pierids, papilionids, lycaenids, hesperids	Drink from puddles and moist soil
Ephestia, Pyrausta	Drinking affects longevity and fecunidty
Noctuid larvae, Pieris larvae,	Drink by mouth in culture

Philadoria, Diataraxia	Drink readily if thirsty (low water content)
Diptera Various tabanids and muscids Calliphora Lucilla Lucilla lavae Syrphid larvae	 Drinks by mouth in hot dry conditions Drinks water if sugar solution not available Drink if dehydrated If dehydrated absorb water by rectal lobes from water surface
Hymenoptera Apis Social wasps Icaria, Odynerus Polistes	 Prefer water with NaCl or NH4Cl to pure water, controlled by amount of water lost, drinks own weight in water in 1 min Drink for control of nest temp and humidity Solitary wasps, drink by mouth Social wasps drink for distribution to larvae
Arachnida Acarina Echinolaelaps Araneida Tarentula, Lycosa	 Drinks when below equilibrium humidity If dehydrated drinks from damp soil

Risk assessment for pesticide residues in guttation fluid
Consumption of residues in drinking water
The primary concern to date has been the consumption of guttation fluid as a source of water. Based on the data available the intake of water by drinking in non-target arthropods may range widely and up to 100% of bodyweight. The only oral toxicity data available for non-target arthropods readily available are those for the honeybee. Therefore the residues identified in guttation fluid, the intake (as a percentage of bodyweight based on a mean 100 mg/ individual) was used to calculate the percentage required to reach the LD50 for a clothianidin, thiamethoxam and imidacloprid (Figure 4). This shows that only a very low volume of the guttation fluid at early stages of development of the plant would be required to cause mortality. However, as studies in Switzerland showed no significant mortality in bee colonies located at the edge of treated maize fields the significance of guttation fluid as a source of water for bees is unclear. No studies have been reported to date on the effects of guttation fluid containing pesticide residues on other non-target arthroprods which have been identified above as potentially using them as a source of water, e.g. coccinelids, syrphids.

Figure 4 Intake of guttation fluid required as a percentage of bodyweight to reach various percentage of the LD50 in the honeybee.



Contact with dried residues
An alternative exposure scenario is contact of non-target arthropods with dried residues from guttation fluid (Figure 5). Data on the toxicity of the three most recently studied residues in guttation fluid were extracted from the EFSA database. These data are based on dried residues following application of the pesticide normally in 200 L/ha over a large surface area.

Table 4 Comparison of residues in guttation fluid with the LR50 from Tier 1 studies for EU registration (from Agritox, AFSSA)

Species	Pesticide	Max concentration in guttation fluid mg ai/L	Study	LR50	Equivalent LR50 in mg ai/L (200L /ha)
Aphidius rhopalosiphi	Clothianidin	25-37; 23 ± 4	Extended lab	1.086 g ai/ha	5.43
Trichogramma cacoeciae	Clothianidin	25-37; 23 ± 4	Extended lab	0.36 g ai/ha	1.8
Typhlodromus pyri	Clothianidin	25-37; 23 ± 4	Semi-field	125.99 g ai/ha	630
Aphidius rhopalosiphi	Thiamethoxam	12 ± 3	Lab single rate	<200 g ai/ha (100% mortality)	<1000
Typhlodromus pyri	Thiamethoxam	12 ± 3	Lab single rate	<200 g ai/ha (100% mortality)	<1000
Orius laevigatus	Thiamethoxam	12 ± 3	Lab single rate	<200 g ai/ha (100% mortality)	<1000
Typhlodromus pyri	Imidacloprid	47 ± 10	Lab	4.23 g ai/ha	21.2
Aphidius rhopalosiphi	Imidacloprid	47 ± 10	Lab	0.0216 g ai/ha	0.108
Typhlodromus pyri	Imidacloprid	47 ± 10	Extended lab	19.13 g ai ha	95.7
Aphidius rhopalosiphi	Imidacloprid	47 ± 10	Extended lab	0.45 g ai/ha	2.25
Coccinella septempunctata	Imidacloprid	47 ± 10	Extended lab	11.38 g ai/ha	56.9
Chrysoperla carnea	Imidacloprid	47 ± 10	Extended lab	10.51 gai/ha	52.55

For leaves where the droplet spreads over the entire surface of the leaf (such as potatoes and beans) the data in Table 4 can be directly compared. Based on the data reviewed above up to 6µl /cm2 leaf area may be present on the surface of the leaf in field beans (Yarwood 1952 cited by Komarnytsky et al 2000) this would be equivalent to an application rate of 600 L/ha and therefore in the same order as the rates used in laboratory studies. These data suggest that the residues present may pose a risk to canopy-dwelling non-target arthropods, particulalry when the data for erxtended laboratory studies, the most realistic for leaf residues, are compared.
For crop species where the fluid gathers as droplets at the edges or tips of leaves, e.g. monocots, a more patchwork pattern of dried residues is likely to be present and the relative proportions of uncontaminated and contaminated parts of the leaf is more likely to influence the exposure of the non-target arthropod. Therefore, where fluid gathers on the tips and edges of leafs exposure by drinking may pose a higher risk although the effects of dew redissolving pesticide residues on the leaf surface should also be considered (Figure 5).

Figure 5 Dried residues following evaporation of guttation fluid

Gaps in current knowledge which limit our ability to undertake an assessment of the risks posed by guttation fluid containing pesticide residues:

1. Extrapolation between crops. Data currently does not allow the effect of crop type on the residues in guttation fluid to be identified. Can residues be extrapolated between crops for representative formulations with active ingredients of varying physico-chemical characteristics?
2. Effects of time since emergence on residues. Although a limited amount of data have been generated in the laboratory, what are the actual residues in a range of representative UK crops and what effects does field conditions (e.g. varying soil types) have on the profile of residues present in guttation fluid?
3. Behaviour of non-target arthropods on plants exhibiting guttation. Do the species identified, e.g. parasitoid wasps, coccinellids, syrphids, honeybees, use guttation fluid as a source of water in relevant crop species?
4.Surface residues. Does the drying of guttation fluid on the surface of leaves result in significant residues of systemic pesticides and thus exposure of non-target arthropods?
4. Toxicity of surface residues to non-target arthropods. What are appropriate methodologies for assessing effects on non-target arthropods for crops, e.g. potatoes, in which guttation results in surface leaf wetting?

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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