

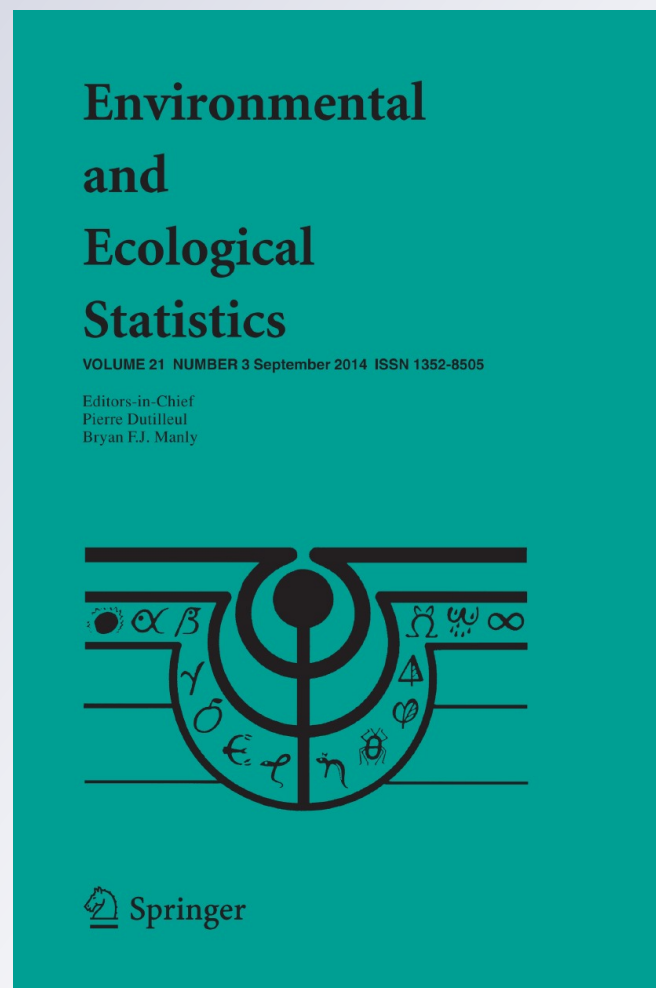
A note on some mathematical models on the effects of Bt-maize exposure

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A note on some mathematical models on the effects of Bt-maize exposure

Francesco Camastra · Angelo Ciaramella · Antonino Staiano

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Abstract Some mathematical models for the estimation of the effects of Cry1Ab and Cry1F Bt-maize exposure in the biodiversity are examined. Novel results about these models are obtained and described in this note. The exact formula for the proportion of population that suffers mortality exposed either to Cry1Ab or Cry1AF pollen is derived. Moreover, regarding Cry1F pollen effects, the species sensitivity of Lepidoptera is discussed.

Keywords Bt-maize · Cry1Ab · Cry1F · Mathematical model · Non-target Lepidoptera · Risk assessment

1 Introduction

In the last years, the cultivation of Genetically Modified Plants (GMP) is very widespread in the world, in particular in America and Asia. On the other side, the debate in the scientific community and in the public opinion about the GMP effects is becoming harder and harder (Mendelsohn et al. 2003; Banks et al. 2008; Sanvido et al. 2011). It is our belief, that one of the causes of the debate depends on the lack of available math-

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ematical models that allow to assess quantitatively the effects of the GMP cultivation on the biodiversity. To this purpose, recently appeared some approaches that discuss the effects of Bt-maize (Felke et al. 2011; Meissle et al. 2011; Szekacs et al. 2012; Yu et al. 2011), and two mathematical models for estimating the effects of Cry1Ab and Cry1F Bt-maize (Perry et al. 2010, 2012), on non-target Lepidoptera. To the best of our knowledge (Lang et al. 2011; Perry et al. 2011), these latter models are the first and the unique mathematical models that assess quantitatively the effects of Bt-crop on the biodiversity. For this reason, we have analyzed in detail the models in (Perry et al. 2010) and (Perry et al. 2012), obtaining novel results. The work is organized as follows: in Sect. 2 the model for the estimation of Cry1Ab Bt-maize effects is discussed and the exact formula for the proportion of population that suffers mortality P is derived; in Sect. 3 the model for the estimation of Cry1F is analyzed; finally, some conclusions are drawn in Sect. 4.

2 Estimation of Cry1Ab Bt-maize effects

Perry et al. (2010) developed a mathematical model for estimating the effects of exposure of three non-target Lepidoptera (e.g., *Inachis io*, *Vanessa atalanta*, *Plutella xylostella*) to Bt-maize pollen expressing the protein Cry1Ab. They computed the estimated proportion of population of a non-target Lepidoptera that suffers mortality, hereinafter denoted by P for convenience. P is given by

$$P = \frac{yzvxa(25eh\sqrt{C} + fD\mu)}{(25e\sqrt{C} + fD)}, \quad (1)$$

where the parameters x (physical effects), a (temporal coincidence), z (maize cropping), v (utilization rate), y (host plant in arable), e (host plant within-crop), f (host plant in margin), C (size of maize fields), D (width of margin) are experimentally measured or taken by the literature; whereas the parameters h (within-crop mortality) and μ , depending on D , have to be properly computed. Following Perry et al., before computing μ , it is necessary to recall the so-called *margin mortality* $g(E)$, where E denotes the distance from the edge of the crop, that is given, for larvae of *Inachis io* and *Vanessa atalanta*, by

$$g(E) = \frac{\exp(-0.359E)}{33.25 + \exp(-0.359E)}; \quad (2)$$

whereas for larvae of *Plutella xylostella* is given by

$$g(E) = \frac{\exp(-0.349E)}{55.33 + \exp(-0.349E)}. \quad (3)$$

That being said, Perry et al declared that μ is obtained by averaging the value of $g(E)$ over the margin. In practice, μ is obtained by the numerical integration of Eqs. (2) or (3), between the values $E = 0$ and $E = D$. In this way, they obtained

an approximate estimate of μ and, consequently of the proportion of population that suffers mortality P . Not having an exact formula to express μ , and hence P , in function of D is a disadvantage; for efficient and rapid prediction of mortalities an exact solution is preferable. We derive such an exact solution in the next section.

2.1 The exact proportion suffering mortality formula

Now, we show that an exact value for μ and hence P can be derivable. The parameter μ is given by

$$\begin{aligned} \mu &= \frac{1}{D} \int_0^D g(E) dE \\ &= \frac{1}{D} \int_0^D \frac{\exp(-\gamma E)}{\delta + \exp(-\gamma E)} dE, \end{aligned} \tag{4}$$

where γ and δ are 0.359 and 33.25 for the larvae of *Inachis io* and *Vanessa atalanta*, whereas are 0.349 and 55.33 for the larvae of *Plutella xylostella*.

The integral of Eq. (4) can be solved exactly:

$$\begin{aligned} \mu &= \frac{1}{D} \int_0^D \frac{\exp(-\gamma E)}{\delta + \exp(-\gamma E)} dE \\ &= -\frac{1}{\gamma D} [\ln(\delta + \exp(-\gamma E))]_0^D \\ &= \frac{1}{\gamma D} \left[\ln \frac{1 + \delta}{\delta + \exp(-\gamma D)} \right]. \end{aligned} \tag{5}$$

Now, plugging last formula in Eq. (1), we obtain the exact formula for computing the proportion of population that suffers mortality P

$$P = \frac{yzvxa \left\{ 25eh\sqrt{C} + \frac{f}{\gamma} \ln \frac{1+\delta}{\delta+\exp(-\gamma D)} \right\}}{25e\sqrt{C} + fD}. \tag{6}$$

The previous equation allows us to predict exactly, rather than numerically, the proportion of population that suffers mortality P in terms of the width of the margin D , and thus to provide a tool for more rapid risk assessment.

We pass to study the dependence on D of the proportion P . When $D = 0$, i.e., when the non-target Lepidoptera is on the crop edge, P becomes:

$$P(0) = yzvxah, \tag{7}$$

and takes the maximum value as $P(D)$ is a monotonic decreasing function. Since the following equation is fulfilled

$$\lim_{D \rightarrow +\infty} \frac{P(D)}{1/D} = \frac{1}{f} yzvx a \left(25eh\sqrt{C} + \frac{f}{\gamma} \ln \frac{1+\delta}{\delta} \right) \quad (8)$$

then $P(D) = O(1/D)$ holds, namely $P(D)$ decreases asymptotically, i.e., for adequately large D , as fast as $1/D$. Hence for very large D , $P(D)$ is negligible, since $\lim_{D \rightarrow +\infty} P(D) = 0$ and its order is $1/D$.

3 Estimation of Cry1F Bt-maize effects

Perry et al. (2012) developed a mathematical model for estimating the effects of exposure of five hypothetical non-target Lepidoptera species to Bt-maize pollen expressing the protein Cry1F. In that work the estimated proportion of population of a non-target Lepidoptera that suffers mortality, P , is given by Eq. (1), that for our convenience, we recall:

$$P(D) = \frac{yzvxa(25eh\sqrt{C} + fD\mu)}{25e\sqrt{C} + fD}.$$

The previous equation combines: (a) *small scale parameters*, namely, e, f, C, D ; (b) *large scale parameters*, namely, y, z, x, a ; (c) *mortality parameters*, namely, h (within crop mortality) and μ (average mortality within a margin of any particular width D). We remind to Sect. 2 for the meaning of the parameters. Small and large scale parameters are taken from literature or experimentally measured. The mortality parameters, instead, have to be properly computed, hence in the following the focus will be on the way this is accomplished. Equation (1) is derived for a range of five hypothetical non-target Lepidoptera species rather than a specific one. To this aim, Perry et al introduce a further *mortality parameter*, m , representing a range of species sensitivities for the hypothetical non-target Lepidoptera. Sensitivity is expressed by the LC_{50} values for maize 1507, i.e., the parameter m represents the lethal concentration value that kills on average half of the larvae of the instar considered, measured in pollen grains per cm^2 . The parameter m affects the way h and μ are computed. Let us focus on a step-by-step mortality parameter derivation (Perry et al. 2012). The starting point is a mortality-dose laboratory-derived bioassay relationship in which a logit-transformed probability of mortality, P , is regressed on a logarithmically transformed dose, d :

$$\text{logit}(P) = \alpha + 2.473 \log_{10} d. \quad (9)$$

Here is where the parameter m comes into play. The intercept α is determined by the sensitivity of the species to the Cry1F protein, for which $\text{logit}(P) = 0$. Five sensitivity values, corresponding to five hypothetical species, are considered in Perry et al. (2012) and denoted as *worst-case, extreme* ($m = 1.265$), *very high* ($m = 14.36$),

Table 1 Intercepts, β_0 , for the linear mortality-distance relationships in Eq. (11), corresponding to the considered species sensitivities

Sensitivity	β_0
Extreme	5.5492
Very high	2.9399
High	0.3297
Above-average	-2.2798
Below-average	-4.8901

high ($m = 163.2$), above-average ($m = 1853$), and below-average ($m = 21057$). These mortality-dose relationships are then combined with a field-derived regression of logarithmically transformed dose, d , on distance E , from the nearest source of the pollen:

$$\log_{10} d = 2.346 - 0.145E, \tag{10}$$

to derive a linear mortality-distance relationship for mortality of larvae in the margin, on the logit scale. So doing, for each species sensitivities the mortality-distance relationships are derived, from Eqs. (9) and (10), as

$$\text{logit}(P) = \beta_0 - 0.3586E, \tag{11}$$

where $\beta_0 = \alpha + 5.8017$ and whose values, for each of the considered sensitivities, are shown in Table 1.

Taking the inverse of the logit function, we return to the natural scale thus obtaining 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:

$$\begin{aligned} g(E) &= \text{logit}^{-1}(P) \\ &= \frac{\exp(\beta_0 - 0.3586E)}{1 + \exp(\beta_0 - 0.3586E)} \\ &= \frac{\exp(-0.3586E)}{\beta + \exp(-0.3586E)}. \end{aligned} \tag{12}$$

In Eq. (12), the corresponding values of $\beta = \exp(-\beta_0)$, for each species sensitivity, are listed in Table 2. The mortality parameters h and μ are derived from $g(E)$. Concretely, Perry et al compute μ by numerically integrating $g(E)$, in Eq. (12), between 0 and D , as described in Perry et al. (2010). To this purpose, we remark that Perry et al.'s numerical computation for μ must be replaced (see Sect. 2.1) with the following formula:

$$\mu = \frac{1}{0.3586D} \left[\ln \frac{1 + \beta}{\beta + \exp(-0.3586D)} \right]. \tag{13}$$

Hence, the formula for estimating the proportion of population that suffers mortality $P(D)$ must be replaced with the following formula:

Table 2 Values of β to compute $g(E)$ in Eq. (12), corresponding to the considered species sensitivities

Sensitivity	β
Extreme	0.0039
Very high	0.0529
High	0.7191
Above-average	9.7747
Below-average	132.9669

Table 3 Within-crop mortality parameter h , corresponding to the considered species sensitivities

Sensitivity	h
Extreme	2.7463
Very high	2.6185
High	1.6037
Above-average	0.0928
Below-average	0.0075

The parameter h cannot be viewed as a probability, for extreme, very high and high sensitivities, since its value is larger than 1

$$P(D) = \frac{yzvxa \left(25eh\sqrt{C} + 2.789f \ln \frac{1+\beta}{\beta + \exp(-0.3586D)} \right)}{25e\sqrt{C} + fD}. \tag{14}$$

This formula is similar to Eq. 6 that expresses the mortality proportion of population exposed to Cry1Ab, hence the same mathematical considerations described in Sect. 2.1 hold for Eq. (14).

Having said that, to estimate the probability of mortality, h , for the five hypothetical larvae within the *Bt*-crop, it is necessary to consider that pollen deposition within a maize crop is 2.757 times that at the edge (Perry et al. 2010). Therefore,

$$h = 2.757 g(0) = 2.757 \frac{1}{1 + \beta}. \tag{15}$$

The values obtained for h , for the considered range of sensitivities, are shown in Table 3. It is necessary to remark that the parameter h cannot be viewed as a probability, for extreme, very high and high sensitivities, since its value is larger than 1. To conclude the model overview, two further parameters have to be introduced, i.e., *mitigation parameter* and *large scale exposure parameter* that we do not describe, since they do not affect our discussion.

3.1 Derivation of the species sensitivity parameter

Now we examine the *within crop mortality* parameter, h , and its assumed values listed in Table 3 of Sect. 3. Three out of five h values, corresponding to the *extreme*, *very high* and *high* sensitivities, violate the definition of probability. Recall that *the*

probability always takes on values in between zero and one (Ross 2009), while the above mentioned h values are larger than one. The joint assumptions that pollen deposition within a maize crop is 2.757 times that at the edge, and the considered range for the species sensitivities do not appear to fit the theoretical soundness of the proposed model. The right side of Eq. (15) should be ≤ 1 , hence Eq. (15) defines a probability if and only if $\beta \geq 1.757$. Therefore, more restrictive assumptions on the sensitivity should be made and/or further argumentation should be given concerning with the assumption that pollen deposition within a maize crop is 2.757 times that at the edge. Obviously, the incorrect values of within-crop mortality h , for extreme, very high and high sensitivities are propagated through the model affecting the correctness of the proportion of population that suffers mortality $P(D)$ computed in Eq. (14) and, consequently, of the entire model. However, assuming the above mentioned Perry et al.'s issues are reasonable, a further inequality on the values of sensitivity m can be added to the Perry et al.'s model in order to guarantee that the parameter h can be viewed as a probability. We pass to derive the inequality. By applying the definition of probability, the following relations must hold:

$$h \leq 1 \Leftrightarrow \frac{2.757}{1 + \beta} \leq 1 \Rightarrow \beta \geq 1.757,$$

and, since $\beta = \exp(-\beta_0)$, we get $\exp(-\beta_0) \geq 1.757$. This implies:

$$\begin{aligned} \beta_0 &\leq -\ln 1.757 \\ &\leq -0.5636. \end{aligned} \tag{16}$$

It comes, from Eq. (11), that $\beta_0 = \alpha + 5.8017$ and therefore, $\alpha \leq -6.3653$. Now, from Eq. (9) we get:

$$\text{logit}(P) - 2.473 \log_{10} d \leq -6.353 \tag{17}$$

and setting $\text{logit}(P) = 0$, it follows:

$$\begin{aligned} -2.473 \log_{10} d &\leq -6.3653 \\ \log_{10} d &\geq 2.5739 \\ d &\geq 10^{2.5739} \\ &\sim 374.89. \end{aligned} \tag{18}$$

Therefore, since at LC50, $m = d$ (see supplementary material S1 and S3 in Perry et al. (2012)), the inequality

$$m \geq 374.89 \tag{19}$$

must hold in order to h be a probability.

We conclude the analysis underlining that in Figure 2 of Perry et al.'s work, the values of the parameter h , related to the values of m that do not fulfill the equation

(e.g. high, very high and extreme sensitivity), would seem to be set to 1.0, although it is not declared in the manuscript. However, the procedure of setting to 1 the parameter h for high, very high and extreme sensitivity has no mathematical and statistical justifications.

4 Conclusions

In this work, some mathematical models for the estimation of the effects of Cry1Ab and Cry1F Bt-maize exposure on non-target Lepidoptera have been examined, deriving novel results. Firstly, it is obtained the exact formula for the proportion of population that suffers mortality exposed either to Cry1Ab or Cry1AF pollen. Besides, the species sensitivity of Lepidoptera has been discussed.

We hope that the derived novel results about mathematical models for the estimation of the effects of Cry1Ab and Cry1F Bt-maize exposure on non-target Lepidoptera can contribute to the debate in the scientific community about the GMP effects.

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Francesco Camastra born in Polignano a Mare (Italy) in 1960, holds a M.Sc in Physics at University of Milan in 1985 and a Ph.D in Computer Science at University of Genova in 2004. Since 2006 he has been Assistant Professor with University of Naples “Parthenope”. He is the co-recipient of Pattern Recognition Award 2008 and the winner of Eduardo R. Caianiello Award 2005. He published 45 papers on referred journals and proceedings and coauthored one book. He is member of two journal editorial boards. At present he is involved in risk assessment of genetic modified plants using machine learning approaches.

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