ISSN 0006-3509, Biophysics, 2007, Vol. 52, No. 1, pp. 52–67. © Pleiades Publishing, Inc., 2007.
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COMPLEX SYSTEMS
BIOPHYSICS

A Spatial Model of the Development of Pest Resistance to a Transgenic Insecticidal Crop: European Corn Borer on Bt Maize

Yu. V. Tyutyunov[a,b], E. A. Zhadanovskaya[a,b], R Arditi[b], and A. B. Medvinsky[c]

[a]*Vorovich Research Institute of Mechanics and Applied Mathematics, Southern Federal University, Rostov-on-Don, 344090 Russia*
E-mail: tyutyunov@rsu.ru, catherine@math.rsu.ru
[b]*AgroParisTech (Institut des Sciences et Industries du Vivant et de l'Environnement), Paris cedex 05, 75231 France*
E-mail: arditi@inapg.fr
[c]*Institute for Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142290 Russia*
E-mail: medvinsky@iteb.ru

Received February 1, 2006

Abstract—A mathematical model was constructed to describe the evolution of resistance to the *Bacillus thuringiensis* toxin (Bt) in an insect pest (European corn borer) population on a transgenic crop (Bt corn). The model comprises a set of partial differential equations of the reaction–diffusion type; local interactions of three competing pest genotypes formed by alleles of Bt resistance and susceptibility are described as in the Kostitzin model, and the spread of insects is modeled as diffusion. The model was used to evaluate the influence of pest characteristics on the efficacy of the high-dose/refuge strategy aiming to prevent or delay the spread of Bt resistance in pest populations. It was shown, by contrast, that a model based on Fisher–Haldane–Wright equations and formally incorporating a diffusion term cannot adequately describe the evolution of Bt resistance in a spatially inhomogeneous pest population. Further development of the proposed demo-genetic model is discussed.

DOI: 10.1134/S0006350907010101

Key words: transgenic insecticidal crop, insect pest resistance, mathematical modeling

THE BIOLOGICAL SYSTEM

Genetically modified (transgenic) insecticidal crops, harboring bacterial genes that encode proteins specifically toxic for pest larvae, were created to control the pest invasion. The European corn borer (ECB; *Ostrinia nubilalis* Hubner, Lepidoptera: Crambidae) is a major pest in many maize-growing countries. Owing to the high tissue levels of *Bacillus thuringiensis* toxin (Bt), the Bt corn is very toxic for the ECB larvae and can almost completely suppress the pest throughout the growing season.

The Bt crop technology is an alternative to the broadly used chemical insecticides and even to the microbiologically produced Bt insecticides. All field-sprayed agents have common essential shortcomings: incomplete coverage of the plant surface, degradation under UV radiation, instability against heating and drying, which result in reduced efficiency. Besides, advanced ECB larvae tunnel deeply into the corn stalks and ears, which makes the surface insecticide treatment useless [1, 2]. The Bt-carrying plants are devoid of such shortcomings and provide for more adequate pest control in the field. However, the risk of rapid evolvement of pest resistance to Bt crops may deprive the transgenic technology of all merits.

Abbreviations: Bt, *Bacillus thuringiensis* (toxin); ECB, European corn borer; FHW, Fisher–Haldane–Wright; HDR, high-dose/refuge; HW(E), Hardy–Weinberg (equilibrium); q, quintal (100 kg).

The possibility of Bt-resistance development in target pests is based on three points:

— insects are well known to become resistant to insecticides, especially when the latter are applied regularly and at high dosages [3];

— the relatively short life cycle of the pest (e.g., the commonly bivoltine ECB can have four generations per year for under favorable conditions [1]) speeds up the elimination of Bt-susceptible insects and favors selection of Bt-resistant ones;

— some insect pest species have been reported to develop Bt resistance in under laboratory conditions [4–6].

The potential hazard of the evolvement of Bt resistance has been discussed long before the appearance of the first insecticidal crop. Comins [7] was the first to theoretically demonstrate how random gene exchange between two pest populations, one subjected to insecticide treatment and the other not, can retard the spread of resistance provided that the resistance allele is recessive. He also formulated the two basic concepts for the later design of a mechanism for resistance management on transgenic crops [8, 9]:

— the incidence of the resistance gene in a toxin-treated population can be attenuated through an active influx of susceptible genes from non-treated plots, which were later termed "refuge;"

— intense insecticidal treatment of crop fields combined with allotment of refuges suppresses the spread of resistance.

The evolution of a resistance gene in the genetic structure of a population is a complicated transitional nonequilibrium process influenced by many factors (the dominance level and the initial frequency of the resistance-conferring allele, the Bt efficiency, the intensity of insect migration within and beyond the toxic region, etc.). This process can be either very fast, taking just a few generations, or conversely, stretched over decades or even centuries, depending on the combination of such factors. Hence, the problem cannot be fully assessed by lab tests or field trials, and mathematical modeling remains one of the main approaches to studying the spatiotemporal dynamics of agroecosystems comprising transgenic crops and insect pests. In particular, mathematical models are built to forecast the duration and rate of Bt-resistance development in pest populations.

In the context of current simulation modeling, the high-dose/refuge (HDR) concept is considered the main strategy of resistance management in pests on transgenic crops [10, 11]. "High dose" means that the Bt toxin content in the GM plants must be so high that only toxin-resistant insects can survive. In more exact terms, the toxin concentration must be 25 times higher than the one necessary to kill all susceptible larvae [12]. The purpose of refuges—defined as plots with any non-Bt plants that are hosts for target pests—is to prevent or attenuate the undesirable effect of the high dose by weakening the selection pressure for resistance and thereby retard the resistance development in the pest population.

Most of the mathematical models assume that Bt resistance is controlled by a single diallelic locus, whereby the susceptibility allele *s* and the resistance allele *r* form three genotypes: homozygotes *ss* and *rr*, and heterozygotes *rs* (*sr*). It is also assumed that the Bt susceptibility of the *rs* and *sr* heterozygotes is the same, i.e., the resistance gene is autosomally inherited. Considering the genetic structure of the pest population subjected to selection for toxin resistance, the efficiency of the HDR strategy is determined by three main conditions [13]:

— the initial frequency of the resistance allele *r* must be low enough so that only the rare *rr* individuals survive on the Bt crop;

— the resistance allele must be recessive so that *rs* individuals prove fully or partly susceptible to the toxin;

— the location and configuration of refuges must be optimal for mating between *rr* insects arising on the Bt field and *ss* insects from the refuge, so that their heterozygous progeny lower the frequency of the resistance allele in every next generation.

If all the three conditions are fulfilled, the development of Bt resistance in pest populations can be delayed for an economically expedient length of time [14–16]. Let us consider each condition as applied to the modeled dynamics of ECB.

Initial *r* allele frequency. Resistance-conferring genes may arise in natural insect populations owing to recurrent mutations and may persist at a low frequency prior to any toxin exposure [3]. The relative scarcity of the Bt-resistance allele in natural ECB populations is confirmed by several works [17, 18]; in

general, this frequency does not exceed 10^{-3}, which is low enough for a HDR strategy to be efficient.

Inheritance of resistance. Whether the resistance allele is recessive has not been verified by laboratory analysis, though partial Bt resistance has been reported to occur in natural ECB populations [17, 18]. On the other hand, resistance to a commercial formulation of a Bt toxin in the laboratory [6] appeared to be inherited as an incompletely dominant autosomal gene; as far as we know, this has not been confirmed by field trials.

Refuge. For successful resistance management, the refuge must produce large numbers of susceptible adult insects relative to the number of resistant ones arising on transgenic fields. The proportion recommended by US FIFRA [12] is 500:1. The refuge size and arrangement relative to transgenic fields are decisive for their efficacy; however, there is still no unanimity on this point.

AN OVERVIEW OF THE PROBLEMS PERTAINING TO THE HIGH-DOSE/REFUGE STRATEGY

Among the major crops, Bt maize is second to Bt soybean; in 2004 it occupied 14% of the global maize acreage (19.3 mln ha) [19]. Such broad distribution and continual action of Bt toxins means long-term strong selection pressure for resistance in pest populations. In the USA, refuges for susceptible pests are mandatory in Bt-maize growing since 1999 [9]. According to EPA recommendations, any host plant can be used as refuge. However, despite the fact that ECB is extremely polyphagous (host range of more than 200), it does not fit into this general scheme. Recent research in the USA [20] and Northern France [21–24] shows that the populations developing on other plants form races differing behaviorally and genetically from the maize populations; hence only usual (non-Bt) maize can provide for normal refuge function.

Corn growers are instructed [9, 25] that the refuge area may vary from 20 to 50% of the crop area depending on the extent of infestation, and that the refuge must be adjacent to the Bt field. They are additionally advised to spray large refuge areas with insecticide to improve the plant yield. The current recommendations are based on simulation modeling of resistance evolvement in pest populations. According to the results available, reduction of the refuge area would accelerate both the extinction of insects and the evolution of resistance [14, 15, 26]. Again, modeling [27] indicates that even rare application of insecticide on a 20% refuge promotes Bt resistance in an ECB population, whereas on a 30% or larger refuge there is practically no such effect. However, growers see no economic interest in organizing refuges and tend to ignore all and any recommendations because the crop yield from refuges is generally lower [28, 29]. As already mentioned, the optimal size and configuration of the refuge is a matter of debate.

After more than a decade, a number of key problems in implementing the HDR strategy remain unsolved. How fast can Bt-crop resistance evolve? What factors are decisive for this process? What is the size of refuge that can retard Bt resistance? What refuge configuration is optimal? Is it possible to completely prevent Bt resistance? These and many other questions still have no definite answers. On the other hand, though lab studies and some mathematical models support the ECB ability to fairly quickly acquire resistance to Bt maize [6, 14, 15, 27], in reality the field surveys since the first planting of Bt maize in 1996 have not detected a single homozygous Bt-resistant moth [18]. The fact that the actual field data defy the laboratory and theoretical forecasts is really striking and calls for novel approaches and models.

AN OVERVIEW OF MODELING METHODS

Modeling of the spread of a Bt resistance gene in a pest population must take into account two main components:

— the spatial inhomogeneity due to partitioning of the pest habitat into plots with common and Bt maize, and

— the genetic structure of the pest population and its evolution.

The first component can be realized in a set of partial differential equations of the reaction–diffusion type, cellular automatons, or compartmental models explicitly describing the gene flows in space.

The second component must include the key elements of the insect ecology and genetics. Some simulation models are built with very detailed assumptions on the population genetics and the life cycle of insect species [14, 15, 26, 27, 30]. Alternatively, in the

framework of a conceptual approach the authors disregard the insect ecology and focus only on the genetic processes described with the classical Fisher–Haldane–Wright (FHW) equations [11, 16, 31, 32]. This conceptual approach encounters at least two problems comprehensively considered elsewhere [33].

The matter is that the FHW design initially pertained to species whose life cycle is an alternation of nonoverlapping diploid and haploid generations (see [33]). In this case, to describe the genetic structure of the entire population it is sufficient to use equations derived for one of the phases; as the description at the haploid (allele) level is simpler, instead of the dynamics of genotype frequencies one can consider the dynamics of allele frequencies. This circumstance hinders the application of the FHW equations to the evolvement of Bt resistance in insects (such as ECB) for which the diplophase is the main and the longest form of vital activity, while the haplophase is significantly reduced and devoid of ecological autonomy (i.e., boils down to the existence of gametes).

Expansion of the FHW applicability is based on an auxiliary hypothesis of a "gamete pool" whereby the mating of diploid organisms with ensuing copulation of gametes (haploid forms) for a separate parent pair is equivalent to panmictic copulation of gametes. In other words, it is assumed that all gametes make an ecologically independent pool [33]; this admits the term "gametic population." Panmixia of diploid organisms immediately after the first generation establishes the Hardy–Weinberg (HW) equilibrium between the genotype and allele frequencies, which holds in further generations.

Note that the "gamete pool" hypothesis also draws in additional assumptions concerning the pest: (i) large population size, (ii) sexual reproduction, (iii) lack of mutations, (iv) lack of selection, (v) lack of migration. In reality, only points (i) and (ii) are true for ECB and many other insect pests. Point (iii) may be sometimes accepted for such populations, but points (iv) and (v) contradict both the properties of the species under study and the nature of the a priori nonequilibrium transitional process of the evolvement and spread of resistance in the pest population. Thus, neither the "gamete pool" hypothesis nor the HWE assumption appear to be adequate to the case.

As far back as 1937, the limited applicability of the conventional population genetics was pointed out by Kostitzin [34]. He noted that in a diploid population the selection operates through genotype competition at the level of densities rather than allele frequencies (see also [33]). Nonetheless, FHW and HW relationships are still broadly used to model Bt resistance in insect populations [11,14–16, 27, 30–32]. To account for spatial inhomogeneity of pest distribution, such models include diffusion terms [16, 31] or migration of a fixed portion of individuals from each cell in a cellular automaton [32]. However, the above reasoning makes one doubt that the diffusion version of Fisherian equations can adequately describe the real process or that a pointwise (panmictic) FHW-based model can correctly predict the delay in pest resistance development (see [11]). Indeed, the estimates thus obtained are pessimistic and thus far not confirmed by practice. Thus only a 10-year delay is predicted even with quite a large (26%) refuge (see, e.g., [16]); note that according to the current standards an acceptable resistance management strategy must sustain the Bt crop efficacy for more than 10 years [12].

Onstad [35] stated that for an ecological model to be realistic it should use all the available information on the object under study. This approach is represented by the maximally detailed simulation models of the evolution of Bt resistance in pest populations [14, 26, 27, 30]. Their predictions appear to be closer to reality: a 56-year delay with a 10% refuge and over 100 years with a more than 20% refuge [14, 27]. Another simulation model [15] gives a delay of 80 generations with a 10% refuge, which makes 40 years for bivoltine ECB, or less for a multivoltine pest; by contrast to the FHW approach, this model also supports the expedience of the HDR strategy.

On the other hand, the practical use of simulation models is hindered by their complexity and the vast number of parameters [36], many of which are very difficult or impossible to determine from available data. In some cases, the detailing becomes absurd, e.g., a model is praised for including 17 600 equations with 41 000 coefficients [35]. This greatly limits the value of simulation models in agroecological studies and management.

Hence there is need for modified conceptual models that would adequately describe the systems dynamics, accounting not only for genetic transformations but also for ecological interrelationships between insect pests and host plants.

To describe the spatiotemporal dynamics of a pest in a spatially inhomogeneous habitat, we propose a conceptual demo-genetic reaction–diffusion model, whereby the local kinetics of competing genotypes is set by a modified Kostitzin model ([34], see also [33, 37]). This approach is alternative to the use of the conventional equations of population genetics and obviates the problems unsolvable by the latter. It allows explicit modeling of the evolution of a resistance-conferring allele in a population with nonoverlapping generations and reduced haplophase, adequately describing the selection for resistance both close to and far from HW equilibrium [37]. In addition, the model can account for the influence of the plant resource on the pest dynamics; this interaction is described by a Lotka–Volterra predator-prey model.

Here, we consider ECB on Bt maize as an example and assess the influence of spatial factors such as pest mobility and refuge size on the efficacy of the HDR strategy of resistance management.

THE MODEL

Population Genetics

As in most of the relevant models [8, 14–16, 30, 32], we assume that the individual resistance to a Bt crop in a pest population is determined by a single locus of two alleles: susceptible (*s*) and resistance-conferring (*r*), which form three genotypes: resistant homozygous (*rr*) and susceptible homo- and heterozygous (*ss*, *rs*). We also assume that locally (i.e., at any space point) the genotypes cross at random. By contrast to the conventional approach, we do not follow the HW principle in determining the gene frequencies in the pest population.

Modeling the Population Dynamics

With the given premises on pest genetics and demography, the model appears as

$$\frac{\partial N_{ss}}{\partial t} = F_{ss}(N_{ss},N_{rs},N_{rr}) + \delta_{ss}\left(\frac{\partial^2 N_{ss}}{\partial x^2} + \frac{\partial^2 N_{ss}}{\partial y^2}\right),$$

$$\frac{\partial N_{rs}}{\partial t} = F_{rs}(N_{ss},N_{rs},N_{rr}) + \delta_{rs}\left(\frac{\partial^2 N_{rs}}{\partial x^2} + \frac{\partial^2 N_{rs}}{\partial y^2}\right), \quad (1)$$

$$\frac{\partial N_{rr}}{\partial t} = F_{rr}(N_{ss},N_{rs},N_{rr}) + \delta_{rr}\left(\frac{\partial^2 N_{rr}}{\partial x^2} + \frac{\partial^2 N_{rr}}{\partial y^2}\right),$$

where functions F_{ij} (i,j is r or s) describe the reproduction/mortality of the genotypes denoted by subscripts and their intraspecific competition:

$$F_{ss}(N_{ss},N_{rs},N_{rr}) = W_{ss}\left[\frac{b}{N}\left(N_{ss} + \frac{N_{rs}}{2}\right)^2\right] - \alpha_{ss}N_{ss}N - \mu_{ss}N_{ss};$$

$$F_{rs}(N_{ss},N_{rs},N_{rr}) = W_{rs}\left[\frac{2b}{N}\left(N_{ss} + \frac{N_{rs}}{2}\right)\left(\frac{N_{rs}}{2} + N_{rr}\right)\right] - \alpha_{rs}N_{rs}N - \mu_{rs}N_{rs}; \quad (2)$$

$$F_{rr}(N_{ss},N_{rs},N_{rr}) = W_{rr}\left[\frac{b}{N}\left(\frac{N_{rs}}{2} + N_{rr}\right)^2\right] - \alpha_{rr}N_{rr}N - \mu_{rr}N_{rr}.$$

Here $N_{ij} = N_{ij}(x,y,t)$ are the densities of the respective genotypes in point (x,y) at moment t; $N = N_{ss} + N_{rs} + N_{rr}$ is the overall population density. It is assumed that all genotypes have the same fertility coefficient (b) but may have different mortality (μ), competition (α), diffusion (δ), and fitness coefficients (W); the latter can also be interpreted as survival of larvae of the corresponding genotype depending on localization in the habitat (usual or Bt maize).

The diffusion coefficients characterize the intensity of nondirectional movements of individuals. We assume that there are no diffusional density fluxes across the boundary, i.e.,

$$\frac{\partial N_{ij}}{\partial x} = \frac{\partial N_{ij}}{\partial y} = 0 \quad (3)$$

at the borders of the entire model field.

Thus, the nonlinear demo-genetic model (1)–(3) is based on the well-known Lotka–Volterra competition equations. Being autonomous, this model does not account for seasonal variations in the environmental conditions and should be regarded as a first approximation of the real agroecosystems. We do not model the different stages of ECB ontogeny, and assume the reproduction/mortality processes to be continuous.

Note that model (1)–(3) implies that each genotypic group is represented by both males and females. The functions of genotype reproduction bracketed in set (2) imply Mendelian inheritance and a constant 1:1 sex ratio in the population. Also note that

demographic processes and spatial movement of the pest take place on the same spatiotemporal scale, while short-range motion of adult individuals is ignored.

When $W_{ij} = 1$, i.e., the whole pest habitat is a refuge, and the genotypes are distributed uniformly, model (1)–(3) is a particular case of the Kostitzin demo-genetic model ([34], see also [37]).

Modeling the High-Dose/Refuge Strategy

To account for spatial inhomogeneity, we assume that the pest habitat is a rectangle $\Omega = [0,L_x] \times [0,L_y]$ composed of an arbitrary number of plots with either Bt maize (further denoted Ω_{Bt}) or usual maize (refuge, Ω_{Ref}).

Like other authors [16, 31], we assume that in the general case the fitnesses of susceptible ECB genotypes on these plots can differ:

$$W_{rr} = 1-c, \quad (x, y) \in \Omega;$$

$$W_{rs}(x,y) = \begin{cases} 1-h_c c, & (x,y) \in \Omega_{\text{ref}}; \\ 1-h_s s-(1-h_s)c, & (x,y) \in \Omega_{\text{Bt}}; \end{cases}$$

$$W_{ss}(x,y) = \begin{cases} 1, & (x,y) \in \Omega_{\text{ref}}; \\ 1-s, & (x,y) \in \Omega_{\text{Bt}} \end{cases}, \qquad (4)$$

where s is the selection coefficient for Bt-resistance, c is the cost paid by the resistant genotype for the advantage on transgenic plots, h_s is the dominance level of the selection, h_c is the level of dominance of the cost; $s, c, h_s, h_c \in [0;1]$.

In a particular case when the selection intensity on Bt plots is maximal (i.e., 100% of susceptible individuals are killed) and the resistance gene is recessive (i.e., all heterozygous individuals are also killed), $s = 1$, $h_s = 1$. Further assuming that there is no cost for resistance, the fitnesses of *rs* and *ss* genotypes on Ω_{Bt} become zero, i.e., the Bt-susceptible progeny does not survive on Bt maize, while the resistant *rr* individuals have maximal fitness $W_{rr} = 1$ both on Bt plots and in the refuge:

$$W_{rr} \equiv 1, \ \forall \ (x, y) \in \Omega;$$

$$W_{ss}(x,y) = W_{rs}(x,y) = \begin{cases} 1, & (x,y) \in \Omega_{\text{Ref}}; \\ 0, & (x,y) \in \Omega_{\text{Bt.}} \end{cases} \qquad (5)$$

This is an extreme case. Here, following the conclusions [18] about the partial survival of heterozygous ECB on transgenic maize plots and about the lack of pronounced resistance cost, we take the parameters used elsewhere [16]: $s = 1$, $c = 0$, $h_s = 0.95$.

Thus, the difference between the transgenic and the refuge plots is determined only by the different survival of the pest larvae in accordance with conditions (4). Note that the interfaces between adjacent Ω_{Bt} and Ω_{Ref} are transparent, the boundary conditions (3) apply only to the outer borders of the habitat.

Ecological Simplifications in Model (1)–(3)

To better understand the influence of the HDR strategy on the evolution of Bt resistance in the pest population, let us assume that the ecological characteristics of all pest genotypes are identical: $\alpha_{ss} = \alpha_{rs} = \alpha_{rr} = \alpha$, $\mu_{ss} = \mu_{rs} = \mu_{rr} = \mu$, $\delta_{ss} = \delta_{rs} = \delta_{rr} = \delta$; i.e., genotypes differ only in their ability to survive on Bt maize. Thereby the set (1)–(2) becomes

$$\frac{\partial N_{ss}}{\partial t} = F_{ss}(N_{ss}, N_{rs}, N_{rr}) + \delta\left(\frac{\partial^2 N_{ss}}{\partial x^2} + \frac{\partial^2 N_{ss}}{\partial y^2}\right);$$

$$\frac{\partial N_{rs}}{\partial t} = F_{rs}(N_{ss}, N_{rs}, N_{rr}) + \delta\left(\frac{\partial^2 N_{rs}}{\partial x^2} + \frac{\partial^2 N_{rs}}{\partial y^2}\right); \qquad (6)$$

$$\frac{\partial N_{rr}}{\partial t} = F_{rr}(N_{ss}, N_{rs}, N_{rr}) + \delta\left(\frac{\partial^2 N_{rr}}{\partial x^2} + \frac{\partial^2 N_{rr}}{\partial y^2}\right);$$

where

$$F_{ss}(N_{ss}, N_{rs}, N_{rr}) = W_{ss}\left[\frac{b}{N}\left(N_{ss} + \frac{N_{rs}}{2}\right)^2\right] - \\ - \alpha N_{ss} N - \mu N_{ss};$$

$$F_{rs}(N_{ss}, N_{rs}, N_{rr}) = \\ = W_{rs}\left[\frac{2b}{N}\left(N_{ss} + \frac{N_{rs}}{2}\right)\left(\frac{N_{rs}}{2} + N_{rr}\right)\right] -$$

$$F_{rr}(N_{ss}, N_{rs}, N_{rr}) = W_{rr}\left[\frac{b}{N}\left(\frac{N_{rs}}{2} + N_{rr}\right)^2\right] - \\ - \alpha N_{rr} N - \mu N_{rr}. \qquad (7)$$

Note that in a non-spatial (point) case with $W_{ij} = 1$, i.e., when the whole pest habitat is a refuge, summation of equations (6) yields a simple logistic equation for the overall population growth: $\frac{dN}{dt} = bN - \mu N - \alpha N^2$ where $N = N_{ss} + N_{rs} + N_{rr}$, b and μ are birth and natural mortality constants, respectively. At $b > \mu$, the relationship $K = (b - \mu)/\alpha$ gives the 'carrying capacity' for the pest population.

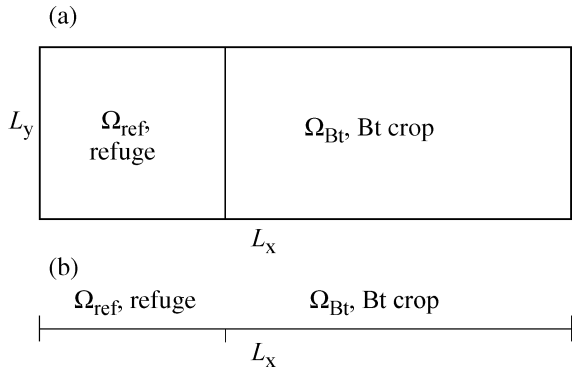

Fig. 1. (a) Single-strip template of rectangular field used in numerical experiments and (b) its 1D simplification without fluxes of pest genotype density along L_y.

Though here we consider only the $b > \mu$ case, note that in the opposite case the 'carrying capacity' should be understood not as K (which becomes negative and corresponds to an unstable equilibrium of the logistic equation) but rather as the zero equilibrium of the model becoming stable at $b < \mu$ (see also [38]).

Translation from the Density to the Frequency Form

Unlike the models based on formally adding the diffusion of the Bt-resistance frequency to the classical FHW equations, the demo-genetic model (6),(3) explicitly describes the spatial transfer and interaction of population densities of ECB genotypes. Translation of this model into allele frequencies makes it easier to see the relationships between the two approaches.

To simplify the subsequent mathematical derivation, let us consider a 1D case of model (6),(3) corresponding to a single-strip template shown in Fig. 1. If in this configuration all genotypes at the initial moment are homogeneously distributed with respect to the L_y direction, there are no density fluxes along this side, the dynamics of the system does not depend on the L_y size and is fully described by a 1D model.

As above, we take that pest genotypes differ from one another only by their survival on Bt maize. Summing all equations (6) and denoting the frequency of each genotype as $u_{ij}(x,t) = \dfrac{N_{ij}(x,t)}{N(x,t)}$, we obtain an equation for the dynamics of the overall population density N:

$$\frac{\partial N}{\partial t} = N(bW - (\mu + \alpha N)) + \delta \frac{\partial^2 N}{\partial x^2}, \qquad (8)$$

where

$$W(u_{ss},u_{rs},u_{rr}) = W_{ss}\left(u_{ss} + \frac{u_{rs}}{2}\right)^2 + 2W_{rs}\left(u_{ss} + \frac{u_{rs}}{2}\right)\left(\frac{u_{rs}}{2} + u_{rr}\right) + W_{rr}\left(\frac{u_{rs}}{2} + u_{rr}\right)^2. \qquad (9)$$

Let us proceed to the frequency form in set (6). To this end, we express the density of each genotype through its frequency and the overall density, $N_{ij} = u_{ij}N$. Then the reproduction/mortality functions (7) can be written as

$$F_{ss}(u_{ss},u_{rs},u_{rr}) = W_{ss}\left[bN\left(u_{ss} + \frac{u_{rs}}{2}\right)^2\right] - \alpha u_{ss}N^2 - \mu u_{ss}N;$$

$$F_{rs}(u_{ss},u_{rs},u_{rr}) = W_{rs}\left[2bN\left(u_{ss} + \frac{u_{rs}}{2}\right)\left(\frac{u_{rs}}{2} + u_{rr}\right)\right] - \alpha u_{rs}N^2 - \mu u_{rs}N;$$

$$F_{rr}(u_{ss},u_{rs},u_{rr}) = W_{rr}\left[bN\left(\frac{u_{rs}}{2} + u_{rr}\right)^2\right] - \alpha u_{rr}N^2 - \mu u_{rr}N. \qquad (10)$$

Denote the reproduction functions as f_{ij}:

$$f_{ss}(u_{ss},u_{rs},u_{rr}) = W_{ss}\left[bN\left(u_{ss} + \frac{u_{rs}}{2}\right)^2\right];$$

$$f_{rs}(u_{ss},u_{rs},u_{rr}) = W_{rs}\left[2bN\left(u_{ss} + \frac{u_{rs}}{2}\right)\left(\frac{u_{rs}}{2} + u_{rr}\right)\right];$$

$$f_{rr}(u_{ss},u_{rs},u_{rr}) = W_{rr}\left[bN\left(\frac{u_{rs}}{2} + u_{rr}\right)^2\right]. \qquad (11)$$

Then the functions of local kinetics (10) appear as

$$F_{ss}(u_{ss},u_{rs},u_{rr}) = f_{ij} - \alpha u_{ij}N^2 - \mu u_{ij}N; \qquad (12)$$

and the initial model can be written in terms of genotype frequencies $u_{ij}(x,t)$ and overall pest density N:

$$\frac{\partial u_{ij}}{\partial t} = \frac{1}{N}f_{ij} - u_{ij}bW + \delta\left(\frac{\partial^2 u_{ij}}{\partial x^2} + 2\frac{1}{N}\frac{\partial u_{ij}}{\partial x}\frac{\partial N}{\partial x}\right) \equiv$$

$$\equiv \frac{1}{N}f_{ij} - u_{ij}bW + \delta\left(\frac{\partial^2 u_{ij}}{\partial x^2} + 2\frac{1}{N}\frac{\partial u_{ij}}{\partial x}\frac{\partial \ln N}{\partial x}\right), \qquad (13)$$

where $u_{ssj} + u_{rs} + u_{rr} = 1$.

Now we proceed from (13) to allele frequencies $p_r = u_{rr} + \frac{1}{2}u_{rs}$; $p_s = u_{ss} + \frac{1}{2}u_{rs}$. Since $p_r + p_s = 1$, it is sufficient to consider the equation for the resistance allele

$$\frac{\partial p_r}{\partial t} = \frac{\partial u_{rr}}{\partial t} + \frac{1}{2}\frac{\partial u_{rs}}{\partial t} = \frac{1}{N}\left(f_{rr} + \frac{1}{2}f_{rs}\right) - $$

$$- p_r bW + \delta\left(\frac{\partial^2 p_r}{\partial x^2} + 2\frac{\partial p_r}{\partial x}\frac{\partial \ln N}{\partial x}\right). \qquad (14)$$

Taking into account $f_{rr} + \frac{1}{2}f_{rr} = p_r b_N (W_{rr}p + W_{rs}p_s)$ as well as equation (8), we obtain

$$\frac{\partial p_r}{\partial t} = bp_r(W_r - W) + \delta\frac{\partial^2 p_r}{\partial x^2} + 2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x};$$

$$\frac{\partial N}{\partial t} = N(bW - (\mu + \alpha N)) + \delta\frac{\partial^2 N}{\partial x^2}; \qquad (15)$$

$$p_s + p_r = 1,$$

where $W_r = W_{ss}p_s + W_{rr}p_r$ is the mean fitness of the resistance allele. The W value can be interpreted as the mean fitness of the entire population and expressed in allele frequencies as $W = W_{ss}p_s^2 + 2W_{rs}p_s p_r + W_{rr}p_r^2$.

The set (15) with boundary conditions

$$\frac{\partial p_r}{\partial t} = \frac{\partial N}{\partial t} = 0 \qquad (16)$$

completely describes the evolution of the frequency of the Bt resistance allele and the dynamics of the overall pest numbers. This model is distinguished from the classical equations of population genetics with diffusion (a spatial FHW model) [16, 31, 32] by the presence of the term $2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x}$, which affects the spatial distribution of the resistance allele and can be interpreted as an 'advective' term describing the directed flow of allele frequency p_r at a rate $-2\delta\frac{\partial \ln N}{\partial x}$ along spatial coordinate x. Such advection arises from the inhomogeneity of the spatial distribution of N and p_r, disappearing if any of the latter is distributed uniformly.

It must be emphasized that in the general case the system (15) does not necessarily evolve at the HW equilibrium: $u_{ss}^* = p_s^2$. $u_{rs}^* = 2p_s p_r$, $u_{rr}^* = p_r^2$. Introducing an additional variable

$$\xi = u_{ss}u_{rr} - \frac{u_{rs}^2}{4}, \qquad (17)$$

that gives the deviation from HWE (see [37]) and expressing genotype frequencies as $u_{ss} = p_s^2 + \xi$, $u_{rs} = 2p_s p_r - 2\xi$, $u_{rr} = p_r^2 + \xi$, we obtain a differential equation for the spatiotemporal dynamics of ξ:

$$\frac{\partial \xi}{\partial t} = b(p_s^2 p_r^2(W_{ss} + W_{rr} - 2W_{rs})\xi W) + \delta\frac{\partial^2 \xi}{\partial x^2} +$$

$$+ 2\delta\frac{\partial \xi}{\partial x}\frac{\partial \ln N}{\partial x} + 2\delta\left(\frac{\partial p_r}{\partial x}\right)^2. \qquad (18)$$

The deviation tends to zero (i.e., the system tends to HWE) only if one of the allele frequencies tends to zero. Otherwise, a polymorphic system with W_{rs} exceeding W_{ss} and W_{rr} evolves beyond HWE; moreover, ξ increases owing to the inhomogeneity of the spatial distribution of allele frequencies.

A TWO-LEVEL DEMO-GENETIC MODEL "PLANT RESOURCE—PEST"

Model (6),(3) as well as its equivalent (15),(16), allows one to solve purposeful problems related to efficient and long-term pest control. Account of the spatiotemporal dynamics of the plant resource expands the applicability of the model and brings it closer to real agroecosystems.

Let the plant (maize) mass increment $R = R(x,t)$ in point x at time t obey the logistic law. Also let the consumption of the biomass by the pest (ECB) be described by a trophic function $g(R)$ defining the individual rations. In the simplest case, $g(R) = aR$ is the Lotka–Volterra linear trophic function. Considering the genetic heterogeneity of the ECB population arising through selection pressure for Bt resistance as well as the possibility of active diffusional movements of the pest within the 1D habitat, we obtain a two-level demo-genetic model describing the dynamic processes of resource–pest interactions:

$$\frac{\partial R}{\partial t} = r_R R(1 - R \,/\, K_R) - aRN;$$

$$\frac{\partial N_{ij}}{\partial t} = eaRf_{ij} - \mu N_{ij} + \delta\frac{\partial^2 N_{ij}}{\partial x^2}, \qquad (19)$$

where $N = \sum N_{ij}$; r_R is the Maltusian coefficient for plant mass increment, K_R is the 'carrying capacity' in respect of the plant resource, a is the coefficient of feed searching efficacy, e is the coefficient of pest

conversion efficacy; f_{ij} sets the proportions of progeny distribution over the three genotypes and the survival of larvae depending on the spatial localization, as above in model (1)–(3):

$$f_{ij}(N_{ss},N_{rs},N_{rr}) = W_{ss}\left[\frac{1}{N}\left(N_{ss}+\frac{N_{rs}}{2}\right)^2\right]$$

$$f_{rs}(N_{ss},N_{rs},N_{rr}) = W_{rs}\left[\frac{2}{N}\left(N_{ss}+\frac{N_{rs}}{2}\right)\left(\frac{N_{rs}}{2}+N_{rr}\right)\right]$$

$$f_{rr}(N_{ss},N_{rs},N_{rr}) = W_{rr}\left[\frac{1}{N}\left(\frac{N_{rs}}{2}+N_{rr}\right)^2\right]. \qquad (20)$$

The other parameters and variables in (19) are similar to those in (1)–(3).

In the frequency form, we get

$$\frac{\partial R}{\partial t} = r_R R(1-R\,/\,K_R) - aRN;$$

$$\frac{\partial p_r}{\partial t} = eaRp_r(W_r - W) + \delta\frac{\partial^2 p_r}{\partial x^2} + 2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x};$$

$$\frac{\partial N}{\partial t} = N(eaRW - \mu) + \delta\frac{\partial^2 N}{\partial x^2}; \quad p_s + p_r = 1,$$

where $W_r = W_{rs}\,p_s + W_{rr}\,p_r$ and

$W = W_{ss}\,p_s^2 + 2W_{rs}\,p_s\,p_r + W_{ss}\,p_r^2$.

Note that the local kinetics term in the balance equation for the allele frequency p_r differs from the continuous FHW form in that the pest fertility coefficient is not constant but depends on the individual rations $g(R) = aR$, increasing with the resource density as specified by the Lotka–Volterra trophic function. In the absence of feed, the pest does not propagate, and the local variations in p_r are caused solely by spatial flows.

As in model (1)–(3), besides diffusion here we have directed p_r fluxes at the advection rate $-2\delta\dfrac{\partial \ln N}{\partial x}$ due to spatial inhomogeneity of pest density and allele frequency.

NUMERICAL EXPERIMENTS

Model Parameter Estimation

In simulations, we used the estimates of the ECB biological characteristics provided by Onstad et al. [27]. The time unit was year (365 days); the space unit, kilometer. The pest was taken to be bivoltine: two generations (from the egg to the winged imago) take one year, the second generation takes the larger part as it includes the winter diapause in the larval stage.

One ECB female on average lays 288 eggs through its lifespan [27]. To determine the fertility coefficient b for equivalent models (6),(3) and (15),(16), we use $b = \dfrac{1}{\tau}\ln\lambda$ where λ is the fertility coefficient in discrete time, τ is the lifespan. The mean annual fertility of ECB is obtained as the weighted sum

$$b = b_1\tau_1 + b_2\tau_2, \qquad (22)$$

where subscripts pertain to the two generations. Then $b = 2\ln\lambda$. Taking the portion of females in the population to be 0.5 so that $\lambda = 144$, we get $b \approx 9.94$ yr^{-1}.

Likewise, we determine the mean annual mortality coefficient μ as the weighted sum for the two generations:

$$\mu = \mu_1\tau_1 + \mu_2\tau_2. \qquad (23)$$

The μ_1 and μ_2 components are estimated assuming that the decline in population density over a certain period τ obeys the exponential law:

$$\frac{N(\tau_i)}{N(0)} = \exp(-\mu_i\tau_i), \quad i = 1, 2. \qquad (24)$$

where $N(0)$ is the pest number at the beginning of the ith period. Knowing the natural survival of the pest [27] in summer (0.077 for larvae of either generation) and in diapause (0.18), we calculate $\mu_1 = -\dfrac{\ln(0.077)}{\tau_1}$ and $\mu_2 = -\dfrac{\ln(0.077\cdot 0.18)}{\tau_2}$. Note that, as in the cited work [27], we disregard the mortality of the egg and adult stages, assuming 100% survival. Thus, $\mu = 6.84$ yr^{-1}.

From the same source [27] we take the maximal number of 22 larvae per plant and the density of 67 000 plants per hectare, which yields a carrying capacity $K = 147.4\cdot 10^6$ larvae per square kilometer. Hence the mean annual competition coefficient is obtained as $\alpha = (b-\mu)/K = 2.1\cdot 10^{-8}$ km^2 yr^{-1} per individual.

For the two-level resource–pest model (19) we additionally estimated the biomass increment coefficient r_R, assuming for maize a mass doubling time of 10 days: $r_R = \dfrac{365}{10}\ln 2 = 25.3$ yr^{-1}, which agrees with the generalized literature data on the growth of dry

above-ground plant mass and the average values observed for cereals [39].

As in the arid zone with irrigation the yield of dry above-ground mass for maize may exceed 400 q/ha (quintal of 100 kg) [39], we assumed a maximum of 500 q/ha. In the chosen units of measurement, the carrying capacity in the plant resource is $K_R = 5 \cdot 10^4$ q km^{-2}.

Other model parameters were selected so that the equilibrium plant resource would be 60% of the capacity, $R^* = 0.6\, K_R$.

The complete development of ECB larvae takes about one month. In this time the larvae can eat 10 times the pupal weight (ca. 5 g), i.e., nearly 50 g. Hence the pest conversion efficacy coefficient can be estimated at $e = 5$ q^{-1}.

The searching efficacy coefficient a will be chosen so as to allow collation of models (6),(3) and (19),(3); namely, in (19),(3) the pest increment eaR at functions f_{ij} in the pest density equation should correspond to the fertility coefficient b in (6),(3). With $R^* = 0.6K_R$, we get $b = eaR^* = 0.6eaK_R$ or $a = \dfrac{b}{0.6eK_R} = \dfrac{9.94}{0.6 \cdot 5 \cdot 5 \cdot 10^4} = 66.27 \cdot 10^{-6}$ km^2 yr^{-1}.

As the diffusion coefficient δ is difficult to estimate from field data, it was varied in simulations.

Simulation Scenario

For numerical experiments, the initial continuous models (6) and (19) with boundary conditions (3) were discretized in space using a uniform grid, in each node of which the spatial derivatives were approximated with central differences. The resulting set of ordinary differential equations at given initial conditions was numerically integrated using a fourth-order Runge–Kutta procedure with automatic time stepping. The space step was chosen to trade off between minimal error and maximal speed of calculation. As a result, we used 100 nodes for a typical cornfield size of 40 km (fixed in all experiments), i.e., a space step of 400 m, while the time step was automatically varied from a day to a fortnight. The stability of the procedure was checked by calculations with a doubled space grid.

It was assumed that at the initial moment, i.e., at planting maize Bt hybrids, there are no resistant homozygotes (*rr*) in the field, while a low frequency of the Bt-resistance allele is maintained in the modest number of heterozygous individuals (*rs*). This assumption is fully consistent with the field studies on natural ECB populations [17, 18]. The initial pest genotype frequencies (insects per square kilometer) were taken to be $N^0_{ss} = 367\,400$, $N^0_{rs} = 1100$ (0.3% of the total density), $N^0_{rr} = 0$; the total $N^0 = 368\,500$ was 0.25% of the carrying capacity K. Individuals of each genotype were initially homogeneously distributed through space; thereby the initial frequency of the r allele in the pest population was $p^0_r = \dfrac{N^0_{rr} + 0.5N^0_{rs}}{N^0} = 0.0015$, which slightly exceeded the value ($< 10^{-3}$) reported for natural ECB populations [17, 18].

For the resource–pest model, the initial density of the plant dry mass was taken to be $R^0 = 1500$ q km^{-2}, also assuming uniform space distribution.

Delay of Bt Resistance in the Pest Population

To recall, the "high dose" defined as the one killing 100% of susceptible homozygotes and 95% of heterozygotes ($s = 1$, $h_s = 0.95$), and no cost was put on resistance ($c = 0$). Thus the fitness (survival) of the three ECB genotypes is set as follows:

$$W_{rr} \equiv 1,\ \forall (x, y) \in \Omega;$$
$$W_{rs}(x, y) = \begin{cases} 1, & (x, y) \in \Omega_{\text{Ref}}; \\ 0.05, & (x, y) \in \Omega_{\text{Bt}} \end{cases} \qquad (25)$$
$$W_{ss}(x, y) = \begin{cases} 1, (x, y) \in \Omega_{\text{Ref}}; \\ 0, (x, y) \in \Omega_{\text{Bt}} \end{cases}$$

The refuge is at the left of the cornfield (Fig. 1).

At a fixed refuge size (20% of the total area) and a fixed diffusion coefficient $\delta = 8$ km^2 yr^{-1}, simulations in model (6),(3) show that from the moment of implementing the HDR strategy the initially homogeneous distribution of genotype densities loses stability, and in three years a spatially nonuniform regime is established in the field (fig. 2a) where the overall pest density in the refuge is somewhat less than the carrying capacity while in the Bt region there is practically no infestation. Note that all over the field the population is represented mainly by the *ss* genotype (no less than 99%). At the given parameter values, this regime persists for nearly 500 yr. Nonetheless, despite the intense influx of Bt-susceptible insects from the refuge, the *rr* genotype has essential advantage on the Bt area, and since the establishment of

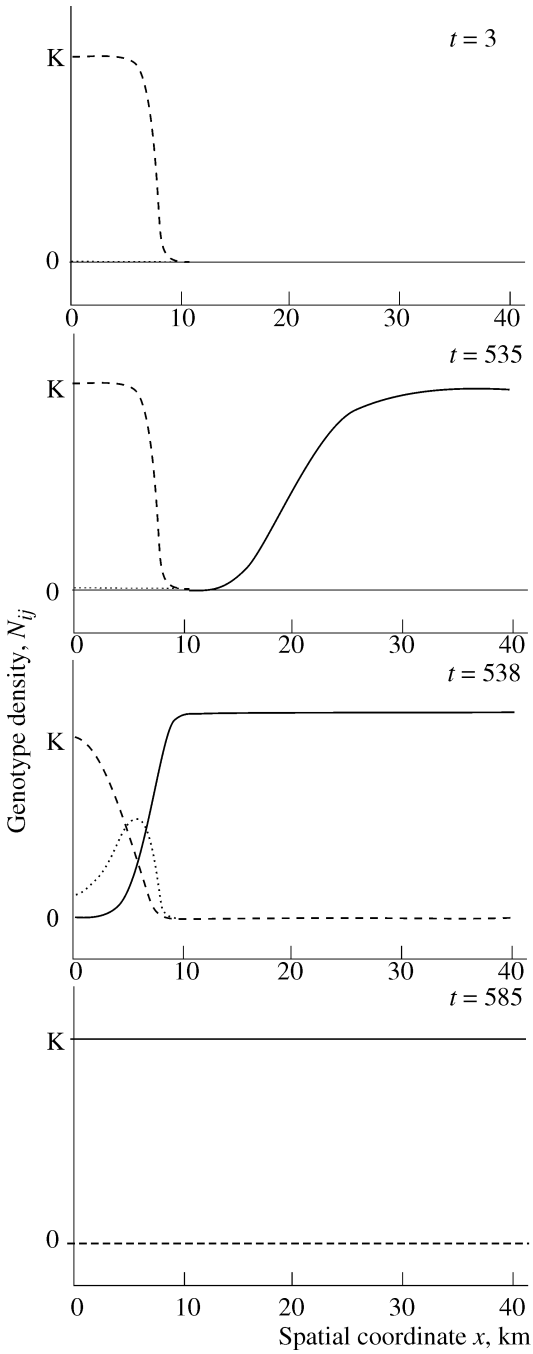

Fig. 2. ECB genotype density distributions (solid line, *rr*; dashed line, *ss*; point line, *rs*) over a 1D model field of $L_x = 40$ km with a 20% refuge at the left (Fig. 1) at indicated times t (years) after planting as predicted by the demo-genetic model (6),(3) assuming pest mobility $\delta = 8$ km^2 yr^{-1}.

spatial inhomogeneity (Fig. 2a) its density (as well as the frequency of the resistance allele) rises very slowly but invariably. Thus the system develops towards another spatially homogeneous state whereby the *rr* genotype overwhelms the susceptible *ss* and *rs* throughout the habitat (Fig. 2d). It is noteworthy that after the resistance gene frequency reaches a certain critical level ($p_r \approx 10\%$), it takes only 5 yr for the *rr* genotype to spread over the whole Bt field (Fig. 2b,c) and about 50 yr to completely displace *ss* and *rs* from the refuge (Fig. 2c,d).

Without a cost for resistance, the spatially uniform distribution $N_{ss}(x) = N_{rs}(x) = 0$, $N_{rr}(x) = K$, $\forall x \in \Omega$ is the only stable steady state of model (6),(3).

We then evaluated the time $T(p_r < 0.1)$ in which the frequency of the Bt-resistance allele in the ECB population reaches 10% in model (6),(3) at different pest mobilities and refuge sizes (Table 1). Both at low mobility ($\delta \le 0.1$ km^2 yr^{-1}) and at complete mixing between the transgenic and the refuge areas ($\delta = \infty$), $T(p_r < 0.1)$ smoothly depends on the refuge size, but there is no essential delay in any case: resistance develops in less than 25 yr. At intermediate mobility ($\delta = 1 \div 10$ km^2 yr^{-1}) the model forecasts dramatic 'jumps' in the delay $T(p_r < 0.1)$ with refuge size variation (however, the monotonic dependence holds at any δ). This estimated delay time amounts to centuries and even millennia (Table 1), corroborating the expedience of the HDR strategy. High pest mobility ($\delta = 10 \div 150$ km^2 yr^{-1}) smoothes out the delay vs. refuge size dependence. Viewed otherwise, Table 1 makes evident that increasing pest mobility at any fixed refuge size first causes a jump in the delay time and then a gradual decline.

For comparison, we performed the same computations at the same parameters in the conventional frequency-based approach (a FHW diffusional version, Table 2). One can see that throughout the δ variation range the time $T(p_r < 0.1)$ monotonically increases with the refuge size. On the other hand, increasing pest mobility at modest refuge size (5–20%) monotonically shortens the delay, whereas with larger refuges the monotony is broken: the delay time first declines with increasing mobility and then tends to rise again. However, all the $T(p_r < 0.1)$ values thus obtained are too low to support this resistance management strategy: mostly less than 10 yr, and within 25 yr at any combinations.

Explicit Account of the Plant Resource in the Demo-genetic Model

To assess the influence of the plant resource, we compared the delays predicted by our two-level

Table 1. The time $T(p_r < 0.1)$ (years) in which the frequency of the resistance allele in the pest population reaches 10% as predicted by the demo-genetic model (6),(3) at different refuge sizes and pest mobilities

Diffusion coefficient (δ) $km^2\ yr^{-1}$	Refuge size, %									
	5	10	15	20	25	30	35	40	45	50
$1\cdot10^{-6}$	12	19	21	24	24	25	25	25	25	25
$1\cdot10^{-5}$	11	13	14	16	17	19	19	21	22	24
$1\cdot10^{-4}$	11	12	13	14	15	16	17	19	19	21
0.01	12	12	13	13	14	14	14	15	15	16
0.1	24	25	25	25	25	25	25	25	25	25
1	25	25	25	25	25	26	26	27	2482	3012
2	25	25	26	723	993	1293	1621	1978	2360	2768
3	95	270	478	708	959	1229	1519	1828	2155	2499
4	85	250	451	668	902	1150	1412	1689	1980	2283
5	77	230	421	627	846	1076	1318	1572	1836	2111
6	70	212	393	589	795	1012	1238	1473	1717	1970
7	64	196	368	554	751	955	1168	1389	1617	1853
8	59	182	345	524	711	906	1107	1316	1531	1753
9	55	170	325	496	676	862	1054	1253	1457	1667
10	52	159	307	472	644	823	1007	1197	1391	1591
50	15	47	97	161	236	318	404	490	578	666
100	11	26	54	89	132	181	235	292	351	412
150	9	19	37	63	93	128	167	210	255	302
…	…	…	…	…	…	…	…	…	…	…
∞	7	7	8	8	9	9	10	11	12	13

demo-genetic model (19),(3) and a bitrophic FHW diffusional model (corresponding to (21),(16) less the advective term $2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x}$, see above) with 10% or 20% refuge. Just as in simulations without the resource, the demo-genetic model predicts far longer times to Bt resistance than the FHW version, as well as a dramatic jump of $T(p_r < 0.1)$ followed by a decline with increasing pest mobility δ (Fig. 3). For complete mixing between the Bt and refuge areas, the two models give a similar outcome: quite quick (within 10 yr) spreading of the *rr* genotype in the pest population (not shown).

DISCUSSION

Here we have demonstrated that formal addition of a diffusion term into a FHW model may lead to serious errors in forecasting the evolution of the genetic structure of a spatially distributed population. The origin of this fallacy is clear. The FHW concept initially concerned species with an ecologically autonomous haplophase [33]. Application of such a model to population dynamics of diploid organisms implies a number of auxiliary conditions that would ensure panmictic reproduction, specifically, uniform spatial distribution of the population and absence of density fluxes. Nonetheless, the approach relying on FHW diffusional models remains quite popular, and it has been used to describe the spatiotemporal dynamics of the resistance allele in ECB populations [11, 14–16, 27, 30–32].

An alternative is the demo-genetic approach put forward by V.A. Kostitzin (1883–1963), a disciple of V.I. Vernadsky and an outstanding Russian mathematician, astrophysicist, and biophysicist. He was the first to recognize that the competition theory developed by Volterra for interaction between species can

Table 2. The time $T(p_r < 0.1)$ (years) in which the frequency of the resistance allele in the pest population reaches 10% as predicted by a FHW-based diffusional model at different refuge sizes and pest mobilities

Diffusion coefficient (δ) km^2yr^{-1}	Refuge size, %									
	5	10	15	20	25	30	35	40	45	50
$1 \cdot 10^{-6}$	12	14	15	18	19	21	22	24	25	25
$1 \cdot 10^{-5}$	11	13	14	16	17	19	19	21	22	24
$1 \cdot 10^{-4}$	10	11	12	14	15	16	17	18	19	20
0.01	9	10	10	10	11	11	12	12	12	13
0.1	9	9	9	10	10	10	10	10	10	11
1	8	9	9	9	9	9	9	9	9	9
2	8	8	9	9	9	9	9	9	9	9
3	8	8	9	9	9	9	9	9	9	9
4	8	8	9	9	9	9	9	9	9	9
5	8	8	8	9	9	9	9	9	9	9
6	8	8	8	9	9	9	9	9	9	9
7	8	8	8	9	9	9	9	9	9	9
8	8	8	8	8	9	9	9	9	9	9
9	8	8	8	8	9	9	9	9	9	9
10	8	8	8	8	9	9	9	9	9	9
50	7	8	8	8	8	8	9	9	9	9
100	7	7	8	8	8	9	9	9	10	10
150	7	7	8	8	8	9	9	9	10	11
…	…	…	…	…	…	…	…	…	…	…
∞	7	7	8	8	9	9	10	11	12	13

also be applied to interaction between genotypes in a diploid population [34]. This approach allows explicit description of the evolutionary selection of the fittest genotype as an immediate result of intraspecies competition. Regretfully, Kostizin's works (which were highly commended by Volterra, see preface in [34]) and particularly his criticism of the unjustified used of Fisherian frequency models, are now known unto few [40, 41], though in Russia his demo-genetic approach was furthered by some authors [33, 37].

By contrast to the FHW-based frequency models, the Kostitzin model describes the population dynamics at the level of genotype densities and thus can most naturally be used in spatial reaction–diffusion modeling. We hope that this circumstance will attract the attention of researchers, which should be spurred by the currently growing interest in studies on spatially distributed ecosystems.

Local interaction of pest genotypes in the demogenetic model (6),(3) is described by Kostitzin equations that are somewhat modified according to the specific features of the modeled system; namely, genotype fitness is considered here in terms of larvae survival coefficients instead of genotype fertility.

It should once again be stressed that the use of differential equations makes model (6),(3) a continuous approximation of the processes of pest reproduction and succession of generations, which under clearly seasonal conditions are actually discrete rather than continuous. However, since the model is intended exclusively for long-term forecasting, its continuity is a natural simplification that should be taken into account when interpreting the results.

An accurate translation of the initial density form (6),(3) into the frequency form (15),(16) reveals its basic distinction from the diffusional version of the

FHW model: along with the diffusive propagation of allele frequency, the demo-genetic model considers a directed gene flux induced by the inhomogeneity of the pest density distribution. This means that a FHW diffusional model would adequately describe the spatial spread of the resistance gene only if the overall density N is uniformly distributed throughout the entire model field Ω.

How large can be the influence of the advective term $2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x}$ in (15) becomes obvious upon comparison of the results obtained with the two models (cf. Tables 1 and 2). With various combinations of the refuge size and pest mobility, the HDR strategy modeled by (6),(3) can delay the spread of resistance by hundreds and even thousands of years (Table 1)—an effect not nearly attainable with the FHW version (Table 2). The delays predicted by the demo-genetic model can easily explain why, despite the broad cultivation of transgenic maize over a decade, no Bt-resistant homozygous ECB has yet been detected.

Thus, our quite simple demo-genetic model, unlike FHW, can reproduce and substantiate the efficacy of the refuge in retarding the evolvement of pest resistance to the Bt crop.

The success of the HDR strategy is determined not just by the existence of a refuge for susceptible insects but by the intensity of their flux from the refuge onto the Bt field, which provides for mating between Bt-resistant insects migrating from the Bt field and the susceptible insects from the refuge, thereby lowering the frequency of the resistance allele in every next generation. It is this flux that allows the system (6),(3) to persist for a long time in the vicinity of an unstable spatially inhomogeneous steady state corresponding to the absence of *rr* and *rs* genotypes (Fig. 2a) before transition to a stable homogeneous steady state $N_{ss}(x) = N_{rs}(x) = 0$, $N_{rr}(x) = K$ (Fig. 2d). It is important that over this time throughout the field the spatial gradients of overall density $N(x)$ and resistance gene frequency $p_r(x)$ are opposite, so the advective flux counteracts the rise in $p_r(x)$ because $2\delta\frac{\partial \ln N}{\partial x}\frac{\partial p_r}{\partial x} < 0$. As the ecological characteristics of all ECB genotypes are identical, in the refuge proper the *rr* genotype is simply outnumbered by the susceptible ones and thus completely displaced by competition. In the Bt area, especially at the refuge border, *rr* is suppressed by *ss* and *rs* coming from the refuge. Remarkably, as soon as the number of resistant homozygotes in the Bt area becomes large enough (Fig. 2b), the advective flux of gene frequency theretofore opposing its diffusive flow reverses its direction and promotes the spatial spread of the resistance allele (Fig. 2c) in the final stage of the transition process.

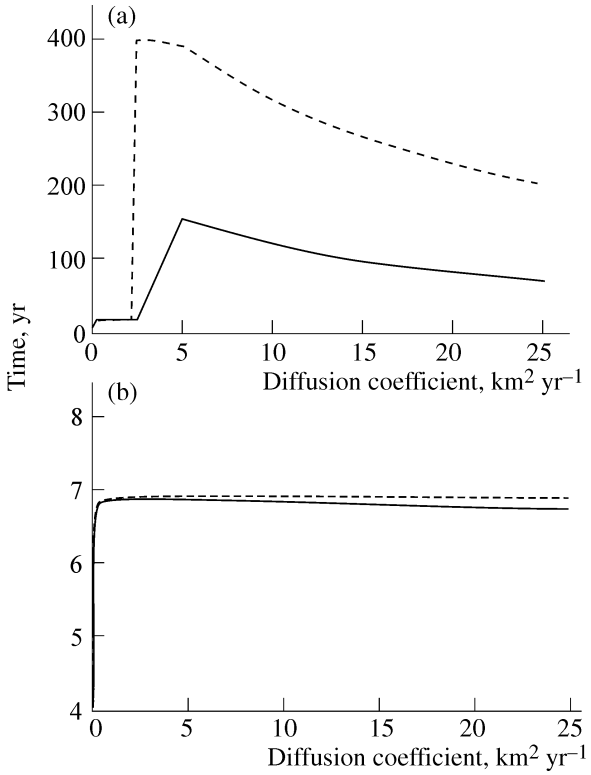

Fig. 3. The time $T(p_r < 0.1)$ (years) in which the frequency of the resistance allele in the pest population reaches 10% as a function of pest mobility (δ) predicted by (a) the two-level demo-genetic resource–pest model (19),(3) and (b) a diffusional model based on FHW equations [i.e.,

(21),(16) without the advective term in the p_r equation] for a field with (solid) 10% and (dashed) 20% refuge.

Thus, a key factor of the effectiveness of the refuge as a source of susceptible insects is the pest mobility. As already noted in analysis of Table 1, very low mobility does not ensure an efflux of susceptible genotypes that would suffice for overwhelming the resistant genotype in the Bt area. In this case, such weak diffusion simply subtracts from the *ss* density in the refuge, increasing the chances of its displacement by *rr* immigrants from the Bt area and thus speeding up the spread of resistance. Interestingly, refuge efficacy also declines when the mobility (i.e., diffusion

exchange between refuge and Bt area) is infinitely high, resulting in system homogenization and panmixia. One can see that in both extreme cases the demo-genetic and the FHW models give similar prognoses (cf. tables).

Incorporation of an explicit description for the spatiotemporal dynamics of maize biomass in the demo-genetic model (19),(3) allows account of the dependence of pest reproduction on the state of the plant resource, thereby making the model more realistic. The results of numerical experiments with (19),(3) qualitatively agree with those for the basic model (6),(3). This confirms the importance of considering the directed gene flux in the frequency form (21),(16). Collating Fig. 3 with the corresponding columns in Table 1, one can see that Bt resistance in the two-level (19),(3) emerges somewhat faster than in (6),(3). Indeed, in the Bt field where infestation is largely suppressed by the toxin the biomass density exceeds the equilibrium $R^* = 0.6K$. This raises the pest feeding rations so that its reproduction rate in (19),(3) becomes higher than in (6),(3). [Recall that in (19),(3) the pest reproduction intensity determines the rate of evolution of the population genetic structure, see (21).] Probably the use of a more realistic trophic function accounting for saturation of the rations with increasing resource, e.g., a Holling type II function $g(R) = aR/(1 + ahR)$, would extend the delay $T(p_r < 0.1)$.

To bring the model closer to reality, one should perhaps also consider the seasonal events in the system, in particular, regular replanting and harvesting (see. e.g., [42]). How can this affect the $T(p_r < 0.1)$ estimates? On the one hand, any events resulting in periodic reduction of plant mass and pest density, especially in the beginning of the crop year, should additionally delay the evolution of resistance. On the other hand, periodical homogenization of the system is likely to accelerate the spreading of the resistance gene. A definite answer awaits further studies.

Practical use of the conceptual model offered here requires field observations and identification of the parameters of a particular agroecosystem. A most important factor determining the success of the HDR strategy is the diffusion coefficient δ, which is hard to specify. Evaluation of this parameter requires monitoring of large-scale movements of the pest density spots rather than rapid motion of adult moths; there are examples of such field studies [43, 44].

ACKNOWLEDGMENTS

We thank D. Bourguet, S, Ponsard, and C. Vacher for fruitful discussions.

The work was supported by the RF Ministry of Education (A04-2.12-358), Russian Foundation for Basic Research (05-04-04000), US CRDF (RO-004-X1), Rostov State University, Institut National Agronomique Paris-Grignon, and the CNRS program "Impact des biotechnologies dans les agro-ecosystemes."

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