для оценки возможностей различных подходов к описанию динамики численности лесных насекомых, выявлению факторов динамики численности, моделированию и прогнозированию популяционной динамики.

В настоящей работе на обширном литературном и собственном материале рассмотрены возможности использования для описания динамики численности сосновой пяденицы различных методов и моделей: метода фазовых портретов, ARMA-модели, модели фазового перехода второго рода, модели потенциальных функций [1, 4—7, 11].

Широкая распространенность вида на гигантской территории Евразии и наличие данных одновременных наблюдений на разных территориях позволяют изучить локальную и глобальную когерентность временных рядов динамики популяций сосновой пяденицы [9], выявить возможное влияние погодных и геофизических факторов на развитие вспышек массового размножения вида.

ЛИТЕРАТУРА

1. *Исаев А. С., Хлебопрос Р. Г., Недорезов Л. В., Кондаков Ю. П., Киселев В. В., Суховольский В. Г.* Популяционная динамика лесных насекомых. М.: Наука, 2001. 374 с.
2. *Исаев А. С., Овчинникова Т. М., Пальникова Е. Н., Суховольский В. Г., Тарасова О. В., Хлебопрос Р. Г.* Динамика численности и устойчивость популяций лесных насекомых на низком уровне плотности (на примере популяций сосновой пяденицы *Bupalus piniarius* L.) //Лесоведение. 2014 (4). С. 3—12.
3. *Пальникова Е. Н., Свидерская И. В., Суховольский В. Г.* Сосновая пяденица в лесах Сибири. Экология, динамика численности, влияние на насаждения. Новосибирск: Наука, 2002. 254 с.
4. *Суховольский В. Г., Пальникова Е. Н., Тарасова О. В., Карлюк А. Ю.* Модель вспышки массового размножения лесных насекомых как фазового перехода второго рода // ДАН. 2005. 403 (4). С. 551—553.
5. *Суховольский В. Г., Исхаков Т. Р., Тарасова О. В.* Оптимизационные модели межпопуляционных взаимодействий. Новосибирск: Наука, 2008. 162 с.
6. *Суховольский В. Г., Тарасова О. В., Красноперова П. А*. Перманентные вспышки массового размножения лесных насекомых: анализ и моделирование популяционной динамики // Математическое моделирование в экологии. Пущино: ИФХиБПП РАН, 2013. С. 249—250.
7. *Isaev A. S., Khlebopros R. G., Kiselev V. V., Kondakov Yu. P., Nedorezov L. V., Soukhovolsky V. G.* Forest insects population dynamics. Новосибирск: Изд. Евразийского энтомологического журнала, 2009. 115 p.
8. *Klomp H*. Dynamics of field population of the *Bulalus piniarius* L. // Adv. Ecol. Res. 1966 (3). P. 207—306.
9. *Palnikova E. N., Soukhovolsky V. G.* Spatial and temporal coherency of forest insects-phillophages' population dynamics // Boreal forests in a changing world: challenges and need for action. Krasnoyarsk, 2011. P. 160—161.
10. *Schwerdtferer F.* Okologie der Tiere. 2. Demokologie. Hamburg—Berlin: Verl. Paul Parey, 1968. 448 p.
11. *Soukhovolsky V., Tarasova O., Krasnoperova P.* Permanent outbreaks of forest insects: population dynamics analysis and modeling // ISEM-2013, Toulouse, France, 2013. P. 108.

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DEVELOPMENT OF A DNA BARCODING REFERENCE LIBRARY FOR FAST AND ACCURATE IDENTIFICATION OF IMMATURE STAGES OF POTENTIAL FOREST INSECT PESTS. NORTH ASIAN LEAFMINERS AS AN EXAMPLE

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SUMMARY

A genetic library is being currently developed for leafmining insects, attacking native and alien woody plants in Siberia, that may allow a quick and reliable identification of the immature stages of potential pests and invaders. Integration of molecular data with ecological characteristics of leafminers, particularly larval and pupal morphology, and diagnostic characteristics of mines will accelerate the detection and tracking of insect pests.

Fast and accurate identification of pest insects are required for an effective plant protection. However, identification of many species remains a challenge, particularly for early undistinguishable life stages such as larva and pupa. The molecular approach of DNA barcoding for species identification, i. e. the use of a standardized sequence of the COI gene of mitochondrial DNA, is becoming an important tool in plant protection allowing rapid and precise identification of most organisms [1]. DNA barcoding reference libraries may significantly facilitate regional inventories of organisms and accelerate detection of invasive species [3].

Among invasive pests, leafminers represent an important group of herbivores that threaten our crops,

gardens, parks, and forest plantations. Indeed, many leafminers are known as important economic pests of agricultural and orchard crops (particularly Diptera and Lepidoptera), ornamental woody plants and forest plantations (Lepidoptera, Hymenoptera, less so Coleoptera) in many countries around the world [2, 4, 5, 11, 15, 16, 18]. Larvae of these insects live in and consume leaf tissue, in this way making distinctive cavities called mines [8]. Many species pupate outside the mines making it difficult to obtain adults for fast and reliable species identification.

Within a project supported by LE STUDIUM® Loire Valley Institute for Advanced Studies (France), we intend to develop a comprehensive genetic library for leafmining insects attacking native and alien woody plants in Siberia, for quick and reliable identification of immature stages of pests and invaders. It will be a significant addition to the present international databases to cover potential forest insects that could expand their range beyond Northern Asia.

In 2008—2013, extensive surveys have been carried out in arboreta, botanical gardens, urban and wild plantations in various regions of Siberia (Tumenskaya, Omskaya, Novosibirskaya, Kemerovskaya, Irkutskaya oblasts, Krasnoyarskiy, Altaiskiykrays, the Republics of Altai, Tuva and Khakassia). Thousands of leaves with mines of different insect species (often with larvae or pupae inside mines) were collected from more than 200 native and alien woody plants and stored in an annotated herbarium. Archiving of herbaria samples attacked by endophagous insects may also provide extremely valuable material for detection of invaders and tracking their colonization history [12]. Hundreds of individuals of leafmining insect species (from the orders Lepidoptera, Diptera, Hymenoptera and Coleoptera) were sampled as immature stages (larvae and pupae) directly from mines and preserved in 96 % alcohol for the following molecular characterization. Thus, this collection serves a valuable material for building genetic library with the added value regarding to host plant data that is missing from field collections of adults.

Lepidopteran specimens (mainly Gracillariidae, Coleophoridae, Eriocraniidae, Incurvariidae, Lyonetiidae etc.) were sequenced with the COI barcoding fragment at the research unit of Zoologie Forestière (UR0633) with the Institut National de la Recherche Agronomique (INRA) in Orléans, France. DNA was extracted from larvae and pupae using QIAGEN DNeasyBlood&Tissue Kit. The COI barcoding fragment was amplified via PCR using the primers HCO/LCO [6]. PCR products were purified using the QIAGENAquick PCR purification kit and sequencing was performed at INRA Orléans. Nepticulidae and Tischeriidae larvae were sequenced at NaturalisBiodiversityCenter (Leiden, Netherlands) [see methods in: 14]. In most cases, one insect specimen was sequenced per one host and one locality. The sequences were visualized using Bioedit [7] and compared with those in BOLD [17]. DNAs of Hymenopteran, Dipteran and Coleopteran samples have been extracted at INRA and are being currently sequenced (in progress).

By now, more than 200 samples of Lepidoptera have been sequenced and identified to 50 species and morphospecies. The identity of 40of these species was obtained with a high confidence (match of 99—100 %) using the BOLD database [17]. Molecular identification of a few insects such as *Micrurapteryx* on *Caragana, Heringocrania* on *Betula, Parornix* and *Phyllonorycter* on *Crataegus* failed probably because either they were not yet included in the genetic database or because they are new species. A total of 11 mining micromoths were recorded for the first time in Siberia (5 species of *Stigmella*, 4 *Phyllonorycter*, 1 *Bohemmania*, 1 *Ectoedemia*). 23 species, known in Siberia previously, were for the first time observed in some Siberian regions (7*Stigmella*, 4 *Phyllonorycter*, 2 *Phyllocnistis*, 2 *Eriocrania*, by 1 species *Caloptilia, Callisto, Coptotriche, Gracillaria, Haploptilia, Incurvaria, Lyonetia, Parornix*). A significant number of new distribution records of mining moths in Siberia is not necessarily an evidence that these insects are expanding their ranges but anyway an indication that this insect group is poorly studied in this part of Northern Asia.

Among the identified insects, 24 species are known as pests of woody plant. The majority of them are representatives of Gracillariidae, a family commonly known by pests and invaders in orchards and urban areas worldwide. 11 species provided noticeable damage to their host plants in Siberia: *Callisto denticulella* to *Malus sp., Gracillaria syringella* to *Syringa vulgaris, Leucoptera malifoliella* to *Cotoneaster melanocarpus* and *C. uniflorus, Micrurapteryx sp.* to *Caragana arborescens* and *C. bosii, Phyllocnistis labyrinthella* and *Phyllonorycter apparella* to *Populus tremula, Phyllonorycter comparella* to *Populus alba, Phyllonorycterissikii* to *Tiliacordata* and *T. sibirica* [10]*, Phyllonorycterpopulifoliella* to *Populus balsamifera, P. nigra* and *P. laurifolia, Stigmella aurora* to *Crataegus sanguinea, Yponomeuta cagnagella* to *Euonymus europaeus* and *Y. evonymella* to *Prunus padus*. Apparently, nearly all of them are known in Europe as native or invasive pests. Two species are known as invaders in the USA: *Gracillaria syringella* [13] and *Yponomeuta cagnagella* [9]. The lime leafminer *Phyllonorycter issikii*, which has invaded Europe expanding its' distribution range westwards from Asia [19], showed a significant genetic divergence in its native range of Russian Far East, suggesting the existence of a sibling species.

So far, insects of the three other orders (Diptera, Coleoptera and Hymenoptera) have been only identified by morphology of their larvae (or pupae) and mines, which suggests the presence of 34 species and morphospecies (13 Diptera, 9 Coleoptera, 12 Hymenoptera). Preliminarily, 13 species and morphospecies from these three orders are known as pests in Siberia and partly in Europe. Four sawflies are known as invaders in the

USA. DNAs of all these samples are being currently sequenced in order to confirminsects's identity. However, genetic databases in these orders are not yet as developed as in Lepidoptera, and this may complicate specific identifications.

By the end of the project, DNA barcodes of all sequenced leafmining species will be deposited at the public online database Barcode of Life Data Systems [17]. This genetic library will allow quick and reliable identification of immature stages of potential pests and invaders that may have a practical importance. Integration of molecular data with ecological characteristics of leafminers, particularly description of morphology of larvae and pupae and diagnostic characteristics of insects' mines will greatly facilitate the reliable determination and tracking of insect pests.

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LITERATURE

1. *Barrett R. D. H., Hebert P. D. N.* Identifying spiders through DNA barcodes // Can. J. Zool. 2005. 83: 481—491.
2. *De Prins J., De Prins W.* Global taxonomic database of Gracillariidae (Lepidoptera). 2011—2014. URL: www.gracillariidae.net [accessed 10-IV-2014].
3. *De Prins J., De Prins W., De Coninck E., Kawahara A. Y., Milton M. A., Hebert P. D. N.* Taxonomic history and invasion biology of two *Phyllonorycter* leaf miners (Lepidoptera: Gracillariidae) with links to taxonomic and molecular datasets // Zootaxa, 2013. 3709 (4): 341—362.
4. *Dempewolf M.* CD-ROM on Agromyzidae (Diptera) of economic importance. Institute for Biodiversity and Ecosystem Dynamics / Zoological Museum Amsterdam, 2006. URL: http://ip30.eti.uva.nl/bis/agromyzidae.php [accessed 7-V-2013].
5. *Digweed S. C., MacQuarrie C. J. K., Langor D. W., Williams D. J. M., Spence J. R., Nystrom K. L., Morneau L.* Current status of invasive alien birch-leafmining sawflies (Hymenoptera: Tenthredinidae) in Canada, with keys to species // Canad. Entomologist. 2009. 141 (3): 201—235.
6. *Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R.* DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates // Mol. Mar. Biol. Biotechnol. 1998. 3: 294—299.
7. *Hall T.* BioEdit: An important software for molecular biology // GERF Bulletin of Biosciences. 2011. 2 (1): 60—61.
8. *Hering M. E.* Biology of the leafminers. Junk's. Gravenhage, 1951. 490 p.
9. *Hoebeke E. R. Yponomeuta cagnagella* (Lepidoptera: Yponomeutidae): a Palearctic Ermine Moth in the United States, with notes on its recognition, seasonal history, and habits // Ann. Entomol.Soc. Am. 1987. 80: 462—467.
10. *Kirichenko N.* The lime leafminer*Phyllonorycter issikii* in Western Siberia: some ecological characteristics of the population of the recent invader // Contemporary Problems of Ecology. 2014. 7 (1): 114—121.
11. *Kuznetzov V. I., Baryshnikova S. V.* Brief catalogue of the mining moths of the fam. Gracillariidae (Lepidoptera) of Russia and adjacent countries. Transactions of Zoological Institute of the Russian Academy of Sciences. Saint Petersburg, 1998. 274: 1—60.
12. *Lees D., Lack H. W., Rougerie R., Hernandez-Lopez A., Raus T., Avtzis N. D., Augustin S., Lopez-Vaamonde C.* Tracking origins of invasive herbivores through herbaria and archival DNA: the case of the horse-chestnut leaf miner // Front Ecol Environ. 2011. 9: 322—328.
13. *Murdoch R. F. M.* The biology of the lilac leaf miner, *Gracillaria syringella* Fabr. // Quaest Entomol., 1967. 247—291.
14. *Nieukerken E. J. van, Doorenweerd C., Stokvis F. R., Groenenberg D. S. J.* DNA barcoding of the leaf-mining moth subgenus *Ectoedemia* s. str. (Lepidoptera: Nepticulidae) with COI and EF1-α: two are better than one in recognising cryptic species // Contr. Zool. 2012. 81: 1—24.
15. *Nieukerken E. J. van, Wagner D. L., Baldessari M., Mazzon L., Angeli G., Girolami V., Duso C., Doorenweerd C. Antispila oinophylla* new species (Lepidoptera, Heliozelidae), a new North American grapevine leafminer invading Italian vineyards: taxonomy, DNA barcodes and life cycle // ZooKeys, 2012. 170: 29—77.
16. *Nieukerken E. J. van, Doorenweerd C., Ellis W. N., Huisman K. J., Koster J. C., Mey W., Muus T. S. T. Bucculatrix ainsliella* Murtfeldt, a new North American invader already common on red oaks (*Quercus rubra*) in Western Europe (Bucculatricidae) // Nota Lepidopterologica. 2012. 35: 135—159.
17. *Ratnasingham S., Hebert P. D. N.* BOLD: The Barcode of Life Data System (http://www.barcodinglife.org) // Molecular Ecology Notes. 2007. 7: 355—364.
18. *Scheffer S. J., Lewis M. L., Joshi R. C.* DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines // Ann. Entomol. Soc. Am. 2006.99 (2): 204—210.
19. *Šefrova H. Phyllonorycterissikii* (Kumata, 1963) — bionomics, ecological impact and spread in Europe (Lepidoptera, Gracillariidae) // Acta Universitatis agriculturae et silviculturae mendelianae brunensis / Sbornik Mendelovy zemědělské a lesnické university v Brné, 2002. 3: 99—104.

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