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HERBIVOROUS INSECTS DIVERSITY AT *MISCANTHUS* × *GIGANTEUS* IN UKRAINE

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Miscanthus × *giganteus* is considered as a perspective energy crop for biomass production in Ukraine where its commercial production has been observed. The herbivorous pest may pose a risk of yield reduction when an energy crop is growing on monoculture. The herbivorous diversity, species composition and potential damage associated with growing *M.* × *giganteus* were studied on seven experimental sites at three locations in Ukraine. The different life stages of herbivorous insects from seven orders representing thirteen families were found on *M.* × *giganteus* during the herbivorous survey and most of the insects had a pest status. Research indicated that crop was an alternate host for key cereal pest the Hessian fly *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). A comparative analysis of the biodiversity of herbivorous insects across research locations was done using statistical analysis. It was found that site location played a significant role in the level of biodiversity and an increase in the insect's herbivores diversity was associated with the type of researched lands. The massive scale commercial use of *M.* × *giganteus* should take into account a responsible consideration of the benefits and risks associated with that crop in order to protect agroecosystems.

Key words: survey of herbivorous insects, pest, Hessian fly, statistical method

M. × *giganteus* is a sterile hybrid of two species: *M. sinensis* and *M. sacchariflorus*, and is native to Southern Asia (Hodkinson *et al*. 2002). Plant belongs to C-4 photosynthetic pathway type and has a capacity for effective and substantial biomass production (Zub & Brancourt 2010; Agostini *et al*. 2015). The biological feature of that crop is its ability to grow at the contaminated and abandoned sites, which makes it appropriate for phytotechnologies (Otepka *et al*. 2011; Pidlisnyuk *et al*. 2014; Nsanganwimana *et al.* 2016). The *M.* × *giganteus* potential for biofuel production has been extensively researched in Europe (Lewandowski *et al*. 2003; Gauder *et al.*2012), including Slovakia (Porvaz *et al.* 2012; Jurekova *et al*. 2013; Gubisova *et al*. 2016) and Czech Republic (Vaprova & Knapek 2010; Strasil 2016). *M.* × *giganteus* perennial rhizomatous grass's feature makes its establishment and reproduction very costly. Recently, significant progress was achieved in the development of *M.* × *giganteus* hybrid seed; the planting of this hybrid seed leads to a more economically feasible crop production than using the rhizomes (Clifton-Brown *et al.* 2017). Nowadays,

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the crop is used commercially for heating and generating electricity in several EU countries. In the US, the crop has been proposed for commercial production in Midwest and Northern States, particularly in locations where the precipitation is not a limiting factor. The number of growing sites has been expended in the US rapidly; however, the commercial production has not been well established yet (Heaton *et al.* 2008).

Currently, no pests of economic importance are found in *M.* × *giganteus* in Europe or the US. The European experience with *M.* × *giganteus* planting showed that the crop has low level of risk from pest damage. However, taking in consideration a hybrid nature of the crop, any pest associated issues that do arise may cause a serious problem (Thomson & Hoffmann 2010). Only few pests have been reported that directly damage the crop (Semere & Slater 2007). The survey of invertebrates of *M.* × *giganteus* in the United Kingdom indicated that there were ‘‘no major pests found’’ (Hugget *et al*. 1999). Results of the two-year research in Germany showed the damage of crop by two-spotted spider mite *Tetranychus urticae.* Koch Gottwald and Adam (1998), Semere and Slater (2007) recorded that aphids were the dominated Homoptera group found on field trials of Miscanthus. This finding may raise the issue of how aphids indirectly affect the crop by transmitting viruses. Several researches indicated that the crop, that may contribute to the distribution of viruses, were transmitted by aphids. It was observed that corn leaf aphid *Rhopalosiphum maidis* (Fitch) may colonize Miscanthus and lay eggs on the established plants. The laboratory studies showed that aphid feeding on *M.* × *giganteus* transmitted viruses: Barley Yellow Dwarf virus (Christian *et al*. 1994; Hugg *et al.* 1999) and the sorghum mosaic virus (Grisham *et al.* 2012).

The results of recent studies in the Northern France, where the cultivation of *M.* × *gigantheus* started in the early 2000s, gave a new argument regarding the concern on increasing the risk of speeding phytoviruses by aphids and Miscanthus acting as reservoir for aphids’ pests from neighbouring food crops. Coulette *et al*. (2013) showed that *M. sacchariflorus* (parental species for M. × *giganteus*) did not appear as an appropriate host for the three aphid species *Aphis fabae* Scop, *Myzus persicae* (Sulzer) and *Rhopalosiphum padi* L. Ameline *et al.* (2015) reported that the host suitability for the four major aphis depends on the degree of specialization to Poaceae and appeared as moderate for specialist *Rhopalosiphum padi* L., low for polyphagous *Aphis fabae* (Scop) and *Myzus persicae* (Sulzer) and as very low for Brassicae specialist *Brevicoryne brassicae* L. In controversy*,* this study illustrated that the cultivation of Miscanthus in large scale might not always aggravate the problem of creating reservoir aphids from adjusting food crops; it could be assumed that *M.* × *giganteus* acted as a barrier crop helping to reduce the risk of transmission and spread of phytoviruses.

The recording of direct increasing damage by several insects in the US may be evidence to suggest that *M.* × *giganteus* has pests; its effect on biomass is still unknown and its severity will depend on the scale and the time of plant’s growth. The fact that all insects reported to feed on *M.* × *giganteus* in the US are pests of corn, sugarcane or sorghum, raises concerns that the production of that crop in the large scale will increase pest numbers in existing food crops. It can be well illustrated by the relationship between *M.* × *giganteus* and corn (Bradshaw *et al*. 2010). *M.* × *giganteus* appeared as a host to the yellow sugarcane aphid and may cause damage in young stands in the field condition. The potential of *Sipha flava* (Forbes) to damage *M.* × *giganteus* in case of a large-scale cultivation was confirmed in the laboratory research (Pallipparambil *et al*. 2014), when the crop served as a host plant for corn rootworm determined as dangerous maize pest (Spencer & Raghu 2009). It was indicated (Gloyna *et al*. 2011) that the larvae of Western corn rootworm, of *Diabrotica virgifera virgifera* (Le Conte) originating from a Central and South Eastern European population, could be developed at *M.* × *giganteus*. Armyworm and stem borer, which were host of corn and sorghum species, were able to feed on the crop as well.

Prasifka *et al.* (2009) showed that *M.* × *giganteus* along with switch grass was a host of fall armyworm *Spodoptera frugiperda* (J. E. Smith) determined as a pest of corn was observed in the infesting plots. Laboratory test showed that *S. frugiperda* larva preferred corn leaves over Miscanthus ones. The pest was able to complete the development on Miscanthus and switchgrass at the green house conditions; however, it did not survive well during the field experiment. As it was reported by Prasifka *et al*. (2012), the stem-boring caterpillars: *Elasmopalpus lignosellus* (Zeller) (Pyralidae), *Diatraea saccharalis* (F.) (Pyralidae), and Mexican Rice Borer *Eoreuma loftini* (Dyar) (Crambidae) might cause *M.* × *giganteus* biomass reduction, and a long-term investment in breeding for host plant resistance might be requested.

Since 2004 *M.* × *giganteus* was under evaluation for commercial production in eight regions of Ukraine (Kvak 2013), an emerging commercial production has been currently observed and the area under cultivation is about 1,500 ha with expectation of significant extending in the nearest future (Pidlisnyuk & Stefanovska 2016). This trend is due to the increasing biofuel demands, energy security concern and political desire to increase the share of bioenergy in the country's energy balance (Geletukha *et al*. 2015). The fact is illustrated in Figure 1.

Growing interest and commercialization of *M.* × *giganteus* production in Ukraine will lead to land use changes and possible cultivation of the crop in monoculture. The land use change is a significant contributor of biodiversity changing (Whittaker *et al*. 2001). Some previous studies indicated a positive effect of *M.* × *giganteus*, growing to biodiversity service (Semere 2007; Gauder *et al*. 2012; Dauber *et al*. 2015), when the crop hosted several arthropods, particular predatory ground beetles and parasitoids – natural enemies of important agricultural pests. Stanley and Stout (2013) indicated that *M.* × *giganteus* supported higher abundance and diversity of pollinators and hymenopteran wasps comprised to traditional food crops. Another study concerned about the possible negative impact of *M.* × *giganteus* growing to the state of biodiversity (Stefanovska *et al*. 2011).

There are several factors that determine species diversity, particular spatial arrangement of habitat elements and the spatial-temporal heterogeneity of the landscape (Schluter & Ricklefs 1994; Lewinsohn *et al*. 2005; Rocca & Greko 2011). In comparison to Western Europe and the US, very limited data exists on that topic while growing *M.* × *giganteus* in Eastern Europe, including Ukraine. The purpose of this study was to do a survey of herbivorous insects in field conditions at different regions of Ukraine and to analyse the abundance, richness and biodiversity of common insect herbivores while growing *M.* × *giganteus* in seven sites at three different locations*:* Vinnytsia, Zhytomyr and Kyiv regions.

MATERIAL AND METHODS

The selected locations represented the areas which have high potential for biofuelcrops' production (Geletukha *et al.* 2015). A total of seven established plots of *M.* × *giganteus* varying in time of cultivation were chosen for herbivorous sampling. Plot sizes varied from 25 m^2 to 100 m^2. Some characteristics of the plots are presented at Table 1. For all the plots, *M.* × *giganteus* was planted manually in the soil depth of 0.05–0.1 m with spacing of 0.70 m × 0.70 m, which was equal to 20 pieces of plant per m^2; planting was done in the period of end of April to middle of May, depending of the annual weather conditions that determined the features of the vegetation season. The way of planting Miscanthus was same for each year of planting. The insect sampling was conducted at 2010 and 2011. Visual observation and sampling was conducted at five subplots randomly located within the planting at a minimum of 10 m from the plot edge to reduce edge effects. Above ground insect samples were collected every four weeks during the growing season using both active and passive sampling methods (Binns *et al*. 2000). Active sampling, through sweep net and stem was performed prior to the passive sampling methods. Stem counts were geared towards sedentary aphids. Sweep net sampling was a direct sampling method that would collect insects on the foliage of the plant. Twenty sweeps were taken down off the alternate crop rows and the specimens were collected and preserved in 90% ethanol solution until identification. In passive samples, Pitfall traps (Prasifka *et al*. 2007) were used for the collection of ground dwelling insects and sticky cards were used for the collection of small arboreal insects. Pitfall traps and yellow sticky cards were set up in a grid pattern with 1 m spacing. The traps were replaced weekly from the beginning of June till the end of July. All the traps were transferred to the laboratory for further insects' identification. For the sampling of Elateridae and Scarabaeidae species, a method of spring soil excavation was implemented. At each plot, the holes 0.50 × 0.59 m at depth 0.50 m were dug. The excavated soil samples were sieved and extensively screened for larvae at all stages of wireworms, grubs, mole crickets and cutworms, which were counted further.

The insect presented in the traps was identified till higher taxon and the selected herbivorous trophic groups were identified till species or at least till genera.

The data obtained from 2010 and 2011 years of insect sampling data are presented together in the same diagrams.

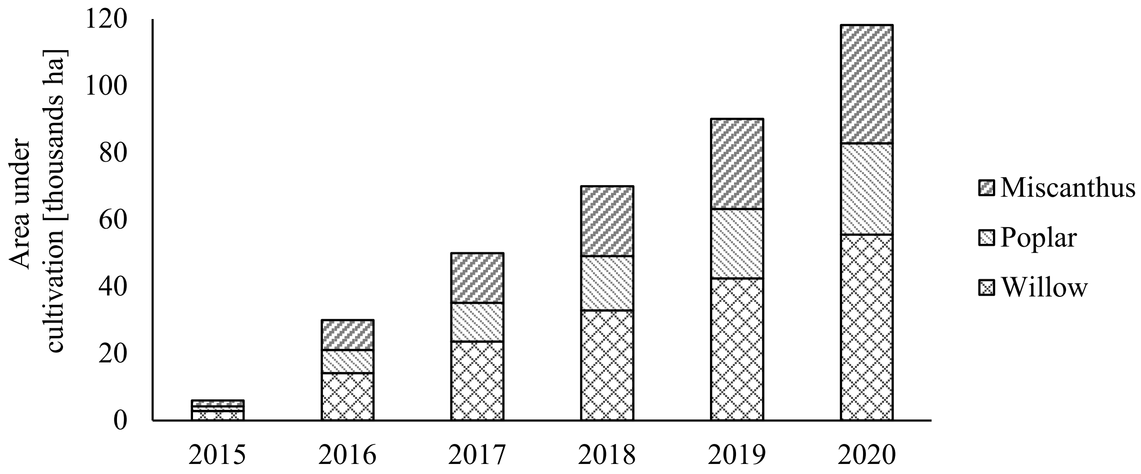

Figure 1. Prediction of energy crops area cultivation (Geletukha *et al*. 2015)

T a b l e 1

Characteristics of the research sites

Site location	Site and plot number	Location (GPS)	Year of the study	Year of the growing	Type of the soil
Zhytomyr	site1, plot 1	50.252385 28.70011	2010 2011	3 4	Podzolic gleyed
	site 1, plot 2	50.252385 28.70011	2010 2011	6 7	
Vinnytsia	site 1, plot 1	48.997958 27.462125	2010 2011	1 2	Flooded grey podzolic soil
	site 1, plot 2	48.997958 27.462125	2010 2011	3 4	
Kyiv	site 1, plot 1	50.42945 30.494808	2010 2011	1 2	Dark grey soil
	site 1, plot 2	50.42945 30.494808	2010 2011	3 4	
	site 2, plot 1	50.415087 30.55705	2010 2011	1 2	

In order to assess the insect biodiversity, the Shannon index (Shannon 1948) was measured.

$$H = -\Sigma p_i \log_2 (p_i) \quad (1)$$

where:
$P_i = n_i/N$
(n_i) – sharing of the specie; (N) – total number of individuals

In order to determine the diversity of herbivorous taxa, we referred to the different research sites and to find the maximum entropy for each taxa of herbivores, the Hartley approach was used (2), in which N – number of taxa, as for the case:

$$H_{max} = \log_2 N \quad (2)$$

The Pielow's evenness index (3) was calculated by dividing the Shannon index ratio (1) by its maximum value (2), in order to align data from one site with other sites:

$$H' = \frac{H}{H_{max}} \quad (3)$$

Parametric statistical method, student's t-test and ANOVA were used to compare herbivore diversity across the sites in 2010–2011.

RESULTS AND DISCUSSION

Results of the survey of herbivorous insects provided at different sites where *M.* × *giganteus* grown are shown at Figure 2. The analysis of outcomes shows the different life stages of insects from seven orders and families observed during the 2010–2011 growing seasons across the sites. Specifically, among the recorded herbivorous generalists and highly specialists for Poaceae family pests were found for all the sites. The soil dwelling polyphagous insect (*Gryllotalpa gryllotalpa* L., *Melolontha melolontha* L. and *Agrotis segetum* S.) were recorded at three sites located in Vinnytsia and Kyiv in the first year of growing *M.* × *giganteus*; no significant plant's injury by insects was observed with one exception of the Hessian fly. Its larvae and pupae were observed inside the stems of the plant in one plot located in Zhytomyr region. It was evident that Aphididae (Homoptera) and Thripidae (Thysanoptera) families were dominated at three locations. The *Rhopalosiphum padii* L. was the most widely represented species for all the researched sites and the Herbivorous trips were found in three sites. That fact is in correlation with results reported by Hurej and Twardowski (2009), who observed this group of sub sucking insects at *M.* × *giganteus* plots in Poland during the first years of cultivation.

Overall, 50 species of herbivorous trips were registered at the agricultural landscapes in Ukraine. In this study, the high population trips' density was observed in three locations (Figure 3). That trend can be due to the weather conditions in the research years, which were very favourable for the trips' development because of warm and dry summer. Leaf hopper, *Psammotettix striatus* L. were additionally recorded in the site located at Vinnytsia region, which was more southern in geographical location in comparison with other sites.

Shannon and Pielow's evenness indexes were used for the comparison of biodiversity of herbivorous insects across research locations. Results of the calculation of average data in 2010–2011 are presented at Figure 4, Table 2 and Table 3. It was hypothesized that the factor of location influenced the biodiversity of herbivorous insects. Following the performing the share of influence of considering and not considering that factors location was determined. The results showed that the impact of the factor 'location 'was 51%. Since *F* Theoretical > *F* Critical (Table 2), the null hypothesis might be rejected, that is, the site location played a significant role in the level of biodiversity. Results also showed that the increase in the diversity of insect herbivores was associated with the type of observed lands. Thus, Vinnytsia site located in more agricultural setting (with many crops types) illustrated a significantly higher level of species diversity in comparison with other locations in Zhytomyr and Kyiv regions, which showed few types of crops.

Analysis of previous research results indicated that *M.* × *giganteus* did not have many herbivorous pests, which in turn confirmed the statement that *M.* × *giganteus* cultivated fields could be served as a refuge for insect herbivores. Hence, the more the fields with various agricultural crops surround Miscanthus field, the more likely it is that the pests from those fields will move to the Miscanthus field. It can be concluded that the introduction of *M.* × *giganteus* in crop rotation may help to improve the environmental sustainability of the agricultural landscapes through the conservation of natural species and territory, and the plant's growth may work as ecological corridors. Profound research is needed for studying the interaction of *M.* × *giganteus* with other trophic groups of insects, particularly entomophagous and soil micro and mezofauna.

The importance of documenting trips, aphids and leafhoppers population data is three-fold. First, the pests may have a direct impact on crop production and profitability. Second, many of these insects are also associated with current crops, such as wheat, and may build up large populations

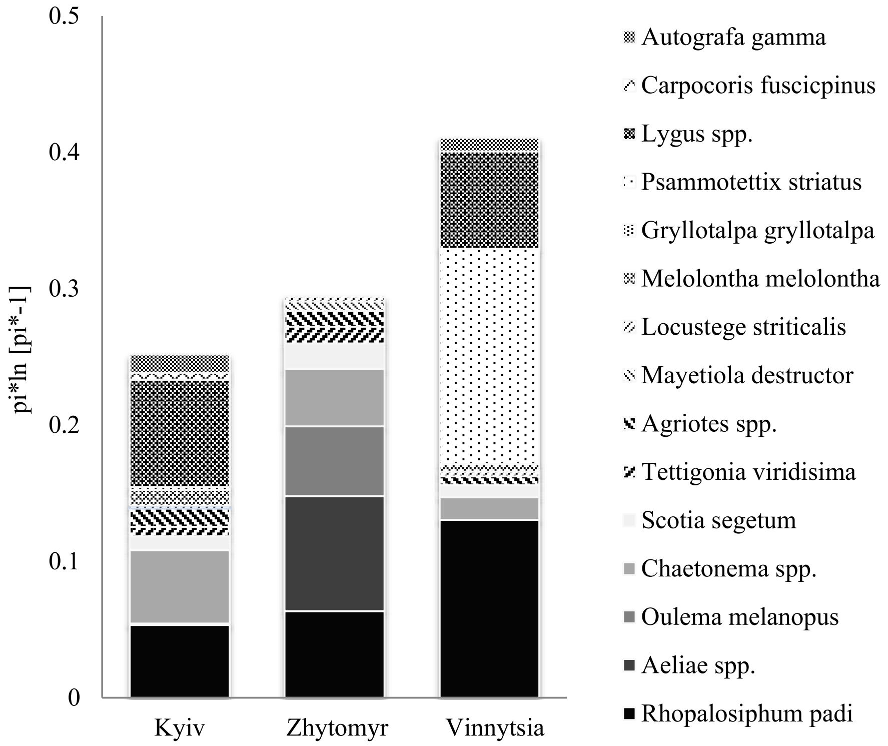

Figure 2. Variation of insect species and genera on *Miscanthus* × *giganteus* at three research locations in 2010–2011 growing seasons

T a b l e 2

Shannon index in sites

Site	Zhytomyr	Vinnytsia	Kyiv, site 1	Kyiv, site 2
1	0.46	0.59	0.42	0.46
2	0.46	0.67	0.44	0.58
3	0.76	0.78	0.47	0.32
4	0.33	0.93	0.52	n/d
M	0.50	0.74	0.46	0.46
±m	0.09	0.07	0.02	0.08

M – mean; SE – Standard error

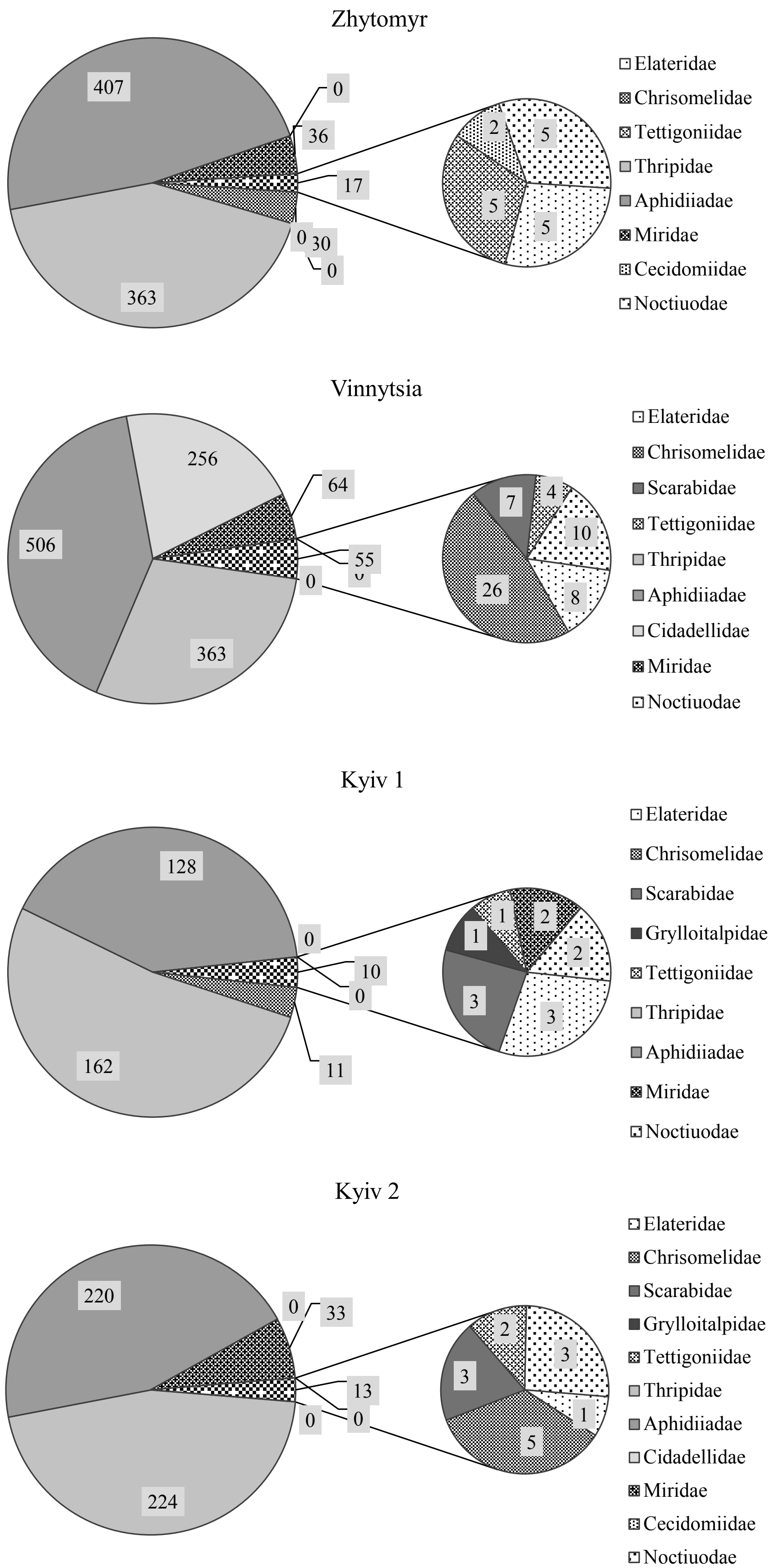

Figure 3. Number of herbivorous individuals in different stages at three locations in 2010–2011 growing seasons

that could then migrate into production fields. Third, two of the most commonly found insects: the aphid *Rhopalosiphum padi* L. and the leafhopper *Psammotettix striatus* L. are both vectors of wheat diseases; thus, *M.* × *giganteus* could potentially serve as harbourage for vectors and disease.

The survey found that *M.* × *giganteus* was an alternate host for a key cereal pest the Hessian fly *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). Since Hessian fly, along with other species from the families Chrolopidae and Antomyidae, is a destructive pest for several cereal crops, there is a potential risk that the insect may reduce the yield of Miscanthus and damage adjacent food crops. Because intercropping is a common practice in Ukraine wheat, barley and oats may be potentially affected by this insect as well. It highlights that the current studies of Hessian fly colonization of Miscanthus plantings should be prolonged for the evaluation of potential risks to other crops and wild species sharing the agricultural landscape.

The fact that *M.* × *giganteus* appeared as a good host for the Western root corn beetle brings a new challenge for the commercial growing of that plant, which may increase the risk of further distribution of *Diabrotica virgifera virgifera* (Le Conte) throughout the country (Andreyanova & Sikura 2010).

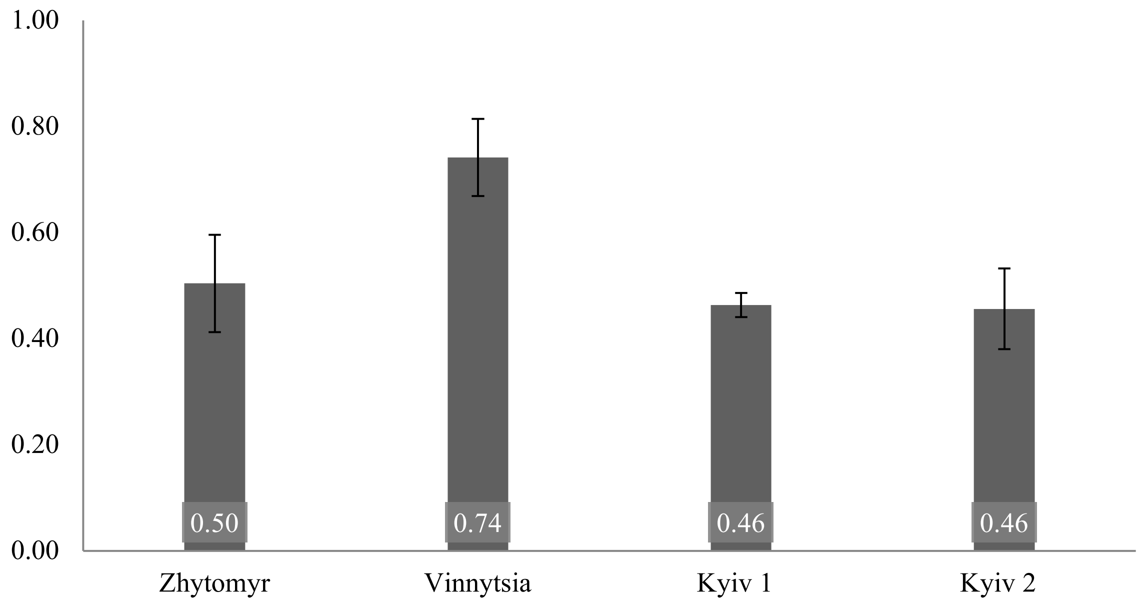

Figure 4. Pielow's evenness index (average value) for three location in 2010–2011 growing seasons

T a b l e 3

Variations between groups as for ANOVA

Variation	SS	df	MS	*Fc*	*P*-value	*Fd*
Between groups	0.211	3	0.07	3.78	0.05	3.59
Inside groups	0.205	11	0.02			
All together	0.416	15				

Fc – *F* calculated; *Fd* – *F* distribution

CONCLUSIONS

The study indicates that intensive involvement of perennial grass *M.* × *giganteus* into the agricultural landscape in Ukraine, dominated by cereal crops from the same family Poaceae (wheat, corn, rye), stimulates numerous indirect interactions between that crop and small grain cereals and can pose a risk to agricultural landscapes as all. The conversion of marginal and abandoned lands, where the growing of *M.* × *giganteus* is profitable into monoculture production areas can bring new environmental challenge. Hence, the massive scale commercial use of *M.* × *giganteus* should take into account a responsible consideration of the benefits and risks associated with that crop, in order to protect the agricultural ecosystems that supply food, feed and increasingly, the fuel.

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