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Epidemiological survey of *Xanthomonas arboricola* pv. *juglandis* and *Gnomonia leptostyla* on natural population of walnut (*Juglans regia*) in eastern Transylvania

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Summary: In Romania, walnut *Juglans regia* L. is an important fruit crop, although most of the fruit production comes from non-grafted walnut trees, which are natural hybrids. Breeding programs have been launched during last 30 years to develop new cultivars with uniform fruit quality. In addition, foreign cultivars have been introduced and tested to establish a valuable walnut genepool. To improve the present assortment of generative rootstocks in walnut and to examine the infestation level, a long term survey was carried out in Eastern Transylvania. The main physical characteristics of fruits and its variation to the infestation level were considered. The cumulative distribution of *Xanthomonas arboricola* pv. *juglandis* and *Gnomonia leptostyla* were relatively low and the maximum value was around 15%. The highest infestation of husk with *X. arboricola* pv. *juglandis* was observed for roundish forms and differences were statistically significant comparing with other phenotypes. Infestation with *G. leptostyla* was similar for roundish, elliptic and thwarting egg-shaped phenotypes, while the husk infestation for egg-shaped phenotypes was not observed. The walnut population studied in our experiment can be considered as a genetically valuable population. More than 20% of them have upper class quality fruits with at least or more than 50% of nutmeat. Do to the large scale climate variation in Eastern Transylvania and the high humidity favourable for pathogen infestations, these population can be considered resistant and well adapted to abiotic and biotic factors.

Key words: abiotic factors, biotic factors, endocarp, infestation level, nutmeat

Introduction

The walnut is a well-represented species of the Romanian fruit-tree flora within Banat, Transylvania and Oltenia region, ranging from the Danube River to the Mureş River. In Romania, the yield of nuts in shell ranged between 128.5 thousand tons in 1938 and 20 thousand tons in 1991. As a rule, over 85% of the yield is obtained on isolated walnut trees which can be found around houses or on private landed properties. Local populations present a great genetic variability due to the seed reproduction used and to an intensive exchange of seminal material with Hungary, Austria and Serbia (*Draganescu* et al., 2006).

In general, the European walnut production still largely depends on trees originated from seedlings. During the last 20 years, an important work on seedling selection has been carried out in local populations of *J regia* throughout Europe. The characteristics of wild walnut trees have been described in Bulgaria, Germany, Greece, Hungary, Italy, Poland, Portugal, Romania, Slovenia, Spain and Ukraine (*Germain* et al.,1983, 1997; *Germain*, 1992, 1999; *Deaconu & Vasilescu*, 1997; *Pieklo*, 1989; *Revin*, 1989; *Solar*, 1989; *Fernández-López & Pereira*, 1997, *Loacker*, et al., 2007). Phenotypes in Romania are described considering their physical characteristics and named after localities where they can be found ex: Sibişel 3, 32, 39, 44, 50, 2, 21, 22, 34, 35, 45; Geoagiu: 53, 66, 67, 1, 2; Homorod 1; Gelmal 1; Romoşel 1; Apold 163; Crăciunel 1, 2, 3, 4 Meza 1, 3, 4, 6, 7, 8, 9, 10; Măgureni 3, 6; Rotunda de Satu Mare, Gubău 22; Gurbău 24; Sărmas 16; Cluj 8; Selistea 26, Cătina 20; Bistriţa 3; Dumitra 2, 9; Viisoara 10.

The walnut is very sensitive to a number of abiotic and biotic factors. The most important abiotic factors are autumn frosts, that sometimes lead to tree death and late spring frosts that have an effect on stem form. The main biotic damage factors are fungus attacks. *Armillaria mellea*, *Phytophthora cinamomii* and *P. cambivora* are important diseases affecting the root system and antracnosis, *Gnomonia leptostyla* causes summer leaf fall. Bacterial diseases are important too. *X. arboricola* pv. *juglandis*, damages leaves and young shoots in humid and mild climate and after several rainy summers some trees might even die (*Fernández-López & Pereira,* 1997). *Erwinia* spp. should be also considered as it damages bark and wood and although it might not kill the trees produces log value depreciation (*Teviotdale* et al., 1985; *López* et al., 1994; *Fernández-López & Pereira,* 1997). Dramatic increases of Gnomonia have been described by Belisario *et al.* in Mediterranean area. Disease incidence, statistically significant, started to be evident from late August to the end of

September. This disease peak was related to the increasing number of leaflets bearing fertile acervuli which supply an enlarging inoculum focus for secondary infections, and to the progressive senescence of leaves (*Belisario* et al., 1998).

Material and method

During our experiences we carried out epidemiological survey of *X. arboricola* pv. *juglandis* and *Gnomonia leptostyla* on natural population (trees growing in a countryside) of walnut (*Juglans regia*) in Eastern Transylvania, Romania. Here were measured the coldest temperature during winter and considered as the coldest region from Romania (- 20 to -25 °C in winter and 25 to 30 °C in summer). Therefore this population may be very resistant to a number of abiotic and biotic factors. The survey was started in 2005 and finished in 2007. We concentrated on walnuts growing in the countryside as a possible seminal material for the National and International Breeding Programme. All individuals were localized with GPS. A number of 20 leaves were collected randomly from each tree in every 30 day in growing season. The husk (also called shuck or shoot) infestations (20 samples from each tree) were also analyzed. The number of spots, lesion size, and the number of acervuli were considered. The bacterium infects male and female flowers, shoots and fruits, causing the loss of a considerable amount of the crop (*Tamponi & Donati* 1989). The fungus attacks the leaves and fruits and can cause more or less severe premature defoliation and the drying of the fruits.

We built up a scale to identify the pathogens distribution in leaf and husk surface. This was the following:

0 – no pots, lesions and acervuli,
1 – 15% of leaf and husk covered by pots, lesions and acervuli,
2 – 25% of leaf and husk covered by pots, lesions and acervuli,
3 – 50% of leaf and husk covered by pots, lesions and acervuli,
4 – 75% of leaf and husk covered by pots, lesions and acervuli,
5 – 90–100% of leaf and husk covered by pots, lesions and acervuli.

The cumulative data of the pathogens frequency were considered and distributed with the number of trees from each locality. The following characteristics were also measured: the shape, extent, mass and the exterior design of the endocarp, the thickness of the endocarp and the proportion between the endocarp and nutmeat.

We carried out analyses of variance (ANOVA) using NUCOSA statistical software to examine the effects of the following variables:

- The thickness and shape of the endocarp.
- The infestation level and the shape of the endocarp
- The infestation level and the thickness of the endocarp
- The effect of infestation to the proportion between the endocarp and nutmeat.

ANOVA may be used to examine the effects of two or more categorical variables (factors), both individually and together, on an experimental response. Back transferred means and $P < 0.05$ were considered as statistically significant differences (*Tóthmérész* 1996).

Results and discussion

Altogether 147 individuals were found in 19 localities. These 147 individuals can be classifying after their fruit phenotype in four different groups. These are:

a) Roundish with 26.35% in the cumulative sample and dominant in nine localities and 40 genotypes.
b) Elliptic with 35.13% in the cumulative sample and dominant in six localities and 51 genotypes
c) Egg-shaped 35.13% in the cumulative sample and dominant in two localities and 51 genotypes
d) Thwarting egg-shaped 3.37% in the cumulative sample and also dominant in two localities and 5 genotypes.

The thickness of the endocarp varied between 1.29 mm for roundish forms and 1.75 mm for elliptic forms. The difference was statistically significant for roundish and elliptic forms (*Table 1*).

Table 1 The thickness of the endocarp for the measured fruit phenotypes

Shape of the endocarp	Roundish	Elliptic	Egg-shaped	Thwarting egg-shaped
The thickness of the endocarp (mm)	1,29 mm	1,75 mm	1,57 mm	1,57 mm
Significances	**$P < 0.01$**	**$P < 0.05$**	**n.s.**	**n.s.**

Explanation: n.s – non significant

Considering the cumulative distribution of *X. arboricola* pv. *juglandis* and *G. leptostyla* (total percentage of leaf and husk covered by pots, lesions and acervuli measured and divided with the number of trees from each locality) we observed that these were relatively low for all localities and the maximum value was 15%. The Eastern Transylvanian walnut population can be divided in four groups considering the infestation level. In group A are included 8 individuals from three locality, free from both bacterial and fungus infestation. In group B (16 individuals) only infestation with *X. arboricola* pv. *juglandis* were observed. *G. leptostyla* were present in four localities here infestation with *X. arboricola* pv. *juglandis* has not been observed (18 individuals). 105 individuals presented various infestation levels with both *X. arboricola* pv. *juglandis* and *G. leptostyla* (*Figure 1*).

The pathogens distribution on husk was the following; the highest infestation with *X. arboricola* pv. *juglandis* was observed for roundish forms (for 40 genotypes) and differences were statistically significant comparing with other phenotypes ($F = 7.5$, $P < 0.01$). Infestation with *G. leptostyla* was similar for roundish, elliptic and thwarting egg-shaped phenotypes, while the husk infestation for egg-shaped phenotypes (for 51 genotypes) has not been observed. The husks of thwarting egg-

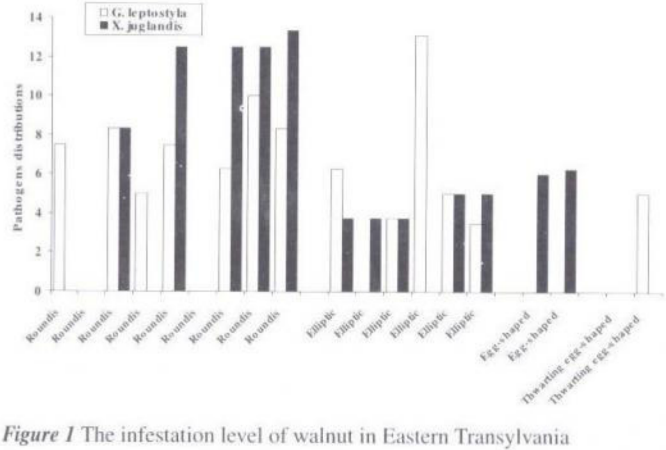

Figure 1 The infestation level of walnut in Eastern Transylvania

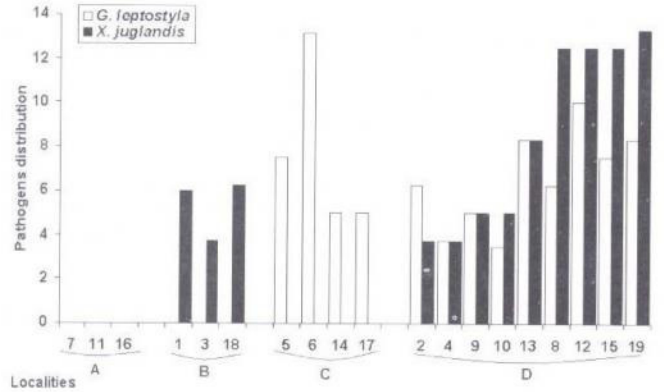

Figure 2 The pathogens distributions on husk for different fruit phenotypes

shaped forms were free of *X. arboricola* pv. *juglandis* infestation (for 5 genotypes) (*Figure 2*).

There were no significant differences between the infestation level and the shape of the endocarp and the thickness of the endocarp. Altogether the low infestation level with *X. arboricola* pv. *juglandis* and *G. leptostyla* has no significant effect upon the quality and quantity of walnut production in Estren Transylvania region.

The trend in plantations and consequently in selection and breeding, is to separate the production of wood from the production of fruit. However, all genetic information issued from walnut breeding programmes is also being used in current programs in Western Europe focused on wood production (FAIR III-CT-96-1887). The clear separation that exists among breeding programs implies the necessity of including the two objectives in the genetic conservation of the species also recommended by other authors (*Fernández-López & Pereira* 1997). Do to the large scale climate variation in Eastern Transylvania (- 20 to –25 °C in winter and 25 to 30 °C in summer) and the high humidity favourable for pathogen infestations, these population could be considered resistant and well adapted to abiotic and biotic factors. Therefore a national and long term breeding programme would be necessary for conserving and improving these genotypes.

Conclusions

The walnut population studied in our experiment can be considered as a genetically valuable population. More than 20% of them have upper class quality fruits with at least or more than 50% of nutmeat. These genotypes could play one important role in a possible breeding programme of walnut to improve the present assortment of generative rootstocks from Transylvania.

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