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HETERODERA ZEAE KOSHY, SWARUP AND SETHI, 1971 (HETERODERIDAE): PROBABILITY OF INTRODUCTION ON CEREALS IN BULGARIA

O. KARADJOVA, Z. ILIEVA, E. PETROVA and V. KRUMOV
Institute of Soil Science, Agrotechologies and Plant Protection „N. Poushkarov", BG - 1080 Sofia, Bulgaria

Abstract

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The present paper assesses the probability of entry and establishment of the corn cyst nematode *Heterodera zeae* in Bulgaria. The species is currently distributed in ten countries in Asia, Africa, Europe and North America and is one of the most economically important pests on corn and other cereal crops in India and Pakistan. In 2009, *H. zeae* was established in close proximity to the Bulgarian border with Greece (Kavala).

Three main pathways of entry of *H. zeae* in the country have been considered. The highest risk is associated with the import of bulbs, rhizomes, tubers and other underground plant parts with attached soil, intended for direct consumption and processing from countries where the pest is distributed. For this pathway, the probability of entry has been assessed as moderate.

A climatic model has been developed to assess the probability of establishment of the pest. It shows that the species can establish on the whole territory of Bulgaria, excluding the mountains Rila, Rhodope and Pirin, the mountains to the west and the north of Struma valley and the Central Balkans where the conditions are unfavourable for its development. The number of generations per year varies from 1.03 in the area of Kyustendil to 1.81 in the southernmost parts of the country.

The overall risk of entry and establishment of *H. zeae* in Bulgaria is low under the current climatic conditions.

The potential spread of *H. zeae* after introduction has been considered briefly to show that the process would be slow due to the expected low reproduction rate of the populations in Bulgaria and the limited natural spread of the species. The risk of economically important losses is low. More significant losses could be expected from corn in warm, dry years, in areas with light, sandy soils.

Key words: Heterodera zeae, entry, establishment, corn, wheat, barley, Climex

Introduction

The corn cyst nematode *Heterodera zeae* was first described in the beginning of the 70's from India (Koshi et al., 1971). Subsequently, it was established in Egypt (Aboul-Eid and Ghorab, 1981), Pakistan (Maqbool, 1981), Thailand (Chinarasri et al., 1995) and Nepal (Sharma et al., 2001). In 2008, the species was found on the island of Madura, Indonesia (Baliadi, 2008), and in 2013 in Afghanistan (Asghari et al., 2013). In the New World, there are only two recorded outbreaks in the eastern states of Maryland and Virginia, USA, where the species is considered invasive (Sardanelli et al., 1981; Eisenback et al., 1993).

The first European record of *H. zeae* is from Protugal (Correia and Abrantes, 2002). In 2009, the corn cyst nematode was established in close proximity to the Bulgarian border, in samples collected from an organic corn farm to the west of Kavala, Greece (Scantar et al., 2012). In the same year, the nematode was included in the Alert List of species with high risk of entry of the European and Meditteranean Plant Protection Organization (EPPO).

The aim of the present study is to assess the risk of entry and establishment of *H. zeae* in Bulgaria. The following tasks have been set:

- to recognize the main pathways of entry of the pest;
- to assess its probability of entry on the territory of Bulgaria;
- to assess the probability of establishment of the species under the current climatic conditions and under a climate change scenario with increase in the mean annual temperature and dry spells.

Corresponding author: oliakaradjova@abv.bg

Material and Methods

In order to evaluate the potential of *H. zeae* for establishment and spread in Bulgaria, an ecoclimatic model was developed using the software application CLIMEX, Version 3 (Sutherst et al, 2007) with data input on climate, geographical distribution and biological parameters of the species. The variables on the biology of the nematede were partly deduced from the available literature (Srivastava and Sethi, 1986; Hutzell and Krussberg, 1990; Ismail et al, 1993; Hashmi and Krussberg, 1995) and adjusted to take into account the current distribution of *H. zeae* (Shahina, 1989; Eisenback and Traut, 1994; Krusberg et al, 1997; Correia and Abrantes, 2005; Abdollahi, 2009a; Ragesh et al., 2009; Ismail, 2009; Srivastava and Jaiswal, 2010; Scantar et al, 2012; Asghari et al., 2013)

The risk of entry and establishment was evaluated using the five point assessment scale developed by EFSA Panel on Plant Health for the potato cyst nematodes (Baker et al., 2012).

The volume, intensity and frequency of movement of consignments along the pathways of entry were evaluated using information from the EUROSTAT database for the period 2008 – 2012. The assessment was based on data on import to Bulgaria from third countries and EU member states where the species is present.

Data on the total cultivated area and crop rotation systems for main hosts of *H. zeae* in Bulgaria were obtained from the Ministry of Agriculture and Food – AGROSTATISTICA and analysed for the period 2010 – 2014. The information was used to assess the probability of transfer of the pest to a suitable host in relation to the major pathways.

Results and Discussion

Biology of *H. zeae*

The development and reproduction of *H. zeae* are favoured by relatively high soil temperatures in the range of 25 - 36ºC (Hashmi et al., 1993; Hutzell and Krusberg, 1990; Verma and Yadav, 1978). However, the optimal conditions for development of the separate life stages vary. The life cycle of the nematode was first studied in detail by Lauritius et al. (1983) on corn root explants. The experiment was carried out at 29.5 ± 0.5ºC based on the report that under laboratory conditions second stage juveniles (J2) hatch most actively at 29 - 31ºC (Koshy et al., 1970). At such temperatures *H. zeae* finishes its life cycle from J2 to mature individual for 22 days. The cycle is prolonged to 42 – 43 days at 25ºC, while at 20ºC it cannot be completed even 99 days after invasion of the host, although eggs with developed J2 have been observed on the 61^{st} day (Hutzell and Krusberg, 1990). Male individuals are rare, copulation has never been observed and probably the reproduction is exclusively parthenogenic (Lauritius et al., 1983; Hutzell, 1984; Hutzell and Krusberg, 1990).

Bajaj et al. (1994) established populations of *H. zeae* with different infectivity and damage potential towards a group of studied varieties, i.e. different biological races of the species. These findings were confirmed by differences in the host ranges of populations from India, USA and Egypt (Ringer et al., 1987; Srivastava and Jaiswal, 2010). Many authors note the heterogeneity of geographically isolated populations in India (Abdollahi, 2009a, b; Szalanski et al., 1997; Umarao et al, 2008; Gavas Ragesh et al., 2009; Grace et al., 2009). Gavas Ragesh et al. (2009) established that some of the populations were isolated at least 4 million years ago through phylogenetic analysis.

In field trials with *H. zeae*, Krusberg and Sardanelli (1989) established that cysts containing eggs with viable J2 can survive for at least 19 months on fallow land with fine soil, containing sand and clay. During laboratory experiments with samples collected from infected fields the same authors observed that *H. zeae* remained infective for 4 years in soil kept at 24ºC and almost 3 years at 2ºC. It has also been established that the cysts have a high rate of survival after 7 months at -18ºC.

The parthenogenetic type of reproduction, the ability to survive unfavorable conditions in the absence of a host (e.g. during transport or storage) and to develop biological races are evidence for the high plasticity of the species and its significant invasive potential.

Host plants

H. zeae parasitizes exclusively on cultivated and wild plants of family Poaceae (Table 1). Depending on their suitability, the host plants in natural habitats are classified in three groups – main, secondary (minor) and random or infected under experimental conditions. The most preferable hosts are corn and wheat.

Hosts from other families, such as tomato, aubergene, garlic, plum, peach, sugarbeet, potato, mango and date have also been reported (Maqbool, 1981; Abd El-Massih et al., 1986; Oteifa et al., 1997; Nasira and Shahina, 2007; Ibrachim et al., 2010) but these results are controversial. Shazad and Ghaffar (1986) have established that *H. zeae* causes stunted growth in tomatoes. A similar obstruction of plant development has also been observed on seedlings of *Prunus amygdalus* (Quasim and Ghaffar, 1986). However, the reported symptoms have not been confirmed by any of the experiments with artificial inoculation of more than 300 plant species of 15 families (Ringer et al., 1987; Shanina, 1989; Srivastava and Jaiswal, 2010), including many wild and weed species, which are considered suitable hosts for *H. zeae* (Ringer et al.,

Table 1
Hosts of *Heterodera zeae* and country of record

I. Natural hosts	Country
1**. *Main hosts***	
Maize (*Zea mays* L.)	India
Wheat (*Triticum aestivum* L.)	Egypt, Portugal, USA (Virginia), Afganistan
2. *Minor hosts*	
Wheat (*Triticum aestivum* L.)	India, Pakistan
Vetiver (*Chrysopogon zizanioides* (L.))	India
Echinochloa colona (L.)	India
African finger millet (*Eleusine coracana* (L.))	India, Egypt, USA
Barley (*Hordeum vulgare* L.)	India, Egypt, Pakistan
Little millet (*Panicum sumatrense Roth ex Roem. & Schult.*)	India
Sugarcane *(Saccharum officinarum* L.)	India, USA
Foxtail millet *(Setaria italica* (L.))	India
Sorghum *(Sorghum bicolor (L.))*	India, Pakistan, USA
Oat *(Avena sativa* L.)	India, Egypt, USA
Rice *(Oryza sativa* L.)	India, USA
Rye *(Secale cereale* L.)	India, Pakistan
II. Hosts at experimental conditions	USA
Eastern gamagrass *(Tripsacum dactyloides* (L.))	
Chapule, teosinte *(Zea diploperennis* H.H.Iltis Doebley & R.Guzman)	
Flourcorn *(Zea mays* L. *subsp. amylacea)*	
Ornamental corn *(Zea mays* L. *subsp. gracillima)*	
Tear grass (*Coix lacryma-jobi* L.)	
Mexican teosinte *(Zea mays* L. *subsp. mexicana)*	
Common reed *(Phragmites australis* (Cav.))	
Meadow foxtail *(Alopecurus pratensis* L.*)*	
Calamagrostis eipgeios (L.)	
Reed canarygrass *(Phalaris arundinacea* L.)	
Ryegrass *(Lolium perenne* L.)	

1987). No cases of infection of these hosts in natural habitats have been described.

Probability of entry

The biological features of *H. zeae* and its host plants exclude the possibility of entry with host plant seeds. The above mentioned reports that the species has been established in the rhizosphere of potatoes, tomatoes, garlic, carrots, seedlings of *Prunus* spp., mango, etc., are evidence that it is possible for *H. zeae* to enter territories out of its distribution range on non-host plants and plant products with attached soil.

It has been established that during harvest of potatoes, carrots, sugarbeat, etc. a significant quantity of soil is moved away from the field attached to plants or small stones clinging to bulbs and tubers (Ruysschaert et al*.,* 2007). MacLeod et al. (2012) have calculated that every year approximately 400 000 tonnes of soil are transported with potatoes on the territory of the EU.

Like most soil nematodes *H. zeae* cannot be transferred with seeds. On the other hand, whole host plants with roots (family Poacea exclusively) are not imported in Bulgaria. Therefore, the trade of hosts is not considered in the present study. The plants and plant products whose import could assist the entry of *H. zeae* in Bulgaria are non-hosts of the parasite. During cultivation of the plants in areas infested with *H. zeae* there is a possibility for transfer of the pest with small quantities of soil attached to the underground plant parts (bulbs, rhizomes, tubers, etc.).

This hypothesis is supported by a previous assessment of the risk of entry of non-European populations of *Globodera* spp. in the EU (Baker et al., 2012). It highlights two important pathways of entry which are also relevant to *H. zeae:*

- Bulbs and tubers for planting (other than Solanaceae, rhizomes, etc)
- Potatoes for consumption and processing from Egypt

In Directive 2000/29/EC, there is a special derogation CD 2000/568/EC with respect to the second pathway. Potatoes are also cultivated along the Nile delta, where *H. zeae* is present (Ibrahim et al., 2010). It is possible for the species to enter the EU during import of plant products from this region.

The following potential pathways of entry of *H. zeae* in Bulgaria have been established:

+ For long distance movement, import of the following from areas where the pest is present:

- Planting material of non-host plants, including bulbs, rhizomes and tubers with attached soil;
- Bulbs, rhizomes, tubers and other underground plant parts with attached soil, intended for direct consumption;
- Infested soil / growing media.

+ For short distance movement:

- Soil attached to machines, inventory, shoes, plants or plant products – entry in Bulgaria along this pathway is very unlikely, but the short distance movement of soil through human activity is of high relevance to spread.

For all pathways of entry, the parasites cannot reproduce during the transport of soil, plants or plant products, but can survive at high or low temperatures and under dry conditions, as described in the part on the biology of the species. The nematodes can establish new populations if transferred to soil in the presence of a host (plants from family Poacae). Few individuals are sufficient to start a population due to their parthenogenic type of reproduction (Lauritis et al., 1983).

Planting material

+ Bulbs, rhizomes and tubers of ornamental plants, with attached soil originating from countries where H. zeae is present

The import of dormant bulbs, corms, tubers and rhizomes of ornamental plants in the EU from countries where *H. zeae* is present is relatively more active from India and, to some extent, from the USA and Thailand. For the rest of the countries within the distribution range of the pest the frequency of import low and the consignments are small. There is regular import to Bulgaria from India and sporadic from the USA (Table 2a). It remains unknown what part of these plants are cultivated near or on soil, infested with *H. zeae.* In the USA the nematode has limited distribution. It is present only in tree regions in Maryland and one region in Virginia.

Regarding trade within the EU, Bulgaria has imported a very limited quantity of consignments from Greece during one of the years of the considered period and there is no import from Portugal (Table 2b). There is intensive trade with

Table 2b
Trade of "Plants for planting - dormant bulbs, tubers, rhisomes of ornamental plants" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to	
Year	Bulgaria	EU27	Bulgaria	EU27
2008	162	98 953		11
2009	242	106 232		2
2010	276	99 158		42
2011	213	109 023		22
2012	2 349	108 395	7	47

* *EUROSTAT (last enterd at 6.12.2013 г.)*
**Zero value in the table – „0" means less than 50 kg

Table 2a
Import of "Plants for planting - dormant bulbs, tubers, rhisomes of ornamental plants" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from Afganistan to		Import from India to		Import from Indonesia to		Import from Nepal to		Import from Thailand to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008		11			45	29 390		1		6		90		470
2009		3			87	42 704		1		3		67		640
2010		6		35	126	48 770		1		2		78		359
2011		2			49	35 290		2		2		80	0**	549
2012		4			79	44 117		2		3		90	0.4	286

other EU member states, but the relative share of goods originating from third countries is unknown.

Thus the risk of entry of *H. zeae* along this pathway is considered very low.

+ Seed potatoes with attached soil originating from countries where H. zeae is present

The import of seed potatoes from third countries to the EU is banned (Directive 2000/29 EU) and this pathway of entry of the pest should be considered closed. However, the EUROSTAT database provides information on import of minor quantities of potato planting material from Egypt, Thailand and the USA (Table 3a). Bulgaria has no such imports during the investigated period. As regards the movement of potato planting material within the EU, Bulgaria has imported relatively small consignments from Greece during 2011 and 2012 (Table 3b) and the risk of entry of the pest along this pathway is considered low.

+ Vegetable and strawberry planting material with attached soil, originating from countries where H. zeae is present

The consignments of vegetable and strawberry planting material imported in the EU from Egypt, India and Indonesia are small and irregular (Table 4a). The import of vegetable planting material from the USA is regular and of high volume, but due the distribution of the species in the country of origin is limited. Therefore, the risk of entry of *H. zeae* in Bulgaria on these commodities from third countries is very low.

The direct import of vegetable and strawberry planting material from Greece to Bulgaria is characterized by relatively high volumes and frequency. In 2008 the total imported volume was 1700 kg, while in 2012 it reached 110 tonnes (Table 4b). This is an unlikely pathway of entry because the distribution of the species in Greece is limited and the methods for production (e.g. soilless technologies, intensive application of chemical plant protection products) of vegetable planting material presumably exclude the presence of soil nematodes. Therefore, the risk of entry of the pest along this pathway is very low.

Plants and plant products for consumption or processing – underground plant parts with attached soil originating from countries where the species is present

+ Bulbs, tubers, corms and edible roots

There is active import of bulbs, tubers, corms and edible roots from third countries to Bulgaria. As regards potatoes for consumption or starch, the import from countries where *H. zeae* is present to the EU is relatively low with small consignments imported from Egypt in 2008 and 2011 (Table 5a). Substantial volumes are moving in internal EU trade (205 461

Table 3a
Import of "Plants for planting - seed potato" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from Thailand to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008						
2009		4				
2010						25
2011		50		25		
2012		194				9

Table 3b
Trade of "Plants for planting - seed potato" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	2 178	594 097		230		2492
2009	2 530	567 388		1 174		1510
2010	2 206	552 287		149		2784
2011	2 656	607 335	24	183		1425
2012	2 086	637 856	45	106		2354

* *EUROSTAT (last accessed on December 12,2013)*
**Zero value in the table – „0" means less than 50 kg

Table 4a
Import of "Plants for planting - vegetable and strawberry seedlings" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from India to		Import from Indonesia to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008						0**		501
2009		0.2		0**		0**		712
2010		0**				0**		688
2011						0**		837
2012		0**		0				803

Table 4b
Trade of "Plants for planting - vegetable and strawberry seedlings" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	69	85 986	2	9		5
2009	89	78 692	78	92		725
2010	76	85 503	75	122		7555
2011	92	85 999	88	151		1209
2012	268	91 211	110	740		1202

* *EUROSTAT (last accessed on December 12,2013)*
**Zero value in the table – „0" means less than 50 kg

Table 5a
Import of "Potatoes for direct consumption or starch" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from Pakistan to	
Year	Bulgaria	EU27	Bulgaria	EU27
2008		484		
2009				
2010				0.1
2011		23		
2012				

tonnes for 2012), but the import to Bulgaria is low (188 tonnes for 2012). The country has not imported underground plant parts for consumption from Greece and Portugal (Table 5b).

This pathway of entry is unlikely due to the low frequency and volume of import from third countries and the low probability of transfer of the pest to a suitable host in the risk assessment area.

+ Sweet potatoes

The most significant imports of sweet potatoes for consumption from third countries to the EU are from the USA and Egypt (Table 6a). In 2012, the import from India has in-

Table 5b
Trade of "Potatoes for direct consumption or starch" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	:	373 693		23		7
2009	:	352 121		21		21
2010	:	288 103		0**		32
2011	23	200 661		3		33
2012	188	205 462				60

* *EUROSTAT (last accessed on December 12,2013)*
**Zero value in the table – „0" means less than 50 kg

creased. The internal EU trade is quite intensive with 4053 tonnes of sweet potatoes traded to Bulgaria in 2011, 467 tonnes of which were imported from Greece (Table 6b)

This pathway of entry is assessed as unlikely because the pest is rarely associated with the pathway at origin and the possibility of transfer to a suitable host in the risk assessment area is limited.

+ *Allium spp.*

The import of onion, garlic and leek for consumption to the EU from third countries where *H. zeae* is present, more specifically from Egypt, India, Thailand and the USA, is regular and of relatively high volume (Table 7a). The internal trade of onions in the EU is also intensive and large volumes enter the territory of Bulgaria (Table 7b). The import from Greece to Bulgaria is frequent.

Small quantities of soil containing cysts of *H. zeae* can be moved with the commodities in trade. Since the products are intended for direct consumption or processing, the probability of the pest to infect a suitable host after entry in Bulgaria is low. For plants and plant products intended for processing there is a very low probability of transfer of cysts of *H. zeae* to the field with waste water. There, the nematodes could infect weeds or crops from family Poaceae. After import of onion, garlic, etc., the roots with attached soil are often removed in warehouses and could be disposed of in the vicinity of the storage facilities or markets. Some of them are adjacent to agricultural fields. Even though there is a general requirement for adequate destruction of the discarded material, there are no explicit measures for waste treatment after packaging or processing.

Entry of *H. zeae* along this pathway is moderately likely because of its frequent association with the pathway at origin due to the regular import from Egypt, where the species develops high density populations. Furthermore, there are no measures in place for the treatment of onion roots, possibly harbouring soil particles with cysts, after their removal in packing houses.

+ Carrots and turnip

The import of carrots and turnip to the EU from third countries where *H. zeae* is present is relatively low. The largest volumes are imported from the USA (Table 8a). The trade with these products within the EU is very intensive. Bulgaria imports consignments from all member states, while the volumes originating from Greece reach 1 561 tonnes (Table 8b).

This pathway of entry is considered unlikely, because the import of carrots and turnip from third countries is mostly from the USA, where the pest has limited distribution and is unlikely to be associated with the pathway at origin. The

Table 6a
Import of "Sweet potato for direct consumption" from third cointries where H. zeae is present (tonnes).

	Import from Egypt to		Import from India to		Import from Indonesia to		Import from Pakistan to		Import from Thailand to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008		2 278						0**		1		25 589
2009		3 042						0.1		3		32 933
2010		4 780		206				0.5		7		41 273
2011		4 236		557				0.6		9		49 193
2012		3 304		867		0		0.7		6		56 803

Table 6b
Trade of "Sweet potato for direct consumption" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	12	18 770	4	26		
2009	11	23 360	2	23		85
2010	315	31 412	4	4		188
2011	4 054	41 403	468	712		662
2012	202	40 839		0.1		381

* *EUROSTAT (last accessed on December 12,2013)*
**Zero value in the table – „0" means less than 50 kg

Table 7a
Import of "Onion, garlic, leek for consumption" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from India to		Import from Pakistan to		Import from Thailand to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008		37,112		3,295		950		1,885		2,301
2009		32,307		3,735		280		3,991		1,675
2010		31,708		5,308				5,513		2,571
2011	60	4 986		350				539		402
2012		5 709		1 126		0**		522		210

Table 7b
Trade of "Onion, garlic, leek for consumption" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	59	102 944	323	1 279		4 465
2009	80	120 774	331	1 154		3 275
2010	46	109 490	472	880		5 219
2011	156	120 924	306	1 882		8 208
2012	1 885	112 909	561	1 655		6 896

** EUROSTAT (last accessed on December 12,2013)*
**Zero value in the table – „0" means less than 50 kg

Table 8a
Import of "Carrots and turnip for direct consumption" from third cointries where *H. zeae* is present (tonnes).

	Import from Egypt to		Import from India to		Import from Pakistan to		Import from Thailand to		Import from USA to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	:	66	:	:		1		0**		2 174
2009	:	62	:	3		3		0.4		2 721
2010	:	3	:	2		7		0**		2 907
2011	:	0**	:	29		9		12		3 094
2012	:	:	:	0.6		6		3		2 942

Table 8b
Trade of "Carrots and turnip for direct consumption" in the EU and from countries where *H. zeae* is present (tonnes).

	Internal trade to		Trade from Greece to		Trade from Portugal to	
Year	Bulgaria	EU27	Bulgaria	EU27	Bulgaria	EU27
2008	12 901	33 656 003	332	1 628		13 695
2009	29 406	35 057 392	1 296	2 250		19 442
2010	471 712	29 806 307	1 317	2 459		11 665
2011	35 981	30 726 948	912	3 037		18 083
2012	54 724	33 917 955	1 561	2 824		24 654

* EUROSTAT (last accessed on December 12,2013)
**Zero value in the table – „0" means less than 50 kg

same is also valid for the distribution of *H. zeae* in Greece and Portugal.

Soil and growing media originating from countries where H. zeae is present

Soil, as a commodity, is banned for import from third countries to the EU. An exception is made for Northern Africa, including Egypt, where *H. zeae* is present. Growing media such as peat, inert mixtures, etc. (excluding artificial media) are produced outside of the distribution range of the species. There is also a requirement that they should be free of pests. The quantity and means of import of soil from Egypt remain unknown. This pathway of entry is very unlikely with medium uncertainty due to insufficient data on the quantity and type of import of soil from Egypt.

Generally the probability of entry of *H. zeae* in Bulgaria along this pathway is low. The risk is associated mainly with soil particles attached to planting material or plant parts intended for consumption and processing. Transfer to a suitable host is most likely in case of inadequate sorage and treatment of infested plant waste.

Potentially endangered areas in Bulgaria

H. zeae is considered to be one of the most economically important nematode pests of corn and other cereal crops in India (Sharma and Swarup, 1984) and Pakistan (Maqbool, 1988).

Even though it has been shown that the parasite damages corn, there are no available data on the severity of the resulting economic losses (Koenning et al*.,* 1999). It has been established that the suppression of crop growth is directly propotional to the population density of the nematode (Srivastava and Sethi, 1984; Ismail et al., 1994). At optimal temperature regime for *H. zeae* the plant mass diminishes at a steady rate (Hashmi et al., 1993). Krusberg et al. (1997) established that crop development and yield can be reduced by 13 to 73%. Losses can be especially severe in years with warm and dry summers in regions with coarse structured soils. Higher content of fine soil particles suppresses the population growth of the parasite.

All areas for production of cereals in Bulgaria are potentially at risk of establishment and spread of *H. zeae*, since the main hosts of the pest, namely corn and wheat, are prevalent (Table 9). Wheat occupies the greatest share of cultivated land in the country (37.6%), while corn fields are located mainly in North Bulgaria and occupy 13.9% of the cultivated land (AGROSTATISTIKA, 2013, 2014). The most common crop rotation system includes wheat, corn, sunflower and barley. The low share of fallow land (2.7%) and the increase in areas under wheat or corn monoculture present a potential risk of higher rate of reproduction of *H. zeae* under favourable conditions. Areas with light soils near rivers are most endangered.

Risk of establishment of *H. zeae* in Bulgaria

The development of *H. zeae* is influenced mainly by the host plant and the soil temperature (Ismail et al., 1993). The maximum and minimum developmental thresholds are 10°C and 40°C, respectively, based on the studies of Hashmi and Krussberg (1995). The optimal temperatures for development vary in the different publications but are in the range of 15 - 30°C (Hutzell and Krussberg, 1990; Bajaj et al., 1986; Srivastava and Sethi, 1985; Srivastava and Sethi, 1986; Hashmi and Krussberg, 1995), while the effective accumulative temperatures (degree-days) for the development of one generation were calculated based on the study of Hutzell and Krussberg (1990). The data were processed with specialized software (Climex V. 3) in order to generate a predictive model. The model is based on the link between the current distribution of the species and the opitimal climatic conditions for its development. CLIMEX uses algorithms to predict the potential distribution of the species, its phenology and, to some extent, population density in a particular area.

CLIMEX uses stress parameters which limit the possibilities of survival of the species under different climatic conditions, hence its geographic distribution. *H. zeae* is vulnerable to cold temperatures and humidity stress (Hutzell and Krusberg, 1990). A parameter reflecting the synergistic effect of temperature and humidity has been included in the model.

Table 9
Area of cereal crops in Bulgaria for the period 2008-2012 (ha).

Crop	2010	2011	2012	2013	2014
Mayze	500 000	399 400	466 800	518 471	480 929
Wheat	1 108 700	1 137 642	1 090 000	1 328 062	2 305 733
Barley	245 400	178 993	191 400	182 457	218 612
Oats	23 000	14 794	16 600	20 835	21 732
Rye	10 900	10 298	12 600	31 506	37 020
Rice	12 000	11 791	9 900	10 200	10 000

*After Agostatistica (2013, 2014)

Maps showing the ecoclimatic indices of *H. zeae* have been prepared. The ecoclimatic index (EI) reflects the potential for development of a population, taking into account the different types of stress during unfavourable seasons. EI serves as a measure of the probability for permanent establishment in a particular area and ranges from 0 to 100. When its value is close to 0, the area is not suitable for the establishment of populations. Values above 30 mean that the climatic conditions favour the survival of the species (Sutherst et al., 2007) and are prerequisites for development of permanent viable populations which can reach economically important densities.

The present model does not take into account the availability of host plants because in Bulgaria these are common crops that cannot be considered as a significant limiting factor.

Figure 2 presents the ecoclimatic indices of *H. zeae* for the country. The model shows that the species can establish in Bulgaria. EI is below 30 in the mountains of Rila, Rhodope, Pirin, the mountains to the west and the north of Struma valley, and the Central Balkan. Therefore, these areas are unfavourable for the development of the pest. Everywhere else in the country, the EI values are sufficient for establishment of permanent populations. The numbers of generations which can develop in one year under

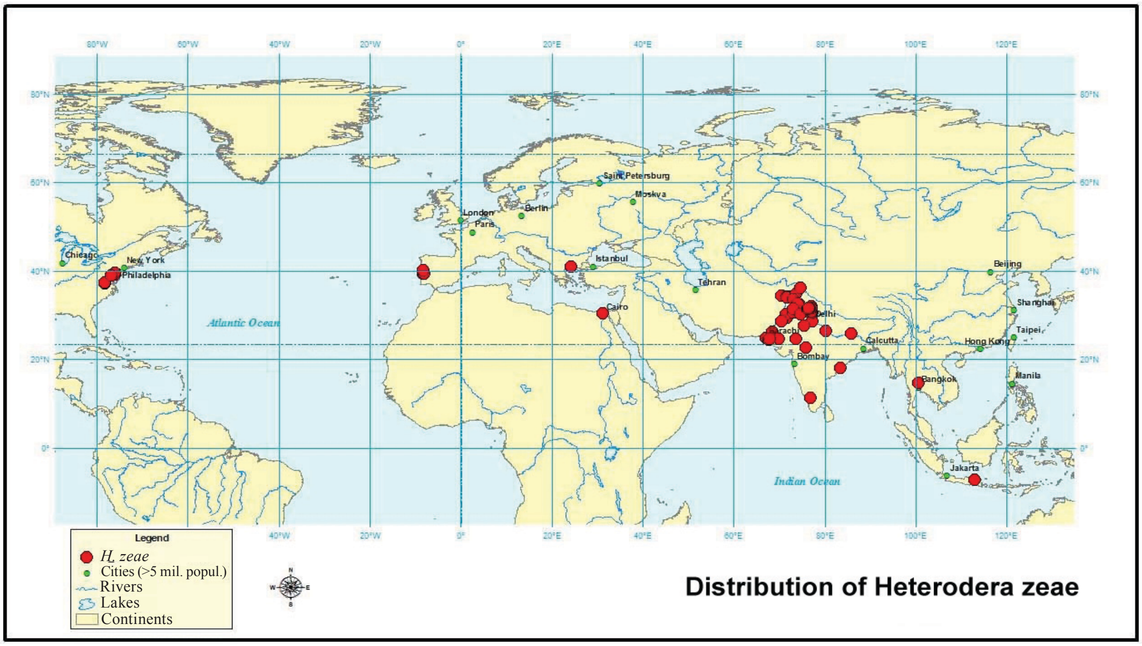

Fig. 1. World distribution of *Heterodera zeae*.

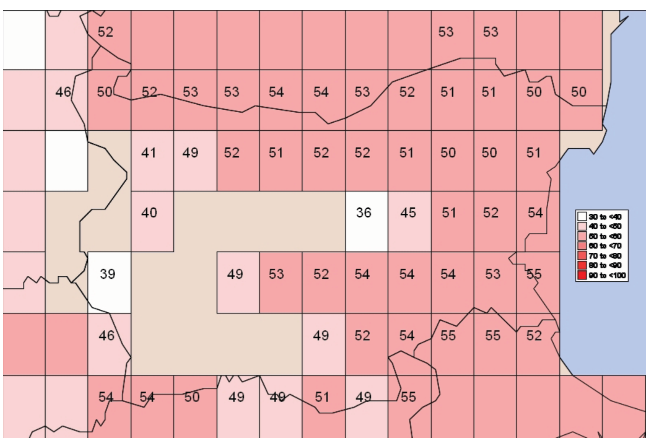

Fig. 2. Ecoclimatic index (EI) map of Bulgaria for *Heterodera zeae*.

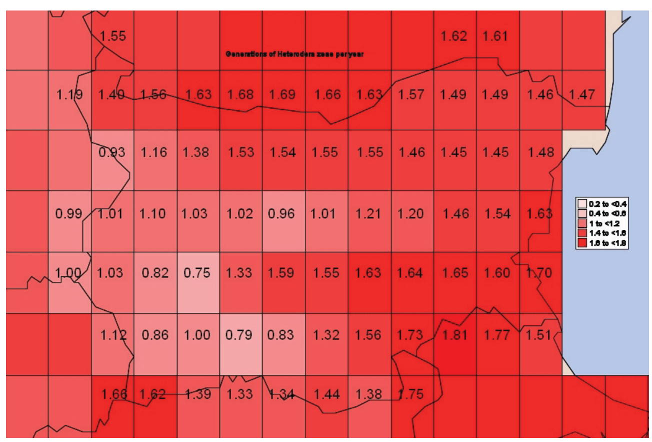

Fig. 3. Number of generations of *Heterodera zeae* per year in Bulgaria.

the local climatic conditions are from 1.03 in the region of Kyustendil to 1.81 in the southernmost parts of Bulgaria (Figure 3).

According to the climate change simulations based on the main emission scenarios of the National Institute of Meteorology and Hidrology at the Bulgarian Academy of Sciences, the mean temperatures in Bulgaria are expected to increase with 2 – 5 degrees by the end of the 21st century (Aleksandrov et al., 2010).

A climatic model has been developed with the inclusion of a parameter 'increase of the mean annual temperature by 2 degrees' in connection to the predicted climate changes. A warmer climate would lead to a change in the potential distribution of *H. zeae*. According to the model, the EI of the species would reach values above 30 (42 – 62). Thus the nematode would be able to develop permanent viable populations on the whole territory of Bulgaria (Figure 4). The climatic changes would also increase the number of generations per year to 1.05 – 2.26 (Figure 5).

The likelihood of establishment after entry would be high because of the abundance of hosts in the risk assessment area which are susceptible for a long period during the year and the relatively favourable climatic conditions. The performed model simulations show that the species is able to permanently establish in most of the country. Initially, local transient populations are expected to develop. However, the population growth will not be very intensive and the damages are not expected to be significant under normal conditions in heavier soils.

Risk of spread in Bulgaria and potential losses

The spread of the species in the country will be slow due to the low reproductive rate and the limited natural spread of the nematodes of 1 – 2 meters per year (Tillitikalla et al., 1995). However, Krusberg et al. (1997) report that the species has spread over 320 kilometers for 11 years solely due to human activity.

In dry and hot summers, the losses from corn cultivated on lighter soils can be significant. The expected prolongation of the summer season, dry spells and warmer temperatures (more pronounced continental element) would lead to an increase in the populations of *H. zeae* and the lossess from its main and even secondary hosts such as barley (Bajaj et al., 1986) or resistant varieties of corn (Ismail et al., 1993).

The likelihood of spread in Bulgaria is unlikely, as under the current climatic conditions the pest would have a very low reproduction rate. Also, its natural spread potential is rather limited. However, *H. zeae* has a high survival potential and host plants are abundant in the country. Thus human activities may facilitate its dispersal.

Conclusion

Four potential pathways of entry of *H. zeae* in Bulgaria have established. The highest risk of entry has been evaluated as moderate for the import of *Allium* spp. for consumption with attached soil from third countries, as there are no specific requirements for treatment of plant wastes after processing.

The endangered area includes all cultivated fields of host plants in the country. The fields of corn and wheat in proximity to rivers are more vulnerable. They are located mainly in the northern part of the country.

A climatic model has been prepared to predict the risk of establishment of *H. zeae*. It shows that the nematode can

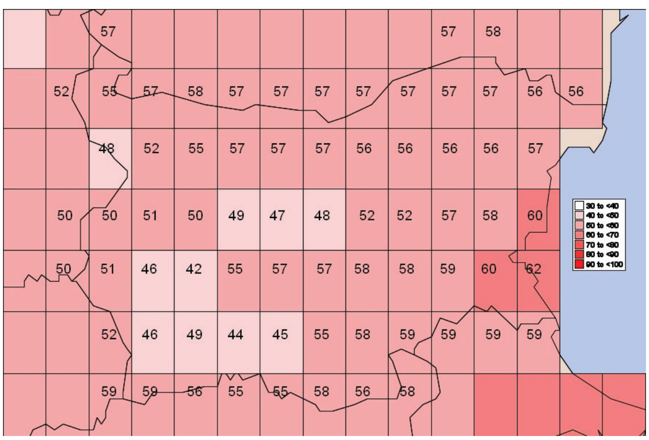

Fig. 4. Ecoclimatic indices of *Heterodera zeae* with 2°C increase in the mean annual temperature for Bulgaria.

Fig. 5. Number of generations of *Heterodera zeae* per year with 2°C increase of the mean annual temperature for Bulgaria.

establish on the whole territory of the country, excluding the mountains of Rila, Rhodope, Pirin, the mountains to the west and the north of Struma valley and the Central Balkan. The number of generations per year varies from 1.03 in the region of Kyustendil to 1.81 in the southernmost parts of Bulgaria. The risk of spread after introduction is low. However, spread can be facilitated by human activities.

The overall risk of introduction of *H. zeae* in Bulgaria and subsequent economic losses is low under the current climatic conditions.

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