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Population Ecology of Lepidopterous Pests

Abstracts

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ВСЕРОССИЙСКИЙ НИИ ЗАЩИТЫ РАСТЕНИЙ РАСХН

ЦЕНТР ИЗУЧЕНИЯ БИОЛОГИИ И УПРАВЛЕНИЯ ПОПУЛЯЦИЯМИ, ИНРА МОНПЕЛЬЕ

ЛАБОРАТОРИЯ ДИНАМИКИ БИОРАЗНООБРАЗИЯ, УНИВЕРСИТЕТ П. САБАТЬЕ, ТУЛУЗА

РОССИЙСКО-ФРАНЦУЗСКОЕ РАБОЧЕЕ СОВЕЩАНИЕ ПО ЗАЩИТЕ РАСТЕНИЙ

Популяционная экология чешуекрылых вредителей

Тезисы докладов

Санкт-Петербург – Пушкин, 28 октября 2004 г.

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All-Russian Institute of Plant Protection RAAS
Centre de Biologie et de Gestion des Populations, *INRA Montpellier*
Laboratoire Dynamique de la Biodiversité, Université P. Sabatier - Toulouse

Научные редакторы А.Н.Фролов, И.Я.Гричанов (ВИЗР)

Editors A.N. Frolov, I.Ya. Grichanov (VIZR)

Foreword

There is a number of lepidopterous species, which causes economically significant harm to agriculture. In particular, such pests as webworm moth, *Loxostege sticticalis*, and corn earworm, *Helicoverpa armigera*, are especially dangerous in Russia and neighbour states, not representing any serious threat for countries of the Western Europe. Others, such as the European corn borer, *Ostrinia nubilalis*, are economically significant objects both in Europe, and in West Asia and North America. Improvement of plant protection and first of all forecasting methods requires careful study of population dynamics, population structure, host-plant relations and microevolutionary processes in harmful pests. The urgency of such works rises due to expansion of areas occupied by genetically modified crops in the world during the last years.

This report reflects materials presented in the course of the Crop Protection Workshop, which was held in the All-Russian Plant Protection Institute during a visit of French experts in the field of entomology to St. Petersburg. Researches conducted in INRA and University of Toulouse are partly devoted to study of ecology of the European corn borer in connection with possible adaptation of the pest to resistance of genetically modified maize. This insect represents an object of intensive investigation in Russia for a long time now. During the Workshop there was a perspective exchange of scientific information and working materials between the Russian and French experts. Therefore I suppose that the given Workshop should promote expansion of fruitful cooperation between entomologists of both our countries.

Vladimir A. Pavlyushin
Academician, Director of All Russian Institute
of Plant Protection RAAS, St. Petersburg, Pushkin

November 28, 2004
St. Petersburg, Pushkin

Brief review of main directions in scientific research on the European corn borer in the All-Russian Institute for Plant Protection

Frolov A.N.

All-Russian Institute of Plant Protection, St. Petersburg, Pushkin

Краткий обзор основных направлений изучения кукурузного мотылька во Всероссийском НИИ защиты растений

Фролов А.Н.

Всероссийский НИИ защиты растений РАСХН,

Санкт-Петербург – Пушкин

The European corn borer (ECB), *Ostrinia nubilalis* Hbn., and its near relatives of the genus *Ostrinia* are widespread insect pests in Russia and neighbour countries (Fig. 1). Many entomologists conducted researches on the ECB, *e.g.* V.N. Shchegolev, I.V. Kozhanchikov, V.O. Khomyakova, I.D. Shapiro, N.A. Vilkova, D.S. Pereverzev and others. The pest was intensively studied in the All-Russian (formerly All-Union) Institute for Plant Protection, especially since 1970-s.

There were at least 4 specific trends of research, namely 1) basic studies (morphology, ecology, life history, biosystematics, microevolution of insects); 2) analysis of population dynamics of the ECB; 3) study of host-plant relations and host-plant resistance; 4) elaboration of other control tactics, viz., pheromone trials, chemical spraying, and agronomical control.

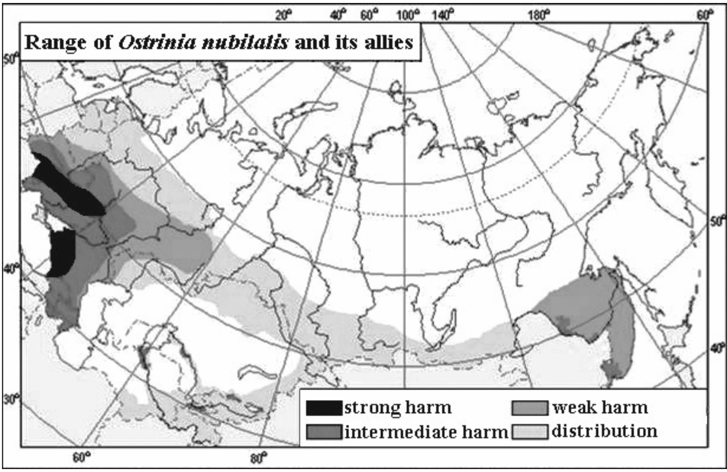

Fig. 1. Distribution and zones of harmfulness of *Ostrinia nubilalis* and allies on the territory of the former USSR (slightly modified after Frolov, Saulich, 2002)

1) Population variability of borers was analysed on the basis of i) morphology of male midtibiae; ii) biological properties of *Ostrinia* races and “biospecies”, with special reference to larval ability to survive on various

host plants; iii) formation of reproductive isolation in the genus *Ostrinia*; iv) peculiarities of genetic structure of allopatric and sympatric populations; v) geographic distribution of populations with specific morphology and biology (Frolov, 1984, 1994a, b, c, 1998).

2) Life table analysis of the European corn borer is now in progress for two geographic populations of the pest, which inhabit different climatic zones of Russia, namely the Krasnodar Territory (2-generations zone) and the Belgorod Region (1-generation zone) (Frolov *et al.*, 1999a; Chumakov, Frolov, 2000; Frolov, 2004a, b). It was shown that there is an obvious periodicity in insect outbreaks (Fig. 2), influenced by some egg and larval parasites. Density-dependence of mortality factors is clear when the second generation develops (Fig. 3).

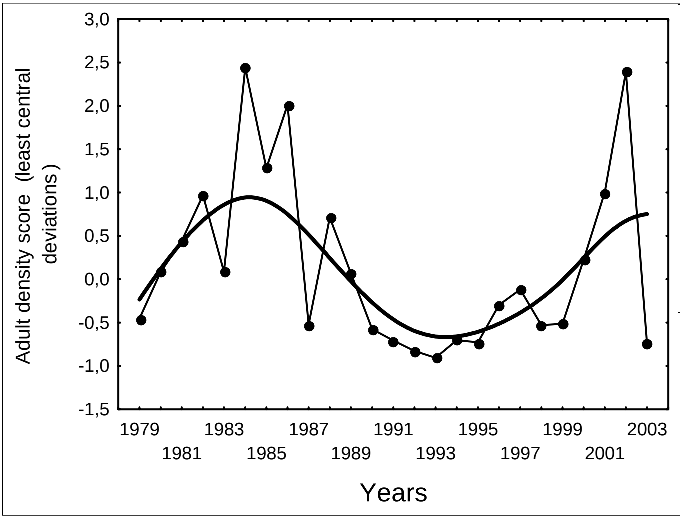

Fig. 2. Variation of the first generation European corn borer adult numbers at the Kuban experimental agricultural station (Krasnodar Terr., 1979-2004).

3) Host-plant relations of the ECB with maize and sorghum were specified on the basis of egg-laying and larval survival estimations. Ecological stability of maize resistance to the pest was studied and genetic factors of maize influencing the level of resistance were also determined. Many thousands of maize inbreds and hybrids were tested for resistance to the pest during decades (Frolov, Chumakov, 1990; Frolov, Khromenko, 1988, 1992, 1993; Sotchenko *et al.*, 1993; Dyatlova, Frolov, 1999; Frolov *et al.*, 2000).

4) Features of the ECB adult spatial distribution were specified, and efficiency of the pest control by chemical spraying of places of adult concentration was confirmed. Trials of pesticides were performed, and application of pheromone traps for the ECB forecasting was described (Frolov *et al.*, 1996, 1999b; Frolov, Chumakov, 1989; Frolov, Pshinka, 1989; Frolov, 1993).

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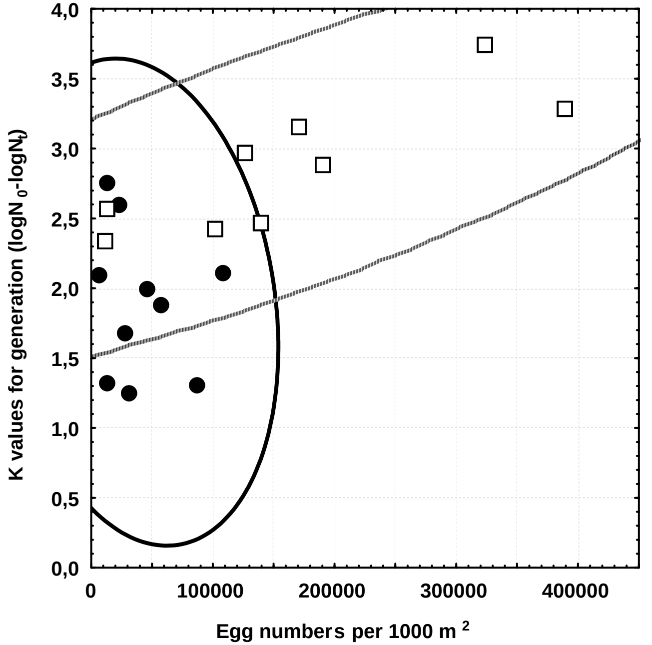

Fig. 3. Egg density and over-all insect mortality for 1st and 2nd European corn borer generations (Krasnodar Terr., 1994-2004)

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References.

Chumakov M.A., Frolov A.N., 2000. Population dynamics factors of the European corn borer *Ostrinia nubilalis* (Hbn.) (Lepidoptera, Pyralidae) in Central Chernozem zone of Russia. *Entomol. Obozr.* 79 (3): 543-549 (In Russian)

Dyatlova K.D., Frolov A.N., 1999. Egg laying and survival of the European corn borer, Ostrinia nubilalis, on sorghum vs. maize in two-generation zone of Russia. *Proceedings of the XX Conf. IWGO.* Adana (Turkey), 4-10 Sept., 1999. p. 80-93.

Frolov A.N., 1984. Biotaxonomic analysis of harmful species of the genus *Ostrinia* Hbn. In: Ethologiya Nasekomykh, Trudy Vsesoyuznogo Entomol. Obshchestva, Leningrad, USSR. Nauka Publ. 66: 4-100. (In Russian)

Frolov A.N., 1993. Genetical peculiarities of maize influencing on efficiency of chemical spraying against the European corn borer. In: Genetica ustoichivosti rastenii k boleznyam i vreditalyam, *Bull. Appl. Bot., Gen. and Plant Breed.* 147: 39-44 (In Russian)

Frolov A.N., 1994a. Geographic variation of population structure in *Ostrinia* spp. living on dicotyledonous host plants and factors determining variation. *Zool. Zhurn.* 73 (3): 47-59 (In Russian)

Frolov A.N., 1994b. Population structure and determinations of evolution within the genus *Ostrinia. Zool. Zhurn.* 73 (3): 60-71 (In Russian)

Frolov A.N., 1994c. Formation of reproductive isolation barriers in the European corn borer, *Ostrinia nubilalis*: differences in host plant exploitation strategies.

Zhurn. Obshch. Biol. 55 (2): 189-97 (In Russian)

Frolov A.N., 1998. Variation in the European corn borer, Ostrinia nubilalis, and allies (Lepidoptera, Pyralidae). *Mem. Soc. r. belge Ent.* 38: 71-105.

Frolov A.N., 2004a. Biotic factors suppressing the European corn borer, *Ostrinia nubilalis. Plant Protection News.* 2: 37-47 (In Russian)

Frolov A.N., 2004b. Natural entomophages as inductors of cyclic population oscillations of the European corn borer in the Krasnodar area. In: Biologicheskaya zashchita rastenii – osnova stabilizatsii agroekosistem, 3: 15-17 (In Russian)

Frolov A.N., Chumakov M.A., 1989. Biological effectiveness of synthetic pyrethroids to the European corn borer. *Bull VIZR.* 73: 31-34 (In Russian)

Frolov A.N., Chumakov M.A., 1990. Early lines of maize resistant to the European corn borer. *Selektsiya i semenovodstvo.* 5: 29-30 (In Russian)

Frolov A.N., Dyatlova K.D., Chumakov M.A., 1999a. Population dynamics of *Ostrinia nubilalis*: specificity in key factors for one and two generation zones of Russia. *Proceedings of the XX Conf. IWGO*, Adana (Turkey), 4-10 Sept., 1999. p. 64-79.

Frolov A.N., Frolova T.A., Garkushka V.G., Tsaregorodtseva O.E., 1999b. The European corn borer: plant damage and yield of field maize. *Agro XXI.* 1: 14-15 (In Russian)

Frolov A.N., Khromenko A.S., 1992. Ecological stability of antibiotic resistance of lines of early-maturing maize to the European corn borer *Ostrinia nubilalis* (Hbn.) (Lepidoptera, Pyraustidae) during leaf whorl stage. *Entomol. Obozr.* 71 (3): 86-97 (In Russian)

Frolov A.N., Khromenko A.S., 1988. Estimation of additive component of variation in the European corn borer by its ability to survive on maize. In: Izmenchivost' nasekomykh-vreditelei v usloviyakh nauchno-tekhnicheskogo progressa v sel'skom khozyaistve. Leningrad, VIZR, p. 30-38 (In Russian)

Frolov A.N., Khromenko A.S., 1993. Damage of early maize diallel crosses by the European corn borer. In: Genetica ustoichivosti rastenii k boleznyam i vreditalyam, *Bull. Appl. Bot., Gen. and Plant Breed.* 147: 44-49 (In Russian)

Frolov A.N., Pshinka E.F., 1989. Damage of early inbreds by the European corn borer and biological efficiency of deltamethrine for protection of seed-farming maize sowings. *Bull. VIZR* 73: 35-43 (In Russian)

Frolov A.N., Saulich M.I., 2002. Distribution of the European corn borer (*Ostrinia nubilalis*). http://www.agroatlas.spb.ru/pests/Metadata/Meta_Ostrinia_nubilalis_ru.htm. (In Russian)

Frolov A.N., Trishkin D.S., Dyatlova K.D., Chumakov M.A., 1996. Spatial distribution of the European corn borer, *Ostrinia nubilalis*, adults at the two-generation area and its relationship to maize attack. *Zool. Zhurn.* 75 (11): 1644-1652 (In Russian)

Frolov A.N., Tsaregorodtseva O.E., Krapivenko T.M., 2000. Effectiveness of maize antibiotic resistance to the first generation of European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera, Pyralidae), and precocity of the plants. *Entomol. Obozr.* 79 (2): 322-327 (In Russian)

Sotchenko Yu.V., Chumakov M.A., Frolov A.N., 1993. Screening for resistance to the European corn borer. *Kukuruza i Sorgo.* 2:15. (In Russian)

Bt resistance and population genetics of the European corn borer: implications for the management of Bt maize

Bourguet D.[1], *Ponsard S.*[2]

[1] *Centre de Biologie et de Gestion des Populations (CBGP), INRA Montpellier, Campus International de Baillarguet, France*

[2] *Laboratoire Dynamique de la Biodiversité, UMR CNRS 5172, Université P. Sabatier - Toulouse III, 118 route de Narbonne, 31 062 Toulouse, France*

Bt устойчивость и популяционная генетика кукурузного мотылька: выводы для управлении Bt кукурузой

Буржэ Д.[1], *Понсар С.*[2]

[1] *Центр изучения биологии и управления популяциями (ЦБУП), ИНРА Монпелье, Международный Университетский городок Бэлларже, Франция*

[2] *Лаборатория динамики биоразнообразия, Университет П. Сабатье, Тулуза, Франция*

Introduction

The last ten years have seen a steady increase in the number of genetically modified crops producing *Bacillus thuringiensis* (Bt) toxins (Bt crops, Navon 2000) with ~ 10 million hectares planted worldwide in 2003 (James, 2003). Increases in sales of these Bt crops have increased the risk that the targeted insect pest species will become resistant to this ecologically valuable class of toxins (Gould 1998; Wolfenbarger, Phifer 2000). In order to manage the evolution of Bt resistance, several countries have implemented the high dose refuge strategy described by Alstad and Andow (1995). In this strategy, refuges are defined as non-Bt plants that can be used by the target pest, planted and maintained in close proximity to Bt-crops (Gould 1998). The principle underlying this system of resistance management is that any resistant insects emerging from Bt crops are more likely to mate with one of the much larger number of susceptible adult pest insects emerging from the refuges than with each other, thereby decreasing the selection of Bt resistance alleles.

An effective high dose/refuge strategy requires three main components. First, the increase in fitness conferred by resistance alleles must be recessive so that individuals heterozygous for a resistance allele are killed by the toxin produced by plant tissues. Second, resistance alleles must be rare so that few homozygotes survive on Bt crops. Third, resistant insects selected on Bt crops should mate randomly, or preferentially with susceptible insects preserved on non-Bt crops.

The most important Bt crop worldwide is Bt maize, producing toxins active against the European corn borer, *Ostrinia nubilalis* (Hübner), the major lepidopteran pest of maize in North America and Europe (Krattiger 1997). Here, we briefly review trials, which are directly related to the three components of the high dose/refuge strategy for managing Bt resistance in the natural populations of *O. nubilalis*.

Selection for Bt resistance

Laboratory selection for Bt resistance has been successful for several insect pest species (Tabashnik *et al.* 2003). The diamondback moth, *Plutella xylostella* (L.), is the only insect to have evolved high levels of resistance in the field as a result of repeated use of formulated Bt insecticide (Tabashnik *et al.* 1990). The potential of *O. nubilalis* to develop Bt resistance has led to numerous studies involving long-term selection with the Cry1Ab and Cry1Ac toxins. In populations from Iowa and Kansas (Huang *et al.* 1997), significant resistance was found after three to seven generations of laboratory exposure to Dipel ES, a composite of Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and Cry2B endotoxins, and after 4 generations of selection with Cry1Ac toxin in Minnesota populations (Bolin *et al.* 1999). Successful selection for resistance has also been reported in two independent selection experiments reported by Chaufaux *et al.* (2001). In these selection, the highest levels of resistance were obtained at generation 7 (14 fold), generation 9 (13-fold), and generation 9 (32-fold) for three different strains. For each strain, the level of resistance fluctuated from generation to generation, but toxin susceptibility significantly decreased over generations for all selected strains.

These results suggest that low levels of resistance are common in populations of *O. nubilalis*. They may be due to the effects of multiple genes, each making a small contribution to overall resistance. Huang et al. 2002 showed that these selected populations are unlikely to survive on transgenic Bt maize. Indeed, in terms of the development of Bt resistance in the field, the principal concern is the presence of rare major resistance alleles, although the multiple effects of minor and/or modifier genes may still contribute to fitness in the field.

Initial frequency of Bt resistance alleles

The F_2 screen, developed by Andow and Alstad (1998), is an elegant method for the estimation of low frequencies of recessive resistance alleles. It consists of four steps: firstly, sampling mated adult females from natural populations and establishing isofemale lines; secondly, rearing and sib-mating the F_1 progeny of each isofemale line; thirdly, screening F_2 neonates to evaluate susceptibility to Bt toxin and fourthly, statistical analysis of the data. Sib-mating the F_1 generation should result in 1/16 of the F_2 larvae being homozygous for a resistance allele if the field-collected female (or her mate) was heterozygous for such a resistance allele. As each female carries at least four haplotypes [two of her own and two from her mate], each isofemale line can be used to characterise at least four alleles. The progeny of 750 isofemale lines must be screened for susceptibility to conclude that the frequency of Bt resistance alleles is < 10^{-3} with 95% confidence (Schneider 1999).

Andow *et al.* (1998, 2000), Andow and Alstad (1999) estimated the frequency of such alleles to be < 0.009 (with a 95% confidence interval) in populations of *O. nubilalis* from Minnesota and 3.9 x 10^{-3} (with a 95% confidence interval) in populations from Iowa. More lines have been screened in several sites of the U.S. Corn Belt and an extensive screening was performed in the south of France. At final, none of the progeny of the 697 isofemale lines derived from the U.S. Corn Belt displayed resistant individuals to Bt maize (Bourguet *et al.* 2003). Similarly, no allele conferring resistance to Bt maize has been detected in the 721 isofemale lines derived from females collected in southern France (Bourguet *et al.* 2003).

Hence, the frequency of alleles conferring resistance to Bt maize is below 10^{-3} with a 95% probability in both the U.S. Corn Belt, eight years after the first planting of Bt maize varieties, and in France where Bt maize has almost not been grown (Bourguet *et al.* 2003).

Dominance of Bt resistance

To date, only one dominance level of Bt resistance selected on *O. nubilalis* has been calculated. Huang *et al.* (1999a), using their strain 70-fold resistant to Dipel-ES, have obtained an incompletely dominant level of resistance. Major cases of resistance to Bt have been shown to involve modification of the toxin receptors. Bt toxins create channels that disrupt ion regulation and the loss of affinity of the receptor for the toxin confers recessive resistance. If the formation of only a few pores is sufficient to cause osmotic swelling, cell lysis and death, then the phenotype of heterozygotes (with 50% sensitive receptors) is likely to be the same as that of susceptible homozygotes (Bourguet, Raymond 1998). For the incompletely dominant resistance found by Huang *et al.* (1999a), it is possible that modification of midgut proteolytic activity, rather than of the toxin receptor, is responsible for resistance (Huang *et al.* 1999b). Indeed modifications of the toxin receptor are generally associated with a much higher resistance levels to the toxin and with recessivity (*e.g.* Gahan *et al.* 2001; Morin *et al.* 2003).

As indicated above, none of the individuals from any of the resistant *O. nubilalis* strains are able to survive on Bt maize (Huang *et al.* 2002), so that the dominance level of resistance to Bt maize cannot be calculated yet.

Gene flow within and between populations

Bourguet *et al.* (2000a) have investigated gene flow within and between maize fields in Europe. Their results suggest an extensive gene flow within and between populations of *O. nubilalis* infesting maize over large scale geographical areas. This intensive gene flow has a double effect. It should result in both the spread of resistance alleles over a large geographic area and a decrease in local resistance to Bt toxins due to the presence of susceptible immigrants from non-Bt maize refuges.

Gould (1998) has suggested that for some generalist pest species, such as *Heliothis virescens*, wild hosts and other crops may make up part of a larger refuge. *Ostrinia nubilalis* is known to be remarkably polyphagous and will attack almost any robust herbaceous wild or cultivated plant with stems large enough to permit the entry of the larvae (Hudon *et al.* 1989). Hence, this pest can establish itself on more than 200 species of plant (Ponsard *et al.* 2004). Nevertheless, to be considered as complementary or alternative refuges, these plants must host European corn borer populations that will randomly mate with those emerging from maize.

Bourguet *et al.* (2000b) and Martel *et al.* (2003) showed that populations collected from maize (*Zea mays* L.) were genetically differentiated from those collected mugwort (*Artemisia vulgaris* L.), hop (*Humulus lupulus* L.). Populations feeding on maize and on mugwort and hop, were referred as to maize-race and mugwort-race, respectively. Both field (Pélozuelo *et al.* 2004) and semi-natural studies (Bethenod *et al.* 2004) have provided evidence that hybrid mating between the two host races is rare. Thomas *et al.* (2003) identified two biological differences that might explain this low level of hybridization. Firstly, mugwort-race moths emerged on average 10 days earlier than maize-race moths, decreasing the likelihood of mating between maize-race and mugwort-race. The genetic divergence between the two host races may also be due to differences in production (by females) and recognition (by males) of E/Z isomeric blend of Δ-11-tetradecenyl acetate, the main component of the sexual pheromones. Indeed, the maize-race uses the so-called Z pheromone blend whereas the mugwort-race uses the E blend (Pélozuelo *et al.* 2004; Thomas *et al.* 2003). Finally, Bethenod *et al.* (2004) showed that females of the two host races displayed striking differences in the relative proportions of egg masses laid on maize and mugwort. Females of the maize race laid their egg masses almost exclusively on maize. Conversely, females of the mugwort-race laid their egg masses preferentially, but not exclusively, on mugwort.

Conclusions

The failure of laboratory selection and F_2 screening to generate highly resistant *O. nubilalis* strains is both good and bad news for pest resistance management. It is bad in that the raw material for evaluating the genetic characteristics of such resistance in *O. nubilalis* cannot be obtained. What are the levels of dominance on Bt maize? Do Bt resistance alleles entail a cost to fitness in the absence of Bt toxins? Does resistance to one Bt toxin confer cross resistance to other Bt toxins? This lack of data limits the scope of theoretical predictions on the sustainability of Bt maize. However, the failure to generate highly resistant *O. nubilalis* strains must also be seen as good news because Bt resistance is probably rare enough in European corn borer populations for the high-dose plus refuge strategy to delay resistance.

The low level of gene flow between the mugwort race and the maize-race and the host plant preference of the females of these two races show that moths emerging from mugwort and hop (wild or cultivated) are unlikely to delay the evolution of resistance to Bt toxins that may be selected on Bt maize. This does not rule out the possibility that other weeds or crops may be useful refuges. However, in the absence of such data, refuges of non transgenic maize should be planted, to extend the durability of Bt maize.

References.

Alstad D.N., Andow D.A., 1995. Managing the evolution of insect resistance to transgenic plants. *Science*, 268: 1894-1896.

Andow D.A., Alstad D.N., 1998. F2 screen for rare resistance alleles. *Journal of Economic Entomology*, 9: 572-578.

Andow D.A., Alstad D.N., 1999. Credibility interval for rare resistance allele frequencies. *Journal of Economic Entomology*, 92: 755-758.

Andow D.A., Alstad D.N., Pang Y.-H., Bolin P.C., Hutchinson W.D., 1998. Using an F2 screen to search for resistance alleles to *Bacillus thuringiensis* toxin in European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology*, 91: 579-584.

Andow D.A., Olson D.M., Hellmich II R. L., Alstad D.N., Hutchinson W.D., 2000. Frequency of resistance alleles to *Bacillus thuringiensis* toxin in an Iowa population of European corn borer. *Journal of Economic Entomology*, 93: 26-30.

Bethenod M.-T., Thomas Y., Rousset F., Frérot B., Pélozuelo L., Genestier G., Bourguet D., 2004. Genetic isolation between two sympatric host plant races of the European corn borer, *Ostrinia nubilalis* Hübner II – assortative mating and host-plant preferences for oviposition. *Heredity*. In press.

Bourguet D., Raymond M., 1998. The molecular basis of dominance relationships: the case of some recent adaptive genes. *Journal of Evolutionary Biology*, 11: 103-122.

Bourguet D., Bethenod M.-T., Pasteur N., Viard F., 2000a. Gene flow in the European corn borer *Ostrinia nubilalis*: implications for the sustainability of transgenic insecticidal maize. *Proceedings of the Royal Society of London B*, 267: 117-122.

Bourguet D., Bethenod M.-T., Trouvé C., Viard F., 2000b. Host-plant diversity of the European corn borer *Ostrinia nubilalis*: what value for sustainable transgenic insecticidal *Bt* maize? *Proceedings of the Royal Society of London B*, 267: 1177-1184.

Bourguet D., Chaufaux J., Séguin M., Buisson C., Hinton J.L., Stodola T.J., Porter P., Cronholm G., Buschman L.L., Andow D.A., 2003. Frequency of alleles conferring resistance to *Bt* maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. *Theoretical and Applied Genetics*, 106: 1225-1233.

Bolin P. C., Hutchison W. D., Andow D. A., 1999. Long-term selection for resistance to *Bacillus thuringiensis* CryIA(c) endotoxin in a Minnesota population of European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology*, 92: 1021-1030.

Chaufaux J., Séguin M., Swanson J. J., Bourguet D., Siegfried B.D., 2001. Chronic exposure of the European corn borer (Lepidoptera : Crambidae) to Cry1Ab *Bacillus thuringiensis* toxin. *Journal of Economic Entomology,* 94: 1564-1570.

Gahan L.J., Gould F., Heckel D.G., 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens. Science*, 293: 857-860.

Gould F., 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology*, 43: 701-726.

Huang F., Higgins R.A., Buschman L.L., 1997. Baseline susceptibility and changes in susceptibility to *Bacillus thuringiensis* subsp. *kurstaki* under selection pressure in European corn borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, 90: 1137-1143.

Huang F., Buschman L.L., Higgins R.A., McGaughey W.H., 1999a. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European Corn Borer. *Science*, 284: 965-967.

Huang F., Zhu K.Y., Buschman L.L., Higgins R.A., Oppert B., 1999b. Comparison of midgut proteinases in *Bacillus thuringiensis*-susceptible and -resistant European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology*, 65: 132-139.

Huang F., Buschman L.L., Higgins R.A., Li H., 2002. Survival of Kansas Dipel-resistant European corn borer (Lepidoptera: Crambidae) on Bt and non Bt corn hybrids. *Journal of Economic Entomology*, 95: 614-621.

Hudon M., LeRoux E.J., Harcourt D.G., 1989. Seventy years of European corn borer (*Ostrinia nubilalis*) research in North America. *Agricultural Zoology Reviews*, 3: 53-96.

James C., 2003. Global status of commercialized transgenic crops: 2002. ISAAA Briefs No. 27: Preview. ISAAA: Ithaca, New York.

Krattiger A.F., 1997. Insect resistance in crops: a case study of *Bacillus thuringiensis* (Bt) and its transfer to developing countries. ISAAA Briefs No.2. ISAAA, Ithaca, New York, pp 42.

Martel C., Réjasse A., Rousset F., Bethenod M.-T., Bourguet D., 2003. Host-plant associated genetic differentiation in Northern French populations of the European corn borer. *Heredity*, 90: 141-149.

Morin S., Biggs R.W., Sisterson M.S., Shriver L., Ellers-Kirk C., Higginson D., Holley D., Gahan L.J., Heckel D.G. , Carrière Y., Dennehy T.J., Brown J.K., Tabashnik B.E., 2003. Three cadherin alleles associated with resistance to *Bacillus* in pink bollworm. Proceedings of the National Academy of Science U.S.A. 100: 5004-5009.

Navon A., 2000. *Bacillus thuringiensis* application in agriculture. *Entomopathogenic Bacteria: From Laboratory to Field Application* (ed. by J.-F. Charles, A. Delécluse, A.C. Nielsen-LeRoux), pp. 355-369. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Pélozuelo L., Malosse C., Genestier G., Guenego H., Frérot B., 2004. Host-plant specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France. *J. Chem. Ecol.* 30: 335-352.

Ponsard S., Bethenod M.-T., Bontemps A., Pélozuelo L., Souqual M.-C., Bourguet D., 2004. Carbon stable isotopes: a tool for studying the mating, oviposition, and spatial distribution of races of European corn borer, *Ostrinia nubilalis*, among host plants in the field. *Canadian Journal of Zoology* 82: 1177-1185.

Schneider J. C., 1999. Confidence interval for Bayesian estimates of resistance allele frequencies. *Journal Economic Entomology*, 92: 755.

Tabashnik B.E., Cushing N.L., Finson N., Johnson, M.W., 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 83: 1671-1676.

Tabashnik B.E., 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology*, 39: 47-79.

Tabashnik B.E., Carrière Y., Dennehy T.J., Morin S., Sisterson M.S., Roush R.T., Shelton A.M., Zhao J.Z., 2003. Insect resistance to transgenic Bt crops: lesson from the laboratory and crop. *Journal of Economic Entomology*, 96: 10031-1038.

Thomas Y., Bethenod M.-T., Pélozuelo L., Frérot B., Bourguet D., 2003. Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hübner I – sex pheromone, moth emergence timing and parasitism. *Evolution*. 57: 261-273.

Wolfenbarger L.L., Phifer P.R., 2000. Biotechnology and ecology – The ecological risks and benefits of genetically engineered plants. *Science*, 290: 2088-2093.

Features of *Loxostege sticticalis* reproduction during the period of its low population density in Krasnodar Territory

Malysh Yu.M.

All-Russian Institute of Plant Protection, St. Petersburg, Pushkin

Особенности размножения лугового мотылька в период его низкой численности в Краснодарском крае

Малыш Ю.М.

Всероссийский НИИ защиты растений РАСХН,
Санкт-Петербург – Пушкин

During 2003 and 2004 we conducted the stationary observations of the *Loxostege sticticalis* number dynamics in wild stations of the Krasnodar Terr.

The works were conducted on four fields. They differentiated by composition of soils, humidity and specific diversity of plants.

Usually three generations of *Loxostege sticticalis* develops in this region, but there were only two in 2004. The peak density of insects was observed during the third flight wave in 2003: about one adult per one step on the average. No any egg, larva or cocoon were discovered on the observed place.

Potential fecundity of adult was determined by dissection of females. It did not practically change within generations during 2003 (160-170 eggs per female). This level was considerably less, than average one for this species. In 2004 the potential fecundity significantly increased (300-400 eggs per female). Percent of gravid females was estimated over generations. It varied from 60 to 100%. The adults laid eggs inside glass jars. However, the average number of eggs laid in 2003 was not large: it varied from 4 to 40 only. In 2004 this index was multiplied in 5-3 times. We noticed that during the mass reproduction the females fully realized their potential fecundity (120-500 eggs per individual (Alekhin, Kuznetsova, 2003)). In 2003 the hatchability of larvae tested in laboratory was low (4-50%), but in 2004 it varied from 55% to 98%.

In 2003 the larvae successfully developed in laboratory. They fed on the cut sprouts of alfalfa, goose-foot, or wormwood. However, repeated attempts to infest host plants with larvae in the field resulted in rapid death of insects. In 2004 the larvae successfully developed in field cages. About 90% of larvae perished, but we succeeded in collection of cocoons and could trace the flight of adult. Second generation larvae did not succeed to develop in field cages and mostly perished during the first and second instars (94%) (Fig. 1).

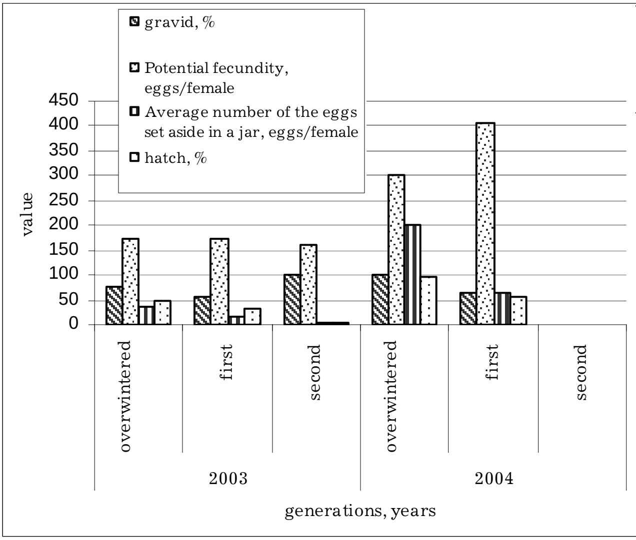

Fig. 1. Reproductive indices of *Loxostege sticticalis* females

The microscopic analysis of dry adults that had been collected in 2003 found that most individuals were infected by microsporidia, *Nosema sticticalis* (Fig. 2), and some of them were also infected by bacteria and fungi. Larvae were infected by microsporidia, bacteria, fungi and viruses (Tables 1, 2).

Microsporidia is a group of obligatory intracellular parasites. *Nosema* increases stress–related overwintering mortality, prolongs larval development, and reduces adult fecundity.

It is known that:

1. Females infected by microsporidia lay 3-5 times less number of eggs;

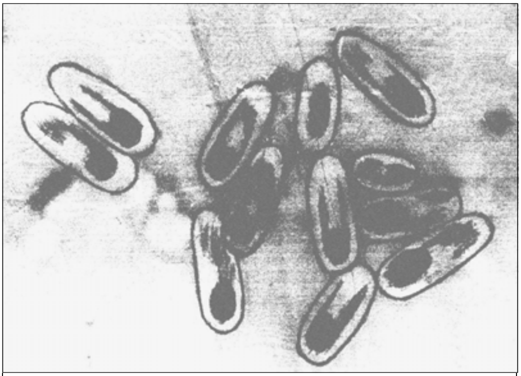

Fig. 2. Spores of microsporidia, *Nosema sticticalis,* isolated from adult of *Loxostege sticticalis* (x1450)

2. Percent of unfertilized eggs is higher in egg masses laid by diseased individuals;
3. No less than 30-50% eggs are infected in progeny of diseased females. Sometimes, 80-90% or even 100% of laid eggs perished from microsporidian infection (Sirotyna, 1954);
4. Most frequently larvae perish from microsporidiosis during the first and second instars (Kramer, 1958; Weiser, 1962).

All features mentioned above were observed during the pest development in both 2003 and 2004. It is possible to assume that microsporidia and other microorganisms play an important role in *Loxostege sticticalis* population dynamics. The diseases not only cause mass mortality of insects of parent generation, but may also promote drop in fecundity and decline of viability in subsequent generations of the pest.

Table 1. Analysis of infection of *Loxostege sticticalis* adults by entomopathogenic microorganisms

Generation	Number of specimen in analysis	Infected by, %			
		Microsporidia, *Nosema sticticalis*	bacteria	fungi	viruses
overwintered	71	16.9	9.9	4.2	0.0
first	27	25.9	22.2	11.1	0.0
second	115	18.3	11.3	4.3	0.0

Table 2. Analysis of dead larvae of *Loxostege sticticalis*

Number of specimen in analysis	Infected by, %			
	microsporidia *Nosema sticticalis*	bacteria	fungi	viruses
13	23.1	38.5	15.4	15.4

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Density and death rate of eggs and larvae of corn earworm in the Krasnodar Territory

Fefelova Yu.A.

All-Russian Institute of Plant Protection, St. Petersburg, Pushkin

Плотность и уровень смертности яиц и гусениц хлопковой совки в Краснодарском крае

Фефелова Ю.А.
Всероссийский НИИ защиты растений РАСХН,
Санкт-Петербург – Пушкин

Corn earworm, *Helicoverpa armigera,* has got the status of extremely dangerous species in south Russia during the last years. Since 2004 we has started to compile life tables for this pest. Observations on dynamics of egg-laying and development of larvae of the first and second generation were carried out on 93 ha of maize field. Dynamics of adult flying was recorded by pheromone traps. Densities of eggs laid were estimated on plots consisted each of 10 plants. Each plant was inspected every 3rd-4th day. Density of larvae was estimated on randomized sample plots of 5 plants. During routing inspections the larvae were also collected for rate specification of their death from diseases and parasites. Density of pupae on maize was determined by soil excavations (on plots 0.5x0.5x0.1 m^3 in size).

Males of the first generation were found in traps since 4th till 21st of June. Substantial egg-laying on plants was recorded since 5th till 29th of June. First hatching of larvae from eggs was registered at 8th of June. Feeding larvae of 3-5 instars were marked since 25th of June.

Density of eggs of the first generation appeared to be rather low, i.e., 0.2 egg per 1 plant on the average. Larvae could not hatch from a half part of the eggs falling-away partly due to interrow cultivations and to rains combined with heavy wind and hailstones.

Death rate of eggs related to *Trichogramma* sp. appeared to be low, but it should be noted, that the density of corn earworm eggs was very low. The higher is density of the host, the more essential can be death rate related to the parasite. The high mortality (87%) of larvae was recorded when the larvae started their feeding on leaves. These findings are in accordance with data published by other workers (Kuznetsova, 1971; Boyarsky, 1982). It is remarkable that the incidence of ichneumonid wasp, *Hyposoter didymator*, was rather high under very low density of the pest larvae (0.01 individuals on a plant).

The actual density of pupae of the first generation was about 0.07 individuals per 1 m^2.

The first males of the second generation were found in traps at 19th of July. Egg-laying on corn was detected since 12th of July till 1st of August.

Hatching of first larvae from eggs was recorded since 16th of July.

The number of eggs of the second generation on maize increased considerably in comparison with that in the first generation. The quantity of falling-away eggs decreased. The survival rate of 1-2 instar larvae increased twice. Obviously, this was connected with more favourable state of host plant for the pest development (tasseling - flowering - the beginning of filling).

Obtained results confirmed that number of corn earworm eggs and larvae was also influenced by natural enemies. 14.4% of eggs were destroyed by predators (Chrysopidae, Coccinelidae, Hemiptera). The mortality of corn earworm eggs caused by *Trichogramma* decreased a little during the second generation in comparison with the first generation.

Parasite *Hyposoter didymator* was more effective, it destroyed about 20% of corn earworm larvae during the second generation.

The density of pupae of the second generation increased substantially, reaching 3.82 individuals per 1 m^2 on the average. It was more than 50-fold increase from the first generation to the second one.

Despite preliminary results, it is obvious, that some natural enemies, first of all *Hyposoter didymator*, represent a significant element of natural regulation of the corn earworm.

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