RESEARCH ARTICLE

Low and high input agricultural fields have different effects on pest aphid abundance via different invasive alien weed species

Attila-Károly Szabó[1,2], József Kiss[2], János Bálint[1], Szidónia Kőszeghi[1], Hugh D. Loxdale[3], Adalbert Balog[1,2]

1 *Department of Horticulture, Faculty of Technical and Human Science, Sapientia Hungarian University of Transylvania, Sighişoara Str. 1C. Tirgu-Mureş, Romania* **2** *Doctoral School of Plant Science, Faculty of Agricultural and Environmental Science, Szent-István University, Gödöllő, Pater Károly Str. 1, Hungary* **3** *School of Biosciences, Cardiff University, The Sir Martin Evans Building, Museum Avenue, Cardiff, Wales, CF10 3AX, UK*

Corresponding authors: *Attila-Károly Szabó* (szabo_ata@yahoo.com); *Adalbert Balog* (adalbert.balog@ms.sapientia.ro)

Abstract

We conducted field surveys to detect the population density of the most important invasive weed species and their associated virus vectoring aphids in crops grown under high input field (HIF) vs low-input field (LIF) conditions, with and without fertilizers and pesticides. The most frequent invasive weed species were *Stenactis annua*, *Erigeron canadensis* and *Solidago canadensis*. These species were hosts predominantly for the aphids *Brachycaudus helichrysi* and *Aulacorthum solani* in both management systems. The 13% higher coverage of *S. annua* under LIF conditions resulted in a 30% higher *B. helichrysi* abundance and ~85% higher *A. solani* abundance compared with HIF conditions. Host plant quality was assessed by measuring peroxidase enzyme activity. There was a significantly increased POD activity at 10 μmol min^{-1} mg protein^{-1} unit in *S. annua* under LIF conditions, suggesting a higher stress by aphids under this management regime. The high colonization intensity of *B. helichrysi* on maize, potato and alfalfa crops were detected from both *S. annua* and *E. canadensis*. We conclude that new and faster methods need to be used to prevent colonization of such virus vectoring aphids and their host plants, even under low input regimes.

Introduction

Invasive pests represent serious threats to crop production as global trade expands and climatic conditions shift (Copping 1998; Agrow 2015; USDA Forest Service 2015). Recent estimates suggest that the losses of crop yield caused by invasive pests, especially by weeds and aphids (Hemiptera, Aphididae), will increase by 25% in the EU by 2020 (USDA Forest Service 2015). Use of conventional chemical pesticides and herbicides to control weeds and arthropod pests represents a further challenge due to pollution, accumulation of toxins, pesticide residues in food, and resistance of the target pests to pesticides (Elbehri 2015). Invasive weeds are particularly important because they may serve as a food source for several local and invasive sap-feeding, virus-vectoring insects, and also because they may represent significant sources of plant pathogenic viruses (Frey et al. 2003; Anastasiu and Negrean 2005; Zimmermann et al. 2015). In the last 25 years, 435 alien weed species from 82 families have been reported from Central Europe (Anastasiu and Negrean 2005). Although weed management strategies involve different methods, including physical (mulching, tilling, burning), chemical and cultural control (high quality seeds, rotate crop, species, herbicide) (Chitsaz and Nelson 1983; Rand and Louda 2004; Uchino et al. 2012; Mabuza et al. 2013), the areas covered by invasive weed species are still increasing (Tunaitienė et al. 2015; Pacanoski 2017). Another important factor that is rarely considered is the effect of these invasive weeds on local sap-feeding pest insect populations such as aphids (Hemiptera, Aphididae) and the influence of the invasive weeds on neighbouring crop plants via aphids (through damage and virus transmission). In terms of the direct and indirect interactions between plants in close proximity, in which the influence of one plant on another can increase (associational susceptibility) or decrease (associational resistance) susceptibility, this can be viewed in the light of the potential importance of the relative abundance of focal and neighbouring plants and their herbivore abundances (Barbosa et al. 2009). Aspects however on natural habitat diversity (i.e. diverse habitat surrounded by natural landscape mosaics) and how management systems (low vs high chemical input) influence associational susceptibility or resistance have rarely been included in such analyses (Steffan-Dewenter et al. 2001). From this standpoint, the effect of the virus vectoring aphids, whose host range naturally includes both local and invasive plant species from the same family (e.g. Asteraceae), needs to be considered in testing associational relations in plant-plant interactions (Bell 1983; Popkin et al. 2017).

The aim of the present study was to: a) assess the population density of the most important invasive weed species when agricultural crops are managed with high-input fertilizers and chemical pesticides (high-input fields, HIF) and without chemical management (low-input fields, LIF); b) identify and compare population densities of the most important aphid species on dominant invasive weeds; and c), detect the most suitable weed as hosts for aphids under different cropping systems by conducting the peroxidase (POD) enzyme activation test during aphid feeding. POD-inducible weed plants would be lower quality hosts, and less likely to confer associational susceptibility

to nearby crops because they would not support large aphid populations. Thus POD enzyme activation is a useful indicator of associational susceptibility or resistance to aphid colonization (Argandoña et al. 2001; Chaman et al. 2001; War et al. 2012; Mai et al. 2016; Scully et al. 2016).

Study area, focal weed and aphid species

Experiments were conducted during the crop growing (vegetative) seasons of 2015 and the 2016 in Central and Eastern Transylvania, Romania in order to assess the population density of the most important invasive weed species and infesting virus-vectoring aphids, both from low- and high-input agricultural crops.

Low-input, traditionally managed fields (LIF). This area belongs to a traditionally managed field (low-input) of the Saxon cultural region encompassing an area of 7,440 km² at altitudes between 230 and 800 mm above sea level (a.s.l.) and characterized by a landscape mosaic of different land-cover types (28% forest, 24% pasture, and 37% arable land, mostly maize, potato and alfalfa). The farming practices in the studied area were predominantly small scale, with no chemical inputs and for subsistence purposes. One consequence of this land use is the exceptional biodiversity and natural value of the farming landscape (Akeroyd and Page 2001). However, the being not economically viable, the abandonment of croplands in this region is frequent, this resulting in the establishment and high abundances of invasive weeds (Zimmermann et al. 2015).

High-input, conventionally managed fields (HIF). This study region contrasts the previously described region by large monocultures and farming landscapes with low levels of natural vegetation and heterogeneity (Eastern Transylvania). The area of about 5,500 km² was intensively treated with synthetic fertilizers and pesticides, major crops were maize, potato and alfalfa (Table 1).

The studied fields from the two regions were situated in the same altitudinal range of about 250 m a.s.l. and under comparable bioclimatic conditions. The distance between the studied areas was about 200 km.

Three weed and two native aphid species were studied, these being the most common species in the study area. The most important weed species, all of the family Asteraceae, were the annual fleabane, *Stenactis* (=*Erigeron*) *annua* (L.), Canadian horseweed, *Erigeron* (=*Conyza*) *canadensis* (L.), and the goldenrod, *Solidago canadensis* (L.). These species are known to use a diverse range of habitats and are considered economically important weeds in Europe (Anastasiu and Negrean 2005). *Stenactis annua* is often a dominant species within the invasive weed communities, and has been reported from almost all European countries, its expansion showing an increase (Edwards et al. 2006; Tunaitienė et al. 2015; Pacanoski 2017). *Erigeon canadensis* is an annual plant native throughout most of North and Central America. It is also widely naturalized in Eurasia (Nandula et al. 2006; Shah et al. 2014; Bajwa et al. 2016). *Solidago canadensis* is a perennial weed native to north-eastern and north-central America, but has established as an invasive plant throughout Europe (Abhilasha et al. 2008; Fenesi et al. 2015).

Table 1. Fertilizer and pesticide input on crops under high intensity management (HIF) in the two study years.

Crop		Treatments
Potato	Fertilizer	N, P, K (15,15,15) 0.2 t/ha
	Herbicide	Sencore (metribuzin70%)
		Titus 25 DF (rimsulphuron)
	Insecticide	Calypso (tiacloprid)
	Fungicide	Banjo (fluazinam)
		Ridomil Gold (mefenoxam, mankoceb)
		Infinito (62.5 g/l fluopicolide + 625 g/l propamocarb clorhidrat)
		Consento (375 g/l propamocarb clorhidrat + 75 g/l fenamidon)
		Acrobat Mz (difenomorf, mankoceb)
Alfalfa	Fertilizer	N, P, K (15,15,15) 0.16 t/ha
	Herbicide	Pallas (piroksulam)
	Insecticide	Fastac (alfa-cipermetrin)
		Falcon Pro (protioconazol 53 g/l + spiroxamină 224 g/l + tebuconazol 148 g/l)
	Fungicide	Amistar Xtra (azoxistrobin)
Maize	Fertilizer	N, P, K-15,15,15 0.15 t/ha
	Herbicide	Adengo (isoxaflutol 225 g/l + tiencarbazon-metil 90 g/l + ciprosulfamide (safener) 150 g/l)

The two native aphid species included in this study where the highly polyphagous leaf-curling plum aphid, *Brachycaudus helichrysi* (Kaltenbach) and the similarly polyphagous foxglove aphid, *Aulacorthum solani* (Kaltenbach). These are particularly important species, not only because of their wide host plant range but also for their diverse virus transmission. The host plant range of *B. helichrysi* includes members of the Asteraceae, e.g. Chrysanthemum, species of *Prunus* and also species of *Solanum*, *Fragaria*, *Trifolium*, *Medicago*, and *Citrus* and maize (Tatchell et al. 1983; Powell et al. 1992; Isac et al. 1998; Popkin et al. 2017). Viruses transmitted by these aphids include plum pox, Potato virus Y and the Beet mild yellowing virus (Isac et al. 1998). Host plants of *A. solani* includes tomato, peppers, tobacco, celery, carrots, tulip bulbs, cucurbits and legumes (Tatchell et al. 1983; Jandricic et al. 2014). Of transmitted viruses, the most important are Potato viruses A, Y and X and Potato leaf roll virus, Cucumber mosaic virus, Soybean dwarf virus, Bean yellow mosaic virus and Turnip yellows virus (Jandricic et al. 2010, 2014).

Material and methods

Invasive weeds and associated aphids assessment

First, we selected two study sites in each region, these being 10 km in a fist and 15 km distant in a second region from each other. In each site we established two transects (at least at 1 km apart) of 10 m long × 1 m wide at an approximately equal distance of at least three major crops (maize, potato and alfalfa). In this way, each transect was surrounded by at least 8–10 ha of high-input, and at least 0.5–3 ha area of low-input, agricultural crops. Each transect was carefully measured and located using GPS. Second, inside each

transect we placed ten 1 m² quadrats. Each of these quadrats was further subdivided in 10 × 10 cm plots (100 for each quadrat) and all plants (native and invasive) were counted and their coverage estimated within them. (Andújar et al. 2010). Third, ten invasive weed plant individuals from each quadrat were randomly collected in plastic bags. The number of invasive plants collected for each species from each quadrat mirrored the coverage of the species within the quadrat. We subjectively decided that we will collect at least eight plants when the coverage of a given species in a quadrat was at least 80% and up to two plants if the coverage of the species was up to 20%. We decided upon these percent coverage thresholds because in each quadrat there was one highly dominant invasive plant species (its coverage having at least 80%) and one species which had a coverage between 15–20%. Therefore from each quadrat, out of the 10 plants at least eight belonged to the dominant species and 1 or 2 to the second dominant species.

Because plants contained aphid colonies, and the exact number of individual aphids was important, all bags were labelled and kept at low temperature (near 0–4 °C in a cool box), then returned to the laboratory, whereupon all samples were stored at –20 °C, and aphids counted and species identified (Blackman and Eastop 2000; Blackman 2010). In total, 100 plant samples were collected per transect and management system (400 samples per management system per collection data). Assessment started at the end of May and repeated fortnightly five times during the summer until the end of the weed vegetative season, whereupon no more aphids were found. The whole procedure was repeated the following year using the same collection methods within the same transects. All aphids were carefully counted under laboratory conditions, and the various species identified).

POD enzyme extraction and activity assays in weeds

Leaf samples used for enzyme analyses were collected each year from each abundant weed species per experimental field, sub-area and transect, starting from the first until the last assessment. Separate young leaves, all containing aphids, were collected from the weeds (n = 10 samples / 1 m² sub-transect = 100 / transect). Samples were also held at –20 °C until enzyme extraction and activity assays.

For extraction, 400 mg of frozen leaves were homogenized in 1 ml of 50 mM phosphate buffer, pH 7.0, using a FastPrep Instrument high-speed benchtop homogenizer (MP Biomedicals). The homogenate was centrifuged at 6,500 r.p.m. for 20 minutes at 4 °C, and the supernatant collected. Protein concentration of the enzyme extract was determined by the Bradford method (Bradford 1976). POD activity was determined by the method of Németh et al. (Németh et al. 2009). The reaction mixture contained 955 µl of 50 mM phosphate buffer, pH 5.5, 10 µl of 0.01 g/l 3.3'-diaminobenzidine and 30 µl of enzyme extract. The reaction was initiated by the addition of 5 µl 0.3% hydrogen peroxide. The increase in absorbance at a wavelength of 480 nm was followed in a spectrophotometer for 5 minutes and 5 and 10 µmol min⁻¹ · mg protein⁻¹ unit of POD activity was separately defined as an absorbance change of 0.01 units·min⁻¹.

Colonization experiment of aphids from weeds to crop plants

The experiment was performed during the vegetative period of 2017 by setting-up 30 blocks of the two most abundant weed species, *E. canadensis* and *S. annua* and the most frequent crop plants cultivated (maize, potato and alfalfa; Fig. 1). Because of a relatively low density of plants and high aphid density variation on *S. canadensis*, this weed was not included in the colonization experiment. Specimens of *E. canadensis* and *S. annua* of the same age (maturity) were collected in April from the field and potted in 8 litre pots.

After acclimatization in May, similar size plants of about 30 cm were selected for experiments. Crop plants of maize, potato and alfalfa were also cultivated in 8 litre pots, and similar sized plants selected in May for experiments. All weed and crop plants were first cleared of any infesting aphids by visual checking of all leaves and shoots. In the case of any aphid colony being detected, these were removed by brushing off colonies from the plants with a soft paint brush. If other insect species were detected, these were also removed. Insect-cleared plants were then allocated for experiments. Altogether 30 experimental blocks were set-up, 15 blocks with *E. canadensis* and 15 with *S. annua* under open field conditions where no other weeds and similar crop plants were present. Weed plants were placed at a distance of 20 m from each other, and six crop plant (two maize, two potato and two alfalfa) were placed around one weed plant to a distance of 50 cm (Fig. 1). Weed plants from each blocks were then artificially colonized with aphids by collecting *B. helichrysi* and *A. solani* from naturally occurring *E. canadensis* and *S. annua* plants. Leaves or shoots of weeds with aphid colonies were carefully removed, the colony of each aphid species reared to five individuals of 4th-instar nymphs by carefully brushing down all other individuals. Thereafter, the plant leaf or shoot was placed to the top of the experimental weed plant already placed in blocks. Each weed plants received two colonies (five aphids of each) from both aphid species.

The established aphid colonies (assessed by careful visual assessment over a 10 minute period as to whether aphids were feeding consistently on plants and not moving) were checked after 24, 48 and 72 hours. If no colony establishments were detected, new colonies were placed on the weeds. The aphids were then left to reproduce for 10 days. The assessment of aphids started after 14 days after aphid colony establishment, such that enough winged individuals were present to leave weeds and colonize crop plants. Aphid numbers were assessed on both weed and crop plants of the same blocks starting from mid-May as follows: two randomly selected blocks (one with *E. canadensis* and one with *S. annua* plants) were sampled by enclosing the infested plant in a transparent polythene bag and then cutting this free with scissors or a knife.

On return to the laboratory, the entire content was stored at –20 °C and the next day all samples were carefully assessed for aphids and their respective numbers counted under a stereo microscope. By this means, all individual aphids were captured and counted. The same procedure was repeated the following day, until the total number of blocks and plants were sampled by cutting all and aphids from plants counted in lab. The entire sampling was done within a two-week period and completed by the end of May, a time when aphid migration to new host plants occurs. All aphids were counted, recorded regarding the weed and crop plants they were collected from, and identified to species level.
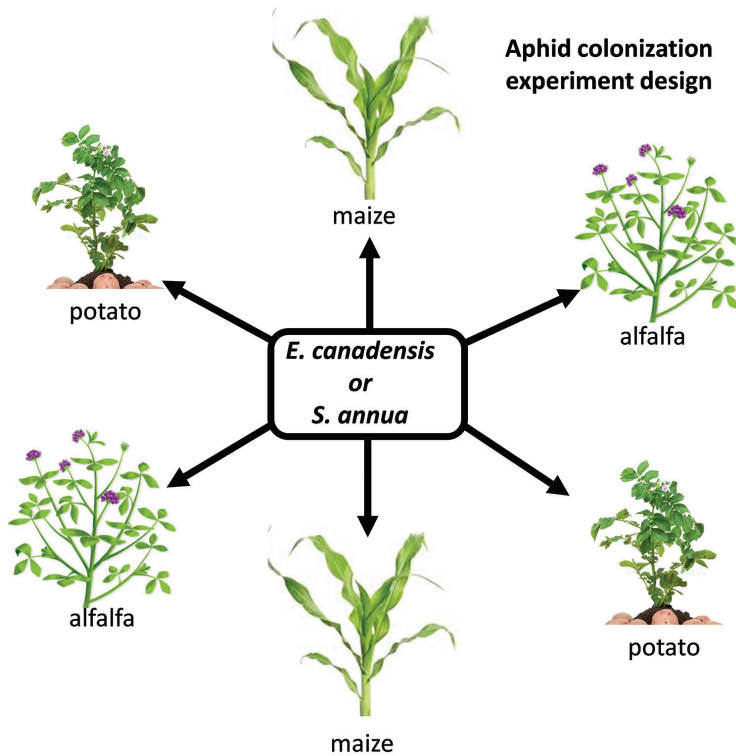
Figure 1. Aphid colonization experiment design, weed plants of *E. canadensis* or *S. annua* surrounded by two maize, two potato and two alfalfa plants.

Data analyses

For weed data, the mean coverage in each 1 m² quadrats was determined by averaging the plant values from 10 × 10 cm plot. Next the inter-annual differences in coverage were tested using multivariate analysis of variance (MANOVA) and mean coverage values obtained for one 1 m² quadrat (40 data / field type / collection dates) were considered. No significant difference in weed coverage was detected between years ($P = 0.12$). Therefore, data from the two years, collected on the same dates, were combined for the analyses. The weed frequency data were tested using Poisson-distributed errors and residuals for normality of errors (Kolmogorov-Smirnov test) and for equality of variance (Levene's test). Because residuals did not meet the assumption of normality, we used the non-parametric Kruskal-Wallis- and Mann-Whitney U test to compare variables. Weed species and management systems (HIF vs LIF) were used as fixed factors and the average weed coverage in 1 m² sub-transect as random factor.

All aphid species were correlated with particular weed species. In the case of one individual weed plant hosting two aphid species, the percentage of the species were considered. This was the case in only 7% of all the samples examined. It was then determined how cropping system differentially affected associational susceptibility to the two aphid

species, *B. helichrysi* and *A. solani*. General linear modelling was used with mean aphid abundance on *S. annua*, *E. canadensis* and *S. canadensis* as response variable. Initial analyses indicated no difference ($P < 0.23$) between study years and aphid abundance averaged across study years were analysed. The model included cropping system type (HIF vs LIF), aphid species (*B. helichrysi* and *A. solani*) and their interaction as explanatory variables. Because aphid abundance is a discrete variable, Poisson-distributed errors were assessed. Aphid abundances on *S. annua* was normally distributed, so factorial ANOVA was used, followed by Tukey testing. Aphid abundance on *E. canadensis* and *S. canadensis* did not meet the assumption of normality, hence the Kruskal-Wallis test was used, followed by the Mann-Whitney U test. Significant ($P < 0.05$) interactive effects (cropping system type x species) suggest that the effect of cropping system depended on aphid species. All analyses were made using R version 3.0.1 (R. Core 2013). Only a small number of other aphid species (e.g. *Macrosiphum* spp.) were detected, and we did not include them in the analyses.

POD activity values obtained were compared between years (MANOVA) considering values from 1 m^2 sub-transects per sampling period. No significant year effect was detected ($P = 0.61$); therefore, data between years were averaged. Thereafter, POD values were compared between cropping system type (HIF vs LIF) for *S. annua*. This was done because only this weed was present during the whole vegetation period in all areas in both years, while the abundance of the other weed species sampled decreased in the HIF regime; thus the POD enzyme data were not compared statistically. POD values at 5 and 10 µmol min^{-1} · mg protein^{-1} unit were analysed separately and compared between fields from the first to the last day of sample collection. Because the residuals meet the assumption of normality, a complete randomized factorial ANOVA of POD specific activity values (5 and 10 µmol min^{-1} · mg protein^{-1} unit) was performed to test for effects of treatment (HIF vs LIF) and time (data collections). The analysis of Tukey test with $P < 0.01$ and LSMEAN (Minimal quadratic means) according to statistic package SAS were included and the average POD quantity / 10 plants / 1 m^2 sub-transect were used. Linear correlation between POD activity level at 5 and 10 µmol min^{-1} · mg protein^{-1} unit and aphid (*B. helichrysi* and *A. solani*) abundances on *S. annua* plants under low and high input management were computed using the SPSS package version 3.14. Correlations were made between data (POD and aphids abundance) of the same sampling periods, and r and P values computed.

Effects of weed plants on *B. helichrysi* colonization toward each crop plant (maize, potato and alfalfa) were tested using repeated measures MANOVA. Interactions were then compared using χ^2 tests on the differences between the covariance matrices and by the root mean square error of approximation. The initial comparison was made between the two aphid species (*A. solani* and *B. helichrysi*) when these were on *E. canadensis*. Because of low density of *A. solani*, comparisons were made separately for those blocks where both aphid species were present (five blocks of *E. canadensis*), and for those where only the *B. helichrysi* persisted. The next analyses followed the comparison between weed species when these served as host plant for *B. helichrysi* only. Comparison between aphids densities found on crop plants (maize, potato and alfalfa) when these were set in blocks with *E. canadensis* or *S. annua* were made using Student's t-tests, following the t-distribution. The statistical analyses were performed in R version 3.0.1 (R Core 2013).

Results

Dominant invasive weed species and their variations between management systems

Three invasive weed species were dominant during the two years field assessment. *Stenactis annua* was the most frequent weed, and dominated both LIF (97.5%) and HIF regimes (84.5%). Two other invasive weed species were present at lower densities. *S. canadensis* was only present in LIF, with an average coverage of 2.5%. No other invasive weeds were detected under this management system during the assessment *E. canadensis* was only present under HIF with an average coverage of 15%. Other weed species, mostly amaranth, *Amaranthus* spp. in HIF regimes with an average coverage of 0.5%, were detected during the end of the vegetation of the previous weed species. Dominance of *S. annua* was significant under both LIF and HIF (Table 2).

Table 2. The most frequent invasive weed average coverage between management systems. LIF = low-input field, HIF = high-input field. Data were compared using Kruskal-Wallis test, followed by Mann-Whitney U test.

Management	Weed species	Aver. Cov.(%)	median	25th/75th quart.	U	P
LIF	*Stenactis annua*	97.50%	98.5	95/99	2.19	0.02
	Solidago canadensis	2.50%	1.5	1/5		
HIF	*Stenactis annua*	84.50%	84.5	83/86	2.16	0.03
	Erigeron canadensis	15%	14.5	13/16		

Aphids and their abundances on invasive weeds

The two important aphid species were detected in high densities on all three dominant invasive weeds. The most frequent species was *B. helichrysi*; its abundance was high and dominated the most frequent weed, *S. annua* under both HIF and LIF regimes (LIF-*B. helichrysi* and LIF-*A. solani* F_{1-40} = 6.4, $P < 0.001$; LIF-*B. helichrysi* and HIF-*A. solani* F_{1-40} = 8.1, $P < 0.001$) (Fig. 2). The next most abundant aphid was *A. solani*, also present on *S. annua* plants under both management systems; its density was significantly higher under LIF compared with HIF (F_{1-40} = 8 $P < 0.001$) (Fig. 2). Higher density of *B. helichrysi* was detected on *S. canadensis* under LIF (U_{1-40} = 3.4, $P < 0.01$) but its density varied greatly between assessment data (Fig. 3A). Furthermore, the dominance of the *B. helichrysi* on *E. canadensis* was detected under HIF conditions (U_{1-40} = 3.1, $P < 0.01$) (Fig. 3B). A very low number of other important aphid species were detected, i.e. about 12 individuals of *Macrosiphum* spp. were collected on *S. canadensis*.

POD enzyme activity on invasive weeds under aphids' feedings

No observable differences in POD enzyme activity were detected for *S. annua* at 5 μmol min^{-1} · mg protein^{-1} unit between HIF and LIF regimes (F_{1-40} = 1.2, $P < 0.2$) (Fig. 4A). When the POD activity was compared for the 10 μmol min^{-1} · mg protein^{-1} unit aliquot
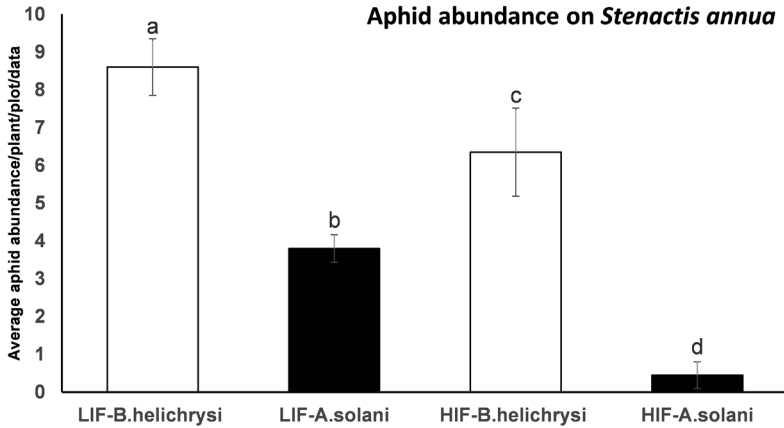
Figure 2. The average *B. helichrysi* and *A. solani* aphid densities on *S. annua* in LIF and HIF. Aphids from 1 m² sub-transect (cumulated and averaged between years) were considered by factorial ANOVA, followed by Tukey testing. Arrows on bars represent standard errors; different letters indicate statistically significant differences at *P* < 0.01 level.
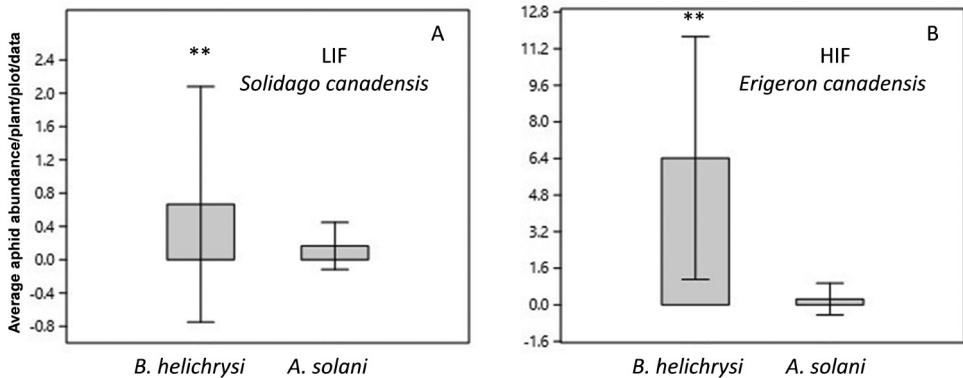

Figure 3 A, B Average *B. helichrysi* and *A. solani* aphid densities on *S. canadensis* and *E. canadensis* in LIF (**A**) and HIF (**B**) field. Aphids from 1 m² sub-transect (cumulated and averaged between years) were considered by Kruskal-Wallis test followed by Mann-Whitney *U* test. Stars above boxplots indicates statistical significant differences at *P*<0.01 level.

sample, there was a significantly higher enzyme activity, suggesting a significantly higher stress by aphids feeding on *S. annua* in LIF system ($F_{1–40}$ = 3.8, P < 0.004) (Fig. 4B). Higher POD enzyme activity at both 5 and 10 µmol unit was detected on *E. canadensis* than on *S. canadensis*, again indicating higher stress as a result of aphid feeding; however, because of low samples numbers no statistics were performed here. There was a strong negative relationship between POD level at both 5 and 10 µmol unit and aphid density (both *B. helichrysi* and *A. solani*) abundances on *S. annua*. No such strong correlation between POD level and aphids abundance for HIF was observed (Table 3).
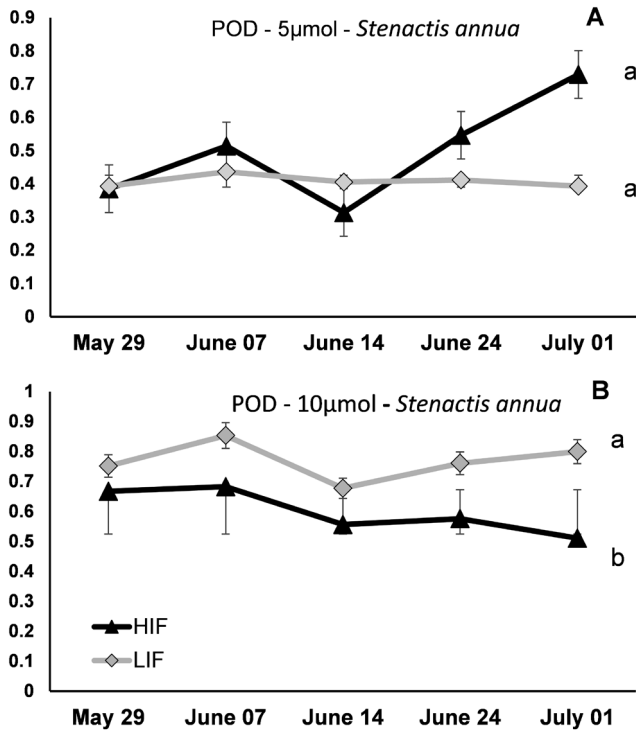
Figure 4 A, B POD enzyme activity at 5µmol (**A**) and 10 µmol min^{-1} · mg protein^{-1} unit (**B**) on *S. annua* and its variation between LIF and HIF. Analysis of Tukey test with $P < 0.01$ and LSMEAN (Minimal quadratic means) were used and the average POD quantity / 10 plants / 1 m^2 sub-transect were considered. Bars represent standard errors. Different letters indicate statistically significant differences at $P < 0.01$ level.

Table 3. Linear correlation between POD activity level at 5 and 10 µmol min^{-1} · mg protein^{-1} unit and aphids (*B. helichrysi* and *A. solani*) abundances on *S. annua* plants under LIF and HIF. Correlation were made between data (POD and aphids abundance) of the same sampling periods.

Correlation	POD 5 µmol				POD 10 µmol			
	B. helichrysi		*A solani*		*B. helichrysi*		*A solani*	
	r	*P*	r	*P*	R	*P*	r	*P*
LIF	−0.67	0.21	−0.74	0.14	−0.76	0.12	−0.72	0.16
HIF	0.42	0.46	0.34	0.56	−0.48	0.4	−0.3	0.61

Colonization of aphids from weeds to crop plants

The number of *A. solani* were low and colonies persisted in five blocks on *E. canadensis* only, which shows a very similar trend with field observations of only 7% of *A. solani* detected together with *B. helichrysi*. The *B. helichrysi* colonies persisted in all blocks on both weed plants. Therefore, comparisons were made separately for those blocks where both aphid species were present, and separately for those where only the *B. helichrysi* persisted. Colo-
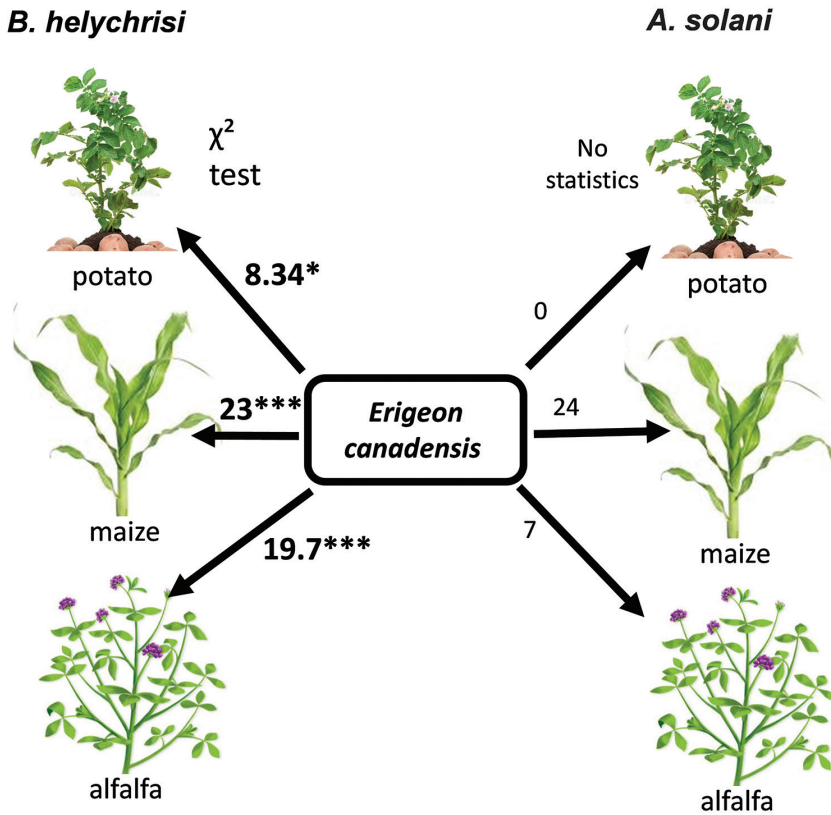
Figure 5. Colonization rate of *B. helichrysi* and *A. solani* from *E. canadensis* towards maize, potato and alfalfa plants. The average number of aphids / plots / plants / sampling data were considered. Interactions were then compared using χ^2 tests on the differences between the covariance matrices, and by the root mean square error of approximation. Numbers represent χ^2-values for significant path coefficients. *$P < 0.05$, ***$P < 0.001$. Because of low *A. solani* numbers, no statistics were possible.

nization of *B. helichrysi* from *E. canadensis* was significant on all crop plants, with a higher number of aphids detected on maize. Low or no colonization of *A. solani* was detected from this weed to crop plants, hence no statistics were here possible (Fig. 5). By comparing the colonization of *B. helichrysi* from both weed species, again a significant effect toward all crop plants was detected (Fig. 6). The number of aphids on maize was significantly higher ($F_{1–14}$ = 5.8, $P < 0.01$) when maize was in close vicinity with *S. annuus*. No differences in aphid abundance were detected for potato ($F_{1–14}$ = 2.5, $P < 0.28$) and alfalfa ($F_{1–14}$ = 1.5, $P < 0.57$) when these plants were in close vicinity with *S. annua* or *E. canadensis* (Fig. 6, bar charts).

Discussion

Here we showed that associational susceptibility can be detected between the most frequent weed and crop plants under the different crop management regimes. The
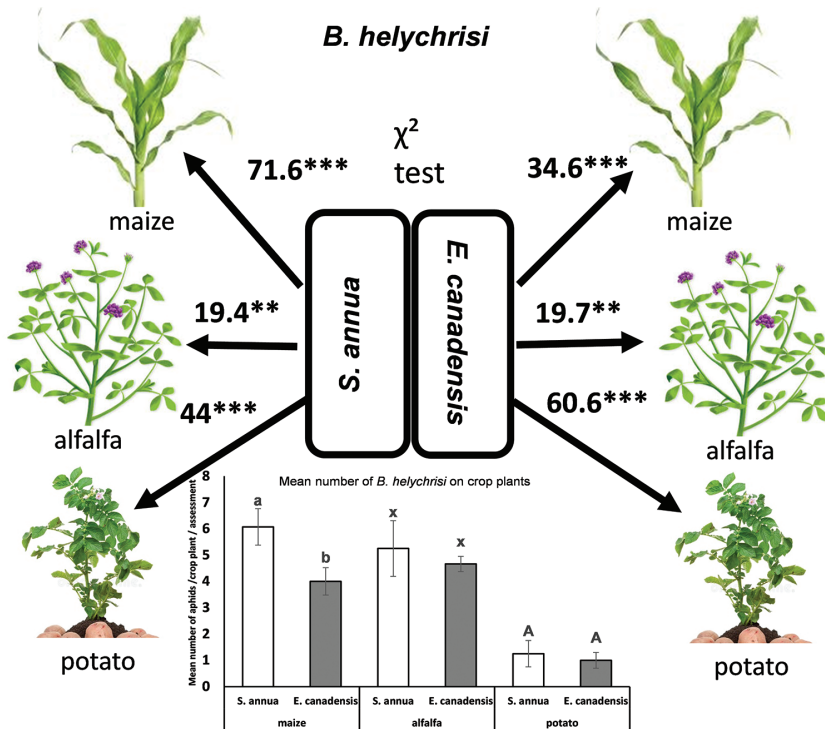
Figure 6. Comparison between colonization rate of *B. helichrysi* from *E. canadensis* and *S. annua* towards each crop plant (maize, potato and alfalfa). The average number of aphids / plots / plants / sampling data were considered. Interactions were then compared using χ² tests on the differences between the covariance matrices, and by the root mean square error of approximation. Numbers represent χ²-values for significant path coefficients. **$P < 0.01$, ***$P < 0.001$. Bar chart represent comparison between aphids densities found on crop plants (maize, potato and alfalfa) when these were set in blocks with *E. canadensis* or *S. annua*. To compare variables, t-tests were used. Different letters indicate statistical significant differences at $P < 0.01$ level. Error bars = ±1 standard error.

high invasive weed density harbours a concomitantly higher aphid population density comprising local species. More precisely, a 13% higher coverage difference of *S. annua* in LIF further resulted in a significantly higher (about 30% higher) *B. helichrysi* aphid abundance under this management system. The same trend can also be detected for *A. solani*, where the 13% higher coverage of *S annua* resulted in an increase of about 85% for this aphid species under LIF compared to HIF (Table 2, Figs 2, 3). Altogether these results also show that the response to cropping system varied according to the aphid species concerned, possibly due to the difference in host plant preference of the two aphid species (i.e. *A. solani* was less polyphagous than *B. helichrysi*), a scenario also supported in the case of lower *A. solani* density on weeds in the field and low colony persistence during the colonization experiment.

The colonization experiment also revealed that *S. annua* and *E. canadensis* can be considered suitable host plants for both aphid species examined, especially for *B. helichrysi*. Significant colonization from both weeds toward the most important crop plants

of this last aphid species were detected. Virus symptoms on potato crops (Potato virus Y and Potato leaf roll virus) were widely observable during the experiment (SZKA pers. obs.). Other previous studies, also reported similar findings, e.g. attack of the weevil, *Rhinocyllus conicus* (Coleoptera, Curculionidae) on the native Wayleaf thistle, *Cirsium undulatum* (Nutt.) Spreng increased three- to fivefold with increasing invasive Musk thistle, *Carduus nutans* (L.) density (Rand and Louda 2004). In a similar vein, frequency-dependence in terms of insect herbivore damage of Carolina horsenettle, *Solanum carolinense* (Asteraceae) was positively influenced by higher herbivore density on neighbouring Canada goldenrod, *Solidago altissima* (Kim 2017). The mechanisms that may influence associational susceptibility, the likelihood of detection of neighbouring plants, and the factors that can directly affect the survival of aphids on these plants may include the natural habitat diversity as well as the lack of chemical management under the LIF regime. The low distance between weeds and crop plants and the high habitat diversity may also clearly influence associational susceptibility of crop plants. This was clear in the present study when the abundance of both aphid species on *S. annua* were higher under LIF. In contrast to the present study, the densities of bean flies, *Ophiomyia phaseoli* (Tryon) and *O. spencerella* (Greathead) (Diptera, Agromyzidae) in Malawi and their rates of parasitism were not changed significantly when the field with non-host plants (bean-maize cultures) were diversified, while fertilizers had significant positive effect on fly densities (Letourneau 1995). In our case, the high habitat diversity and non-use of chemical pesticides in LIF probably had the most important effect on associational susceptibility and can explain the higher aphid abundance on *S. annua* under LIF (Fig. 2).

No clear associational susceptibility was however detected when comparing POD enzyme activity on *S. annua*. Higher POD activity of *S. annua* by *B. helichrysi* feeding was confirmed at 10 μmol unit only under LIF, but no such differences were detected at 5 μmol unit between LIF and HIF (Fig. 4A, B). High POD activity strongly suggests that *S. annua* would be a lower quality host, and less likely to confer associational susceptibility to nearby crops because these would not support large aphid populations, as also demonstrated by Dicke (1998). In our study, the relatively strong negative relationship between POD levels at both 5 and 10 μmol unit and aphids (both *B. helichrysi* and *A. solani*) abundances on *S. annua* under LIF were detected (Table 3). This clearly argues for a significantly higher stress by aphids feeding on *S. annua*, a lower quality host, thereby supporting a lower aphid density on this weed plant, a result not confirmed following aphid abundance assessment (Fig. 2). We explain this apparently contradictory result again by the fact that the habitat effect (via landscape mosaics diversity) had a stronger effect on aphid density, and recolonization of *S. annua* by aphids was faster than the repulsive effect of the high POD activity, a scenario that needs to be further tested.

The idea that plant-induced POD activity increases as a consequence of sap-feeding insect activity was first suggested by Felton et al. (1989; reviewed by War et al. 2012b). Furthermore, the evidence for an anti-herbivore role of POD derives from the discovery that the herbivore defence-inducing signal molecules systemin and methyl jasmonate (MeJA) induce POD activity levels in tomato leaves (Constabel et al. 1995; Constabel and Barbehenn 2008; Mai et al. 2016). Hence, the increased POD level

indicating an intensive feeding process, especially from the aphid nymphs that injure plant cells, is consistent with high aphid preferences toward these particular plants (Dicke 1998; Argandoña et al. 2001b; Balog et al. 2017). Other studies have reported that other plant species, i.e. barley, *Hordeum vulgare* L. infested with greenbug aphids, *Schizaphis graminum* (Rondani) increased the total soluble POD activity in cv. Frontera, with a maximum level of hydrogen peroxide activity, H_2O_2, observed after 20 minutes of infestation (Argandoña et al. 2001b). No influence of landscape diversity on POD activity as a consequence of aphid feeding have been detected until now, and the present study only suggests that such effect may exist. Therefore, additional empirical and laboratory studies are required to test possible landscape effects on plant molecular mechanisms influencing associational susceptibility and/or resistance.

Conclusion

The relevance of our study is threefold: environmental, crop management, as well as aphid control. In terms of environmental management, although low-input management farming systems are widely studied (Akeroyd and Page 2011; Fischer et al. 2012; Mikulcak et al. 2013) and are supposedly low-cost, effective systems (i.e. no or low management costs) with high biodiversity and cultural values (Hartel et al. 2013), the abandonment or absence of management may cause serious problems with increased virus vector aphid densities. Damage produced in this way may overcome the costs of any environmentally friendly weed controls. This effect, caused by aphids via invasive weeds, therefore needs to be considered when low-input management systems are directly compared with high-input ones in terms of costs and environmental values.

From a crop management perspective, new management systems and new assessment methods are necessary to evaluate the possible effect of weeds on vegetable and cereal crops due to aphid activity, both physical (i.e. direct feeding damage) and more importantly, via transmission of one or more plant pathogenic viruses.

Lastly, from the standpoint of aphid control and associated virus transmission, the complete lack of any management needs to be reconsidered. This is because high aphid density and possible virus infestation can make the cultivation of some crops under low-input systems difficult, if not impossible. These crops (potato) are, however, considered low-cost and low-input crops, and hence are widely cultivated under low-input management regimes. From our study, it is clear that cultivation methods, including invasive weed control, need to be synchronised and vector controls reconsidered, even if no other management is planned.

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