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Estimating the effects of Cry1F *Bt*-maize pollen on non-target Lepidoptera using a mathematical model of exposure

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Summary

1. In farmland biodiversity, a potential risk to the larvae of non-target Lepidoptera from genetically modified (GM) *Bt*-maize expressing insecticidal Cry1 proteins is the ingestion of harmful amounts of pollen deposited on their host plants. A previous mathematical model of exposure quantified this risk for Cry1Ab protein. We extend this model to quantify the risk for sensitive species exposed to pollen containing Cry1F protein from maize event 1507 and to provide recommendations for management to mitigate this risk.

2. A 14-parameter mathematical model integrating small- and large-scale exposure was used to estimate the larval mortality of hypothetical species with a range of sensitivities, and under a range of simulated mitigation measures consisting of non-*Bt* maize strips of different widths placed around the field edge.

3. The greatest source of variability in estimated mortality was species sensitivity. Before allowance for effects of large-scale exposure, with moderate within-crop host-plant density and with no mitigation, estimated mortality locally was < 10% for species of average sensitivity. For the worst-case extreme sensitivity considered, estimated mortality locally was 99·6% with no mitigation, although this estimate was reduced to below 40% with mitigation of 24-m-wide strips of non-*Bt* maize. For highly sensitive species, a 12-m-wide strip reduced estimated local mortality under 1·5%, when within-crop host-plant density was zero. Allowance for large-scale exposure effects would reduce these estimates of local mortality by a highly variable amount, but typically of the order of 50-fold.

4. Mitigation efficacy depended critically on assumed within-crop host-plant density; if this could be assumed negligible, then the estimated effect of mitigation would reduce local mortality below 1% even for very highly sensitive species.

5. *Synthesis and applications*. Mitigation measures of risks of *Bt*-maize to sensitive larvae of non-target lepidopteran species can be effective, but depend on host-plant densities which are in turn

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affected by weed-management regimes. We discuss the relevance for management of maize events where *cry1F* is combined (stacked) with a herbicide-tolerance trait. This exemplifies how interactions between biota may occur when different traits are stacked irrespective of interactions between the proteins themselves and highlights the importance of accounting for crop management in the assessment of the ecological impact of GM plants.

Key-words: *Bt*, crop management, Cry1F, ecological impact, exposure, genetically modified maize, mathematical model, mitigation measures, non-target Lepidoptera

Introduction

The intensification of arable agriculture over the last 50 years has been associated with substantial losses of biodiversity (Memmott 2009). Lepidoptera are popular indicators of biodiversity (Merckx *et al.* 2010), but are known to be in decline throughout Europe (Van Swaay *et al.* 2010), partly as a result of farming practice (Stoate *et al.* 2009). The introduction of genetically modified (GM) *Bt*-maize crops presents a potential risk to the larvae of non-target Lepidoptera. Genetic modification remains a controversial area for environmental policy (Memmott *et al.* 2010). The global area planted commercially with GM crops was 148 million hectares in 2010 (James 2010); insect-resistant *Bt* crops, developed to provide protection against target pests by the introduction of a *Bacillus thuringiensis* Berl. (*Bt*) gene encoding various insecticidal Cry proteins, represent more than one-third of this. Current lepidopteran-resistant *Bt*-maize events (*Bt*11, MON810 and 1507) express Cry1 proteins in most plant tissues, including pollen (Mendelsohn *et al.* 2003). The potential risk to the larvae of non-target Lepidoptera mentioned earlier is the ingestion of harmful amounts of pollen arising from *Bt*-maize fields and deposited on their host plants in and around cropped areas (Losey, Rayor & Carter 1999; Felke *et al.* 2010).

A number of laboratory, field and theoretical exposure studies have assessed the potential risks of *Bt*-maize pollen to non-target Lepidoptera (reviewed by Lang & Otto 2010). Most of these were performed in the USA on the monarch butterfly *Danaus plexippus* L. and Cry1Ab protein and estimated that the amounts of maize pollen potentially ingested by monarch larvae on their host-plants in and around *Bt*-maize fields are unlikely to adversely affect a significant proportion of this species (Sears *et al.* 2001). The justification was that *Bt* expression in pollen was low, there was variable and limited overlap between pollen shed and larval activity periods, that only a portion of the monarch population fed on host-plants in or near cornfields and there was limited adoption rate of *Bt*-maize at that time. Extrapolating observations made on the monarch butterfly to other species is problematic because of between-species variability in acute sensitivity to specific Cry1 proteins (Wolt *et al.* 2003; Wolt, Conlan & Majima 2005), plant–insect phenological coincidence (Schmitz, Pretscher & Bartsch 2003), host-plant habitat occupation and adult dispersal capacity.

Compared with other wind-pollinated species, maize pollen grains are relatively large (an average diameter of 90 μm) and heavy (0·25 μg), settle to the ground rapidly (Aylor, Schultes & Shields 2003), have a short dispersal distance (Jarosz *et al.* 2005) and therefore, a limited spatial distribution (Pleasants *et al.* 2001). These characteristics limit the spatial range of mortality studies to the *Bt* crop itself and the margins of the field in which it is grown. Field data on some aspects of exposure, particularly plant–insect phenology, pollen consumption and subsequent mortality in the field, are particularly sparse in Europe, though some studies on certain non-target lepidopteran species in specific locations in Europe provide relevant information on exposure (Darvas *et al.* 2004; Gathmann *et al.* 2006). Darvas *et al.* (2004) analysed the habitats of 187 protected lepidopteran species in Hungary and reported that of these, 30 species may have host plants in the margins of maize fields. Of these 30, two species, *Vanessa atalanta* L. and *Inachis io* L., had a host plant (*Urtica dioica*) that might be exposed to significant deposition of maize pollen; on this basis, Hungary proposed a ban on the cultivation of *Bt*-maize MON810.

Perry *et al.* (2010) developed an 11-parameter mathematical model of exposure of larvae of non-target Lepidoptera (*V. atalanta*, *I. io* and *Plutella xylostella* L.) to *Bt*-maize MON810 pollen. This model integrated a relationship between mortality and pollen dose based on laboratory bioassays with a relationship between dose and distance from a maize crop based on field measurements. Hence, Perry *et al.* (2010) derived predictions of mortality within a *Bt*-maize crop and at various distances from it into the field margins. The model structure distinguished between parameters relating to worst-case local exposure at small spatial and temporal scales (within-field and within the duration of anthesis) to large-scale effects (within-region; within-season; utilization rate of GM technology; allowance for physical effects and larval behaviour). Importantly, it provides a novel structure by which exposure may be quantified for other GM crops, a variety of traits and a range of non-target lepidopteran species. The model generated realistic data for three widespread European species in 11 representative maize ecosystems in four European countries and demonstrated that the likely impact of maize MON810 pollen on non-target lepidopteran populations is low.

Here, we consider the risks associated with maize 1507, which expresses the insecticidal Cry1F *Bt*-protein in its pollen, rather than the Cry1Ab protein expressed by MON810. Maize 1507 could pose a greater risk for non-target Lepidoptera than MON810, because: (i) while Lepidoptera are on average five times less sensitive (Wolt, Conlan & Majima 2005) to Cry1F than to Cry1Ab, the *Bt*-protein content expressed in the pollen of maize 1507 is more than 350 times that expressed in the pollen of maize MON810 (Mendelsohn *et al.* 2003; US

EPA 2005); (ii) reported species sensitivities of laboratory populations, quoted as the average lethal concentration (units: grains of pollen per cm^2 leaf) that kills half of the susceptible larvae (LC50), range widely from 0·065 to 410 μg Cry1F per gram diet (Wolt, Conlan & Majima 2005), leaving open the possibility that some species of conservation concern might be highly sensitive (Lang & Otto 2010); (iii) more recent studies have shown considerable additional variability in LC50 values, because of differences in toxin batches, methodologies (Saeglitz *et al*. 2006) and origin of test populations (Gaspers *et al*. 2010).

Therefore, some lepidopteran species of conservation concern might require specific risk assessment and management (EFSA 2010). The planting of border rows consisting of strips of non-*Bt* maize was recommended as an appropriate management measure to guard against the possible evolution of insect resistance in target pests to *Bt* which would additionally mitigate possible exposure of non-target Lepidoptera (EFSA 2009), though no recommendations concerning the appropriate size of such strips could be given at that time. (It should be noted that in this paper, the phrase 'non-Bt-maize' is intended to denote maize that does not express a Cry1 protein which is active against Lepidoptera). In North America, it is typically recommended that at least 20% of the total crop area of maize should be planted with non-*Bt* maize in order to create refugia designed to delay the evolution of resistance to Cry proteins amongst target pest species (MacIntosh 2010).

Weed management in the field and its margins will affect the risk from *Bt*-maize pollen, as it may modify the abundance of host plants. For example, *Polia bombycina* Hufnagel larvae occur from July onwards on *Sonchus* spp. and other host plants, close to the time of pollen shed for many maize varieties. The moth is rare in Europe and is a priority species in the UK Biodiversity Action Plan; the benefits of agri-environment schemes for this moth have been discussed by Merckx *et al*. (2010). *Sonchus* spp. are widespread in maize fields, but their abundance is known to be affected by management in GM herbicide-tolerant cropping systems (Heard *et al*. 2003). Increasingly, two or more traits present in single GM crop events are combined (stacked) by conventional breeding, resulting in 'stacked events' in which, for example, insect-resistance and herbicide-tolerance traits are both expressed. Currently, many jurisdictions (e.g. Australia, Canada, New Zealand, USA, UK) focus safety concerns for stacked events (Taverniers *et al*. 2008) on whether the proteins interact. However, interactions between biota may occur under different weed-management regimes, irrespective of interactions between the genes themselves or the proteins that they express.

In this study, we extended the Perry *et al*. (2010) model, to assess the potential larval mortality of five hypothetical non-target lepidopteran species. These represent a very wide range of sensitivities to the Cry1F protein so that this study is ecologically relevant to a wide range of lepidopteran species in arable maize ecosystems. A second factor considered was a range of simulated mitigation measures consisting of non-*Bt* maize strips of different widths, planted around the field edge. This factor has not been studied or quantified before. The third factor examined was host-plant density within the crop, for which two abundances were compared. This has allowed an examination of a range of exposure scenarios to quantify mortality and to develop recommendations for management.

Materials and methods

The model and notation are similar to that of Perry *et al*. (2010), except that here we assume: (i) a single, typical region instead of 11 different regions; (ii) five hypothetical species of different sensitivities instead of three actual species of Lepidoptera; (iii) two values of within-crop host-plant density for this typical region; and (iv) a range of mitigation measures consisting of nine different widths of strips of non-*Bt* maize. A fuller description of the model is provided in Appendix S1 (Supporting information).

The spatial arena (Fig. 1) is a square field of size $C = 15$ ha, with a margin on all sides of width $D = 2$ m. For simplicity, the host plants of each species are assumed to have the same density of $f = 0{\cdot}75$ m^{-2} in the margin and one of two values, $e = 0$ or $e = 0{\cdot}01$ in the crop. The rationale for comparing complete absence of host plants with a small, but non-zero density is provided in Appendix S2 (Supporting information). For the moment, we just consider the simple case without mitigation ($w = 0$ in Fig. 1). These parameters, C, D, e, f and w, listed in Table 1, are termed 'small-scale' because mortality is estimated in two phases: first locally, using these small-scale parameters, and then globally, using 'large-scale' parameters. By locally, we mean spatially within the crop and its immediate margins, and temporally

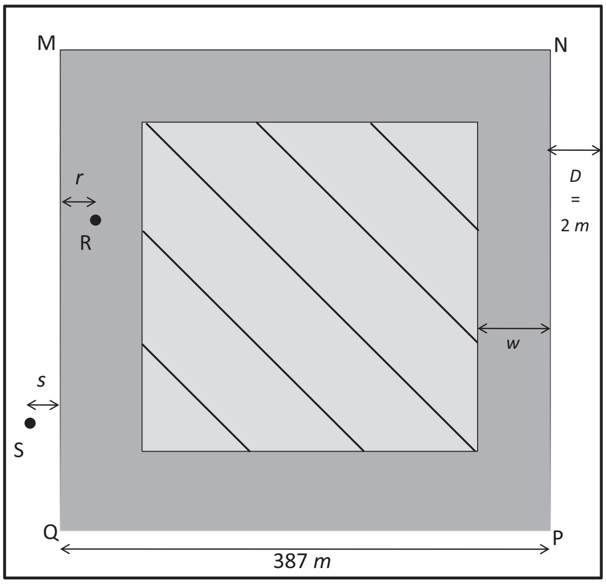

Fig. 1. The spatial arena for the model (not to scale) is a square field MNPQ (two shades of grey), surrounded on all four sides by a margin (white). The crop in the field interior (shaded lighter grey and hatched) is *Bt*-maize event 1507, expressing Cry1F protein in its pollen. Surrounding this is a strip (shaded darker grey, unhatched) of a non-*Bt* maize variety of width w m, the outer border of which is the field edge. Using the notation of Perry *et al*. (2010), the field size is $C = 15$ ha, with side *c*. 387 m, and the margin has width $D = 2$ m. For this field size, a value of $w = 20{\cdot}5$ m would result in an area of the strip equivalent to 20% of the field area. Larva S is in the margin at a distance s from the edge of the field; it is a distance, $E = s + w$ from the *Bt*-maize. Larva R is within the non-*Bt* maize at a distance r from the edge of the field ($r < w$); it is a distance $E = w - r$ from the *Bt*-maize (see Table 1 and text).

Table 1. Parameter values used in the model. The model estimates larval mortality for five hypothetical species of non-target Lepidoptera over a range of sensitivities (parameter *m*), two values of host-plant within-crop density (parameter *e*) and a range of levels of mitigation (parameter *w*). Mortality may be estimated separately for local, small-scale exposure and after allowance for large-scale exposure effects (parameter *L*)

Parameter	Type (units)	Values or derivation
Parameters concerned with mortality		
LC 50, *m*	Dose (pollen grains cm^{-2})	Assumed, for five hypothetical species: 1·265, 14·36, 163·2, 1853, 21 057
Mortality in margin and in non-*Bt* maize, *g*(*E*)	Probability (–)	Calculated in eqn 2
Within-crop mortality, *h*	Probability (–)	Calculated in eqn 3
Small-scale parameters, all values assumed		
Host plant within-crop, *e*	Density (m^{-2})	0, 0·01
Host plant in margin, *f*	Density (m^{-2})	0·75
Size of maize fields, *C*	Area (ha)	15
Width of margin, *D*	Distance (m)	2
Width of non-*Bt* strips, *w*	Distance (m)	0, 3, 6, 9, 12, 15, 18, 21, 24
Large-scale parameters, all values assumed		
Host plant in arable, *y*	Proportion (–)	*typically* 0·5
Maize cropping, *z*	Proportion (–)	*typically* 0·2
Utilization rate, *v*	Proportion (–)	*assumed worst-case* 0·8
Physical effects, *x*	Proportion (–)	*typically* 0·5
Temporal coincidence, *a*	Proportion (–)	*typically* 0·5
Large-scale exposure, *L*	Proportion (–)	Product of *yzvxa*; typically *c*. 0·02, but could range from 0·001 to 0·125

within the period of pollen shed. By globally, we mean averaged over an entire landscape or regional scale and over a whole growing season. However, in this study, we report local estimates of mortality which are worst-case values, before any allowance for exposure effects occurring at larger scales that may reduce mortality. Additionally, the values of the large-scale parameters are subject to a wide range of influences occurring at landscape levels and are therefore environment specific.

Derivation of the model begins with a laboratory-derived bioassay relationship in which logit-transformed probability of mortality, *P*, is regressed on logarithmically transformed dose, *d*. The mortality–dose relationship assumed here has the same slope, 2·473, as that assumed by Perry *et al.* (2010) (see Appendix S1, Supporting information), so

$$\mathrm{logit}(P) = \alpha + 2{\cdot}473 \log_{10} d. \qquad \text{eqn 1}$$

The intercept, α, in eqn 1 is determined by the sensitivity of the species to the Cry1F protein, expressed through *m*, the LC50, for which logit(*P*) = 0. There are five LC50 values considered here (Wolt, Conlan & Majima 2005 and see Table 1), which form a geometric series with 11·4-fold increments: *m* = 1·265, 14·36, 163·2, 1853 and 21 057. These represent values for the larvae of five hypothetical species with differing sensitivities, denoted, respectively, as 'worst-case, extreme', 'very high', 'high', 'above-average' and 'below-average'; the rationale for these values is provided in Appendix S3 (Supporting information). The mortality–dose relationship above is then integrated with a field-derived regression of logarithmically transformed dose, *d*, on distance, *E*, from the nearest source of the pollen: $\log_{10} d = 2{\cdot}346 - 0{\cdot}145E$, to derive a linear mortality–distance relationship for mortality of larvae in the margin, on the logit scale. Backtransformed to the natural scale, the estimated probability of mortality, *g*(*E*), for a larva at distance *E* into the margin from the nearest source of pollen at the edge of the field (Fig. 1), is given by:

$$g(E) = \exp(-0{\cdot}35853E)/[\beta + \exp(-0{\cdot}35853E)], \qquad \text{eqn 2}$$

wherevalues of β for different sensitivities are calculated as: 0·003893, extreme; 0·05290, very high; 0·7190, high; 9·774, above-average; and 132·9, below-average (Fig. 2). The estimated probability of mortality, *h*, for a larva within the *Bt* crop (Fig. 1) is calculated from:

$$h = 2{\cdot}757g(0) = 2{\cdot}757/(\beta + 1) \quad \text{(also see Fig. 2).} \qquad \text{eqn 3}$$

The relative contribution towards overall estimated mortality in crop and margin is proportional to the product of two quantities. The first is the number of larvae within each component, which is itself the product of the density of larvae and the area represented in that component, where the larval density is assumed to be proportional to the host-plant density in each component. The second is simply the estimated mortality rate for an individual larva within that component. Overall mortality depends on the magnitude of parameters *f* and *e* relative to one another, and not on their absolute values.

When there is mitigation ($w > 0$), similar calculations are used, but mortality calculated for larvae in the margin must use an appropriate value of *E* calculated to allow for the fact that the *Bt*-maize is a distance *w* metres further away (e.g. larva S in Fig. 1). Mortality for larvae within the non-*Bt* maize is also calculated using eqn 2 for *g*(*E*), again with an appropriate value of *E* (e.g. larva R in Fig. 1).

The local, worst-case estimates of mortality from the small-scale parameters used earlier may then be modified to allow for large-scale exposure effects. These are represented by the five large-scale parameters listed in Table 1 together with their chosen values; the rationale is provided in Appendix S4 (Supporting information). Estimates of global estimated mortality are calculated by multiplying each estimated local mortality rate by the value *L*, the product of parameters *yzvxa* (Perry *et al.* 2010).

Results

Estimated local percentage mortality was calculated for the nine values of the non-*Bt* maize strip width, *w*, for an individual larva in each of the three components of the spatial arena,

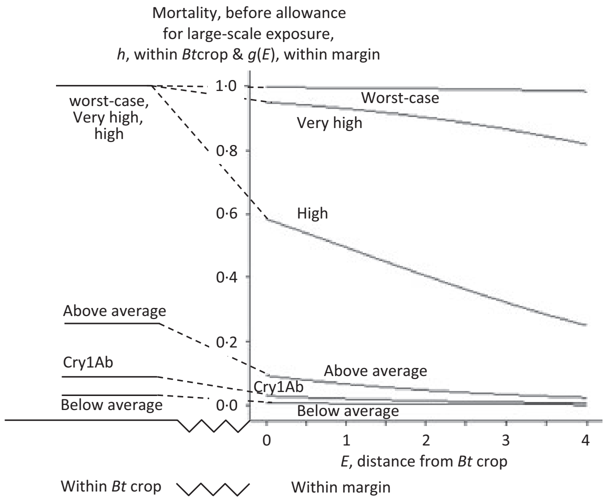

Fig. 2. Probability of mortality for an individual lepidopteran larva exposed locally to Cry1F protein expressed in the pollen of *Bt*-maize event 1507, within the maize crop (denoted h and assumed constant within the crop), and at a particular location within a margin [denoted $g(E)$] where mortality depends upon E, the distance of the larva from the *Bt*-maize. Mortality is worst case, assuming no mitigation ($w = 0$) and before allowance for effects of large-scale exposure. Mortality is shown for five different sensitivities to Cry1F, expressed as LC50 values for maize 1507, in pollen grains per cm^2: 21 057 'below average' sensitivity; 1853 'above average'; 163·2 'high'; 14·36 'very high'; 1·265 'worst-case extreme' sensitivity. Mortalities are calculated from eqns 2 [for $g(E)$] and eqn 3 (for h), but illustrated only for $E = 0$–4. For the range of sensitivities above, the value of $g(E)$ declines to $< 0{\cdot}05$ for values of E, respectively, greater than 0, 2, 10, 17 and 24 m, and declines to $< 0{\cdot}01$ for E greater than 0, 7, 14, 22 and 29 m. For mitigation ($w > 0$), values of E must be adjusted appropriately in eqn 2 (see Fig. 1), but mortality within non-*Bt* maize and margin may still be calculated from $g(E)$. Also shown, for comparison, is the corresponding relationship for exposure to Cry1Ab protein of maize event MON810 (Perry *et al.* 2010) for the LC50 value of 5800 pollen grains per cm^2 estimated for the butterflies *Inachis io* and *Vanessa atalanta*.

before allowance for large-scale exposure, and for each of the five different sensitivities, for a host-plant within-crop density of $e = 0{\cdot}01$; results are displayed in Fig. 3. For $e = 0$, there are no host plants, and therefore, no larvae is assumed to be exposed in either the *Bt* or non-*Bt* components of the crop and therefore, no mortality; estimated individual larval mortality in the margin is unaffected and has the same values as shown in Fig. 3 for $e = 0{\cdot}01$. For $e = 0{\cdot}01$, as expected, mortality is always greatest, for any particular width of strip, for the field interior 'within *Bt*-maize' component, next greatest for the non-*Bt* maize component and least for the margin, representing the dependence of mortality on distance from the *Bt* crop. Furthermore, the mortality of larvae within the *Bt*-maize field interior is assumed to be unaffected by the presence or absence of the non-*Bt* maize strips, so estimates in Fig. 3a for the field component 'in *Bt* crop' are unaffected by the value of w. Mitigation always depresses mortality in the field margin, and the degree of this depression is dependent directly on the strip width, w. However, the efficacy of the mitigation is also clearly dependent on the field component and sensitivity of the species, with the greatest proportional reductions in mortality observed for the margin and for species with lower sensitivities.

After weighting the values above by the appropriate expected number of host plants in each component, the estimated overall mortality in the spatial arena is shown in Table 2, for $e = 0$ and $e = 0{\cdot}01$. For the scenario $e = 0{\cdot}0$, there are, by assumption, no host plants in the field and therefore no contribution towards mortality from the 'in *Bt* crop' or the 'non-*Bt* crop' components. The entries in Table 2 are therefore identical to those for the margin component. For the scenarios with $e = 0{\cdot}01$, the density of host plants is much greater in the margin than within the crop interior (×75-fold). However, by contrast, the interior crop area is much larger than the margin (×48-fold, when $w = 0$), and as $h > g(E)$, the overall mortality is always greater than the mortality in the margin,

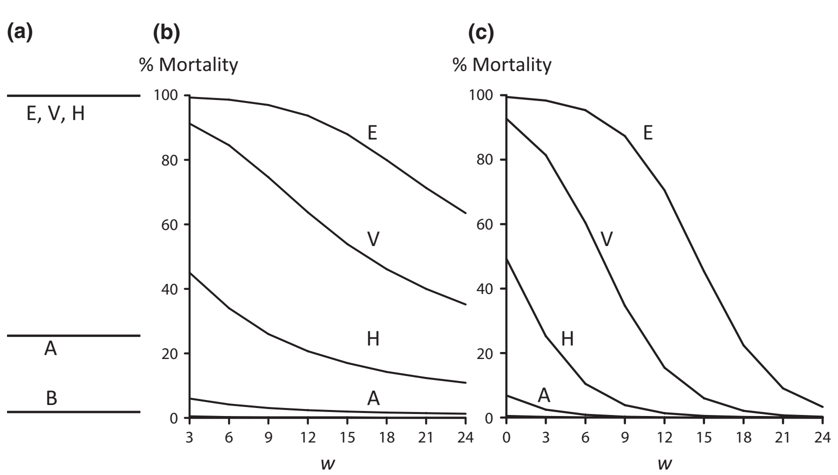

Fig. 3. Estimated local percentage mortality (y-axis) calculated for nine values of the non-*Bt* strip width, w (x-axis), for an individual larva in each of the three components of the spatial arena, before allowance for large-scale exposure, and for each of the five different sensitivities (B, below-average; A, above-average; H, high; V, very high; E, extreme), for a host-plant within-crop density of $e = 0{\cdot}01$. (a) Mortality within the *Bt* maize component, which is unaffected by the value of w; (b) mortality within the non-*Bt* maize component (undefined for $w = 0$); (c) mortality within the margin component. For (b) and (c), the mortality for below-average sensitivity is not labelled on the graphs because it is $< 0{\cdot}5\%$ for all values of w.

Table 2. Estimated local percentage mortality over entire field (*Bt*-maize, non-*Bt* maize and margin), before allowance for large-scale exposure, for the five different sensitivities and the nine non-*Bt* maize strip widths, for a crop with no host-plants ($e = 0$) and for a moderate within-crop host-plant density ($e = 0{\cdot}01$)

	Sensitivity (LC50 pollen grains per cm^2)									
	21 057 (below average)		1853 (above average)		163·2 (high)		14·36 (very high)		1·265 (extreme)	
w, Non-*Bt* strip width	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$
0	0·5	1·1	6·9	14·2	49·3	69·1	92·7	95·6	99·4	99·6
3	0·2	0·9	2·5	11·3	25·3	53·8	81·4	88·6	98·3	99·0
6	0·1	0·8	0·9	10·0	10·5	43·9	60·4	75·5	95·3	97·1
9	0·0	0·8	0·3	9·4	3·9	38·8	34·7	59·3	87·3	92·2
12	0·0	0·7	0·1	9·0	1·4	36·2	15·5	46·9	70·5	81·7
15	0·0	0·7	0·0	8·7	0·5	34·6	6·0	40·0	45·4	66·1
18	0·0	0·7	0·0	8·4	0·2	33·3	2·1	36·7	22·4	51·4
21	0·0	0·6	0·0	8·1	0·1	32·1	0·7	34·7	9·1	42·3
24	0·0	0·6	0·0	7·8	0·0	31·0	0·3	33·4	3·3	37·8

sometimes considerably so (Fig. 2). For species with high or greater sensitivity, the estimated mortality is considerably greater than was the case for the three lepidopteran species in the model of Perry *et al.* (2010) for the Cry1Ab protein.

However, the most striking result revealed in Table 2 is that the model predicts that the efficacy of mitigation proposed depends critically on the density of host plants within the crop. When there is near absence of host plants in the crop ($e = 0{\cdot}0$), there is a considerable reduction in mortality because of mitigation for each increment in the width of the non-Bt maize strips. For example, for the 'high'-sensitivity category, mitigation with 3-m-wide strips halved predicted mortality compared with no mitigation. By contrast, for a host plant density of $e = 0{\cdot}01$, strips of width 6, 12 and 21 m would be required to reduce predicted local mortality below 50% for the high, very high and extreme sensitivity categories, respectively. For these categories, even 24-m-wide strips would not reduce predicted mortality below 30%. This occurs because the mortality within the *Bt*-maize crop is estimated to be almost 100% and this is unaffected by mitigation; overall mortality is therefore only limited by those larvae that survive outside the *Bt* crop. Therefore, as w increases, mortality approaches a sizeable lower asymptote, and increased levels of mitigation have little further effect.

To allow for the large-scale effects of exposure, each of the overall worst-case local mortality rates in Table 2 would be reduced by multiplication with L. Here, the large-scale parameter values described in Table 1 would give a value of about $L = 0{\cdot}02$. However, there is considerable variability in the parameter L, particularly between species. L could easily be as small as 0·005 or as large as 0·125. Table 3 shows, for a range of four values of L, how estimates of the local mortality in Table 2 translate to estimates of global mortality. For a typical value of $L = 0{\cdot}025$ with full mitigation ($w = 24$), the estimated global mortality rate could be reduced to $< 1\%$, even for species of extreme sensitivity. For a worst-case value of $L = 0{\cdot}125$, species of above-average sensitivity are predicted to suffer global mortality $> 1\%$ if there is no mitigation (Table 3).

Discussion

If the assumptions of this model are correct, then the estimated mortality of non-target lepidopteran larvae because of Cry1F pollen may be substantial in the field. Mitigation by appropriate management is possible, but its efficacy depends sensitively on within-crop host-plant density, itself a function of agricultural management (Meissle *et al.* 2010) and weed ecology. For example, *Verbascum* spp., host plants of the butterfly *Melitaea trivia* Den. & Schif. which is considered near-threatened within the EU-27 countries (Van Swaay *et al.* 2010), is rare in agricultural landscapes (Fried *et al.* 2009, see further examples in Appendix S2, Supporting information).

For maize, weed control to prevent competition with early growth of the crop is crucial for good crop establishment (whether or not it is a *Bt*-maize variety) and this can limit host-plant availability to non-target lepidopteran larvae. If the host-plant population is relatively large within the crop, then there is a complex trade-off for larvae between the advantage of more habitat (host plants) potentially leading to a higher population, and the disadvantage of a greater mortality within the crop than elsewhere. The resolution of this trade-off may depend on the extent to which host-plant habitat is limiting. However, where it is likely that there are few or no host plants within the crop and host plants occur in the field margins, the conclusion is clear that mitigation could potentially reduce mortality in species with above-average sensitivity to the Cry protein. By contrast, where there are higher numbers of host plants and larvae in the field at anthesis, then other measures to restrict within crop exposure to *Bt* pollen would be required, such as reducing the proportion of *Bt* crops in the landscape.

The sowing of strips of non-*Bt* maize around field edges is not the only form of mitigation possible, but is similar to some field-margin management used in agri-environment schemes in Europe for reversing biodiversity declines in agricultural landscapes (Donald & Evans 2006). How practical the sowing of strips of non-*Bt* maize around field edges is for a grower depends on the other management and conservation practices

Table 3. Estimated global percentage mortality over entire field (*Bt*-maize, non-*Bt* maize and margin), after allowance for four different levels of large-scale exposure effects ($L = 0{\cdot}001, 0{\cdot}001, 0{\cdot}005, 0{\cdot}025, 0{\cdot}125$), for the five different sensitivities, and for full ($w = 24$) and no ($w = 0$) mitigation with strips of non-*Bt* maize, for a crop with no host-plants ($e = 0$) and for a moderate within-crop host-plant density ($e = 0{\cdot}01$). Values larger than 1% are shown in bold type

		Sensitivity (LC50 pollen grains per cm^2)									
		21 057 (below average)		1853 (above average)		163·2 (high)		14·36 (very high)		1·265 (extreme)	
L	Mitigation	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$	$e = 0{\cdot}0$	$e = 0{\cdot}01$
0·001	None	All values < 0·1									
	Full										
0·005	None	< 0·1	< 0·1	< 0·1	< 0·1	0·2	0·3	0·5	0·5	0·5	0·5
	Full	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	0·16	< 0·1	0·17	< 0·1	0·19
0·025	None	< 0·1	< 0·1	0·2	0·4	**1·2**	**1·7**	**2·3**	**2·4**	**2·5**	**2·5**
	Full	< 0·1	< 0·1	< 0·1	0·2	< 0·1	0·8	< 0·1	0·8	< 0·1	0·9
0·125	None	< 0·1	0·1	0·9	**1·8**	**6·2**	**8·6**	**11·6**	**12·0**	**12·4**	**12·5**
	Full	< 0·1	< 0·1	< 0·1	**1·0**	< 0·1	**3·9**	< 0·1	**4·2**	0·4	**4·7**

on the farm and in the region. Additional management factors to consider for GM *Bt* crops include the provision of refugia for the target pest (MacIntosh 2010) and isolation measures for coexistence requirements (Messéan *et al.* 2009). One alternative approach might seek to optimize benefits from habitat creation (POST 2010) for specific non-target lepidopteran species of conservation concern. The sowing of appropriate host-plant seed into the field margins would increase the ratio of host-plant density in the margin relative to that in the *Bt* crop and thereby improve the efficacy of mitigation. Such a form of agri-environmental stewardship (Sotherton 1991) has already proved effective in helping to maintain farmland biodiversity (Cordeau, Reboud & Chauvel 2010).

Whether mitigation is required is a decision for regionally based risk managers considering local conservation objectives. Our study relates to one specific component of mortality at a defined point in the lepidopteran life cycle, and these estimates should be placed into context using life-table data. Small declines in lepidopteran populations are difficult to detect in practice (Aviron *et al.* 2009) because of the natural fluctuations and trends in lepidopteran populations. Nevertheless, an estimate of local mortality represents an important indicator, which is unhampered by the additional component of uncertainty inherent in larger-scale parameters.

The optimal management strategy for mitigation will depend on the arable ecosystem concerned, which affects both small- and large-scale parameters. Regarding small-scale parameters, for arable ecosystems such as some of those in southern and central Europe (see Appendix S2, Supporting information), it may be reasonable to assume that within-crop host plants are almost completely absent at the time of anthesis, although for others such as the Po Valley in Italy such an assumption would be wrong. If this assumption is valid, then mitigation as described here with $w = 24$ m strips is recommended, as it would reduce estimated mortality locally below 4% for all species, even for the extreme cases. By contrast, for a host-plant density that may be moderate for arable ecosystems such as some of those occurring towards the north and west of Europe ($e = 0{\cdot}01$, see Appendix S2, Supporting information), such mitigation could not, for a considerable proportion of species, reduce local mortality rates to less than about one-third. Whether the resulting global mortality rates (Table 3) would be deemed acceptable depends critically on the value of the parameter L and also on local protection goals. In some cases, it might be necessary to restrict the cultivation of *Bt*-maize crops that express a relatively large amount of lepidopteran-specific Cry protein in their pollen, such as 1507, in these arable ecosystems. Alternatively, isolation distances may be required from neighbouring areas where there are identified Lepidoptera of conservation concern.

Our conclusions attempt to balance a precautionary viewpoint, which endeavours not to underestimate likely mortality and its consequences (Perry *et al.* 2011), with a pragmatic approach that accounts for natural fluctuations in populations and for normal pest management within the maize ecosystem (see Beringer 2000 and Marvier *et al.* 2007). Wolfenbarger *et al.* (2008) viewed *Bt* crops as a tool for integrated pest management with resulting environmental impact on non-target organisms that depends on the management applied within agricultural production systems. Clearly, there may be benefits in reduced insecticide use in *Bt* crops compared with non-*Bt* crops (James 2010), but the ecological effects of insecticide management within *Bt*-maize systems raise complex issues of trade-off (Brookes 2009) that are beyond the scope of this study. First, *Bt* uptake is higher in regions where insecticides are more heavily used. Second, it is uncertain that farmers who do not currently use insecticides would not use *Bt*; conversely, *Bt* farmers may still use insecticides. Finally, conventional insecticides are usually applied at different times to pollen dehiscence and thus, affect different species or developmental stages.

Of course, there are considerable uncertainties in predictions from any ecological model (Gray 2004). For simplicity, this study does not specifically estimate sub-lethal effects, for which predictions would be highly uncertain. Sub-lethal effects may well occur with Cry1F, and alone are capable of driving a population to extinction (Hallam, Canziani & Lassiter 1993). However, to place this into context, the additional mortality because of sub-lethal effects is likely to be small compared with that because of increases in the level of sensitivity of the magnitude considered here.

Other issues not covered in this study are bimodal distributions of field margins; effects of aggregated pollen deposition (Perry *et al.* 2010); and dilution effects (see Appendix S5, Supporting information).

Estimates of mortality in the model of Perry *et al.* (2010) were most sensitive to the value chosen to represent the slope of the logit regression of mortality on dose from laboratory bioassays (see Perry *et al.* 2011). The predictions of this model are also highly sensitive to this parameter (eqn 1). At this stage, we cannot predict whether future, more accurate estimates of this slope parameter from bioassays will alter the conclusions from this study. Unpublished data suggest that the parameter value chosen here is satisfactory for neonate larvae of *Vanessa cardui* L. However, it is clear that information is required for other Lepidopteran species as such slope estimates are equally necessary as those of LC50 values; they should be routinely calculated and reported, together with estimates of their variability.

Our results that show that mortality may be sensitively dependent on host-plant density have implications for management recommendations for *Bt*-maize plants expressing the Cry1F protein stacked with a herbicide-tolerance trait. Weed management for these maize crops should consider carefully the value of weeds within fields to higher trophic taxa (Firbank *et al.* 2003), as they will help to sustain diverse arthropod communities, and should integrate the timing and amounts of herbicide applied with the management requirements of the *Bt* component of the crop. Such crops will allow different weed-management practices to be used, having differential effects on weed diversity and abundance. They will therefore impact on biodiversity in general and the abundance of host plants of non-target lepidoptera in particular. This exemplifies how interactions between biota may occur when different traits are stacked irrespective of interactions between the proteins themselves, and highlights the importance of accounting for crop management (EFSA 2010) in the assessment of the ecological impact of GM plants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Detailed description of the model with equations and parameters.

Appendix S2. Background material considered which aided the choice of the small-scale parameters: C, D, e, f, and w.

Appendix S3. Background material considered which aided the choice of parameters governing the range of sensitivities of species.

Appendix S4. Background material considered which aided the choice of the large-scale parameters: y, z, v, x, a and therefore of their product, L.

Appendix S5. Background material considered concerning potential dilution effects.