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OCCURRENCE AND SPREAD OF THE PARASITIC MICROSCOPIC FUNGI ON WALNUT (*JUGLANS REGIA* L.) ON VARIOUS LOCALITIES OF SLOVAKIA

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Abstract: During 2000-2003 the health condition of *Juglans regia* L. in the urban environment of the 43 localities of Slovakia was evaluated in relation to the location of the trees and the assessment of occurrence, spread and harmfulness of parasitic fungi. We detected on stem the following pathogens: *Melanconium juglandinum* Kunze, *Cytospora juglandina* Sacc., *Gibberella baccata* (Wallr.) Sacc., *Phoma juglandis* (Preuss.) Sacc., *Nectria cinnobarina* (Tode ex Fr.) Fr. with conidial state Tubercularia vulgaris Tode. On branches: *Melanconium juglandinum* (Kunze), *Cytospora juglandina* Sacc., *Gibberella baccata* (Wallr.) Sacc., *Phoma juglandis* (Preuss.) Sacc., *Nectria cinnobarina* (Tode ex Fr.) Fr. with conidial state Tubercularia vulgaris Tode, *Diplodina juglandina* Hollós, *Dothiorella gregaria* Sacc. On leaves: *Gnomonia leptostyla* (Fr.) Ces. et de Not. and anamorph *Marssonina juglandis* (Lieb.) Magn., *Ascochyta juglandis* Boltsh., *Microstroma juglandis* (Bér.) Sacc., *Mycosphaerella juglandis* K. J. Kessler, anamorph *Cylindrosporium juglandis* F. A. Wolf, *Cryptosporium nigrum* Bon. and on fruits: *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. teleomorph *Glomerella cingulata* Stoneman (Spauld. & H. Schrenk) and *Septoria epicarpii* Thüm.
The degree of leaf damage was determined by *Marssonina juglandis* to *Juglans regia* on selected localities.

Key words: *Gnomonia leptostyla*, health condition, *Juglans regia*, harmfulness of parasitic fungi

Slovakya'nın çeşitli lokalitelerindeki ceviz ağaçlarındaki (*Juglans regia* L.) mikroparazitik funguslar ve yayılmaları

Özet: 2000-2003 yıllar arasında Slovakyada 43 lokalitenin kentsel kısımlarında bulunan ceviz ağaçlarının (*Juglans regia* L.) sağlık koşulları, ağaçların bulundukları yerler ile parazitik mikrofungusların bulunuşları, yayılmaları ve zararlarının belirlenmesi ile ilgili olarak,değerlendirilmiştir. Ağaçlarda tespit edilen patojenler şunlardır: gövdelerde; *Melanconium juglandinum* Kunze, *Cytospora juglandina* Sacc., *Gibberella baccata* (Wallr.) Sacc., *Phoma juglandis* (Preuss.) Sacc., konidiyal durumdaki *Tubercularia vulgaris* Tode ile birlikte *Nectria cinnobarina* (Tode ex Fr.) Fr., dallarda; *Melanconium juglandinum* (Kunze), *Cytospora juglandina* Sacc., *Gibberella baccata* (Wallr.) Sacc., *Phoma juglandis* (Preuss.) Sacc., konidiyal durumdaki *Tubercularia vulgaris* Tode ile birlikte *Nectria cinnobarina* (Tode ex Fr.) Fr., *Diplodina juglandina* Hollós, *Dothiorella gregaria* Sacc., yapraklarda: *Gnomonia leptostyla* (Fr.) Ces. et de Not. Ve anamorf *Marssonina juglandis* (Lieb.) Magn., *Ascochyta juglandis* Boltsh., *Microstroma juglandis* (Bér.) Sacc., *Mycosphaerella juglandis* K. J. Kessler, anamorf *Cylindrosporium juglandis* F. A. Wolf, *Cryptosporium nigrum* Bon. ve meyvelerde: *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. teleomorf *Glomerella cingulata* Stoneman (Spauld. & H. Schrenk) ve *Septoria epicarpii* Thüm.
Belirli lokalitelerde *Marssonina juglandis* tarafından yapraklara verilen tespit edilmiştir.

Anahtar kelimeler: *Gnomonia leptostyla*, sağlık koşulu, *Juglans regia*, parazitik fungus zararları.

Introduction

The distribution area of walnut (*Juglans regia* L.) trees in Slovakia covers almost the whole territory of the country, except the alpine zone at altitudes above 900 m a.s.l. According to Radócz (2002) this tree is very important and have a great prospect in planting areas which exploited as cultivated land.

Programm of European union for progress of country prefers the tree species of a multiple utilisation. Important place belongs to *Juglans regia* L. and *Castanea sativa* Mill. The trees are common in orchards and they are very frequent on sloppy terrains – performing an erosion control and a landscaping function. The wood of both tree species is valuable, but they are seldom planted specifically for timber.

Production of fruits is not sufficient to comply with the current demand in our country. The main cause is a bad state of trees grown in their majority from a material obtained using generative methods. The walnuts are very sensitive to unfavourable environmental conditions (site factors, climatic factors) and also susceptible to diseases and attacks by pests. The high disease rate in this woody plant is also influenced by the fact that the trees in our country are grown outside their natural distribution range. The walnut is a fruit tree, with considerably high demands on warm climate but this woody plant suffers frequently from frost injuries in our country. The frost-damaged trees have lowered resistance against various noxious factors attacking all tree parts. Fungi are very frequent parasites on walnut trees. Lot of literature dates dealing with fungi parasites on walnut trees, which originate from generative reproduced organs. (Bose 1961, Barry 1964, Nagy and Koch 1965, Záchej 1977, Čača 1981, Paulechová 1988). Many of trees notice occurrence of the brown spots on the leaves of walnut. This disease - anthracnose is caused by the parasitic fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not.

The aim of our work was to evaluate the health condition of walnut (*Juglans regia* L.). In addition, we evaluated the occurrence, spread and harmfulness of the parasitic microscopic fungi (especially the fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not. and anamorph *Marssonina juglandis* (Lieb.) Magn. on the various localities of Slovakia and proposed optimal protective measures.

Material and methods

The evaluation of occurrence and distribution of pathogens on walnut trees in Slovakia was performed on a base of the inventory of the occurrence of parasitic fungi on stems, branches and assimilatory organs of walnut trees in various localities in the country (Table 1, Figure 1). The health condition of walnut trees (*Juglans regia* L.) was evaluated between years 2000-2003.

A general health condition and a degree of damage of some parts of the woody species was evaluated. We took samples from damaged trees for laboratory testing by common phytopathological methods. The degree of leaf damage was evaluated by 100-leaves method. Calculation of the disease severity on *Juglans regia* leaves by fungus *Marssonina juglandis* was made using of Towsend, Heuberger formula (1943).

$$P = \frac{S(n \cdot v)100}{5 \cdot N}$$

P = degree of damage (%)
n = number of leaves in each category of four-point scale
v = numeric value of the damage category
N = total number of leaves (100)
S = sum of trees

Damage degree was evaluated with the tree species differing from 10 to 100 years. Selection of the location was made to, that observed trees which were represented in various classes of the functional greenery (greenery of the streets, surrounding public and private buildings, in alley). From every locality one-hundred leaves were evaluated from 5 trees by the following points scale.

0. .....leaves and tree healthy, no symptoms
1. …...……1 - 30 spots
2. .....31 - 60 spots
3. .....…61 - 90 spots
4. .....91 - 120 spots
5. .....……121 spots and more

Table 1. Localities with trees of *Juglans regia* L in Slovakia

N.	Locality	Location	Code DFS	Age of trees – years	Number of trees
1.	Arborétum Mlyňany	Park	7676	50 – 80	10
2.	Bratislava – Koliba	Private greenery	7869	20 – 80	10
3.	Čeľadice	Private greenery	7675	10 – 40	10
4.	Drážovce	Vineyards	7674	10 – 100	5
5.	Hliník nad Hronom	Private greenery	7478	10 – 40	10
6.	Horné Lefantovce	Private greenery	7574	10 – 40	10
7.	Horné Plachtince	Private greenery	7781	10 – 100	10
8.	Horné Príbelce	Private greenery	7881	10 – 100	10
9.	Hostie	Private greenery	7576	20 – 30	10
10.	Hrnčiarovce	Private greenery	7674	10 – 50	10
11.	Jarok	Private greenery	7773	10 – 50	10
12.	Jasov	Private greenery	7391	20 – 80	10
13.	Kynek	Colony of private gardens	7674	20 – 80	5
14.	Nitra – Golianova st.	Street planting	7674	20 - 80	7
15.	– Hlohovecká st.	Alley	7674	80	80
16.	– Hornozoborská st.	Private greenery	7674	40	8
17.	– Jašíkova st.	Street planting	7674	35	1
18.	– Kamenná st.	Private greenery	7674	40	6
19.	- Ľ. Okánika st.	Street planting	7674	70	5
20.	– Malá Kamenná st.	Private greenery	7674	60	6
21.	– Metodova st	Street planting	7674	20 – 50	3
22.	– Mojtova st	Street planting	7674	50	2
23.	– Moskovská st.	Street planting	7674	60	2
24.	– Nábrežie mládeže	Street planting	7674	50	20
25.	– Schurmanová st.	Street planting	7674	40	7
26.	– Tatarkova st.	Street planting	7674	50	3
27.	– Višňová st.	Private greenery	7674	20	5
28.	– Vráble	Alley	7674	30 – 40	50
29.	– Brezový háj	Park	7674	40	2
30.	– Krvavé Šenky	Alley	7674	30 – 40	100
31.	Martin	Private greenery	6979	20 – 40	10
32.	Medzev	Private greenery	7291	20 – 80	10
33.	Modra	Private greenery	7670	10 – 30	10
34.	Modrý Kameň	Private greenery	7782	10 – 100	10
35.	Myjava	Private greenery	7271	10 – 100	10
36.	Pezinok	Private greenery	7670	10 – 30	10
37.	Poproč	Private greenery	7291	20 – 80	10
38.	Radošina	Orchard	7473	35 – 80	60
39.	Senné	Private greenery	7397	10 – 80	20
40.	Slepčany	Private greenery	7675	10 – 80	10
41.	Stredné Plachtince	Private greenery	7781	10 – 100	10
42.	Štúrovo	Private greenery	8178	10 – 60	10
43.	Vieska pri Púchove	Street greenery	7373	50 – 80	10

Results and Discussion

Our aim was to evaluate the disease severity of *Juglans regia* on various localities on Slovakia with regard to the presence of fungus *Marssonina juglandis*. During our observation we detected the occurrence of various microscopic fungi and their agressivity from various localities (Table 1, Figure 1.) on stem, branches, leaves and fruits on *Juglans regia* L. The fungi were detected according to Brandenburger (1985), Ubrizsy (1952) and Příhoda (1959). The results are given in Table 2.

Table 2. Occurence of fungi and their agressivity on stem, branches, leaves and fruits on *Juglans regia* L.

Name of fungus	Damage of host			
	Stem	branches	leaves	fruits
Gnomonia leptostyla *(Fr.) Ces. et de Not.*			+	
Anamorph Marssonina juglandis *(Lieb.) Magn.*			+	
Ascochyta juglandis *Boltsh.*			+	
Melanconium juglandinum *Kunze*	+	+		
Cytospora juglandina *Sacc.*	+	+		
Gibberella baccata *(Wallr.) Sacc.*	+	+		
Microstroma juglandis *(Bér.) Sacc.*			+	
Phoma juglandis *(Preuss.) Sacc.*	+	+		
Nectria cinnobarina *(Tode ex Fr.) Fr.*	+	+		
Conidial state Tubercularia vulgaris *Tode*	+	+		
Mycosphaerella juglandis K.J. Kessler Anamorph *Cylindrosporium juglandis* F. A. Wolf.			+	
Diplodina juglandina Hollós		+		
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. teleomorph *Glomerella cingulata* Stoneman (Spauld. & H. Schrenk)				+
Dothiorella gregaria Sacc.		+		
Septoria epicarpii Thüm.				+
Cryptosporium nigrum Bon.			+	

***Marssonina juglandis* (Lib.) Magn.**

On the lower side of *Juglans regia* L. and *J. nigra* L. leaves this fungus causes roundish or polygonal, dark olive-grey spots (Figure 2,3). In case of severe attacks it could cause serious defoliation. From spots it was possible to isolate conidia of the fungus, which were formed in oblate, wrinkled and small, brown centres. The dimensions of conidia were: 20 - 25 x 5 µm. The conidia could cause extensive secondary infections, especially with wet climatic condition in summer. The perithecia were produced on the fallen leaves and the ascospores from them are responsible for fresh infections in the following spring. *Gnomonia leptostyla* (Fr.) Ces et de Not. is the teleomorph of fungus *Marssonina juglandis* (Lib.) Magn. This fungus caused the leaf-blotch.

***Ascochyta juglandis* Boltsh.**

The infection appeared in a form of great number of large, roundish spots, which continually enlarged and gradually coalesced, darkened and became greyish-brown, 1 to 10 mm in diameter. Than the spots withered, in the middle the spots fell out, so that the leaves appeared as perforated. Dimensions of conidia were 10 - 13 x 4 - 5 µm.

***Melanconium juglandinum* Kunze**

Melanconium juglandinum Kunze, the perfect stage of which is *Melanconis charthusiana* Tul., has been recorded commonly on dying branches of *Juglans regia*. This fungus forms on the twigs black pustular fruit bodies (acervuli), from which very large numbers of spores extruded in black tendrils. Acervuli are gregarious,

prominent, conical to discoid and covered by the epidermis. Conidia were formed under the epidermis, they were cupolated, discoid and black. They were formed on conidiophores, which were scutate, round and sootily-black. Dimensions of conidia were 17-27 x 12 – 17 µm. The results of the study suggest that the fungus infects the host preferably during late spring-summer (June-August). Once inside the host, the parasite can progressively encroach on host tissue during the whole year. This implies that the parasite could take advantage of low host defences during winter dormancy.

***Cytospora juglandina* Sacc.**

Twig dieback of walnut were common. It is often primarily due to frost and the fungi which colonize the dead twigs are secondary. Such fungi recorded in Slovakia include *Cytospora juglandina* Sacc. The dimensions of conidiophores were 10 - 14 x 1 µm, the dimensions of conidia were 4,5 – 6,5 x 1 µm.

***Gibberella baccata* (Wallr.) Sacc., the conidial stage of *Fusarium lateritium* Nees.**

This fungus is widespread on species: *Acer, Ampelopsis, Brassica, Citrus*, *Fraxinus, Pyrus, Populus, Rosa, Salix* and *Ulmus.* Conidia were 3 - 5 cellular, dimensions of conidia: 33 - 55 x 3,4 - 3,9 µm. The ascus contained 8 ascospores with dimensions of 15 - 19 x 6 - 7 µm.

***Microstroma juglandis* (Bér.) Sacc.**

On the lower side of leaves of *Juglans regia* L. and *Carya ovata* L. this fungus caused small (10 µm) white, polygonal spots or a yellow-leaf blotch. The strong infection manifested itself by wither and prematurely dryness of leaves. Conidia were oviform, with oblonged endings and dimensions: 5 - 8 x 2 - 3 µm. The fungus is a foliar pathogens of walnut and causes diseases of lesser importance.

***Phoma juglandis* (Preuss.) Sacc.**

This fungus very often occurred on the dry walnut branches, stems and fruits, but it is not responsible for drying of the branches. The fungus can grow saprophytically in tissue of plants and is know to be secondary invader of diseased tissues, perhaps feeding on fungal saprophytes or pathogens of diseased tissues. Conidia were unicellular, colourless. Dimensions of conidia were 8-10 x 3 µm.

***Tubercularia vulgaris* Tode.**

Tubercularia vulgaris Tode is the conidial stage of fungus *Nectria cinnabarina* (Tode ex Fr.) Fr. The fungus secondarily colonized dead twigs, which were often primarily damaged by frost. On walnut there were formed the purple-red, downy sporodochium. Conidiophores were divided transversely. They contained unicellular conidia with dimensions of 55 - 8 x 1,5 - 3 µm.

***Diplodina juglandina* Hollós**

This fungus often occurred on the dry walnut branches. The dimensions of conidia were 10 - 12 x 2 – 3 µm.

***Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., teleomorph *Glomerella cingulata* Stoneman (Spauld. & H. Schrenk)**

The spots on green fruits of walnuts were roundish to oval, slender, greyish-brown in colour with a rot rim. Conidiophores in small, more or less disc-shaped masses which break through the surface of leaves and other substrata forming acervuli. Acervuli containing masses of spores and dark setae were observed within lesions, and conidia from the acervuli produced pur culture of fungus. Conidia were cylindrical to elliptical in shape and measured 12 – 15 x 2,5 - 4 µm.

***Mycosphaerella juglandis* K. J. Kessler, anamorph *Cylindrosporium juglandis* F. A. Wolf.**

The fungus *Mycosphaerella juglandis* K. J. Kessler, anamorph *Cylindrosporium juglandis* F. A. Wolf. was occured on *Juglans regia* L. and also on another *Juglans* species. Its foliar pathogens of walnut and causes diseases of lesser importance. The attacked leaves were wither and dry prematurely. It is important in young walnut plantations, where it causes premature defoliation, thus reducing growth and nut production. Affected wal-

nut trees appear healthy with good foliage color until July. Then, from a distance, affected trees begin to look yellowish. Closer examination reveals leaf scorch, vein-pattern necrosis and lesion fleck symptoms. Symptom-bearing leaves become in creasingly chlorotic and by mid August may have dropped. Pycnidia bearing co-nidiophores on undersurfaces of leaf spot lesions and become apparent about two weeks after lesions. Conidio-spores develop in fruit bodies under the cuticle. They contained colourless conidia with dimensions: 4,5 – 6 x 2,5 – 3,5 µm.

***Dothiorella gregaria* Sacc.**

Pitchy black spots formed on the barky branches. Later they were pressed under the bark, what formed de-pression with black liquid. After cracked the bark of branches, the black liquid ran out. The timber under the bark was dark discolored, too. The lesions were gradually enlarged and during vegetation caused dieback of branches. Dimensions of pycnidia were 180 - 260 µm, dimensions of fusiform conidia were: 20 – 26 x 5 – 7 µm.

***Septoria epicarpii* Thüm.**

The spots on green fruits of walnuts were roundish to oval, later irregulral, greyish-brown in colour with black rim. On the spots occure pycnidia occurred separately or in groups. The dimensions of conidia were 20 - 35 x 4 - 5 µm.

***Cryptosporium nigrum* Bon.**

On the leaves of *Juglans regia* L. this fungus formed dark spots with small black centres of conidia. Their dimensions were 8 - 14 x 1,5 - 2,6 µm.

The number of damaged leaves of walnut and degree of damage by the fungus *M. juglandis* are given in Table 3.

Table 3. Occurrence of fungus *Marssonina juglandis* (Lib.) Magn. and disease severity on *Juglans regia* leaves in various localities in Slovakia

Locality / year	Number of damaged leaves and degree of damage of *Juglans regia* leaves							
	0	1	2	3	4	5	Number of leaves	Degree of damage (%)
Arborétum Mlyňany / 2001	4	15	20	43	15	3	100	64.7
Arborétum Mlyňany / 2002	4	15	16	32	22	11	100	71.5
Bratislava / 2001	5	25	18	27	17	8	100	62.5
Bratislava / 2002	9	9	20	22	25	15	100	72.5
Hliník nad Hronom / 2001	2	28	18	27	17	8	100	63.2
Hliník nad Hronom / 2002	2	8	10	32	29	19	100	83.7
Nitra- Krvavé Šenky / 2001	1	17	14	41	21	6	100	70.5
Nitra- Krvavé Šenky / 2002	0	6	6	38	32	18	100	87.5
Modrý Kameň/ 2001	5	15	26	32	14	8	100	64.0
Modrý Kameň / 2002	4	5	5	29	41	16	100	86.5
Myjava / 2001	6	6	38	25	16	9	100	66.5
Myjava / 2002	0	5	6	17	46	26	100	95.5
Nitra / 2001	3	20	27	33	14	3	100	61.0
Nitra / 2002	2	10	5	20	38	25	100	89.2
Radošina / 2001	3	17	24	37	12	7	100	64.7
Radošina / 2002	3	7	3	28	27	32	100	91.2
Nitra -Vráble / 2001	1	19	24	37	12	7	100	65.2
Nitra -Vráble / 2002	5	5	9	30	33	18	100	83.7
Zvolen / 2001	1	20	38	27	7	7	100	58.7
Zvolen / 2002	0	6	16	30	36	12	100	83.0

The results indicate, that degree of leaf damage of walnut on the following localities was relatively high: from 58,7% - Zvolen to Nitra - Krvavé Šenky 70% (year 2001) and from 71,5% - Arboretum Mlyňany to 95,5% - Myjava (year 2002). The differences in degree of damage between the evaluated years 2001-2002 on all measured localities was higher in year 2002. The number of attacted leaves in 4 and 5 scales (year 2002) was significantly higher. The most higher differences in number of damaged leaves from scale 4 was in Myjava, Modrý Kameň, Nitra, Zvolen and from scale 5 was in Myjava, Nitra and Radošina.

Interesting was the influence of the climatic condition on the attack between the years 2001 and 2002. Year 2001 was very wet. Rainfall was oversized. Maximum rainfall fells in June (182 mm), minimum in May (36 mm) and in October (17 mm). The rainfall was teritorial delimited. Decrease appears from north to south. The driest area was on south-west of Slovakia from April to June. Yearly amount was 845 mm on the whole Slovakia and on observed localities from 598 mm to 1005 mm. Oversized average air temperature (10,7 °C) in year 2001 was mainly the result of the first half of the year, because it was warm. May, July, August and October were very warm similarly as the whole vegetative period.
(http://www.shmu.sk/cms/mak/s4/klimat.zhodnotenie.roka.2001.html).

Year 2002 was drier and there was standard amount of rain fall. Yearly amount on Slovakia was 606,3 mm and on observed localities from 586 to 670 mm. Average yearly temperature was 9,3 °C, on observed localities from 8,9 °C to 9,6 °C, during vegetative period from 15,0 °C to 15,5 °C.

It is know, that during wet years the susceptible walnut trees prematurely lose leaves. Therefore they are sizeable diminished and during dormant period they are less resistant against different diseases. Also ascospores and conidia germinate better at relative higher air humidity.

Data on many species of parasitic microscopic fungi on walnut tree species can be found in foreign mycological and phytopathological literature. Ubrizsy (1952), Brandenburger (1985) and Bánhegyi et al. (1985) listed many species of parasitic microscopic fungi occur on the stem, leaves and branches of walnut. Out of the species we recorded: *Ascochyta juglandis* Boltsh., *Cytospora juglandina* Sacc., *Diplodina* sp., *Gibberella baccata* (Wallr.) Sacc., *Melanconium juglandinum* Kunze, *Microstroma juglandis* (Bér.) Sacc., *Nectria cinnabarina* (Tode ex Fr.) Fr. and *Phoma juglandis* (Preuss.) Sacc.

The fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not was recorded with conidial stage *Marssonina juglandis* (Lib.) Magn. on all localities on the leaves, annual shoots and young fruits of *Juglans regia* L. The obtained results corresponded with data reported by several authors: Peiker (1964), Hladík (1966), Ubrizsy and Vörös (1968), Bergougnoux and Grospiere (1975), Čača (1981), Tóth 1985, Bánhegyi et al. (1985), Michalíková (1988), Paulechová (1988). According them the most frequent damage of asimilative organs of walnut caused the fungus *Gnomonia leptostyla* and their conidial stage *Marssonina juglandis*.

Plant protection measures proposed in this paper include generally accepted principles (Příhoda 1959, Peiker 1964, Brandenburger 1985, Juhásová 1993). Prevention of infection is possible by taking suitable plant protection measures. At the towns the mechanical protection is recommended. The source of infection – the foliage from the previous years – is necessary to remove. The infection pressure and degree of damage reduce. Chemical protection can be used for emergency reasons in towns only rarely. The prevention is not often used in ornamental gardening. The health condition of the seedlings is insufficiently controlled. The source of disease are infected trees and the fungi can spread out to other localities. By the evaluation of the health condition of tree species it was determined that application of suitable cultural procedures in public greenery is important.

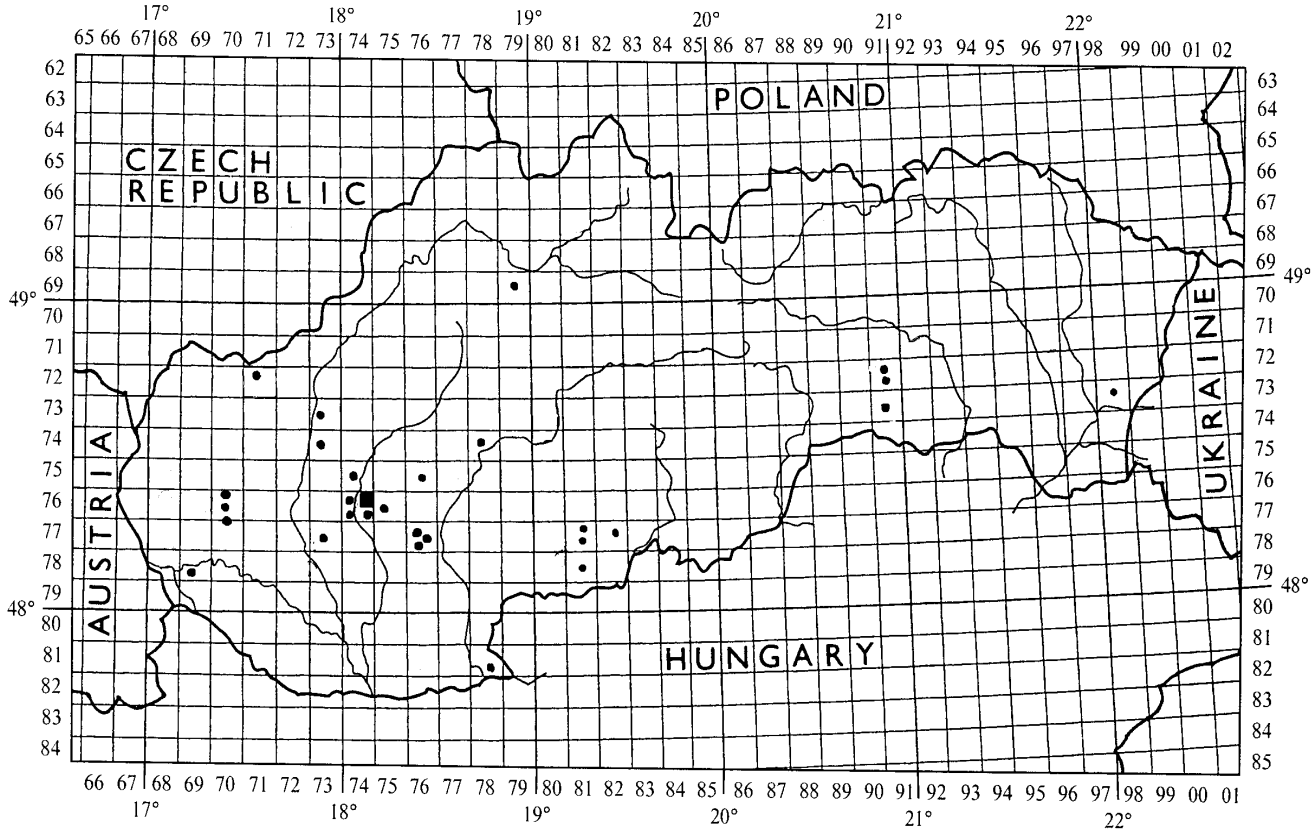

Figure 1. Localities with trees of *Juglans regia* L. in Slovakia

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