Scale insect communities (Hemiptera: Coccoidea) of Hungarian highways: biocoenology and ecology

PhD thesis

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1. BACKGROUND AND OBJECTIVES

Scale insects are highly specialized plant parasites which belong to order Hemiptera, suborder Sternorrhyncha. From the Siberian tundra to the tropical area they are worldwide spread serious crop pests. At world level, the number of known species is more than 7,300 and nowadays new species are described not only from the tropics, but from Europe also (BEN-DOV et al. 2013). They are characterized by sexual dimorphism, adult females are neotenous forms and live their lives bound to the host plant, while adult males are winged. Due to the cryptic life style and unusually small size they are almost impossible to track with/by visual inspections during quarantine inspections. The scale insect species level identification is possible only on slide mounted female specimens.

Hungary, with detected 274 scale insect species is currently the third best explored country after France and Italy in Central Europe (PELLIZZARI and GERMAIN 2010). The local outdoor fauna is represented by 224 species and respectively 50 species listed as ornamental pests in greenhouse environment or accidentally introduced with various imported ornamental plant species or tropical fruits (KOZÁR et al. 2013c).

The climate change and the intensive international trade have produced high number of entomological problems in the recent decades worldwide. The introduced insect pest species represent serious problems in agriculture not only in orchards and greenhouse environment but also in urban ecosystems on ornamental shrubs and trees or in vineyards (KOZÁR and NAGY 1986, VOLNEY and FLEMING 2000, RIPKA 2005, 2010, PELLIZZARI and GERMAIN 2010, SZEŐKE and CSÓKA 2012, KISS et al. 2013). Therefore monitoring of climate change related distribution area shift of introduced scale species and detection of introduction pathways are important research issues of practical pest management as well as the deeper knowing of scale insect biology and local distribution which can subserve even for recognition and ecological assessment of further protected habitats (RUIZ and CARLTON, 2003, KOZÁR and SZENTKIRÁLYI 2005, CROWL et al. 2008, KOZÁR el al. 2004b, 2013c).

Concerning to the spread of scale insect species through Europe, in the recent years alien scale insect species – such as *Ceroplastes japonicus* Green (Coccidae) or *Pseudococcus comstocki* (Kuwana) (Pseudococcidae) – have started a slow expansion from Mediterranean region to Central Europe (PELLIZZARI et al. 2012b). Moreover it become the recurring accidentaly or single introduction of new horticultural and ornamental scale insect pest species (SEFROVA and LASTUVKA 2005, LONGO 2010, MALUMPHY and BADMIN, 2012, KOZÁR et al. 2013 a, c). Several possible European spreading and/or expansion routes of scale insects are raised nevertheless various introduction opportunities are human-mediated and human activity may increase the natural extension of some scale insect species (PELLIZZARI and GERMAIN 2010).

Forman et al. (2002) *Road Ecology: Science and Solutions* book gives several European, American and Australian examples for appearance and spreading of non-native plant and insect species alongside roads and highways. The significance of road ecology researches is emphasized by many thematic investigations designed over the past decades across Europe which are now focus not only on the negative environmental impact of roads (such as road kill or habitat fragmentation), but explore the newly created habitats plant and insect diversity and their interactions with natural habitats.

Hungary due to its special geographical position it can be considered as a European "distribution hub". The highway network consist of M3 and M7 highways, which are located in Southwest-Northeast direction between Italy and Ukraine (Rome-Kiev axis) and M1 and M5 highways, which are part of east Belgium and Greece transport route in Northwest-Southeast direction (Brussels-Athens axis). These two international highway system intersect each other in Budapest region and M0 highway represent the connection between them. Therefore Hungarian highway system can be excellent object for studying patterns of various arthropod invasions, which should be precede by an exploratory examination for each investigated taxa.

Green corridors settled alongside the highways, rest stops and filling stations are mosaic like man-made environments which provide a suitable and diverse habitat for insects. The "introduced" topsoil cover of road side slopes and rest stops laid during the landscaping works, give a chance for millions of insect, plant and fungi propagules. In the landscaped environment of rest stops, gas stations and restaurants are planted various herbaceous, woody and potted ornamentals. Some of these ornamentals are imported plant material. Occasionally, the rest areas have a constant connection with the neighboring natural habitats; they are blend into the landscape. The before mentioned aspects of the highway rest stops and margin zones facilitate the appearance and settlement of scale insects.

Based on these observations, it is likely that in man-made habitats of the highway rest stops a unique scale insect community is present. We suppose that scale insect community of woody vegetation would be similar to the urban habitat ones, dominated by ornamental pest species, while in the case of herbaceous vegetation common and rare scale species were predicted. The present investigation aims to explore the structure of scale insect communities and their relationship with the neighboring natural habitats in the case of Hungarian highway rest stops, and on the other hand to define the most frequent scale insect vectors (soil, nursery plant material).

The research aimed the following:

- To explore the structure of scale insect communities (species composition and dominant species) of woody and herbaceous vegetation of Hungarian highway rest stops.
- To determine the effect of different local and landscape level variables on scale insect communities of woody and herbaceous vegetation of Hungarian highway rest stops.
- To define the spreading/expansion routes of dominant scale insect species.
- Distribution maps and characterization of 15 most common Hungarian scale insect species living on herbaceous plants.
- Observations and recommendations for the treatment of highway rest stops/rest areas: with special regard to scale insect species spreading with ornamental plants, monitoring of insect spreading and proposal for further protected areas.

2. MATERIAL AND METHODS

2.1. Scale insect community structure research on Hungarian highways

2.1.1. Sampling sites and methods

The investigation was performed from 2009 to 2012 on highways M0, M1, M3, M5 and M7 on 44 sampling sites (Table 1.). Herbaceous and woody plant samples were collected twice a year, in May and September. From each sampling site 10 herbaceous plant, 2x10 cm bark samples and 15cm long twig samples were taken after the visual plant and site survey.

Table 1. : Hungarian highway sampling sites (2009-2012)

M0	M1	M3	M5	M7
0.km**	Sasfészek	Szilas	M0xM5**	Budaörs_Tesco**
Anna-hegy	Óbarok	Kisbagi*	Inárcsi	Érd_SOS**
Csepel	Harkályosi*	Ecsédi*	Örkényi	Velencei*
Ferihegy**	Turul*	Borsókúti	Lajosmizse	Pákozdi*
Alacska	Grébics*	Rekettyési	Kecskeméti	Töreki*
Dunakeszi**	Bábolnai	Geleji	Petőfiszállás*	Táskai*
	Arrabona	Polgári*	Szatymazi	Szegerdői*
	Börcs*	Görbeházi	Röszkei	Sormási*
	Hanság*	Hajdúnánási*		Letenyei**
	Mosoni	Nyíregyházi*		
		Záhony**		

* Highway rest stops
** Sampling site alongside or near the highway
No asterix sampling site: filling station and rest stop

Scale insect infection rate was determined using KOZÁR and VIKTORIN (1978) 1-4 scaling method in both host plant groups. Sampling sites were documented with photos. In year 2009 and

2010 we focused on herbaceous vegetation while in year 2011 and 2012 on woody vegetation (coniferous and deciduous trees and shrubs).

The specimens were slide-mounted according to method given by KOSZTARAB and KOZÁR (1988). Identification of scale insects are almost based entirely on adult females morphological characters but in the case of some genus second larval stage and wingless male specimens are suitable for species level identification. Identification keys used were the following: DANZIG (1980, 1993), KOSZTARAB and KOZÁR (1978, 1988), KOZÁR (1984), KOZÁR and KONCZNÉ BENEDICTY (2007), KOZÁR et al. (2013b) TANG (1991), WILLIAMS (1985, 1962).

The scale insect nomenclature of BEN-DOV et al. (2013) and KOZÁR et al. (2013b) were used. Slide mounted material was deposited in the scale insect collection of the Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary.

2.1.2. Statistical analyses

Scale insect data were recorded in MBSD (Multi-taxa Biodiversity and Spatial-information Database) multi-user Microsoft Access database. Due to the different sampling methods scale insect data regarding to species and detection number were divided into two groups: scales associated with woody and herbaceous vegetation. In case of herbaceous vegetation another grouping was performed: species living on roots and on leaf/leaf sheaths.

The characterization of scale insect communities were based on relative frequency and KJ index (revised presence index from 1 to 5 - marked in parentheses after each species name) of the detected species. It was determined the proportion of mono-, oligo- and poliphagous species for all scale species number and detection data in both vegetation groups.

The species diversity of scale insect communities from each highway were compared using Rényi diversity profiles. Rényi diversity profile is a diversity ordering technique which operates with diversity profile curves, based on a scale parameter value. Diversity profile curve is sensible at low scale parameter values to the rare species presence while at high scale parameter values for dominant species. Using diversity profiles sampling sites can easily ordered from high to low diversity and when the profiles intersect they are non-comparable (TÓTHMÉRÉSZ 1997). We applied cluster analyses method using Jaccard index to quantify the similarity of different scale insect communities from each highway.

Four local variables were designated regarding to the sampling sites: age, size, site-profile (slope or flat) and built-up index. The age of sampling sites were calculated by subtracting the foundation year from 2013. The sampling site size was measured using an online Google Earth tool. Sampling sites were grouped by site-profile categories: slope (1) or flat (0); e.g. sampling sites such as Turul rest stop (M1 highway) or Ecséd rest stop (M3 highway) were characterized with slope profile. Sampling sites were categorized with a build-up index: restaurant/motel (1) and other (0).

Correlation between the age of sampling sites and scale insects detection data associated with herbaceous plant species was presented using Lowess smoothing (CLEVELAND and DEVLIN 1988). Correlation analyses between sampling site age and the detection number of six most frequent root-inhabiting scale insects (*Atrococcus achilleae, Chaetococcus sulci*, *Fonscolombia europaea*, *Lecanopsis turcica*, *Rhizoecus albidus* and *R. kazachstanus*) were made in order to prove the effect of soil vector.

The landscape level variables in 500 m and 1000 m radius circle around each sampling site were calculated using Google Earth maps calibrated to 1 km precision. Five landscape level variables were defined: agricultural field, pasture, forest/orchard, industrial zone and residential area.

Canonical Correspondence Analysis (CCA) was used to reveal the relation between sampling site related variables and species composition (PODANI 1997). CCA was performed with three environmental variables: local and landscape level variables in 500 and 1000 m radius circle. The dependent variables were processed also in three groups: total scale insect detection data, woody and herbaceous associated scale insect detection data.

2.2. Distribution maps of 15 most common scale insect species associated with herbaceous vegetation from Hungary

Based on the scale insect checklist of *Distribution maps of scale insect species* (Homoptera: Coccoidea) *Hungary* (KOZÁR 2005) book, the 13 most common, native scale insect species were selected, living on herbaceous hosts and two recently denoted scale species were also added which had no distribution map presented in the aforementioned book (for species list see Table 2.).

All registered data regarding to these 15 scale insect species were recorded in database and a new distribution map templates was prepared by adding the highway network lines to the originally used UTM map template. All updated distribution maps represent the negative scores of the species detection on the highway sampling sites as well.

Sampling sites were graphically represented through species composition in a multi-dimensional space using NMDS method because of the insufficient background data available for the sampling sites (PODANI 1997).

Table 2. : List of the 15 most common scale insects associated with herbaceous vegetation from Hungary

Family	Species	Preferred plant part	Distribution map
Coccidae	*Eriopeltis festucae* (Fonscolombe, 1834)	leaf	2003, 2012
	Lecanopsis turcica Borchsenius, 1952	root collar	2003, 2012
Eriococcidae	*Anophococcus agropyri* (Borchsenius, 1949)	leaf	2003, 2012
	Kaweckia glyceriae (Green, 1921)	leaf sheaths	2003, 2012
Margarodidae	*Dimargarodes mediterraneus* (Silvestri, 1906)	root	**2012**
Pseudococcidae	*Atrococcus achilleae* (Kiritchenko, 1936)	root	2003, 2012
	Balanococcus boratynskii Williams, 1962	leaf sheaths	2003, 2012
	Chaetococcus sulci (Green, 1934)	root collar	2003, 2012
	Fonscolombia europaea (Newstead, 1897)	root	2003, 2012
	Heterococcus nudus (Green, 1926)	leaf sheaths	2003, 2012
	Phenacoccus hordei (Lindeman, 1886)	leaf sheaths	2003, 2012
	Rhizoecus albidus Goux, 1936	root	2003, 2012
	Rhizoecus kazachstanus Matesova, 1980	root	**2012**
	Trionymus aberrans Goux, 1938	leaf	2003, 2012
	Trionymus perrisii (Signoret, 1875)	leaf sheats	2003, 2012

3. RESULTS

3.1. Scale insect community structure on Hungarian highways

3.1.1. General characterization of scale insect communities

The investigation of Hungarian highways M0, M1, M3, M5 and M7 was carried out in the years 2009-2012 and 689 scale insects were collected. A total of 647 microscopic slides were made and 664 scale insect specimens were identified. During the four year survey 100 scale species belonging to 10 families were recorded, from woody and herbaceous host plant species (**Table 3**.).

Table 3. : Scale insect species number of woody and herbaceous vegetation for each Hungarian highway (2009-20012)

Family	**Woody plants**					**Herbaceous plants**					*Total of species number by families*
	M0	**M1**	**M3**	**M5**	**M7**	**M0**	**M1**	**M3**	**M5**	**M7**	
Asterolecaniidae	-	2	1	1	1	-	-	-	-	-	3
Cerococcidae	-	-	-	-	-	-	-	-	-	1	1
Coccidae	2	3	4	4	3	2	3	2	2	3	11
Cryptococcidae	-	1	1	1	-	-	-	-	-	-	1
Diaspididae	10	9	9	7	9	-	-	-	-	1	19
Eriococcidae	-	1	1	-	-	4	5	6	2	6	14
Kermesidae	-	-	1	-	-	-	-	-	-	-	1
Margarodidae	-	-	-	-	-	2	-	-	1	2	2
Ortheziidae	-	-	-	-	-	-	1	-	-	-	1
Pseudococcidae	1	1	1	-	1	24	17	17	15	22	47
Total of species number for each highway	*13*	*15*	*18*	*13*	*13*	*30*	*26*	*15*	*20*	*36*	
Total of species number on Hungarian highways											*100*

Because of the man-made aspects of the rest stop sampling sites, investigation of scale insect community was divided into two groups based on host plant character: woody and herbaceous vegetation. Regarding to the whole amount of scale insect species detected, it was revealed that scale insect communities on highways were dominated by species of Pseudococcidae (50%), Diaspididae (27%), Coccidae (9.8%) and Eriococcidae (9.6%) families. The other six families were represented by only low species number and abundance.

The scale insect community associated with woody vegetation was dominated by the scale families Diaspididae by 17 and Coccidae by 5 species. Considering to the host plant specificity of the total number of detected scales were 11% oligophagous and 14% poliphagous species. The majority of oligophagous scale species were associated with coniferous host plants such as *Pinus* spp., *Juniperus* spp., *Thuja* spp. or *Picea* spp. and probably these were introduced to the rest stops by the previously infested plant nursery material. A low number of cosmopolitan, polyphagous (generally fruit tree pest) species were found such as San Jose scale, *Diaspidiotus perniciosus,* or the mulberry scale *Pseudaulacaspis pentagona*. These species were introduced in the highway rest stops with infested hosts such as *Syringa*, *Prunus*, *Pyrus* and *Ulmus* species, all of them originated from different ornamental plant nurseries.

Dominant species of scale insect community associated with woody host plants were *Carulaspis juniperi* (5), *Leucaspis loewi* (5), *L. pusilla* (5), *L. pini* (2), *Unaspis euonymi* (4), *Physokermes hemicryphus* (3), *Diaspidiotus perniciosus* (5), *Parthenolecanium corni* (5), *Eulecanium tiliae* (3) and *Lepidosaphes ulmi* (3).

In the scale insect community associated with herbaceous vegetation the dominant scale families were Pseudococcidae with 43 and Eriococcidae with 13 species. Host plant specificity in this community considering to the total number of detected scales was 30% oligophagous and 27% poliphagous species. Oligophagous scale species were collected mainly on common grass species such as *Festuca*, *Elymus*, *Bromus* and *Lolium* species. In the case of poliphagous species *Atrococcus achilleae*, the yarrow mealybug seems to be a very characteristic highway species and it was listed from more than 19 host plants. Root inhabiting scale species were dominant among scale insect species associated to the herbaceous vegetation both by their detection number and species number.

Dominant species of scale insect community associated with herbaceous host plants were *Atrococcus achilleae* (5), *Chaetococcus sulci* (5), *Rhizoecus albidus* (5), *Kaweckia glyceriae* (5), *Trionymus perrisii* (5), *Fonscolombia europaea* (5), *Phenacoccus hordei* (5), *Heterococcus nudus* (5), *Lecanopsis formicarum* (4) and *Trionymus multivorus* (2). Only *Kaweckia* and *Trionymus* are polyphagous species and the other oligophagous species are root-inhabiting on different grass species. *Heterococcus* and *Phenococcus* species can be found on leaf/ leaf sheaths of grasses.

3.1.2. Scale insect checklist of Hungarian highway network – new data

The 26 scale insect species were newly added to the scale insect checklist of Hungarian highway network. These are the following:

- M0 highway, on woody vegetation: *Diaspidiotus gigas* (Thiem & Gerneck, 1934) (2009, Csepel, *Populus nigra*; 2010, 0. km, *Populus* sp.), *Diaspidiotus zonatus* (Frauenfeld, 1868) (2009, 0. km, *Quercus* sp.); on herbaceous vegetation: *Spilococcus artemisiphilus* Tang, 1988 (1) (2009, Csepel, *Lotus corniculatus*), *Volvicoccus stipae* (Borchsenius, 1949) (2) (2010, Dunakeszi, *Stipa* sp.), *Trionymus graminellus* (Borchsenius, 1949) (2) (2010, 0.km, *Festuca* sp.).
- M1 highway, on woody vegetation: *Trionymus newsteadi* (Green, 1917) (1) (2009, Harkályos, *Quercus* sp.) *Diaspidiotus gigas* (Thiem & Gerneck, 1934) (2009, Börcs, *Populus* sp.), *D. ostreaeformis* (Curtis, 1843) (2009, Moson, *Fraxinus* sp.), *D. pyri* (Lichtenstein, 1881) (2009, Hanság, *Fraxinus* sp.); on herbaceous vegetation: *Rhizococcus gnidii* Signoret, 1875 (2010, Turul, *Veronica* sp.), *Ripersiella periolana* Goux, 1936 (2009, Turul, *Achillea* sp.), *Rhizococcus micracanthus* Danzig, 1975 (2010, Turul, *Rhinanthus* sp.).
- M3 highway, on woody vegetation: *Kermes quercus* Linnaeus, 1758 (1) (2012, Kisbag, *Quercus* sp.) *Diaspidiotus ostreaeformis* (Curtis, 1843) (2009, Rekettyés, *Prunus* sp.); *Dynaspidiotus abietis* (Schrank, 1776) (2010, Rekettyés, *Picea pungens*), *Phenacoccus piceae* Löw, 1883 (2009, Záhony, border-crossing station, *Picea abies)*; on herbaceous vegetation: *Anophococcus granulatus* (Green, 1931) (1) (2009, Hajdúnánás, from soil), *A. pannonicus* (Kozár & Konczné Benedicty, 2011) (1) (2009, Ecséd, *Bromus* sp.), *Phenacoccus avenae* Borchsenius, 1949 (2) (2009, Hajdúnánás, *Festuca* sp.).
- M5 highway, on herbaceous vegetation: *Phenacoccus avenae* Borchsenius, 1949 (2) (2009, Szatymaz, *Dactylis glomerata*), *Spilococcus furcatispinus* (Borchsenius, 1937) (1) (2009, Lajosmizse, *Festuca* sp.)
- M7 highway, on herbaceous vegetation: *Poaspis lata* (Goux, 1939) (1) (2009, Töreki, *Dactylis glomerata*), *Scythia craniumequinum* Kiritchenko, 1938 (1) (2010, Érd_SOS, *Stipa* sp.), *Cerococcus cycliger* Goux, 1932 (1) (2010, Érd_SOS, *Thymus* sp.); *Acanthomytilus jablonowskii* Kozár & Matile-Ferrero, 1983 (2010, Érd_SOS, *Chrysopogon gryllus*), *Diaspidiotus labiatarum* (Marchal, 1909) (1) (2010, Érd_SOS, *Festuca* sp.); *Rhizococcus desertus* Matesova, 1957 (1) (2010, Érd_SOS, *Festuca* sp., *Scabiosa* sp.), *Mirococcopsis borchsenii* (Ter-Grigorian, 1864) (1) (2010 Érd_SOS, Velence, *Festuca* sp.).

3.1.3. Diversity profiles and similarity

Based on Rényi diversity profiles referring to the species list of each investigated highway we may conclude that M7 highway had the highest scale insect diversity, while M5 highway could be characterized with the lowest diversity. The diversity profile curves of M0, M1 and M3 highways intersect each other therefore their scale insect diversity were non-comparable.

Cluster analysis based on Jaccard similarity index (paired group) for the two host plant groups associated scale insect communities for each highway, showed in either cases medium or slightly different similarity. For the woody host plants a compiled urban species list, while for the herbaceous hosts the scale insect list of natural loess steppe habitats (Mezőföld) was used as outgroup.

In the case of woody vegetation scale insect communities could be distinguish two separated branches on relatively narrow range of 0.48-0.4 similarity, in brief: [(M3,Urban)M7][(M5,M0)M1]. Urban category and M3 highway showed the highest similarity. In the case of herbaceous vegetation scale insect communities also two branches could be observed, separated from each other on relatively narrow range of 0.3-0.2 similarity, in brief: [(M7,M0)(M1,M5)][M3,Mezőföld].

3.1.4. Effects of local variables: site profile, build-up index and age

Effects of local variables were tested as follow: effect of site-profile on species number and detection number of herbaceous vegetation associated scales and the effect of build-up index on detection number of woody vegetation associated scales. For the site-profile effect, in the case of herbaceous host plants was observed positive significant effect on the species number (ANOVA, F=6.00; p=0.01) and detection number of scales (ANOVA, F=4.13; p=0.04). For the build-up index effect, in the case of woody hosts was observed positive significant effect on the detection number of scale insects (ANOVA, F=5.08 p= 0.02).

The role of the soil as scale insect vector can be answered with the presence of root-inhabiting scales in different aged sampling site. We assumed based on literature data (KOZÁR et al. 1999) that the newly established rest areas can be described by low species richness, dominated by root-inhabiting scale insects, which were probably introduced to the sampling sites together with the topsoil laid during the final landscaping work steps. No significant correlation were found between age of sampling sites and species number of root-inhabiting scales, but using Lowess smoothing method it could be observed temporal change trend of species richness. If we considered only the six most common root-inhabiting species detection number (*Atrococcus achilleae, Chaetococcus sulci, Fonscolombia europaea, Lecanopsis turcica, Rhizoecus albidus* and *R. kazachstanus)* in correlation with sample site age, it was not significant.

3.1.5. Canonical Correspondence Analysis

Canonical Correspondence Analysis showed weak effects of the local and landscape level variables on the total detected scale insect data. Regarding to local and landscape level variables (500 and 1000 m radius circle) weak correlation were revealed in the case of site age and meadows with total detected scale insect data. Unexplained correlation were observed for herbaceous vegetation associated scale detection data and industrial zone, while in the case of woody vegetation associated scale detection data, the industrial zone, forests and orchards slightly increased the total number of scale detection. Weak effects can be distinguished, however, in the case of woody vegetation associated scales detection number.

3.2. Distribution maps of 15 most common scale insect species associated with herbaceous vegetation from Hungary

A total of 845 sampling data were recorded regarding to the selected 15 species over the past ten year (2003-2012). The documented sampling site categories were highway habitats (rest stops/filling stations/margin zone), loess steppes, grasslands/meadows, sandy grasslands, urban habitats and alkaline grasslands. The best explored sampling sites were those from the highway network, followed by the Mezőföld loess steppe habitats and grassland habitats (with various involved sampling sites such as fresh meadows, ruderal vegetation). The lowest data were originated from sandy grasslands, urban and saltmarsh habitats.

The investigated 15 most common, herbaceous plant associated scale insects are mesophilous and steppe-inhabiting mesophilous species with Palearctic distribution, and they are very tolerant to disturbed habitats even with man-made one.

According to the results of NMDS ordination, the involved different habitats (sampling sites) could not be separated depend on the species composition but scale species could be separated based on their feeding site on the host plant.

4. DISCUSSION AND CONCLUSIONS

Highway margins as green corridors and the different sized rest stops provide mosaic like habitats designed with various vegetation elements. Our four year investigation supports the premise that alongside the highways and in the rest areas a unique structured scale insect community is present. Woody vegetation associated scale insect communities are similar to those from urban habitats therefore mainly ornamental and fruit tree pest species were identified whereas on herbaceous vegetation mesophilous species with predominantly on grass hosts and only sporadically were reported rare steppe or xerophilous steppe species.

The dominant scale species of both coniferous and deciduous associated scale insect community were the same with species cited from Hungarian urban habitats such as juniper scale *Carulaspis juniper* (Bouché, 1851) on evergreen/coniferous host plants or euonymus scale *Unaspis euonymi* (Comstock, 1881) the most common and important pest of *Euonymus* species (VINIS 1977, KOSZTARAB and KOZÁR 1978, RIPKA et al. 1996). *Carulaspis juniperi* are familiar worldwide pest of thujas and junipers (MILLER and DAVIDSON 2005, BEN-DOV et al. 2013) reported from both in urban and natural habitats from many European countries (LAGOWSKA 1998, FETYKÓ et al. 2010, GOLISZEK et al. 2011, SIMON and KALANDYK-KOLODZIEJCZYK 2011). From the highway rest areas it were documented heavy local infestations of juniper scale with the characteristic damage signs such as chlorotic or yellow branches eventually branch die. Despite the fact that juniper scale was the most common armoured scale on thujas and junipers another potentially invasive species was recorded the *C. carueli* (Signoret, 1896) or minute juniper scale. This species has a Mediterranean distribution and is well adapted to the hot and dry climate (BEN-DOV et al. 2013). From two sampling sites Szilas (M3 highway) and Csepel (M0 highway) was recorded on infested thuja and juniper shrubs, planted in green area of restaurants. In both sites the infested plant material was imported from Italy. *C. carueli* in Hungary is listed as urban pest and it was found regular on potted ornamental coniferous host (FETYKÓ and SZITA unpublished).

Leucaspis spp. are another important coniferous pest species on highway rest stops ornamental plants. *L. loewi* Colveé, 1882 and *L. pusilla* Löw, 1883 are frequently may co-exist, but *L. loewi* remains the most frequently reported armoured scale on *Pinus* species. Severe infestation of *L. pini* (Hartig, 1839) causing dieback of young *Pinus nigra* hosts were recorded from Szegerdő and Sormás rest stops on M7 highway. In both cases young trees certainly were previously infested with scales in plant nursery (KOZÁR et al. 2012).

The Nasonov's mealybug, *Planococcus vovae* (Nassonov, 1908) in the last few years became a common urban pest on thuja and false cypress in Hungary (FETYKÓ 2010). From Europe it was signaled typically from synanthropic habitats (FRANCARDI and COVASSI 1992, GOLAN and JASKIEWICZ 2002, MASTEN-MILEK et al. 2008, FETYKÓ et al. 2010, SIMON and KALANDYK-KOLODZIEJCZYK 2011). Its massive appearance in urban environment infesting various coniferous ornamental plants is attributed to the introduction and distribution of unnoticed infested plant material (MASTEN-MILEK et al. 2008, TALEBI et al. 2008, FETYKÓ et al. 2010). In our case it was found in Csepel (M0 highway) on juniper host plant, together with *C. carueli* species.

Carulaspis carueli, *Leucaspis pini* and *L. loewi* armoured scale species were reported in every case from newly established green areas. For *C. carueli* and *P. vovae* the country of origin of the plant material was easy to identify. Both species are listed by DAISIE database as invasive scales. The further appearance on highway rest stops could be possible but the dispersion in the country via or alongside highway verges seem to be unrealistic due to the lack of host plants.

During the visual survey of the second woody host plant group, on deciduous ornamentals only low infestations were recorded caused by common, cosmopolite fruit tree pest scales such as *Parthenolecanium* sp. or lecanium scale on *Ulmus, Tilia, Acer* and *Quercus* hosts or *Diaspidiotus* armored scales on *Prunus, Pyrus* and *Quercus* hosts. Whereas *Parthenolecanium* and *Diaspidiotus* species are listed by the literature as important fruit tree pests (BEN-DOV et al. 2013) often were reported as ornamental pest in urban habitats (BOGNÁR and VINIS 1979, LAGOWSKA 1998, RIPKA et al. 1996, RIPKA 2005, SIMON and KALANDYK-KOLODZIEJCZYK 2011). The presence of some rare scale insect species such as *Trionymus newsteadi* (Green, 1917) (2009, M1-es, Harkályosi pihenő, *Quercus* sp.) or *Kermes quercus* Linnaeus, 1758 (2012, M3-as, Kisbagi, *Quercus* sp.) on woody hosts can be explained by the continuous connection of sampling sites with natural habitat patches.

Only sporadical were detected severe scale infestations on young deciduous ornamental trees. In Kecskemét sampling site on M5 highway was discovered a heavy infestation of nut scale, *Eulecanium tiliae* Linnaeus, 1758 on a young *Ulmus* tree row near the filling station. This well localized infestation of same aged, young trees denote a previously infestation originated from plant nursery. Nut scale was cited as typical nursery pest from Poland (SOIKA and LABANOWSKI 2003) while in Hungary is forest and ornamental tree pest (BOGNÁR and VINIS 1979, KOZÁR and KOSZTARAB 1980, KOZÁR 1989).

Several poliphagous cosmopolite scale species were observed on deciduous ornamental trees in the investigated sampling sites but no severe infestations or tree diebacks were recorded.

The mulberry scale, *Pseudaulacaspis pentagona* (Targioni-Tozzetti, 1886) and San Jose scale, *Diaspidiotus perniciosus* (Comstock, 1881) can be mentioned as significant invasive scale present in the highway margin zone, but based on our recent knowledge the spreading/dispersion are unlikely. Their appearance on the rest stops deciduous ornamentals are most probably caused by a previous plant nursery infestation.

From local variables the build-up index had a positive significant effect on the detection number of scales and this can be attributed to the high number of medium and severe infested ornamental hosts from both categories deciduous and coniferous. On the newly settled ornamentals (around filling stations, motels, in park areas) due to the extreme conditions (e.g. sunlight, water and nutrient deficiency) low or medium scale infestations intensified over the years and this is reflected in the increasing number of scale detection but not in an increasing species number.

Herbaceous vegetation associated scale insect community was dominated by the following species: *Atrococcus achilleae* (Kiritchenko, 1936) (5), *Chaetococcus sulci* (Green, 1934) (5), *Rhizoecus albidus* Goux, 1936 (5), *Kaweckia glyceriae* (Green, 1921) (5), *Trionymus perrisii*

(Signoret, 1875) (5), *Fonscolombia europaea* (Newstead,1897) (5), *Phenacoccus hordei* (Lindeman, 1886) (5), *Heterococcus nudus* (Green, 1926) (5), *Lecanopsis formicarum* Newstead, 1893 (4) and *Trionymus multivorus* (Kiritchenko, 1935) (2). Most of these species are oligophagous, living on root of grass species typical for lawns such as *Elymus, Festuca and Lolium* species (KOSZTARAB and KOZÁR 1978, 1988, KOZÁR 1989). Despite the fact that *Chaetococcus sulci, Kaweckia glyceriae and* a *Rhizoecus albidus* scales were marked as rare species by KOZÁR and KONCZNÉ BENEDICTY (1998) and were signaled only from natural protected areas, lately they were reported as quite common species (KOZÁR et al. 1999, KOZÁR and KONCZNÉ BENEDICTY 2002, KOZÁR et al. 2002, 2004a, 2009, FETYKÓ et al. 2012) and currently were listed as frequent and dominant scale insect species of highway margins and rest areas (KOZÁR 2009, NAGY and KOZÁR 2010 a, b). These observations can be explained by the mesophilous character and Palearctic distribution of this species which are probably present on the whole territory of Hungary (KOSZTARAB and KOZÁR 1978, KOZÁR et al. 2009, BEN-DOV et al. 2013).

Whereas the vast majority of the investigated sampling sites were situated in agricultural landscape and dominated by domestic lawn, in the case of few sampling sites a continuous connection with natural habitats could be observed. Due to this connection of the sampling sites, habitat specific plant species were found characteristic to sandy grasslands (M5 highway), loess steppes (M7 highway) or xerophilous grasslands (M0 and M1 highways). These well-defined herbaceous plant patches assure a suitable habitat for rare steppe and xerophilous-steppe scale species. From this type of small habitat patches two root-inhabiting mealybug species were reported, both new to the Hungarian fauna: *Spilococcus artemisiphilus* Tang, 1988 (2009, M5-ös, Lajosmizse, *Festuca* sp.) and *Spilococcus furcatispinus* (Borchsenius, 1937) (2009, M0-ás Csepel, *Lotus corniculatus*). From sampling site Érd_SOS (M7 highway) *Acanthomytilus jablonowskii* Kozár & Matile-Ferrero, 1983 and *Diaspidiotus labiatarum* (Marchal, 1909) were recorded, both rare steppe species and *Cerococcus cycliger* Goux, 1932 xerophilous steppe scale species, these were previously found only in nature reserves such as Sas-hegy or Aggteleki National Park (KOZÁR et al. 1977, FETYKÓ et al. 2013), moreover they were cited as rare xerophilous species from Mediterranean region of Europe (FOLDI 2001, MATILE-FERRERO and PELLIZZARI 2002).

Our investigation results are confirmed by the conclusions of a recently made highway research work of SCHMIDT at al. (2010) which concluded that highway margins and slope zones can offer proper habitat for loess, sandy or rock grassland vegetation. For scale insects, at the same time, this type of herbaceous vegetation plots may ensure a long-term survival site as we observed in the case of some rare steppe or xerophilous-steppe species which were reported from highly disturbed highway rest stop slopes like Turul (M1 highway) or Érd_SOS (M7 highway). These characteristic highway verges and slopes could represent potential reservoir for several valuable plant and insect species.

Diversity of scale insect communities on highways was compared based on Rényi diversion profiles. M7, the oldest highway had the highest diversity for both rare and common scale species. This could be explained by the continuous connection (Érd_SOS, Töreki) to natural habitats of some sampling sites therefore a high number of rare species were detected. M5 highway had the lowest diversity because of the low species and detection number of scale species.

Similarity analyses of species composition for each highway showed medium or slightly different similarity (Jaccard-index <0.48). In the case of woody vegetation the M3 and M7 highway species composition was the most similar with the complied *urban* outgroup while species composition of herbaceous vegetation M3 highways showed the highest similarity with *Mezőföld* outgroup due to the high number of eriococcid species.

Based on our four year investigation it can be conclude that mealybugs and eriococcids were dominant by high species and detection number in the scale insect community associated with herbaceous vegetation of highway rest stops. The management regime of the lawns on the highway rest stops (*Festuca*, *Lolium* and *Poa* species) supports the persistence of root-inhabiting oligophagous scale species. Scale species living on leaf/leaf sheath were represented by low species number and detection number, most probably due to the intensive lawn mowing which does not guarantee the survival of these species. During the visual survey of the sampling sites these scale species were typically found in protected spots like small shrub groups with some tall grasses or unmowed patches. We observed rarely the change of feeding/living site from leaves near to the ground level (pl. *Heterococcus nudu*s or *Trionymus* spp.).

The site-profile of sampling sites means if the typical collecting place of a given rest stop was slope or flat. The influence of this feature on the species number and detection number of scales associated with herbaceous host plants was tested, which resulted that slopes increase the species and detection number of scales. Slopes can provide favorable microclimatic conditions for root-inhabiting scale species rather than flat sites because the soil can quickly dried out after heavy rains and do not remain stagnant water spots like on flat sites.

No significant correlation was found between age of sampling sites and species number of root-inhabiting scales. Applying Lowess smoothing method, a trend of temporal change of species richness could be observed. According to the previous literature data (KOZÁR et al. 1999) we suppose that newly established rest stops could be characterized by very low species richness (even none). Nevertheless our investigations revealed continuous presence of some dominant root-inhabiting scales in so called ” young” 5-10 year old sampling sites. A very slight increasing tendency of root-inhabiting scale insect species number could be noticed between sampling sites age 15 and 25 as same as in the case of 30 year old or older sampling sites.

The regeneration of herbaceous plant cover on disturbed highway margin zones and slopes were estimated to 30-40 year interval (SCHMIDT et al. 2010) while this time interval is also very realistic in the case of stabilizing of scale insect communities of highway rest stops/margin zones which are enclosed mainly by a mosaic like agricultural landscape. Literature data cited as first scale insect inhabitants of secondary pastures established on abandoned agricultural fields the poliphagous, root-inhabiting *Atrococcus cracens* Williams, 1962 and *Fonscolombia europaea* mealybug species, while on leaf /leaf sheaths the monophagous *Longicoccus festucae* (Koteja, 1971) and the poliphagous *Trionymus perrisii* (Signoret, 1875) mealybug species were reported (KOZÁR et al. 1999). In the case of young (5-10 year old) rest stops the dominant scale species were dominant the root-inhabiting *Chaetococcus*, *Fonscolombia* and *Rhizoecus* species, whereas on leafs/leaf sheaths *Kaweckia* and *Trionymus* species. The low species richness of aforementioned secondary pastures was probably due to the weak migration ability of scales and is in concordance with the long regeneration interval of the vegetation cover. The relatively high presence of root-inhabiting scale species in the highway rest stops/margin zone are very likely to be a human activity related introduction perhaps via soil covering (which is the last step of landscaping work). The stabilization of scale insect communities in these disturbed habitats are probably strongly promoted by the continuous connection of rest stops vegetation with the neighboring natural habitats or by the presence of remaining natural vegetation patches. Our assumption seems to be correct because the species richness was high on those sampling sites which were connected to natural habitats such as Turul rest stop on highway M1, Örkény rest stop on highway M5 or Érd_SOS highway margin site on highway M7.

Highway rest stops herbaceous vegetation was dominated by root-inhabiting scale species, especially by two *Rhizoecus* species. *Rhizoecus* species were marked by the literature as pioneer scale species of regenerating habitats (BROZA and IZHAKI, 1997) and they are in strong symbiotic relationship with ant species (MALSCH et al. 2001) moreover some of them are severe pest species usually introduced and distributed with the soil of potted plants worldwide (JANSEN 2003, MALUMPHY and ROBINSON 2004). With respect to introduced arthropod species in highway rest stop habitats VONA-TÚRI et al (2013) revealed the presence of *Armandillidium nasatum* Budde-Lund 1885, a common greenhouse pest originated from Mediterranean region which probably was introduced by the soil of potted ornamental trees on the Csepel rest area, highway M0. This species indicates that human activity contributes seriously to the introduction and distribution of greenhouse species such in this case with the soil of potted ornamentals. According to the results of our investigations the vector role of the soil for root-inhabiting scale insects was assumed but it requires further investigations.

CCA analyses of scale insect communities of highway rest stops reveal only weak effects of local and landscape variables. Only in the case of scale communities associated with woody vegetation were observed a slight increase of detection number as effect of industrial zone, forest and orchard variables. This can be explain by high number of potentially scale infested ornamental plants settled in the man-made environments such as thuja, juniper, spruce or euonymus species whereas for the effect of forests and orchards they might increase the number of scale species on the rest stops deciduous plants and also the detection number of it. Our results were same with the previous ones where low effect of local and landscape factors on the scale insect communities were reported in the case of secondary pastures (Kozár et. al. 1999) and natural environments (Kozár et al. 2009).

Highways habitats seem to be best explored one environment for herbaceous vegetation associated 15 most common scale insect species from Hungary. From the sixth most common root-inhabiting scales the detection rates revealed that the poliphagous and mesophilous legless festucue mealybug *Chaetococcus sulci* was the most frequent mealybug in all investigated habitat types.

NMDS ordinations regarding to sampling site data and detection data of 15 most common herbaceous vegetation associated scales showed that none of this species could be used as habitat indicator species. According to the relative high detection frequency in highway green environments we can consider the following scale species as highway-species: *Atrococcus achilleae*, *Chaetococcus sulci*, *Heterococcus nudus*, *Kaweckia glyceriae*, *Phenacoccus hordei*, *Rhizoecus albidus* and *R. kazachstanus.*

Altogether we may conclude that in the highway rest stops and margin zones uniquely structured scale insect communities occurred due to the mosaic-like man-made environment. The scale infested coniferous and deciduous ornamental trees planted in the highway rest stops contribute to the rapid dispersal/ spread of some pest species across the country. The presences of invasive and non-native scale species were generally due to the use of imported ornamental trees and shrubs. In highway environments woody ornamental vegetation deserves a special attention in order to track the appearance of some important invasive poliphagous scale species like the Comstock mealybug *Pseudococcus comstocki* (Kuwana) (Pseudococcidea) or the horse chestnut scale *Pulvinaria regalis* Canard (Coccidae).

Suggestions

• We propose the use of ornamental plants originated from local nurseries and growers, where it is possible. Special attention should be paid to the evergreen/coniferous shrubs and trees which came from unknown foreign sources.

• We recommend a careful visual survey and professional selection of imported plant material for the landscaping work of newly established rest stops, filling stations, parks and other recreational area of motels and restaurants.

• We suggest the elimination of all heavily infested woody plant material, trees and shrubs especially coniferous species from the rest stops/filling stations, and use of pesticide treatment in the case of localized infestation foci.

• Long-term protection and conservation of highway margin zones with valuable vegetation (loess or steppe) which could contribute to the protection, respectively implementation of ecological corridors for rare plant and insect species.

• Further development of a national network of invasive insect monitoring system.

5. NEW SCIENTIFIC RESULTS

Scale insect communities of 44 highway rest stops were investigated between 2009 and 2012. Based on collected and analyzed scale insect material my new scientific results are the following:

- This four year highway research completes the Hungarian highway scale insect checklist with 26 newly detected scale insect species.
- Four scale insect species were recorded at the first time for the Hungarian fauna: *Mirococcopsis borchsenii* (Ter-Grigorian, 1964), a *Spilococcus artemisiphilus* Tang, 1988 a *Spilococcus furcatispinus* (Borchsenius, 1937) and *Trionymus graminellus* Borchsenius, 1949 from herbaceous host plants.
- Two, DAISIE data base registered invasive scale insect species were detected on imported coniferous ornamental plants: *Carulaspis carueli* (Signoret, 1896) and *Planococcus vovae* (Nassonov, 1908) (host plant *Thuja* sp. and *Juniperus* sp.).
- Site profile as local factor was proved to have a positive effect on the species number and detection number of root-inhabiting scale insects associated with herbaceous host plants.
- Both of local and landscape level factors were proved to have a weak effect on the highway scale insect communities species and detection number.
- Infested nursery plant material was the most important and relevant scale insect vector regarding to the dominant scale insect species of woody ornamental plants.
- We conclude that in Hungary, highway margins as green corridors do not facilitate natural spreading/dispersion of invasive scale insect species.
- The next scale insect species characterized with high tolerance for disturbed habitats were categorized as highway-species: *Atrococcus achilleae* (Kiritchenko, 1936), *Chaetococcus sulci* (Green, 1934), *Fonscolombia europaea* (Newstead, 1897), *Heterococcus nudus* (Green, 1926), *Phenacoccus hordei* (Lindeman, 1886), *Rhizoecus albidus* Goux, 1936 and *Rhizoecus kazachstanus* Matesova, 1980.

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