



# BENEFITS AND RISKS FROM ENTOMOPHAGA MAIMAIGA INTRODUCTION AND SPREAD

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# Forest cover in Serbia

Area	Total area (10 <sup>6</sup> ha)	Total forest cover (10 <sup>6</sup> ha)	Forest cover (%)
Serbia	7.7	2,25	29,1
Central Serbia			37,6
AP Vojvodina			7,1

Source: National Forest inventory, 2009

## Forest ownership in Serbia

Type of ownership	Forest area (10 <sup>6</sup> ra)	Forest area (%)
State owned forests	1,190	53,0
Private forests	1,058	47,0



## Forest types in Serbia

Forest types	Forest area (10 <sup>6</sup> ha)	Forest area (%)
Pure broadleaved stands	1.328.000,0	59,0
Mixed broadleaved stands	660.800,0	29,3
Mixed broadleaved and coniferous stands	54.000,0	2,4
Mixed coniferous stands	14.000,0	0,6
Pure coniferous stands	195.600,0	8,7

Coppice forests are dominant in Serbia (64%)

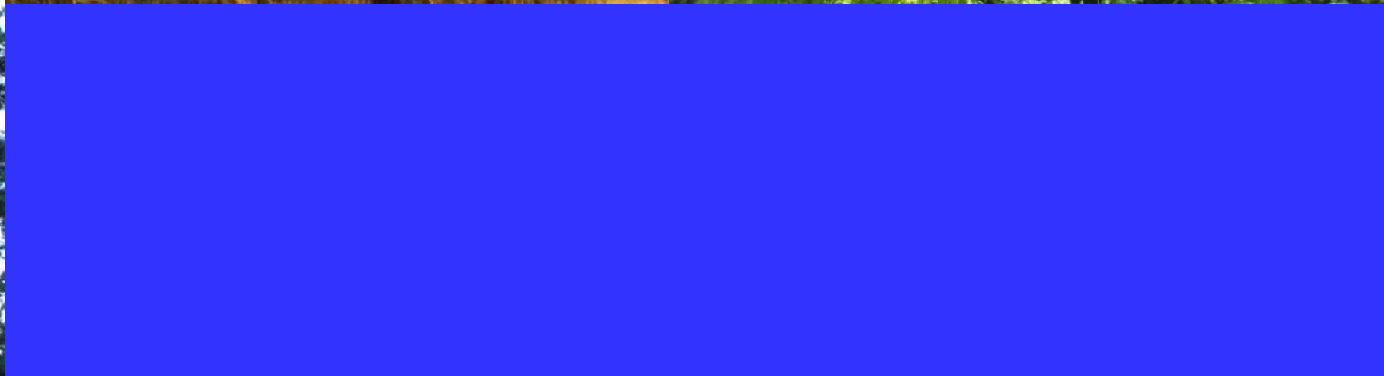
Natural high stands (27,5%)

Artificially established stands – Poplar and willow plantations (6.1%)

## Totaly 49 Tree species in Serbia are recorded

Tree species	Percentage in total volume (%)	Volume increment (%)
<i>Fagus moesiaca</i>	40,5	30,6
<i>Quercus cerris</i>	13,0	11,4
<i>Quercus petraea</i>	5,9	-
<i>Quercus frainetto</i>	5,8	-
<b><i>Carpinus</i></b>	4,2	-
<b><i>Robinia pseudoacacia</i></b>	3,1	-
<i>Quercus robur</i>	2,5	-
<b><i>Fraxinus angustifolia</i></b>	1,6	1,7
<b><i>Picea abies</i></b>	5,2	6,7
<b><i>Pinus nigra, P. sylvestris</i></b>	4,5	9,8
<b><i>Abies alba</i></b>	2,3	2,2

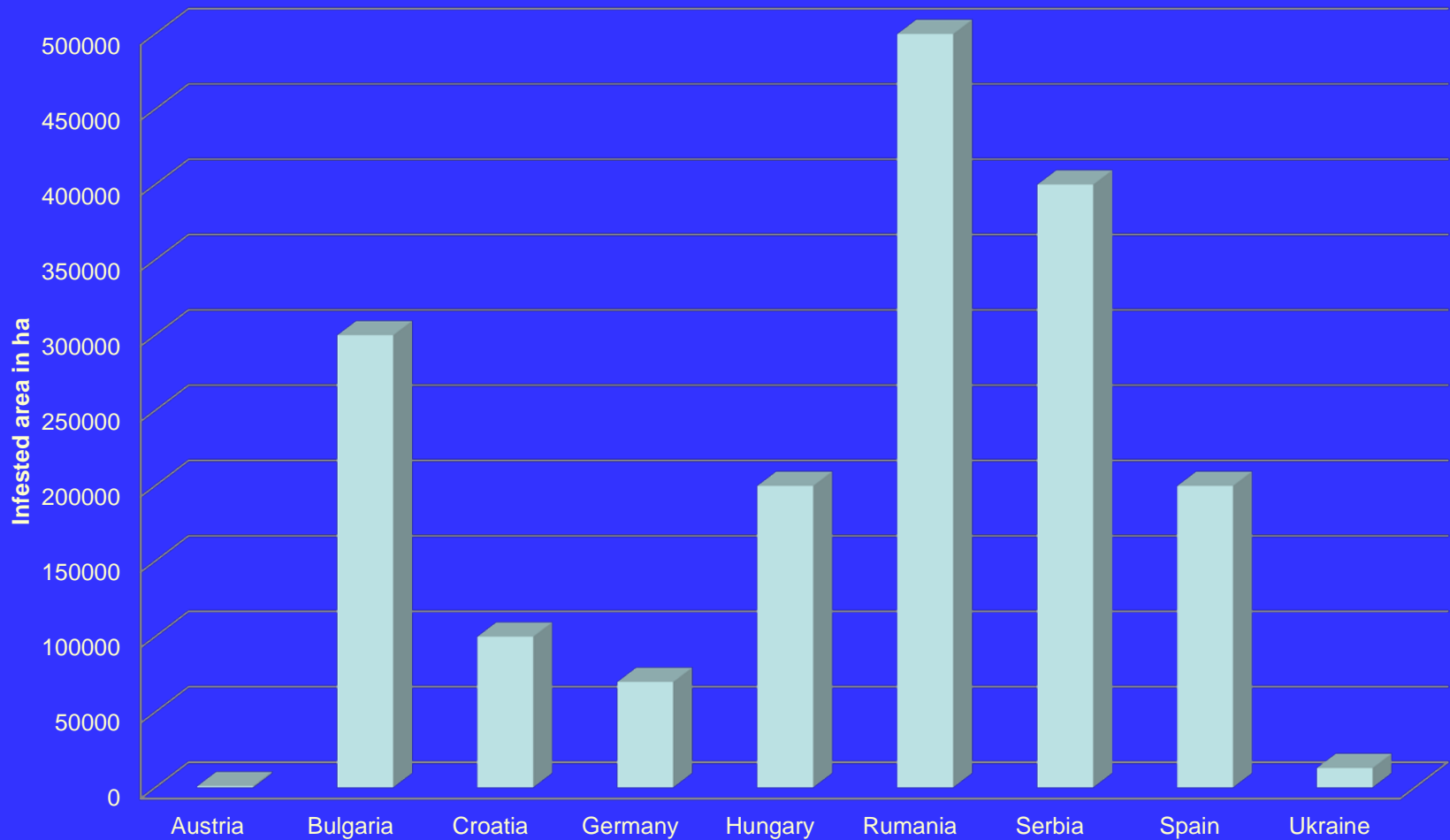






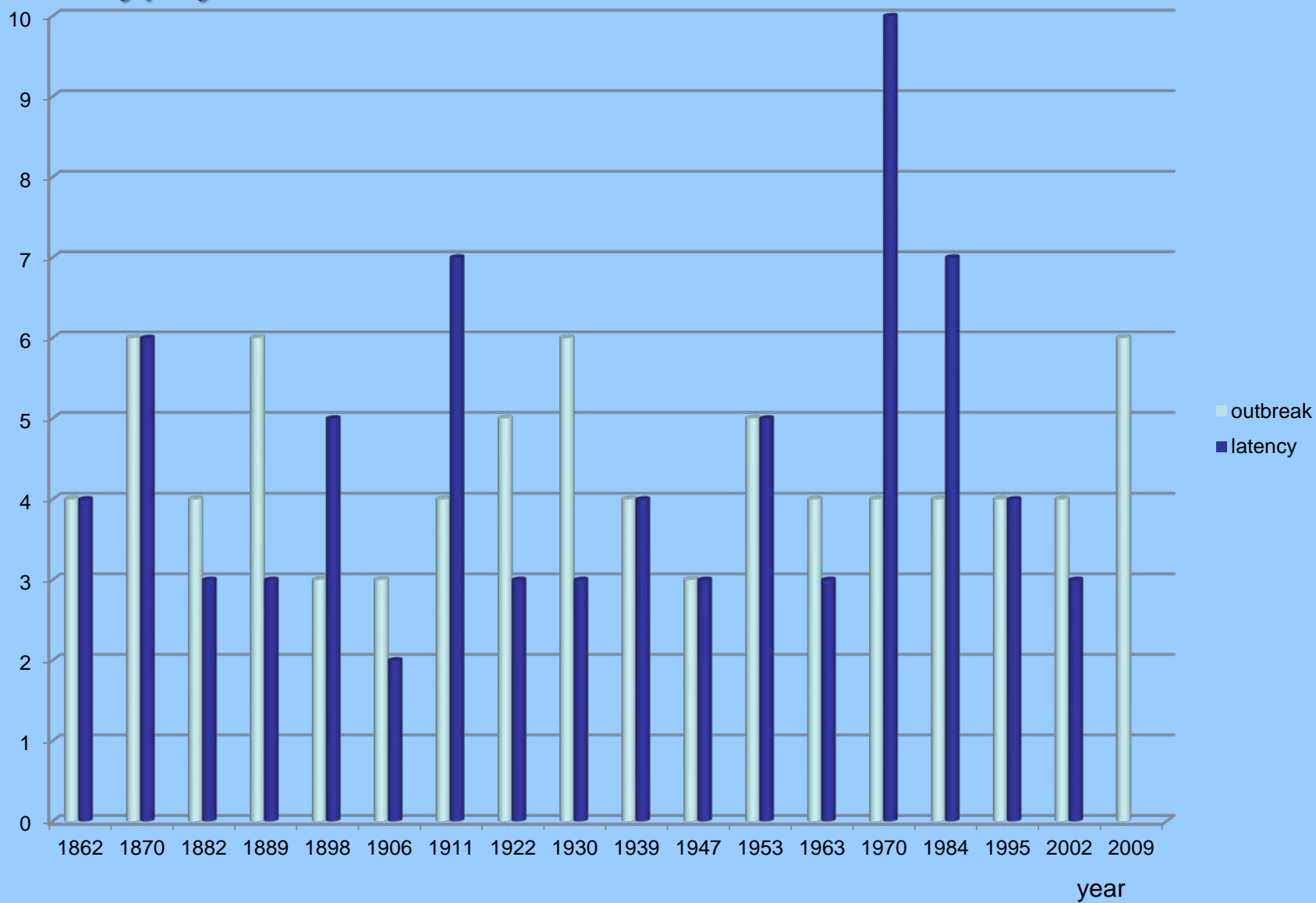


# Gypsy moth in Europe

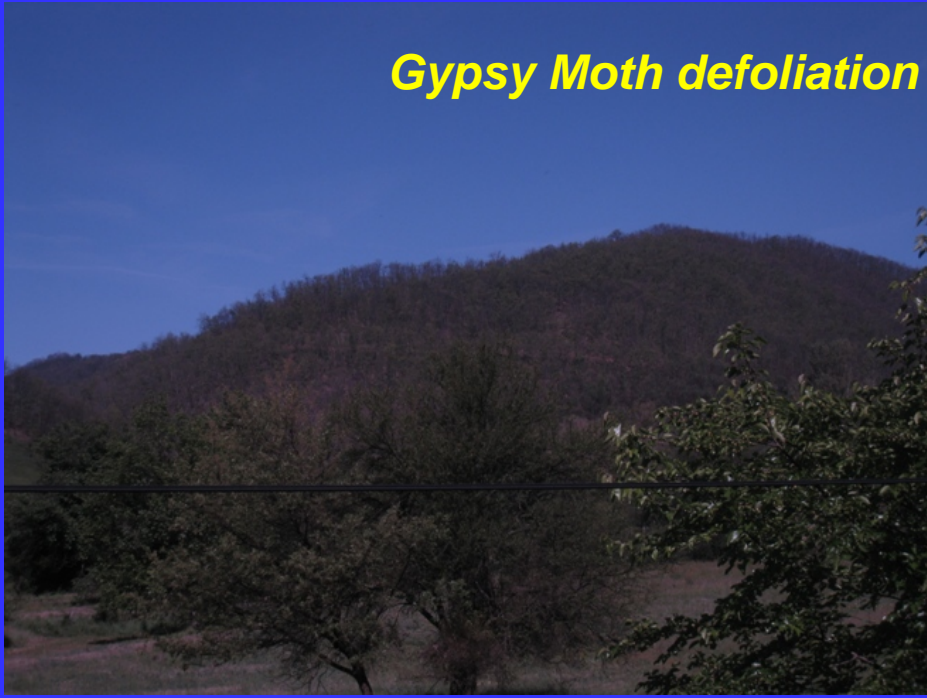


Csoka et al., 2014

# Gypsy Moth outbreaks in Serbia from 1862 to 2014



*Gypsy Moth defoliation in eastern Serbia, 2013*



# *Gypsy Moth defoliation in eastern Serbia, 2013*



***Gypsy Moth defoliation in eastern Serbia, 2013***

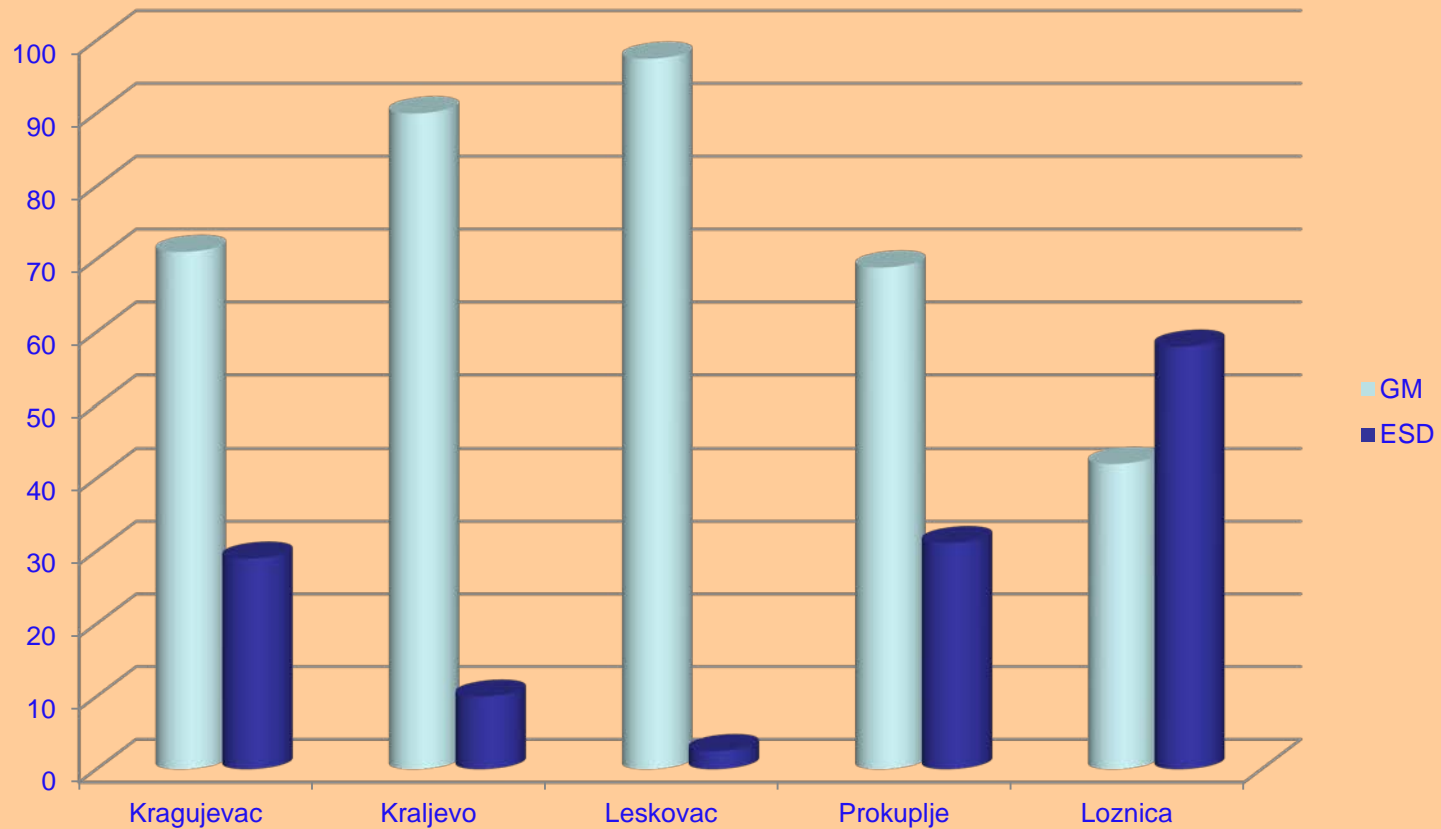




Gypsy moth control - Arial application with Btk in 2014



# Relationship of GM and early spring defoliators (ESD)





# NATURAL ENEMIES OF GYPSY MOTH IN SERBIA



# Natural enemies of GM at non treated plots in 2014

<i>LOCALITY</i>	<i>Com</i>	<i>No. Lar.</i>	<i>NPV %</i>	<i>Nem. %</i>	<i>Cot. m %</i>	<i>Ich.</i>	<i>Tach. %</i>	<i>Tot. mort.</i>	<i>GM (%)</i>
<i>F.E.Lesk., Gor. Jablanica</i>	<i>18a</i>	<i>38</i>	<i>36,84</i>	<i>2,63</i>	<i>2,63</i>	<i>0</i>	<i>2,63</i>	<i>44,73</i>	<i>36,84</i>
<i>F.E.Lesk., Gor. Jablanica</i>	<i>63b</i>	<i>50</i>	<i>40</i>	<i>2,00</i>	<i>18,00</i>	<i>0</i>	<i>6,00</i>	<i>66,00</i>	<i>34,00</i>
<i>F.E. Lesk., Z.A.S.</i>	<i>79</i>	<i>19</i>		<i>5,26</i>	<i>26,32</i>	<i>0</i>	<i>31,58</i>	<i>63,16</i>	<i>36,84</i>
<i>F.E. Lesk., Rad.cesma</i>	<i>54</i>	<i>47</i>	<i>61,70</i>	<i>0</i>	<i>4,25</i>	<i>4,25</i>	<i>8,51</i>	<i>78,71</i>	<i>21,29</i>
<i>F.E.Nis, Rtanj</i>	<i>3</i>	<i>61</i>	<i>31,15</i>	<i>4,92</i>	<i>6,55</i>	<i>0</i>	<i>26,23</i>	<i>68,85</i>	<i>31,15</i>
<i>P.F.Kucevo Duboka CM</i>		<i>81</i>	<i>12,35</i>	<i>0</i>	<i>23,46</i>	<i>0</i>	<i>20,98</i>	<i>56,79</i>	<i>43,21</i>



# Natural enemies of gypsy moth eggs in Serbia

- ***Anastatus japonicus***  
(Hymenoptera: Eupelmidae)
- ***Ooencyrtus kuwanae***  
(Hymenoptera: Encyrtidae)
- ***Eremioscelio lymantriae***  
(Proctotrupoidea: Scelionidae)
- ***Anechura bipunctata***  
(Dermaptera: Forficulidae)
- ***Trombidium* spp.** (Acari)
- ***Carabus latus*, *Megatoma* spp., *Julistus* spp.**

Nonveiller, 1958



# Natural enemies of Gypsy moth larvae in 2014

## *Braconidae*

- *Cotesia melanoscelus*
- *Apanteles glomeratus*

## *Ichneumonidae*

- *Casitaria* sp.

## Diptera: Tachinidae

*Exorista larvarum*

*Parasetigena silvestris*

*Compsilura concinnata*

*Blepharipa pratensis*



*Casitaria* sp.



# Parasitoids of larval stage

*Cotesia melanoscelus* (4-26%)

Tachinidae (2,63 – 31,58 %)



# Predators of Gypsy moth

- *Silpha quadripunctata*
- *Carabus coriaceus*
- *C. cancelatus*
- *C. cavernosus*
- *Calosoma sycophanta*
- *C. inquisitor*



**Nematoda  
(2.00-5.6%)**



**NPV (12,35 - 61.7%)**



*Entomophaga maimaiga*  
introductions from 2011-2013

NPV





## ***Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae)**

An entomopathogenic fungus was found causing an extensive epizootic in outbreak populations of the gypsy moth, *Lymantria dispar*, throughout many areas of the northeastern United States.

- Appearance of fungus in North American gypsy moth populations was **coincident with an abnormally wet spring**.
- Most fungal-infected gypsy moth larvae were killed in mass during the fourth and fifth stadium and were characteristically found clinging to the trunks of trees with their heads pointed downward.
- The fungus produces thick-walled resistant resting spores within dried gypsy moth cadavers and infectious conidia when freshly killed larvae are held in a wet environment.

## *Entomophaga maimaiga* Humber, Shimazu & Soper

- was recovered from gypsy moth, *Lymantria dispar* (L.), populations from Massachusetts, Connecticut, New Hampshire, Vermont, eastern New York, northeastern Pennsylvania, and New Jersey in 1989 and 1990.
- No *E. maimaiga* was recovered from cadavers of gypsy moth larvae collected in western New York, western Pennsylvania, West Virginia, or Virginia in either year despite high levels of rainfall in these regions in May of both years. In 1990, *E. maimaiga* was recovered in many areas that bordered the 1989 distribution (Maine, central Pennsylvania, central New York, Delaware, and northeastern Maryland) where it had not been recovered in 1989, possibly due to spread of the disease.

## *Entomophaga maimaiga* in USA – effect on nontarget hosts

- In laboratory: 279 nontarget Lepidoptera belonging to 34 species in 8 families were collected and reared from areas with low density native gypsy moth populations, and *E. maimaiga* infections were not found in these nontarget hosts.
- A survey of lepidopteran cadavers collected from 1989 to 1995 containing entomophthoralean spores documented *E. maimaiga* infections in 3 species of lymantriids.
- The Lepidoptera-specific North American endemic entomopathogen *Entomophaga aulicae* (Reichardt in Bail) Humber, which is morphologically identical to *E. maimaiga*, was found in 1 geometrid species, 1 notodontid, 2 species of arctiids, and 1 introduced lymantriid, but in none of the gypsy moth larvae tested.

## *Entomophaga maimaiga* in USA

- To establish *E. maimaiga* along the leading edge of spreading gypsy moth populations,  $6 \times 10^5$  *E. maimaiga* resting spores were released around the bases of oaks in 0.01–ha plots in Maryland, Pennsylvania, Virginia, and West Virginia.
- *E. maimaiga* was released in 34 plots in 1991 and 7 plots in 1992, with 15 control sites in 1991 and 3 control sites in 1992. During 1991, *E. maimaiga* infections were found in 28 plots, with infection levels >40% in 6 plots (17.6%).
- Infection levels were greater in plots where *E. maimaiga* resting spores were watered weekly. Low levels of infection were found in 4 of the 15 control plots.
- In 1992, *E. maimaiga* infections were detected in 40 of the 41 release plots as well as in the majority of control plots. Infection levels in 1992 *E. maimaiga* release plots averaged  $72.4 \pm 10.7\%$  (mean  $\pm$  SE) and were associated with declining egg mass densities.

## *Entomophaga maimaiga* spread

- During 1991, *E. maimaiga* spread up to 350 m from centers of release plots, and in 1992, *E. maimaiga* was abundant 1,000 m from release plots.
- Because of the abundance of *E. maimaiga* in 1992 control plots and at 1,000 m from 1992 release plots, 228 sites were surveyed to reevaluate the distribution of this fungus. *E. maimaiga* was found at all sites sampled in Virginia and Maryland and in the majority of West Virginia sites, demonstrating **spectacular spread by this pathogen to the south and west**;
- *E. maimaiga* now occurred at the southern limit of the gypsy moth distribution in the northeastern United States.
- **Such rapid spread was completely unexpected and has never before been documented for an entomopathogenic fungus.** We hypothesize that both our *E. maimaiga* release sites and preexisting *E. maimaiga* populations to the north and east may have been sources for the fungal inoculum that spread.

# Introductions of *Entomophaga maimaiga* to Europe

## Bulgaria

1996 ● 1999 ● 2001 ● 2005 ● 2008 ● 2009 ● 2010 ● 2011  
12 sites

## Serbia

2011 ● 2012 ● 2013  
90 sites

# First records of EM by countries

Bosnia-Hercegovina	2013 (natural spread)
<b>Bulgaria</b>	<b>1996</b> (introduction)
Croatia	2013 (natural spread)
Georgia	2005 (natural spread)
Greece	2012 (natural spread)
Hungary	2013 (natural spread)
Macedonia	2012 (natural spread)
<b>Serbia</b>	<b>2011</b> (introduction+natural spread)
Slovakia	<b>2010</b> (natural spread)
Turkey	2011 (natural spread)

РЕПУБЛИКА БЪЛГАРИЯ  
МИНИСТЕРСТВО НА ОКОЛНАТА СРЕДА И ВОДИТЕ  
София 1000, ул. "Уилям Гладстон" № 67, тел.8472-2000

Изх. № 04-00-5893  
София, ..... 16.03 .....1999 г.

ДО  
БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ  
ИНСТИТУТ ПО ЗООЛОГИЯ

ДО  
МИНИСТЕРСТВО НА ЗЕМЕДЕЛИЕТО, ГОРИТЕ И  
АГРАРНАТА РЕФОРМА  
НАЦИОНАЛНО УПРАВЛЕНИЕ ПО ГОРИТЕ

На Ваш № 234/РД-07/08.09.1998 г.

На основание чл.22 от Правилника за приложение на Закона за защита на природата, във връзка с писмо № 05-08-46/01.03.1999 г. на Министерство на земеделието, горите и аграрната реформа, писмо № 272/40-03-09 от 16.10.1998 г. на БАН, *Министерството на околната среда и водите* не възразява да бъде интродуцирана *Entomophaga maimaiga* в България за контролиране на плътността на гъботворката.

Моля, в срок до 15 април да представите в МОСВ проект за интродуцирането, като опишете подробно технологията, районите, методите на контрол и отчитане на резултатите.

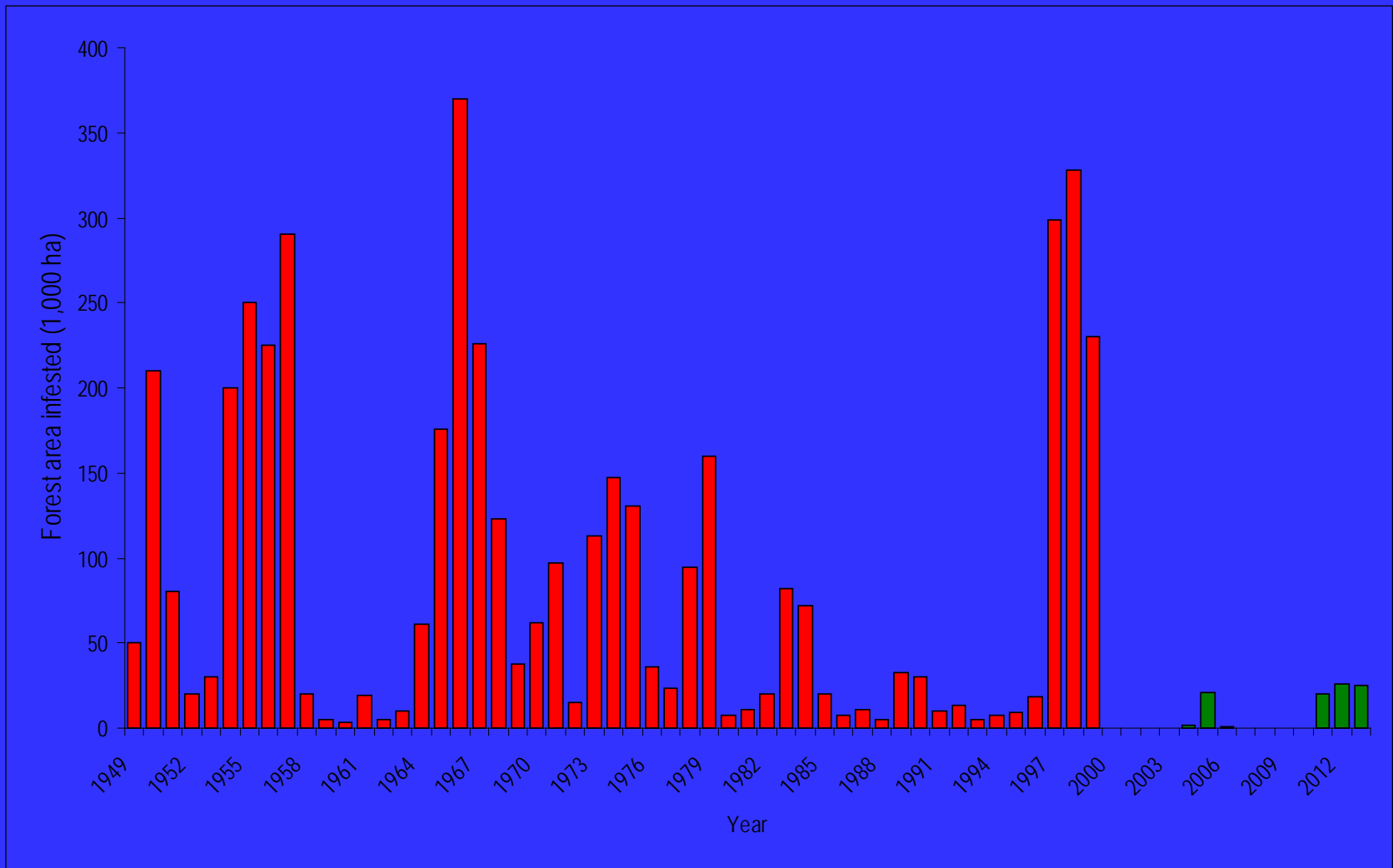
Районите в които ще бъде интродуцирана *Entomophaga maimaiga* да бъдат съгласувани с Националната комисия по лесозащита. Резултатите от опита да бъдат представени до края на 1999 г. за обсъждане и оценка през Националната комисия по лесозащита и МОСВ.

МИНИСТЪР



- Permit for the introduction of *Entomophaga maimaiga* in Bulgaria issued by Ministry of Environment and Water Management in 1999.





Forest area infested yearly by GM in Bulgaria **before** and **after** the introduction of EM Csoka et al., 2014

In 2012 EM caused collapse of GM outbreak in the culmination phase on 8,000 ha forest land in Serbia.

Similar „premature collapse“ happened in Hungary in several thousand hectares of forests in 2013.

## Impact of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) on Outbreak Gypsy Moth Populations (Lepidoptera: Erebidae): The Role of Weather

- *E. maimaiga* is prevalent in gypsy moth populations throughout North America.
- To understand how weather-related variables influence gypsy moth—*E. maimaiga* interactions in the field, fungal infection rates were measured at 12 sites in central Pennsylvania over 3 years, concurrently measuring rainfall, soil moisture, humidity, and temperature.
- Fungal mortality was assessed using both field-collected larvae and laboratory-reared larvae caged on the forest floor.

## Impact of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) on Outbreak Gypsy Moth Populations (Lepidoptera: Erebidae): The Role of Weather

- It was found **significant positive effects of moisture-related variables** (rainfall, soil moisture, and relative humidity) on mortality due to fungal infection in both data sets
- It was found **significant negative effects of temperature** on the mortality of field-collected larvae.
- Lack of a clear temperature relationship with the mortality of caged larvae may be attributable to differential initiation of infection by resting spores and conidia or to microclimate effects.

# INTERACTIONS BETWEEN ENTOMOPHAGA MAIMAIGA AND INDIGENOUS TACHINIDS IN BULGARIA

- Host mortality caused by tachinids 0-48% (average 9.2%).
- Tachinids died in pupal stage with *E. maimaiga* azygospores 86.5%
- High mortality was due to competition between pathogen and parasitoid.

Geogriev G. et al., 2013: Phytoparasitica, Vol. 41, Issue 2, pp. 125-131

## INTERACTIONS BETWEEN ENTOMOPHAGA MAIMAIGA AND INDIGENOUS TACHINIDS IN SERBIA

- Host mortality caused by tachinids 0.7-37.1%
- Tachinids died in pupal stage with *E. maimaiga* azygospores 97.15%

High mortality was due to competition between pathogen and parasitoid.

- Azygospores of *E. maimaiga* were observed on 69.5% of tachinid pupa.

## INTERACTIONS BETWEEN ENTOMOPHAGA MAIMAIGA AND INDIGENOUS TACHINIDS IN USA

- Natural enemies and environmental factors likely both influence the population cycles of forest defoliating insects.
- A significantly negative spatial association was detected between rates of fungal mortality and parasitism.
- Potentially indicating a displacement of parasitoids by *E. maimaiga*.

# SUMMARY

- **PROOVED EFFICACY OF ENTOMOPHAGA MAIMAIGA MAIMAIGA AS BIOLOGICAL CONTROL AGENT**
- **DEPENDANT OF CLIMATE CONDITIONS**
- **MANY OPENED QUESTIONS CONCERNING INTERACTIONS BETWEEN ENTOMOPHAGA MAIMAIGA AND INDIGENOUS NATURAL ENEMIES**



# Thank you for your attention

