Submitted to Acta Biotheoretica manuscript No.
(will be inserted by the editor)

Role of spatial and temporal refuges in the evolution of pest resistance to toxic crops.

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Abstract Toxic plants have been used for years in agriculture to control major crop pests. However, the continuous exposure of targeted pests to toxins dramatically increases the rate of resistance evolution (Gassmann et al. 2009a, Tabashnik et al. 2008). To prevent or delay resistance, non toxic host plants can be used as refuges. Our study considers spatial and temporal refuges that are respectively implemented concurrently or alternatively a toxic crop. A conceptual model based on impulsive differential equations is proposed to describe the dynamics of the susceptible and resistant pest populations over time. The mathematical study enlightens threshold values of the proportion of the spatial refuge and key parameters that should help to understand evolution of pest resistance to toxic crop.

Keywords impulsive differential equations · population dynamics · pest resistance · toxic plants.

1 Introduction

Integrated pest management (IPM) has been promoted for the last fifty years (Stern et al. 1959). Its main goal is to control pests through the use of different environmental friendly means. Toxic plants, genetically modified or selected for antibiosis properties, produce toxins that are harmful to agricultural pests (Betz et al. 2000). For example, varieties of corn and cotton expressing insecticidal Bt (*Bacillus thuringiensis*) proteins have been widely deployed to control lepidopteran pests (James 2008). Pest resistance is a major concern for the sustainability of such a technology as the Hessian fly for wheat varieties with antibiosis properties (El Bouhssini et al. 2001), the cereal stem borer,

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Busseola fusca, the fall armyworm, Spodoptera frugiperda, and the corn earworm, Helicoverpa zea, respectively evolved field resistance to toxins in transgenic Bt corn and cotton crops (van Rensburg 2007, Tabashnik et al. 2000, Tabashnik et al. 2008, Tabashnik et al. 2009).

In this context, resistance pest management became a major ecological and economical issue (Dale et al. 2002; Gassman et al. 2009b). Strategies to manage pest resistance to toxic crops rely on the availability of refuges of non-toxic host plants that produce susceptible individuals that mate with the rare resistant survivors from toxic crops (Mallet and Porter 1992; Roush 1998).Theoretical work on this topic showed that it is possible to delay the emergence of resistant pests when genes conferring resistance to pests are recessive (Comins 1977). Recessive resistance implies that susceptible homozygous individuals and heterozygous ones have a susceptible phenotype, and only resistant homozygous individuals have a resistant phenotype. Consequently, as resistant individuals mate with susceptible individuals, their progeny is susceptible. Based on this result, refuges have been highly promoted through the "high dose plus refuge" strategy that was implemented for *Bt*-crops in North America (Alstad et al. 1995). The efficiency of this strategy is based on three concepts: low frequencies of resistant genes in natural population, toxin expressed by toxic plants at high level to kill susceptible pests, recessive resistance. Reports of EPA (United States Environmental Protection Agency) (see for example EPA 1998) recommended variable refuge size depending on the considered crop: for example a refuge of 5% of the whole crop if no insecticidal sprays are used, growing to 20% if insecticidal sprays are applied, are recommended for *Bt*-cotton crop.

Different mathematical models have been proposed to describe the evolution of the frequency of the different genotypes and/or phenotypes (Cerda and Wright 2004; Alstad and Andow 1995; Caprio 2001) or to reflect the environmental consequences of the propagation of pest resistance, using genetically structured population models or not (Hillier and Birch 2002a, 2002b; Tyutyunov et al. 2008; Medvinsky et al. 2004; Medvinsky et al. 2007). All these studies highlight some key factors in the evolution of resistance: *net* population growth rate, high expressed toxin level by toxic plants and the presence of spatial and/or temporal refuges.

In this paper, a mathematical model based on semi-discrete/impulsive differential equations (Mailleret and Lemesle 2009) is proposed to address the evolution of pests resistance. We focus on the influence of temporal and spatial refuges on the evolution of pest resistance. We describe the rotation of a crop composed of a mixture of toxic and non toxic plants (spatial refuge) with a completely non toxic crop (temporal refuge). On each cropping period, the dynamics of pests with susceptible phenotype, *i.e.* susceptible homozygous and heterozygous individuals, and those with resistant phenotype, *i.e.* resistant homozygous individuals, are detailed. These dynamics are modelled in the simplest manner to highlight the key factors involved in the evolution of pest resistance. In the present study, we present model equations along with mathematical analysis and modelling assumptions. Simulation results are discussed in the light of pest resistance management.

2 Mathematical modelling and analysis

We consider a cropping system which consists in the rotation of two different types of crops along the time. Within a cropping season, two time periods are described:

during the first one (length T_1 time units), a crop composed of a mixture of toxic and non-toxic plants is grown, while, during the second (length T_2), only non-toxic plants are cultivated. The former and latter plant types are not necessarily the same and the two periods length T_1 and T_2 may be different as well. At the end of each time period, the crops are harvested. This kind of agriculture is typical of some sub-saharan Africa cropping systems that grow successively cotton plants (possibly a mixture of non-toxic and toxic *Bt* ones) and food crops in irrigated perimeters (Nibouche et al. 2007).

Assuming that space is homogeneous, our model describes the dynamics of resistant and susceptible pest populations in response to changes in agricultural lanscape. During each cropping period we model the pests' dynamics continuously in time, while we assume that between cropping periods processes, harvests and plants change, may be considered as discrete events. Moreover, some susceptible pests may immigrate into the system between two consecutive cropping sesons (and not period). We model this immigration as a discrete inter-season event. The next cropping season is assumed to follow the same pattern.

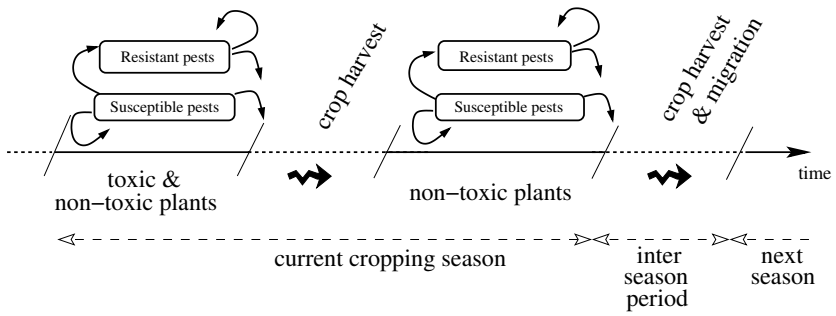

Fig. 1 Description of the cropping system with respect to time as well as the interactions and dynamics of resistant and susceptible pests. Details on the different phenomena are given in the text.

Susceptible and resistant pests have different growth and death rates depending on the host plant. Although the first part of our analysis does not require these hypotheses, it remains important to keep in mind these biological mechanisms. As a consequence of plant toxicity, susceptible pests have a higher *net* rate of increase, *i.e.* the difference between growth and death rate, on non-toxic plants than on toxic plants. Conversely, resistant pests have a lower *net* rate of increase on non-toxic plants than on toxic plant. This property is linked to the fact that resistant pests are actually poorly adapted to non-toxic plants compared to susceptible ones so that the development of resistance penalize their growth on non-toxic host plants (see *e.g.* (Bird and Akhurst 2007) for cotton bollworm and (Betz et al. 2000) for more general topics).

We model the apparition of resistant pests as a continuous random process: a (small) fraction ϵ of the susceptible pests' progeny is assumed to be resistant to toxic plants. In the following, we refer to ϵ as the mutation rate. The pests' resistance is supposed to be recessive so that the resistant pest progeny is also resistant. This latter hypothesis is consistent with the resistance mechanism observed on *Bt* toxic plants (Gould 1998).

Let $t_k \in [0, T_1 + T_2]$ be the time elapsed since the beginning of the k-th cropping season of the system ($k = 1, 2, \dots$). Denote with N_s and N_r the densities of susceptible and resistant pests at time t_k

Consider a first cropping period of the season, *i.e.* $t_k \in [0, T_1)$, and let $\rho \in [0, 1]$ be the proportion of toxic plants that were sowed together with $(1 - \rho)$ non-toxic plants. Within this period and assuming linear pests' growth and death, we get:

$$\begin{aligned} \dot{N}_s &= (1-\rho)\left[(1-\epsilon)g_{P_1} - m_{P_1}\right] N_s + \rho\left[(1-\epsilon)g_{R_1} - m_{R_1}\right] N_s, \\ \dot{N}_r &= (1-\rho)\epsilon g_{P_1} N_s + \rho\epsilon g_{R_1} N_s + (1-\rho)\left[\tilde{g}_{P_1} - \tilde{m}_{P_1}\right] N_r + \rho\left[\tilde{g}_{R_1} - \tilde{m}_{R_1}\right] N_r, \end{aligned} \tag{1}$$

for all $t_k \in [0, T_1)$ and with dot standing for the derivative with respect to t_k. The first and second terms in the $\dot{N}_s$ equation represent the growth and death rates on non-toxic plants (in proportion $(1 - \rho)$) and on toxic plants (in proportion ρ), respectively. g_{P_1} and m_{P_1} (*resp.* g_{R_1} and m_{R_1}) are the intrinsic growth and mortality rates of the susceptible pests on the non-toxic (*resp.* toxic) plants. Since only a proportion $(1 - \epsilon)$ of the susceptible pests' progeny is susceptible, the susceptible pests' intrinsic growth rates are multiplied by $(1-\epsilon)$. The first and second terms in the $\dot{N}_r$ equation represent the proportion ϵ of the susceptible pests progeny that evolved resistance. The two last terms represent resistant pests' growth (at rates $\tilde{g}_{P_1}$ and $\tilde{g}_{R_1}$) and mortality (at rates $\tilde{m}_{R_1}$ and $\tilde{m}_{P_1}$) on non-toxic and toxic plants, respectively. Since the resistant pests progeny is also resistant, no term needs multiply the resistant pests' growth rates.

For the sake of clarity, we re-arrange the terms so that the first cropping period model can be rewritten:

$$\begin{aligned} \dot{N}_s &= (1-\epsilon)g_s(\rho)N_s - m_s(\rho)N_s, \\ \dot{N}_r &= \epsilon g_s(\rho)N_s + g_r(\rho)N_r - m_r(\rho)N_r, \end{aligned} \tag{2}$$

for all $t_k \in [0, T_1)$.

At the end of the first cropping period ($t_k = T_1$), crop harvest occurs, what causes also a fraction of the pests to be removed from the system. Following (Ives et al. 2000) and (Nundloll et al. 2008), we model crop harvest as a discrete event:

$$\begin{aligned} N_s(t_k = T_1) &= (1-p_1)N_s(t_k = T_1^-), \\ N_r(t_k = T_1) &= (1-p_1)N_r(t_k = T_1^-), \end{aligned} \tag{3}$$

with $p_1 \in [0, 1)$ the proportion of pests eliminated by the harvest and T_1^- denoting the instant right before harvest.

The pests' dynamics during the second cropping period are modelled in a similar manner than in the first cropping period, apart from the fact that only non-toxic plants are grown. We have:

$$\begin{aligned} \dot{N}_s &= (1-\epsilon)g_{P_2}N_s - m_{P_2}N_s, \\ \dot{N}_r &= \epsilon g_{P_2}N_s + \tilde{g}_{P_2}N_r - \tilde{m}_{P_2}N_r, \end{aligned} \tag{4}$$

for all $t_k \in [T_1, T_1 + T_2]$. In these equations, g_{P_2} and m_{P_2} are the intrinsic growth and death rates of the susceptible pests, $\tilde{g}_{P_2}$ and $\tilde{m}_{P_2}$, the growth and death rates of the resistant pests. Again, $(1-\epsilon)$ and ϵ describe the proportion of susceptible and resistant pests in the susceptible pests' progeny.

At the end of the second cropping period, crop harvest occurs and an inter cropping season time period follows. During this period, we assume that pests may suffer from mortality within the system, as no more plants are cultivated, but also that new

susceptible pests may also migrate into the system. No resistant pests can migrate into the system because we consider that the plants outside the system are non-toxic, and that therefore pest resistance should not be selected for in the environment (Carrière et al. 2004). Notice however that this assumption may not hold if the environment is composed of toxic crops similar to the one we consider here.

We approximate the different phenomena occuring during the inter cropping season, *i.e.* crop harvest - pests mortality - potential susceptible pests immigration, as a discrete process such that the pests densities at the beginning of the $(k+1)$-th cropping season depend on the pests densities at the end of the k-th season in the following way:

$$\begin{aligned} N_s(t_{k+1}=0) &= (1-p_2)N_s(t_k=(T_1+T_2))+i_s, \\ N_r(t_{k+1}=0) &= (1-p_2)N_r(t_k=(T_1+T_2)), \end{aligned} \tag{5}$$

with $p_2 \in [0,1)$ the proportion of pests eliminated by harvest and natural mortality between the cropping seasons and i_s the number of susceptible pests that have migrated into the crop.

In the rest of the paper, we denote by $(\mathcal{M})$ the complete semi-discrete/impulsive model (Mailleret and Lemesle 2009) composed of the sub-models (2), (3), (4) and (5). We have the following result for model $(\mathcal{M})$.

Proposition 1
Model $(\mathcal{M})$ possesses a periodic orbit if and only if $\rho_{min} < \rho < \rho_{max}$ with

$$\rho_{min} = \frac{((1-\epsilon)g_{P_1}-m_{P_1})T_1+((1-\epsilon)g_{P_2}-m_{P_2})T_2+\ln((1-p_1)(1-p_2))}{((1-\epsilon)g_{P_1}-m_{P_1}-((1-\epsilon)g_{R_1}-m_{R_1}))T_1},$$

$$\rho_{max} = \frac{-(\tilde{g}_{P_1}-\tilde{m}_{P_1})T_1-(\tilde{g}_{P_2}-\tilde{m}_{P_2})T_2-\ln((1-p_1)(1-p_2))}{(\tilde{g}_{R_1}-\tilde{m}_{R_1}-(\tilde{g}_{P_1}-\tilde{m}_{P_1}))T_1}.$$

Moreover, this orbit is globally asymptotically stable on $\mathbb{R}^2_+$.

Remark 1 It is biologically sensible that $(1-\epsilon)g_{P_1}-m_{P_1} > (1-\epsilon)g_{R_1}-m_{R_1}$ and $\tilde{g}_{R_1}-\tilde{m}_{R_1} > \tilde{g}_{P_1}-\tilde{m}_{P_1}$. Indeed the former condition simply means that susceptible pests grow more efficiently on non toxic plants than on toxic plants, and the latter that resistant pests are more adapted to toxic plants than to non-toxic plants and thus grow more efficiently on toxic plants than on non-toxic ones.

Remark 2 The condition $\rho_{min} < \rho < \rho_{max}$ is non-trivial only if $0 \leq \rho_{min} < \rho_{max} \leq 1$. The threshold ρ_{min} is linked to the susceptible pest dynamics only. $\rho_{min} \geq 0$ indicates that if only *non-toxic* plants were grown during the first cropping season, susceptible pests would thrive so that a susceptible pest control problem exists and toxic plants should be used. Indeed if ρ_{min} were negative, susceptible pests would die out due to crop harvests. $\rho_{min} \leq 1$ means that, if only *toxic* plants were grown during the first cropping season, susceptible pests would disappear thanks to the combined effects of plant toxicity and crop harvests. The threshold ρ_{max} on its part is linked to the resistant pests dynamics. $\rho_{max} \geq 0$ indicates that, if only *non-toxic* plants were grown during the first cropping season, resistant pests would die out due to their poor adaptation to the non-toxic plants and to crop harvest. $\rho_{max} \leq 1$ means that, if only *toxic* plants were grown during the first cropping season, resistant pests would thrive.

Proof Notice first that $\mathbb{R}_+^2$ is invariant by model $(\mathcal{M})$.

In the following we take advantage of the fact that model $(\mathcal{M})$ is a combination of linear differential and difference equations so that it is possible to compute explicitly its solutions.

Consider a season k and integrate the continuous sub-model (2) along $t_k \in [0, T_1)$; this yields:

$$N_s(t_k = T_1^-) = \exp(a_1 T_1) N_s(t_k = 0),$$
$$N_r(t_k = T_1^-) = \frac{\epsilon g_s(\rho)}{a_1 - b_1} \left(\exp(a_1 T_1) - \exp(b_1 T_1)\right) N_s(t_k = 0) + \exp(b_1 T_1) N_r(t_k = 0),$$

with $a_1 = (1-\epsilon) g_s(\rho) - m_s(\rho)$, and $b_1 = g_r(\rho) - m_r(\rho)$.

Using this result together with sub-model (3) gives:

$$\begin{aligned} N_s(t_k = T_1) &= (1-p_1)\exp(a_1 T_1) N_s(t_k = 0), \\ N_r(t_k = T_1) &= (1-p_1)\frac{\epsilon g_s(\rho)}{a_1 - b_1}\left(\exp(a_1 T_1) - \exp(b_1 T_1)\right) N_s(t_k = 0) \ldots \\ &\qquad \cdots + (1-p_1)\exp(b_1 T_1) N_r(t_k = 0). \end{aligned} \tag{6}$$

The same can be done for sub-models (4) and (5):

$$\begin{aligned} N_s(t_{k+1} = 0) &= i_s + (1-p_2)\exp(a_2 T_2) N_s(t_k = T_1), \\ N_r(t_{k+1} = 0) &= (1-p_2)\frac{\epsilon g_{P_2}}{a_2 - b_2}\left(\exp(a_2 T_2) - \exp(b_2 T_2)\right) N_s(t_k = T_1) \ldots \\ &\qquad \cdots + (1-p_2)\exp(b_2 T_2) N_r(t_k = T_1). \end{aligned} \tag{7}$$

with $a_2 = (1-\epsilon) g_{P_2} - m_{P_2}$, and $b_2 = \tilde{g}_{P_2} - \tilde{m}_{P_2}$.

Combining equations (6) and (7) we find the relation that maps the pests densities at the beginning of the k-th cropping season onto the pests densities at the beginning of the $(k+1)$-th season; we have

$$\begin{aligned} N_s(t_{k+1} = 0) &= i_s + A N_s(t_k = 0), \\ N_r(t_{k+1} = 0) &= B N_s(t_k = 0) + C N_r(t_k = 0), \end{aligned} \tag{8}$$

with

$$\begin{aligned} A &= (1-p_1)(1-p_2)\exp(a_1 T_1 + a_2 T_2), \\ B &= (1-p_1)(1-p_2)\frac{\epsilon g_s(\rho)}{a_1 - b_1}(\exp(a_1 T_1) - \exp(b_1 T_1))\exp(b_2 T_2) \ldots \\ &\qquad \cdots + (1-p_1)(1-p_2)\frac{\epsilon g_{P_2}}{a_2 - b_2}\exp(a_1 T_1)(\exp(a_2 T_2) - \exp(b_2 T_2)), \\ C &= (1-p_1)(1-p_2)\exp(b_1 T_1 + b_2 T_2). \end{aligned}$$

Model (8) is a discrete linear dynamical system. It possesses a single equilibrium such that

$$N_s^*(t_k = 0) = \frac{i_s}{1 - A}, \text{ and } N_r^*(t_k = 0) = \frac{B i_s}{(1-A)(1-C)}. \tag{9}$$

Since B is clearly positive, this equilibrium has non-negative components, *i.e.* is reachable from non-negative initial conditions which are of biological interest, if and only if $A < 1$ and $C < 1$. After a little algebra, the former condition is shown to be equivalent

to $\rho_{min} < \rho$ while the latter is equivalent to $\rho < \rho_{max}$, *i.e.* the combination of the two conditions yields $\rho_{min} < \rho < \rho_{max}$.

Since A and C are positive and are the eigenvalues of the discrete linear system (8), equilibrium (9) is (globally asymptotically) stable if and only if $A < 1$ and $C < 1$, *i.e.* if and only if $\rho_{min} < \rho < \rho_{max}$. The non-negativity and stability of equilibrium (9) are thus equivalent properties; this is a classical result of positive discrete time systems theory, in fact a consequence of the Perron Frobenius theorem on non-negative matrices (Berman and Plemmons 1979).

Equilibrium (9) of model (8) actually corresponds to a periodic orbit of model ($\mathcal{M}$). It is easy to show through Poincaré maps arguments that the (global asymptotic) stability of (9) for model (8) is equivalent to the (global asymptotic) stability of the corresponding periodic orbit of model ($\mathcal{M}$). □

One can deduce from Proposition 1 the following result that is important with respect to pest management issues.

Corollary 1
If $\rho < \rho_{min}$ or $\rho > \rho_{max}$, at least one pests population N_s or N_r grow unbounded.

Proof Suppose $\rho < \rho_{min}$, then $A > 1$. Since

$$\frac{N_s(t_{k+1} = 0)}{N_s(t_k = 0)} \geq A,$$

for all k, the susceptible pests population grow unbounded (and the resistant pests population does as well).

Suppose now that $\rho > \rho_{max}$, then $C > 1$. Since

$$\frac{N_r(t_{k+1} = 0)}{N_r(t_k = 0)} \geq C,$$

for all k, the resistant pests population grow unbounded. □

Remark 3 In real life it is unrealistic to consider that a population may grow unbounded. Corollary 1 is actually a consequence of the linear modelling framework we considered. Hence "pests grow unbounded" should be interpreted loosely as "the cropping system is not able to prevent pests to multiply and provoke dramatic damages to the plants". In other words, if $\rho < \rho_{min}$ or $\rho > \rho_{max}$ the cropping practices described above are not able to control the pests expansion.

Proposition 1 and Corollary 1 show that a necessary and sufficient condition to control pest expansion is to cultivate during the first cropping period a proportion ρ of toxic plants that is no smaller than a value ρ_{min} and no larger than a value ρ_{max}. If there is no immigration of susceptible pests between cropping seasons, *i.e.* $i_s = 0$, such a strategy conduces to suppress both pests populations on the long term. However, if the system experiences re-colonizations by susceptible pests between cropping seasons, *i.e.* $i_s > 0$, the cropping practices, even if the proportion of toxic plants ρ is appropriately chosen, cannot suppress the pests but only contain their expansion. This situation should however be understood as a success of the pest management strategy since otherwise the pests population would have not been controlled at all. Indeed, from Corollary 1, a too low proportion of toxic plants ρ does not permit to thwart the susceptible pests, and a too large ρ would make the resistant population thrives. This

latter property is actually what has lead to recommend the use of non-toxic plant refuges in toxic crops, see *e.g.* (EPA 1998).

In the considered cropping system, two main forces act jointly to control pests populations: on the one hand, crop harvests impose recurrent demographic bottlenecks to both pests populations, and on the other hand toxic plants penalize the overall rate of increase of the susceptible pests populations (although they might also favour the growth of resistant pests). In particular, the use of a proportion of toxic plants $\rho > \rho_{min}$ does not guarantee by itself that the susceptible pest population does not proliferate during the first cropping period of a season, although it is the main goal of the use of a mixture of toxic and non-toxic plants. Indeed from sub-model (2), the contribution of the susceptible pest population to the *net* rate of increase of the total pest population is $(g_s(\rho) - m_s(\rho))$. Hence, if this coefficient is negative, *i.e.* if

$$\rho > \frac{g_{P_1} - m_{P_1}}{g_{P_1} - m_{P_1} - (g_{R_1} - m_{R_1})} \triangleq \bar{\rho}$$

the net contribution of the susceptible pest population to the total pest population is negative, what is exactly the situation that is sought after. Then, the recommendation on the proportion ρ of toxic plants should be

$$\max(\rho_{min}, \bar{\rho}) < \rho < \rho_{max}, \tag{10}$$

rather than $\rho_{min} < \rho < \rho_{max}$. Such a condition ensures, that (i) pests are controlled, and (ii) the mixture of toxic and non-toxic crops in the first cropping period acts efficiently.

A last remark should be made. In the preceding discussion it was implicitly assumed that $\rho_{min} < \rho_{max}$ (*resp.* $\max(\rho_{min}, \bar{\rho}) < \rho_{max}$). This is not necessarily the case and they are situations for which $\rho_{min} > \rho_{max}$, so that it is not possible to find a proportion of toxic plants ρ that makes the control of pests possible. For example, when $\rho_{min} \approx 1$, susceptible pests are not very sensitive to the toxic plants or they grow very efficiently during the second season; when $\rho_{max} \ll 1$, resistant pests grow very fast in the presence of toxic plants only.

3 Simulation analysis

We performed some numerical simulations to present graphically the dynamical properties of model $(\mathcal{M})$. Depending on the species and host plants, pest insects have very variable demographic characteristics. Since our goal in this section is to illustrate the general properties of the model, we chose demographic parameter values (see Table 1) that roughly correspond to some insect species, but that does not specifically fit a given insect species. In particular, we assume that resistant pests are poorly adapted to non-toxic plants ($\tilde{g}_{P_i} - \tilde{m}_{P_i} < \tilde{g}_{R_i} - \tilde{m}_{R_i}$) and that toxic plants are efficient on susceptible pests ($(1-\epsilon)g_{P_1} - m_{R_1} > (1-\epsilon)g_{R_1} - m_{R_1}$); all of these are coherent with our biological hypotheses (see the beginning of §2 and Remark 1).

The proportion of resistant newborns in the susceptible pests progeny ϵ is difficult to estimate, but very low. (Gould et al. 1998) reported an order of magnitude of 0.001; as a benchmark, we used $\epsilon = 0.01$. We assumed the proportion of the pests eliminated by the first crop harvest $p_1 = 90\%$ and the harvest/mortality coefficient $p_2 = 95\%$. The between season immigration of susceptible pests i_s has been taken equal to 1 unit

Plant type	Pests demographic parameters (in month^{-1})			
Non-toxic plants (model (2))	g_{P_1}	$\tilde{g}_{P_1}$	m_{P_1}	$\tilde{m}_{P_1}$
	5	1	2	3
Toxic plants (model (2))	g_{R_1}	$\tilde{g}_{R_1}$	m_{R_1}	$\tilde{m}_{R_1}$
	2	3	4	1
Non-toxic plants (model (4))	g_{P_2}	$\tilde{g}_{P_2}$	m_{P_2}	$\tilde{m}_{P_2}$
	0.7	0.6	0.5	0.7

Table 1 Pests demographic parameter values used in the numerical simulations.

of pest density. The periods T_1 and T_2 are assumed to be measured in months with $T_1 = 4$, $T_2 = 8$ so that a full cropping season $T_1 + T_2$ corresponds to one year.

Figure (2) illustrates the typical dynamics of resistant and susceptible pests within a cropping season for two values of the proportion of toxic plants ρ. With the considered parameter values, we have $\rho_{max} = 0.88$, $\rho_{min} = 0.41$ and $\bar{\rho} = 0.6$. We contrasted two values of $\rho \in (\rho_{min}, \rho_{max})$, depending whether ρ was lower or larger than $\bar{\rho}$, *i.e.* depending whether or not the proportion of toxic plant is large enough to control the susceptible pests population within the first cropping period.

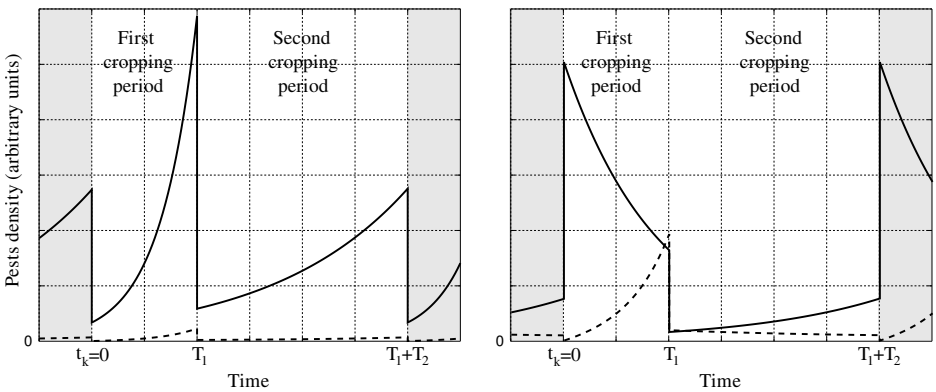

Fig. 2 Typical dynamics of resistant and susceptible pests (dashed and continuous line respectively) during one cropping season (white area). Left: $\rho = 0.45$; right: $\rho = 0.65$. There is not enough toxic plant in the mixture as $\rho_{min} < \rho = 0.45 < \bar{\rho}$; the pest management strategy is efficient as $\bar{\rho} < \rho = 0.65 < \rho_{max}$.

Figure (3) illustrates the dynamics of model ($\mathcal{M}$) on a 10-years term for two values of ρ. For ρ satisfying condition (10) both resistant and susceptible pest populations are controlled. For $\rho > \rho_{max}$ the cropping practices are still able to maintain susceptible pests population under control, but not the resistant ones that invade the system.

To finish with, we considered a criterion that is commonly used in experimental studies to characterize resistance in pests population, the frequency of resistant individuals within a population: $\frac{N_r}{N_s+N_r}$. We computed it at equilibrium for the multi-season model (8), *i.e.* it is the frequency of resistant individuals in the population at the beginning of a cropping season, and show its variations with respect to the proportion of toxic plants ρ. Our analysis highlights the fact that the relationship between the resistant individuals frequency and the proportion of toxic plants is not monotonic: as

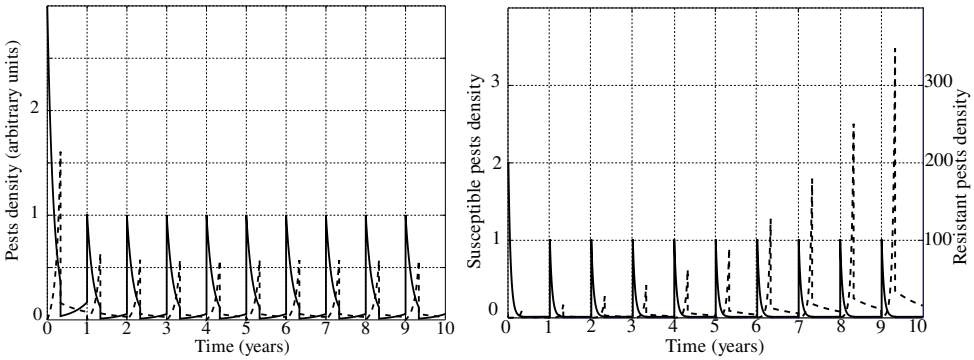

Fig. 3 Long term (10-years) dynamics of resistant and susceptible pests populations with respect to time (dashed and continuous line, respectively; units are arbitrary). Left: $\rho = 0.70 \in (\bar{\rho}, \rho_{max})$, both pest populations are controlled. Right: $\rho = 0.95 > \rho_{max}$, susceptible pests are still under control, but resistant pests are able to invade the system.

ρ approaches ρ_{max}, the resistant pests take over the susceptible population and goes to fixation. As the proportion of toxic plants decreases, *i.e.* as the proportion of 'refuges' increases in the crop, the resistance frequency decreases towards a minimum which is reached for some optimal crop composition. This minimum frequency, approximately 0.001 in the simulation, is much lower than the resistance frequency in the susceptible pests population progeny $\epsilon = 0.01$. As ρ still decreases, the resistance frequency counter-intuitively increases so that resistant individuals can reach a frequency that is above the resistance frequency in the susceptible pests population progeny $\epsilon = 0.01$ (more than two times ϵ in the simulation). Hence, it appears that increasing the proportion of refuges in a crop composed of a mixture of toxic and non-toxic plants may actually in some cases favour pest resistance. Although this conclusion is quite controversial, it is worth noting that (Mohammed-Awel et al. 2007) reached a similar one using a fairly different modelling framework. There is though a common hypothesis in the work by (Mohammed-Awel et al. 2007) and the one reported here, which is immigration of susceptible pests individuals within the system. This might be an important topic for future research on the durability of toxic plants based pest control strategies with respect to the management of pest resistance.

4 Discussion

Managing the evolution of pest resistance to toxins is one of the key issue of pest management strategies based on the use of toxic crops. It strongly relies on the identification and understanding of the main factors underlying pests resistance dynamics. In the present contribution, we developed a model that is intended to address such questions. We considered a particular cropping system where two crops grow alternatively, one being composed of a mixture of toxic and non-toxic crops, the other of non-toxic crops only. Our choice of being simple and assume linear processes as a first approximation, allowed to concentrate on one important factor which is the proportion ρ of toxic crops in the toxic/non-toxic crop mixture. We exhibited two critical thresholds on the proportion ρ, $\max(\rho_{min}, \bar{\rho})$ and ρ_{max}, between which both susceptible and resistant pests are controlled on the long term. These thresholds essentially depend on

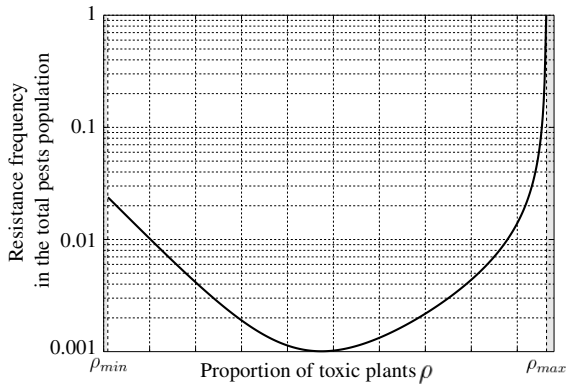

Fig. 4 Variations of resistant pests frequency in the total (susceptible + resistant) pests population with respect to the proportion of toxic plant $\rho \in (\rho_{min}, \rho_{max})$ (white area, semi-log scale). Resistant pests frequency is given at equilibrium, at the beginning of a cropping season.

the demographic parameters of the pests on the different host plants. We noted that, depending on the demographic parameters, $\max(\rho_{min}, \bar{\rho})$ may be larger than ρ_{max}, a situation in which it is not possible to control pests through the use of toxic plants. When pest control is possible and ρ is between the two critical thresholds, it was shown that increasing the proportion of refuges does not always diminish pest resistance in terms of frequency within the total pest population. An optimal proportion of refuges within the crop that minimizes resistance frequency actually exists. This controversial result was also put forward by (Mohammed-Awel et al. 2007) in a somewhat different model, and is possibly due to the explicit consideration of a cropping system recolonization process by susceptible pests. Another point stressed by our modelling is the importance of a "temporal refuge", *i.e.* the rotation of a mixed toxic/non-toxic crop and a purely non-toxic crop, in the management of resistance. Indeed, as we replace the temporal refuge, *i.e.* the purely non-toxic crop, by another mixed toxic/non-toxic crop with some fixed composition, an analysis similar to the one performed in §2 would show that resistant pests would proliferate even if ρ was smaller than ρ_{max}. Hence the temporal refuge allows to better control of resistant pests, what is consistent with the studies of (Sisterson et al. 2005) and (Storer 2002).

Nevertheless, our model remains a rough approximation of the involved biological processes since we chose to describe the main population dynamics through linear processes only. Generalizing growth and death processes with non linear functions would be interesting but would likely not change the qualitative result of the mathematical study: the existence of an attractive periodic orbit. Liu and Chen (2005) indeed proposed a quite general result on non linear impulsive systems and proved the existence of an attractive periodic orbit under the following hypotheses: the continuous part should be cooperative (*i.e.* the off-diagonal terms of the Jacobian should be positive, which would be true with respect to the interactions we consider) and strongly concave (*i.e.* the difference between the function and its derivative should be positive, which is not the case here because of our linear operators). For most biological systems, these properties are likely to be met so that our conclusions should apply to a more general and realistic non-linear version of model ($\mathcal{M}$).

Another remark should be made on the hypotheses linked to pest reproduction: it was assumed that there was no time delay between the reproduction of the adult pests and birth of the newborn pests. Such a modelling would suit well pests like aphids that reproduce through parthenogenesis and give rise to almost adults newborns continuously throughout the season. If aphids are sensitive to (genetically modified) toxic plants expressing some lectin toxins (Chen 2008) they are barely affected by *Bt*-plants that remain the main toxic plants used worldwide. The typical insect pests organisms sensitive to *Bt*-plants are Coleoptera and Lepidoptera species, but their life cycle is in general fairly different from the aphid's one. Actually these species' adults reproduce through the production of eggs that will take a non-negligible time to maturate and hatch. In this respect, we shall probably consider to add a delay in the terms corresponding to reproduction in models (2) and (4).

As a last remark, our modelling, based on pure demographic parameters, could be improved by adding quantitative genetics processes to derive a demo-genetic model (see *e.g.* (Tyutyunov et al. 2008)). Our interpretations on the threshold values $\max(\rho_{min}, \bar{\rho})$ and ρ_{max} would be refined and new biological insights may be obtained. This will be the subject of some future work.

Acknowledgements V. L. is grateful to Réseau National des Systèmes Complexes for financial support through the "D&GEI" project. L.M. is grateful to Agropolis Fondation and the Réseau National des Systèmes Complexes for financial support through the "Mod-PEA" project. Authors thank S. Nibouche (UMR PVBMT, Universté de La Réunion, France), T. Brévault (UR 102, CIRAD, France) for insightful discussions on the subject and the reviewer for very constructive comments.

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