SUPPORTING INFORMATION
ECOLOGY LETTERS

Intraspecific chemical diversity among neighboring plants correlates positively with plant size and herbivore load but negatively with herbivore damage

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MATERIAL AND METHODS

Glucosinolate analysis

Samples were freeze-dried until constant weight and ground to a fine powder. Ten to fifteen mg of freeze-dried and pulverised material per plant was used for glucosinolate analysis. Glucosinolates were extracted with 1 ml of 80% methanol solution containing 0.05 mM intact 4-hydroxybenzyl glucosinolate as internal standard and were desulfataed with arylsulfatase (Sulfatase from *Helix pomatia*, Sigma-Aldrich) on a DEAE Sephadex A 25 column. The eluted desulfoglucosinolates were separated using high performance liquid chromatography (Agilent 1100 HPLC system, Agilent Technologies, Waldbronn, Germany) on a reversed phase C-18 UPLC column (Zorbax Eclipse XDB-C18, 50 x 4.6 mm, 1.8um, Agilent Technologies) with a water-acetonitrile gradient (2-6.5% acetonitrile from 0-3 min, 6.5-24.5% acetonitrile from 3-9min, followed by a washing cycle; flow 1.1 ml min-1). Detection was performed with a photodiode array detector and peaks were integrated at 229 nm. We used the following response factors: aliphatic glucosinolate 2.0, indole glucosinolate 0.5 (Burow et al. 2006) for quantification of individual glucosinolates in micromoles per dry mass of leaves ($\mu mol \cdot g^{-1}$ DM). Glucosinolates were identified by comparing the retention times and UV absorption spectra with those of known standards (Reichelt et al. 2002). The following glucosinolates (Gls) were detected in order of elution: 3-methylsulfinylpropyl Gls (glucoiberin), R-2-hydroxy-3-butenyl Gls (progoitrin), 4-methylsulfinylbutyl Gls (glucoraphanin), 2-propenyl Gls (sinigrin), 3-butenyl Gls (gluconapin), indol-3-ylmethyl Gls (glucobrassicin), 4-methoxy-indol-3-ylmethyl Gls (4-methoxyglucobrassicin), 1-methoxy-indol-3-ylmethyl Gls (neoglucobrassicin).

Statistics

All statistical analyses were performed in R (R Core Group, v 3.2.3). For all models we applied an ANOVA type II using the function *Anova* (*car* package), which allows the interpretation of the main effects and their interactions independently and is preferred over ANOVA type III for unbalanced designs (Langsrud 2003). This function performs F-tests for general linear models and Wald's chi-square tests for mixed models. Tukey post-hoc tests were performed whenever there was a significant effect of a factor variable with more than two levels. Plots of model predicted values were done with the *effects* R package; they indicate the predicted response to one factor when all the other factors are held constant.

Invertebrate community. The effects of the diversity treatment on the invertebrate community characteristics (abundance and diversity of herbivores and carnivore abundance, respectively) was assessed using linear mixed models (LMM, *lmer* package) with a repeated-measures structure. In these analyses, plant population origin of the focal plant, diversity treatment, time (in weeks) and their interaction terms were entered as fixed factors and plant ID as a random factor. To analyze abundance we used Generalized Linear Mixed Models (GLMM) with a Poisson error distribution. The GLMMs for abundance of herbviores and *Brevicoryne brassicae* were analyzed with and without plant size (log-transformed) as a covariate. The GLMMs for carnivores included abundance of herbivores as a covariate. Using similar GLMMs, we analyzed the data on abundance of the aphid *B. brassicae*, and its main parasitoid *D. rapae*, separately.

Plant traits. The effects of diversity treatment and plant population on plant size were determined with a LMM using the logarithm of plant size as the response variable and diversity treatment, plant population and time (week) as fixed factors. Plant ID was entered as a random factor in the model. For analyzing variation in plant damage, we used a similar model but added the invertebrate community attributes. We included the diversity of herbivores and the natural logarithm of the abundances of carnivores and leaf chewing herbivores (Table 1), and their interactions with plant diversity treatment as fixed factors. The response variable was the logit transformation of plant damage, calculated as:

$$D(logit) = \log \frac{D+0.05}{1-D+0.05}$$

where D is proportion of damage, instead of using the arc-sine transformation as suggested by Warton & Hui (2011).

To test if total glucosinolate concentration differed among plant populations and with the number of plant populations within a plot we used a linear model. For analyzing differences in the glucosinolate composition among plant populations we performed a Partial Least Squares regression with Discriminant Analysis (PLS-DA, *mixOmics* package, González et al. 2011) which reduces the dimensions of the multivariate data taking into account the separation by groups (in this case plant populations) and allows to explore which variables contribute most to the differences among groups.

Effects of plant chemistry. We estimated the spatial variation in glucosinolate concentrations within plots as the coefficient of variation in total glucosinolate concentration among the core nine plants within plots (expressed as CV_{conc}). We used this estimate to analyze how glucosinolate variation among neighbouring plants affected the herbivore community and plant damage levels using multiple regression models. The CV_{conc} increased when more plant populations were combined within a plots ($F_{(1, 58)}$=40.34, P<0.0001) and there were also differences in CV_{conc} among plant-population combinations ($F_{(3, 30)}$=4.93, P=0.0067; Fig. S1). Since dicultures and tricultures had overlapping mean CV_{conc} values, which were higher than those in monocultures (Fig. S1), we grouped dicultures and tricultures into a single level (polyculture) and included the diversity treatment as a two levels factor (monocultures – polycultures).

To test if total glucosinolate concentration differed among plant populations and with the number of plant populations within a plot we used a linear model. For the regression models, we used the mean values per plant across the entire monitoring season for plant damage and herbivore abundance/diversity, and for the foliar glucosinolates we used the data that were measured once at the end of the season. For the models analyzing herbivore abundance and diversity as the response variable, the explanatory variables were diversity treatment, total glucosinolate concentration per plant, the CV_{conc} per plot, the first component of the PLS-DA on glucosinolate data, their interactions with diversity treatment and the log transformation of plant size as a covariate. Only the first component of the PLS-DA for glucosinolates was included since it accounted for most of the variation in glucosinolate composition. For the model analyzing plant damage (logit transformed), the explanatory variables were the diversity and the natural logarithm of abundance of leaf chewing herbivores, the diversity treatment, total glucosinolate concentration per plant, the CV_{conc} per plot, the first component of the PLS-DA on glucosinolates and their interactions with diversity treatment.

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Reichelt, M., Brown, P.D., Schneider, B., Oldham, N.J., Stauber, E., Tokuhisa, J., *et al.* 2002. Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. *Phytochemistry* 59: 663–671.

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Table S1. Herbivore taxa identified during the monitoring period and their feeding type and host specialization.

Order	Family	Species	Specialization	Feeding type
Lepidoptera	Pieridae	*Pieris rapae*	Specialist	Leaf chewer
		Pieris brassicae	Specialist	Leaf chewer
	Plutellidae	*Plutella xylostella*	Specialist	Leaf chewer
	Pyralidae	*Evergestis forficalis*	Specialist	Leaf chewer
	Noctuidae	*Mamestra brassicae*	Generalist	Leaf chewer
		Autographa gamma	Generalist	Leaf chewer
		Lacanobia suasa	Generalist	Leaf chewer
Coleoptera	Chrysomelidae	*Phaedon cochleariae*	Specialist	Leaf chewer
		Phyllotreta atra	Specialist	Leaf chewer
		Phyllotreta undulata	Specialist	Leaf chewer
	Coccinellidae	*Subcoccinella* sp.	Generalist	Leaf chewer
Hemiptera	Aphididae	*Brevicoryne brassicae*	Specialist	Phloem feeder
		Myzus persicae	Generalist	Phloem feeder
	Aleyrodidae	*Alyrodes proletella*	Specialist	Phloem feeder
Thysanoptera	Thripidae	*Thrips tabaci*	Generalist	Cell content feeder
Diptera		Leaf miners	Generalist	Tissue feeder
Pulmonata		Snails/Slugs	Generalist	Leaf chewer

Table S2. Carnivore taxa included in the monitoring period, both parasitoids and predators were included.

Order	Family	Species / Group	Feeding type	Host
Hymenoptera	Braconidae	*Diaeretiella rapae*	Parasitoid	Aphid
		Praon sp.	Parasitoid	Aphid
		Cotesia rubecula	Parasitoid	Caterpillar
		Cotesia glomerata	Parasitoid	Caterpillar
		Microplitis mediator	Parasitoid	Caterpillar
	Ichneumonidae	*Diadegma semiclausum*	Parasitoid	Caterpillar
Coleoptera	Coccinellidae	*Coccinella* spp.	Predator	
Diptera	Syrphidae	Hoverflies	Predator	
	Cecidomyiidae	gall midges	Predator	
Neuroptera	Chrysopidae	Lacewings	Predator	
Araneae/Opiliones		Spiders and opiliones	Predator	

Table S3. Effects of glucosinolate composition (PLS 1), total concentration, variation between neighbouring plants (CV (conc)) and plant diversity treatment (Diversity) on herbivore diversity and plant damage. The linear model for analyzing plant damage included as well herbivore abundance and herbivore diversity (H) as explanatory variables.

Effect	Sum Sq	d.f.	*F*	*P*
Herbivore diversity (H)				
PLS 1	0.00	1	0.01	0.928
Concentration	0.02	1	0.56	0.454
CV (conc)	0.02	1	0.91	0.339
Diversity	0.02	1	0.64	0.425
Plant size	**2.06**	**1**	**76.78**	**<0.001**
PLS 1 × Diversity	0.01	1	0.39	0.533
Conc × Diversity	0.00	1	0.00	0.996
CV (conc) × Diversity	**0.15**	**1**	**5.63**	**0.018**
Residuals	13.20	491		
Plant damage (logit)				
Abundance (log)	0.01	1	0.03	0.862
H	**3.03**	**1**	**13.61**	**<0.001**
PLS 1	**2.62**	**1**	**11.78**	**<0.001**
Concentration	0.16	1	0.73	0.394
CV (conc)	0.31	1	1.41	0.236
Diversity	0.27	1	1.23	0.269
Abundance (log) × Diversity	0.67	1	2.99	0.084
H × Diversity	0.07	1	0.32	0.571
PLS 1 × Diversity	0.05	1	0.24	0.623
Conc × Diversity	0.22	1	0.97	0.326
CV (conc) × Diversity	**2.93**	**1**	**13.20**	**<0.001**
Residuals	108.49	488		

The effects for the linear models are based on an ANOVA type II approach. Effects in bold are significant at $P<0.05$.

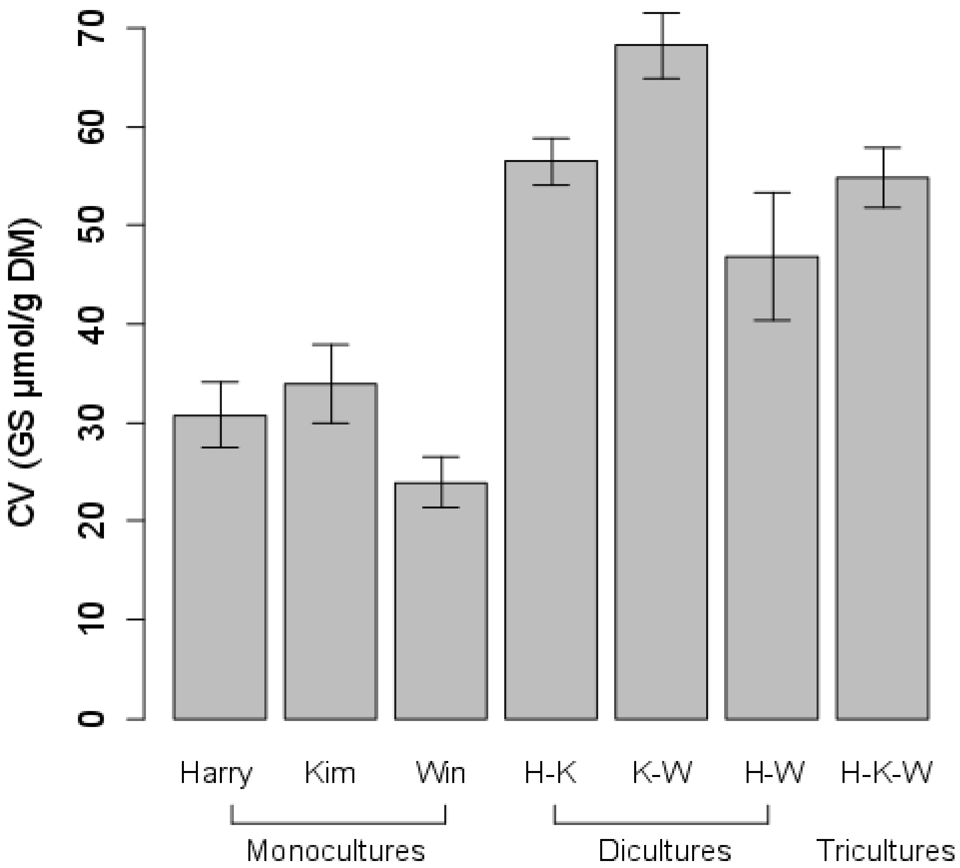

Figure S1. Mean coefficient of variation in glucosinolate (GS) concentration within a plot per plant population-diversity treatment combination (n=7). The labels on the X-axis refer to the plant populations Old Harry (Harry, H), Kimmeridge (Kim, K) and Winspit (Win, W) that formed the mono- di and tri-cultures. Error bars denote ±1 s.e.m.

RESULTS.

The following graphs represent the predicted values of each model including all level interaction terms with time (time of monitoring). First the type of applied statistical model, explanatory variables and the response variable are given (with random effects in parentheses), followed by the corresponding type II ANOVA table and the graph. (Type II tests include the significant values of any term without taking into account higher order terms, therefore main effects are interpretable independently of the interaction terms). Each panel in the graphs indicates the predicted values for each time point across the monitoring season (although time was considered as a continuous variable in the models). The model results are shown at the upper-left panel for the first time point and at the bottom-right panel for the last one.

Generalized linear mixed model (Poisson distribution):

Herbivore abundance ~ Plant population * Diversity treatment * Time + (1|Plant ID)

```
Response: Herbivore abundance (no plant size in the model)
AIC: 24683
                                        Chisq Df          P
Plant population                      19.1959  2  6.787e-05 ***
Diversity treatment                    8.4153  1   0.003721 **
Time                                 855.0789  1  < 2.2e-16 ***
Plant population×Diversity treat       0.7461  2   0.688642
Plant population×Time                 43.1743  2  4.215e-10 ***
Diversity treat×Time                  34.3895  1  4.512e-09 ***
Plant pop×Diversity×Time              67.5736  2  2.121e-15 ***
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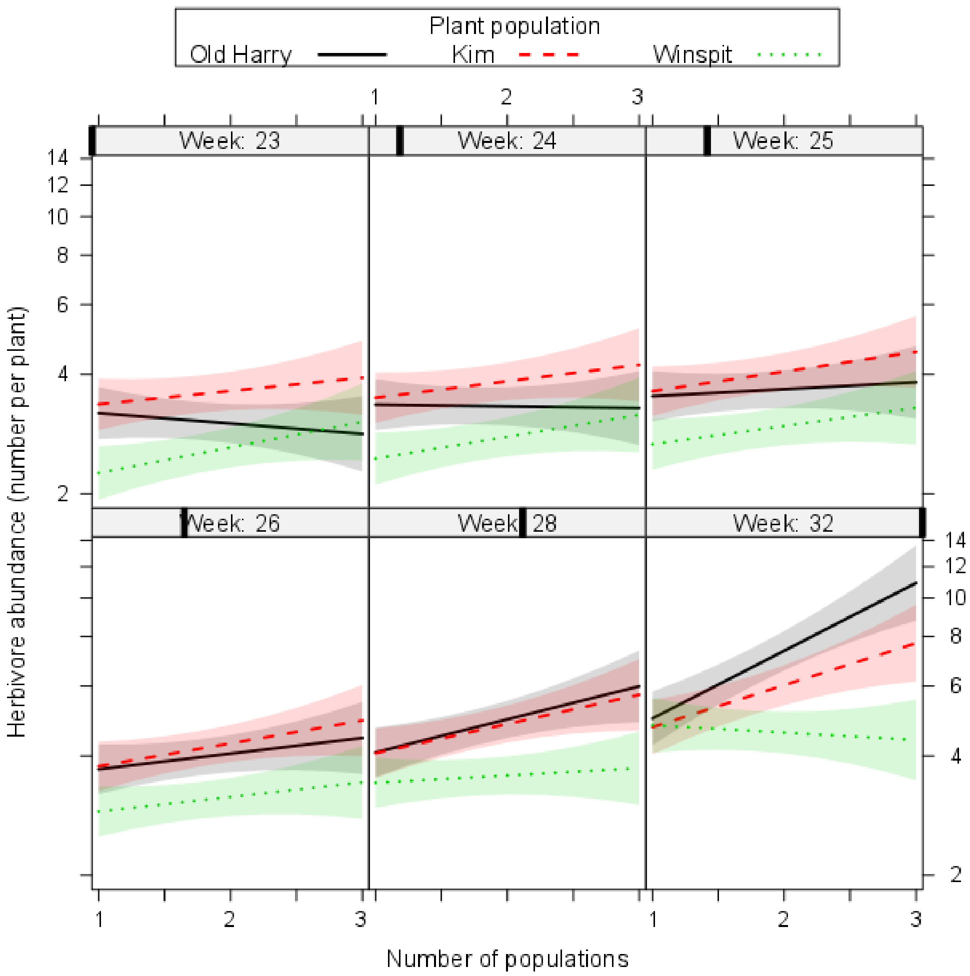

Figure S2. Predicted values of herbivore abundance (excluding the cabbage aphid *B. brassicae*) from the model analyzing the effects of plant diversity, plant population, time and their interactions. The type II ANOVA table is shown above.

Generalized linear mixed model (Poisson distribution):

Herbivore abundance ~ Plant population * Diversity treatment * Time + Plant size + (1|Plant ID)

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Response: Herbivore abundance (plant size as covariate)
AIC: 23955
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	Chisq	Df	P	
Plant population	4.7455	2	0.09322	
Diversity Treat	3.9187	1	0.04775	*
Time	237.2012	1	< 2.2e-16	***
Plant size (log cm^3)	727.4200	1	< 2.2e-16	***
Plant population×Diversity Treat	2.1166	2	0.34704	
Plant population×Time	72.3435	2	< 2.2e-16	***
Diversity Treat×Time	39.8224	1	2.781e-10	***
Plant pop×Diversity×Time	60.8776	2	6.034e-14	***

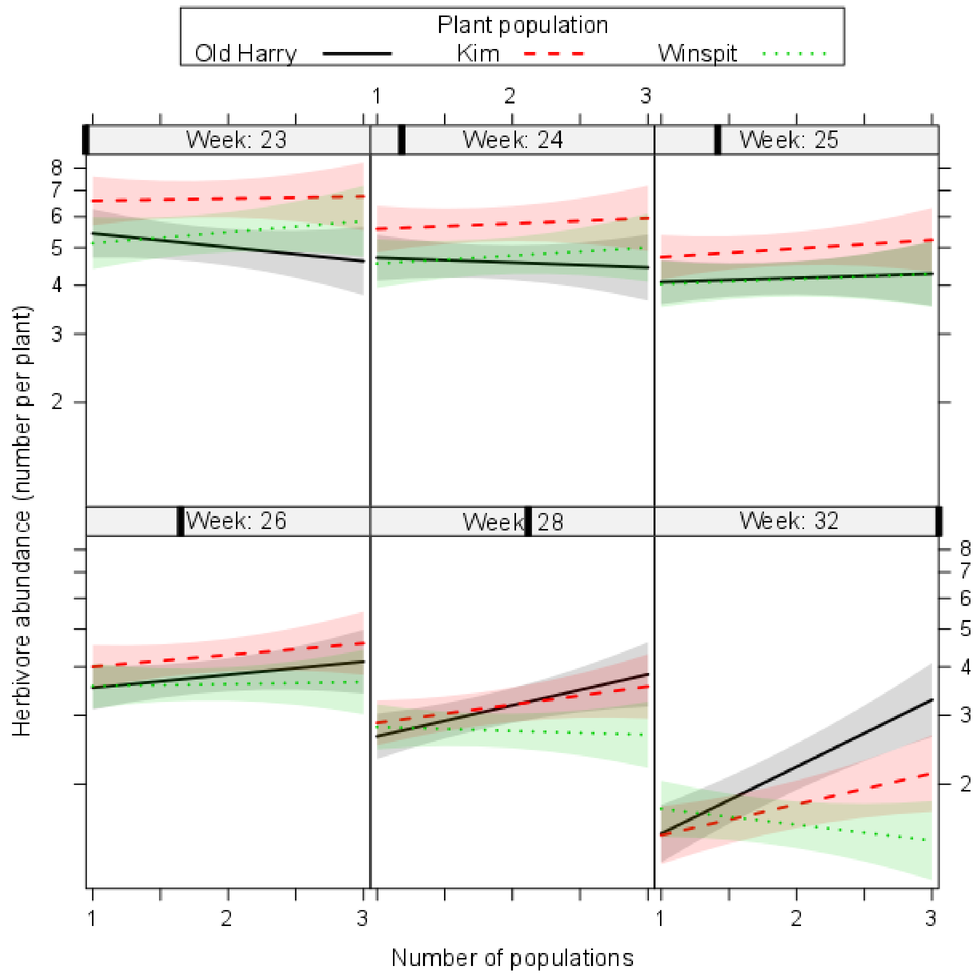

Figure S3. Predicted values of herbivore abundance (excluding the cabbage aphid *B. brassicae*) from the model analyzing the effects of plant diversity, plant population, time and their interactions, including plant size as a covariate. The herbivore abundance seems lower later in the season, since there are less herbivores per volume of plant. The type II ANOVA table is shown above.

Generalized linear mixed model (Poisson distribution):

B. brassicae abundance ~ Plant population * Diversity treatment * Time + (1|Plant ID)

```
Response: Brevicoryne brassicae abundance
AIC: 55018
                                          Chisq Df          P
Plant population                        43.2197  2  4.121e-10 ***
Diversity treatment                      0.2992  1     0.5844
Time                                 13809.9336  1  < 2.2e-16 ***
Plant population×Diversity treat         0.4660  2     0.7921
Plant population×Time                 1000.0011  2  < 2.2e-16 ***
Diversity treat×Time                    15.2744  1  9.297e-05 ***
Plant pop×Diversity×Time               495.5483  2  < 2.2e-16 ***
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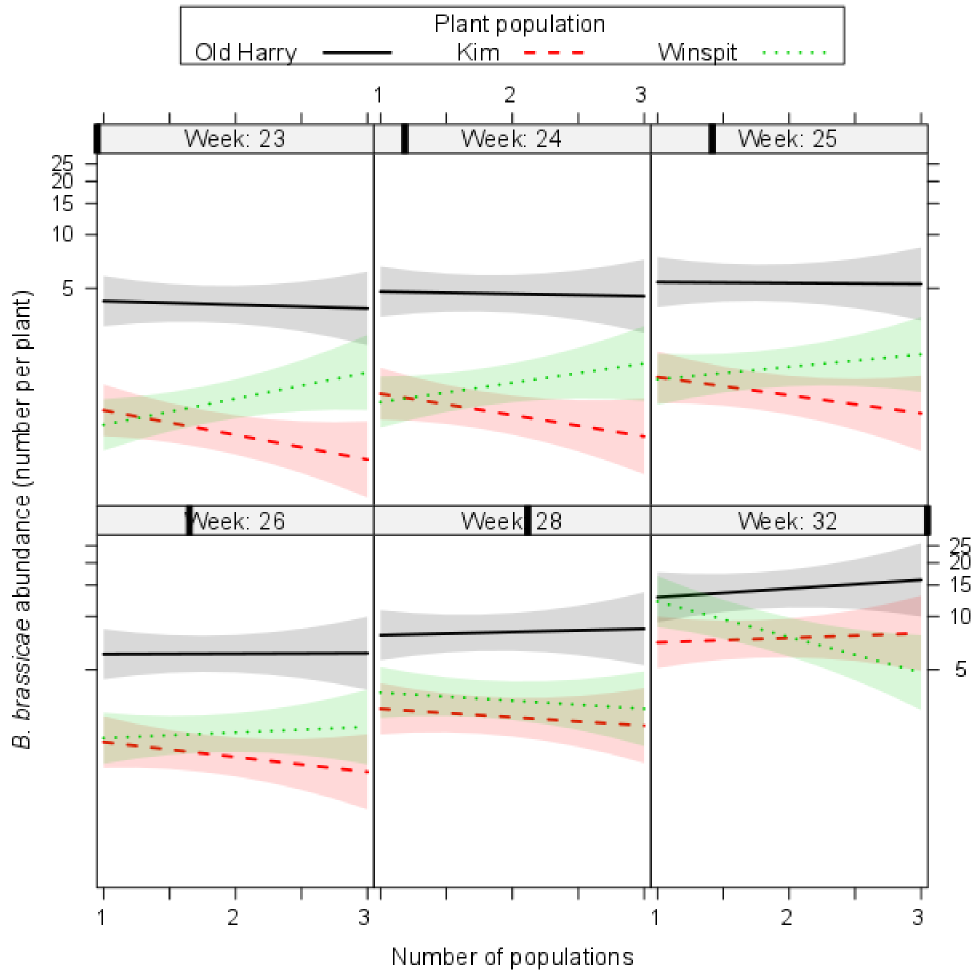

Figure S4. Predicted values of abundance of the cabbage aphid *B. brassicae* from the model analyzing the effects of plant diversity, plant population, time and their interactions. The type II ANOVA table is shown above.

Generalized linear mixed model (Poisson distribution):

B. brassicae abundance ~ Plant population * Diversity treatment * Time + Plant size + (1|Plant ID)

Response: *Brevicoryne brassicae* abundance
AIC: 47083

	Chisq	Df	P	
Plant population	40.6741	2	1.471e-09	***
Diversity treatment	3.4159	1	0.06457	
Time	2103.2467	1	< 2.2e-16	***
Plant size (log cm^3)	6798.1887	1	< 2.2e-16	***
Plant population×Diversity treat	2.4079	2	0.30000	
Plant population×Time	203.4601	2	< 2.2e-16	***
Diversity treat×Time	17.7931	1	2.463e-05	***
Plant population×Diversity×Time	398.7476	2	< 2.2e-16	***

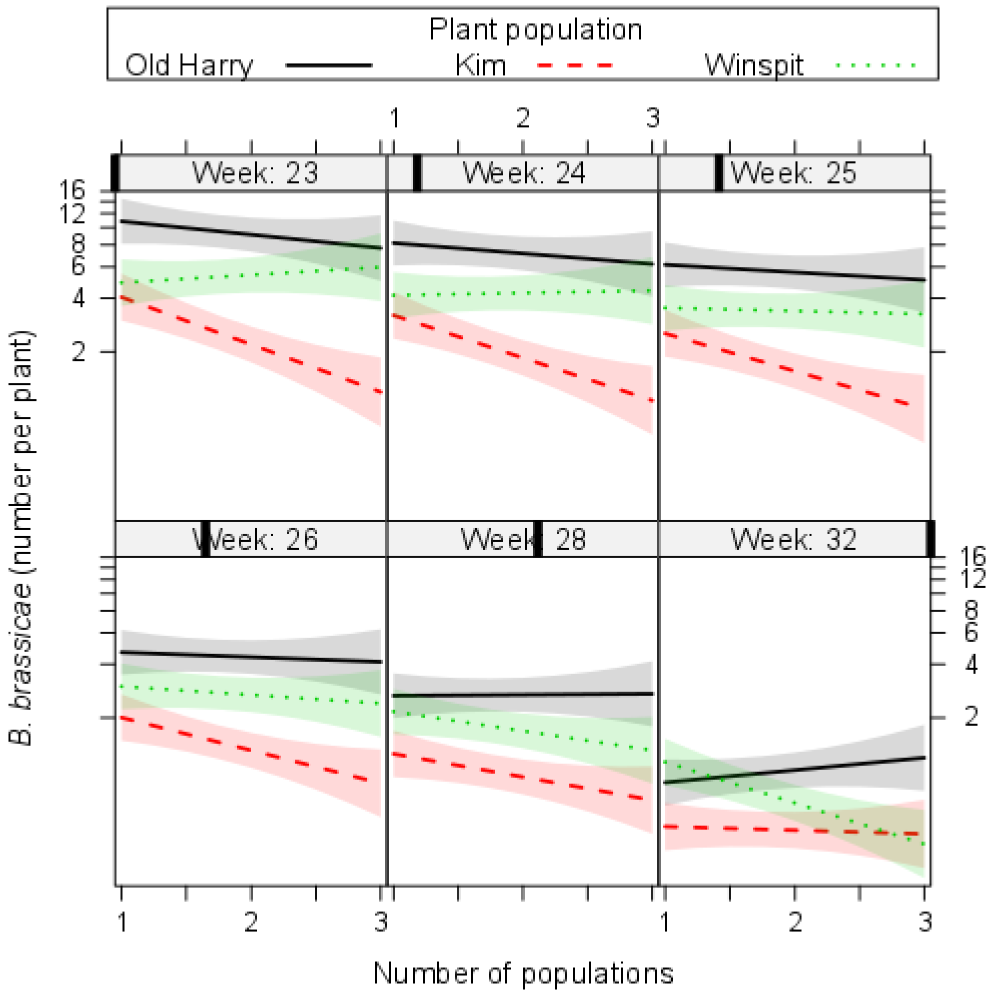

Figure S5. Predicted values of abundance of the cabbage aphid *B. brassicae* from the model analyzing the effects of plant diversity, plant population, time and their interactions, including plant size as a covariate. The aphid abundance seems lower later in the season, since there are less herbivores per volume of plant. The type II ANOVA table is shown above.

Generalized linear mixed model (Poisson distribution):
Carnivore abundance ~ Plant population * Diversity treatment * Time + Herbivore abundance + (1|Plant ID)

```
Response: Carnivore abundance

                                      Chisq Df          P
Plant population                     8.8732  2  0.0118360 *
Diversity treatment                  3.6426  1  0.0563188
Time                              3720.1235  1  < 2.2e-16 ***
Herbivore abundance (log)          164.6713  1  < 2.2e-16 ***
Plant population×Diversity treat     1.3550  2  0.5078832
Plant population×Time               17.9410  2  0.0001271 ***
Diversity treat×Time                24.9992  1  5.735e-07 ***
Plant population×Diversity×Time      6.1025  2  0.0473008 *
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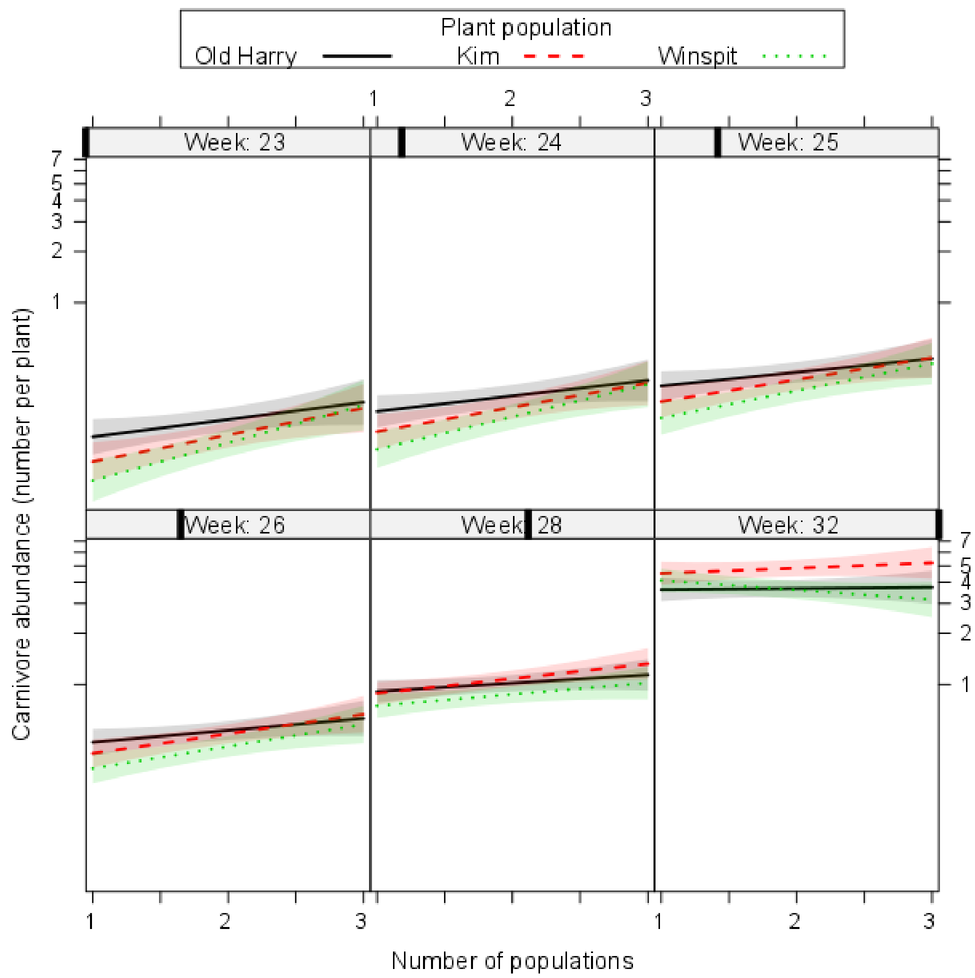

Figure S6. Predicted values of abundance of carnivores from the model analyzing the effects of plant diversity, plant population, time and their interactions, including herbivore abundance as a covariate. The type II ANOVA table is shown above.

Generalized linear mixed model (Poisson distribution):

D. rapae abundance ~ Plant population * Diversity treatment * Time + *B. brassicae* abundance + (1|Plant ID)

Response: Abundance of the parasitoid *Diaeretiella rapae*

	Chisq	Df	P	
Plant population	20.8613	2	2.951e-05	***
Diversity treat	0.4773	1	0.4897	
Time	2990.8896	1	< 2.2e-16	***
Abundance of *B. brassicae* (log)	444.1542	1	< 2.2e-16	***
Plant population×Diversity treat	1.1240	2	0.5701	
Plant population×Time	2.6309	2	0.2684	
Diversity treat×Time	99.7666	1	< 2.2e-16	***
Plant population×Diversity×Time	33.3787	2	5.648e-08	***

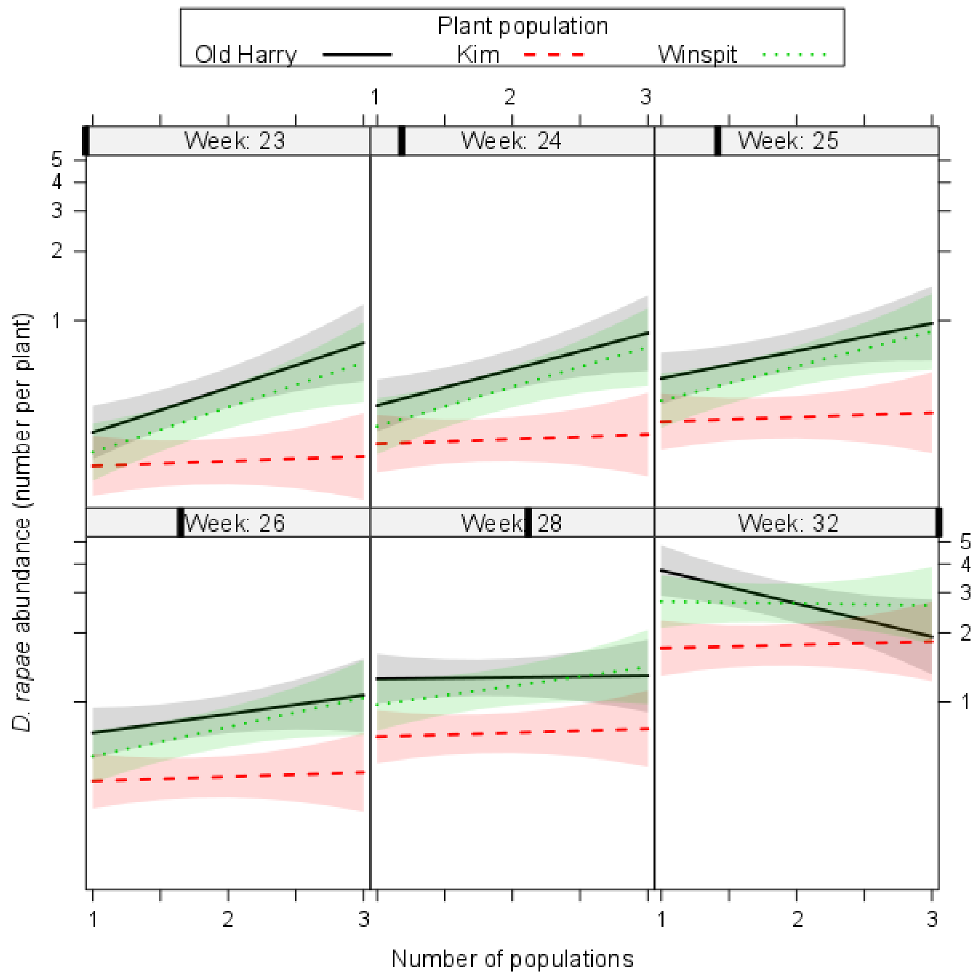

Figure S7. Predicted values of abundance of the parasitoid *D. rapae* from the model analyzing the effects of plant diversity, plant population, time and their interactions, including the host abundance (*B. brassicae*) as a covariate. The type II ANOVA table is shown above.

Linear mixed model:

Herbivore diversity ~ Plant population * Diversity treatment * Time + (1|Plant ID)

Response: Herbivore diversity (Shannon's H)

	Chisq	Df	P	
Plant population	9.6189	2	0.008152	**
Diversity treat	0.6931	1	0.405104	
Time	149.5861	1	< 2.2e-16	***
Plant population×Diversity treat	4.4273	2	0.109302	
Plant population×Time	1.6118	2	0.446694	
Diversity treat×Time	1.1187	1	0.290206	
Plant population×Diversity×Time	7.6398	2	0.021930	*

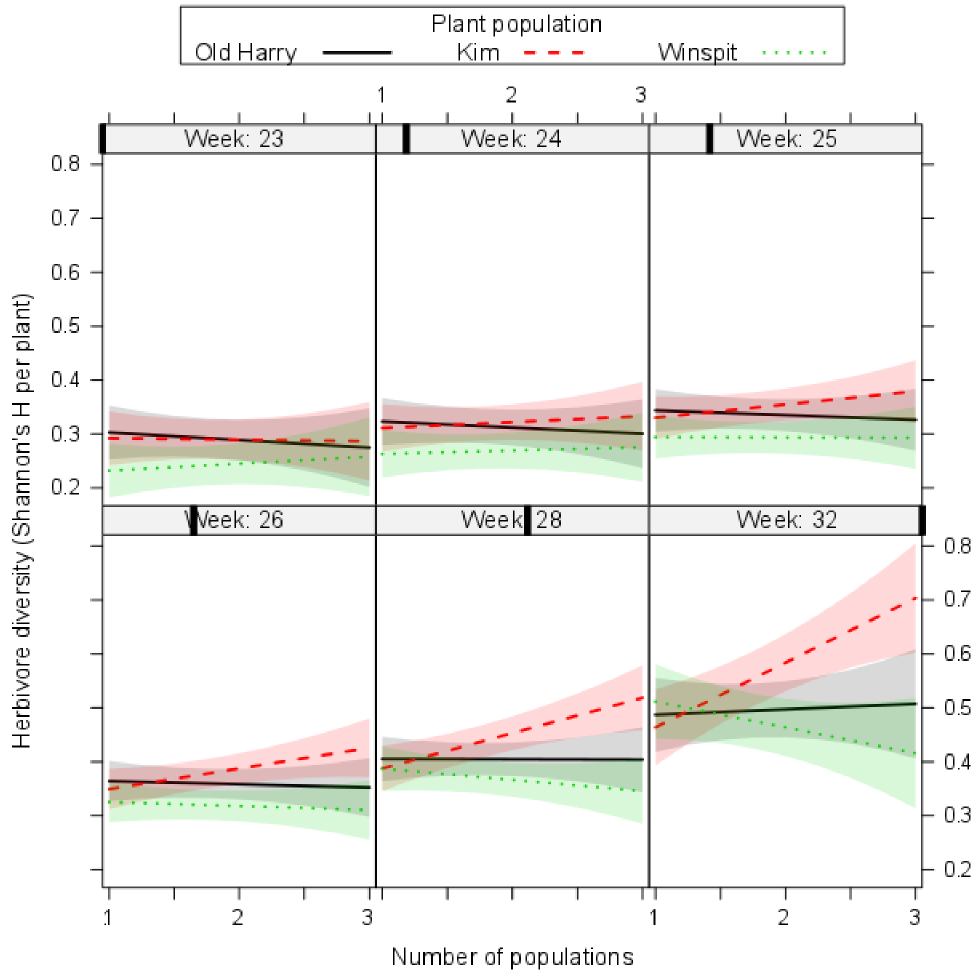

Figure S8. Predicted values of herbivore diversity from the model analyzing the effects of plant diversity, plant population, time and their interactions. The type II ANOVA table is shown above.

Linear mixed model:

Plant size ~ Plant population * Diversity treatment * Time + (1|Plant ID)

Response: Plant size (log cm^3)

	Chisq	Df	P	
Plant population	57.7191	2	2.927e-13	***
Diversity treat	8.0199	1	0.004627	**
Time	22920.9296	1	< 2.2e-16	***
Plant population×Diversity treat	2.5781	2	0.275533	
Plant population×Time	24.3077	2	5.268e-06	***
Diversity treat×Time	2.1351	1	0.143958	
Plant population×Diversity×Time	3.2625	2	0.195689	

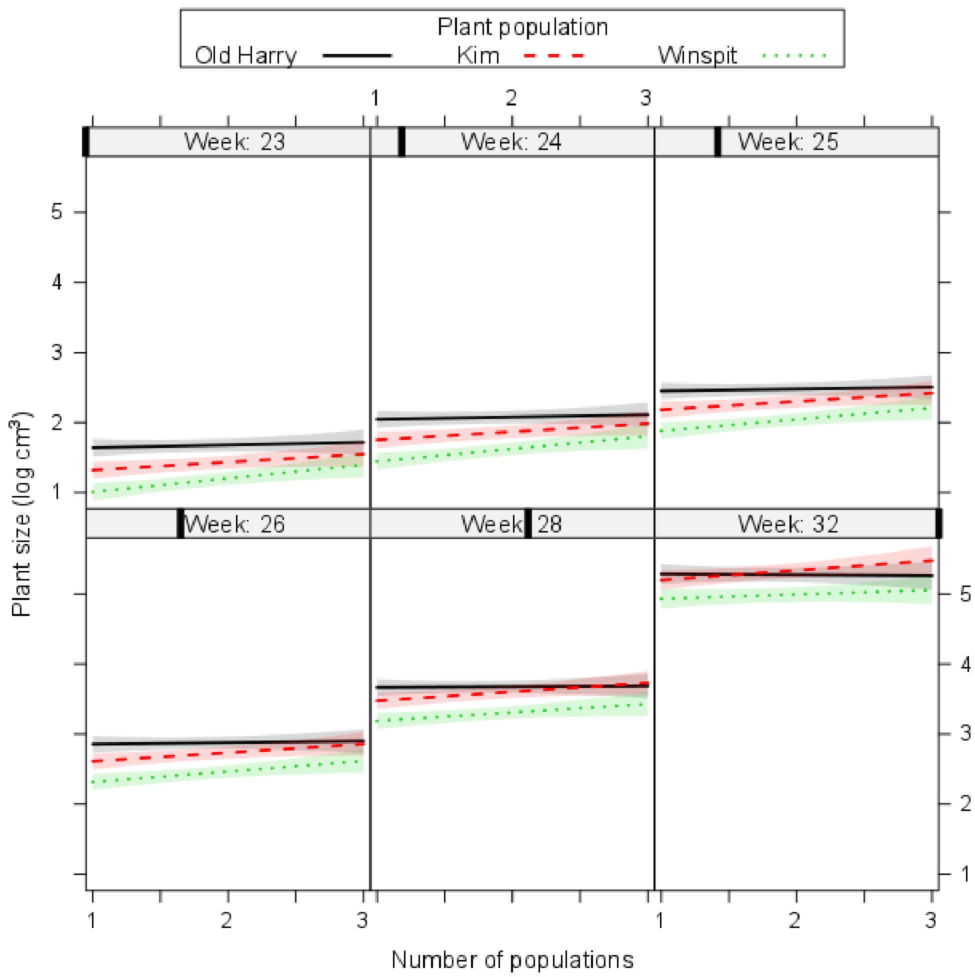

Figure S9. Predicted values of plant size from the model analyzing the effects of plant diversity, plant population, time and their interactions. The type II ANOVA table is shown above.

Linear mixed model:

Plant damage ~ Plant population * Diversity treatment * Time + Diversity treatment * (Herbivore abundance + Herbivore diversity + Carnivore abundance) + (1|Plant ID)

Response: Proportion of damage (logit)

	Chisq	Df	P	
Plant population	11.8236	2	0.0027073	**
Diversity treat	4.4788	1	0.0343181	*
Time	29.0904	1	6.908e-08	***
Herbivore Abundance (log)	4.8607	1	0.0274751	*
Herbivore Diversity (H)	13.2340	1	0.0002749	***
Carnivore Abundance (log)	0.4091	1	0.5224437	
Plant population×Diversity treat	0.5182	2	0.7717298	
Plant population×Time	16.1335	2	0.0003138	***
Diversity treat×Time	7.4534	1	0.0063315	**
Diversity treat×Herb abundance	1.5156	1	0.2182849	
Diversity treat×Herb diversity	0.1205	1	0.7284657	
Diversity treat×Carn abundance	1.7216	1	0.1894905	
Plant population×Diversity×Time	2.1259	2	0.3454408	

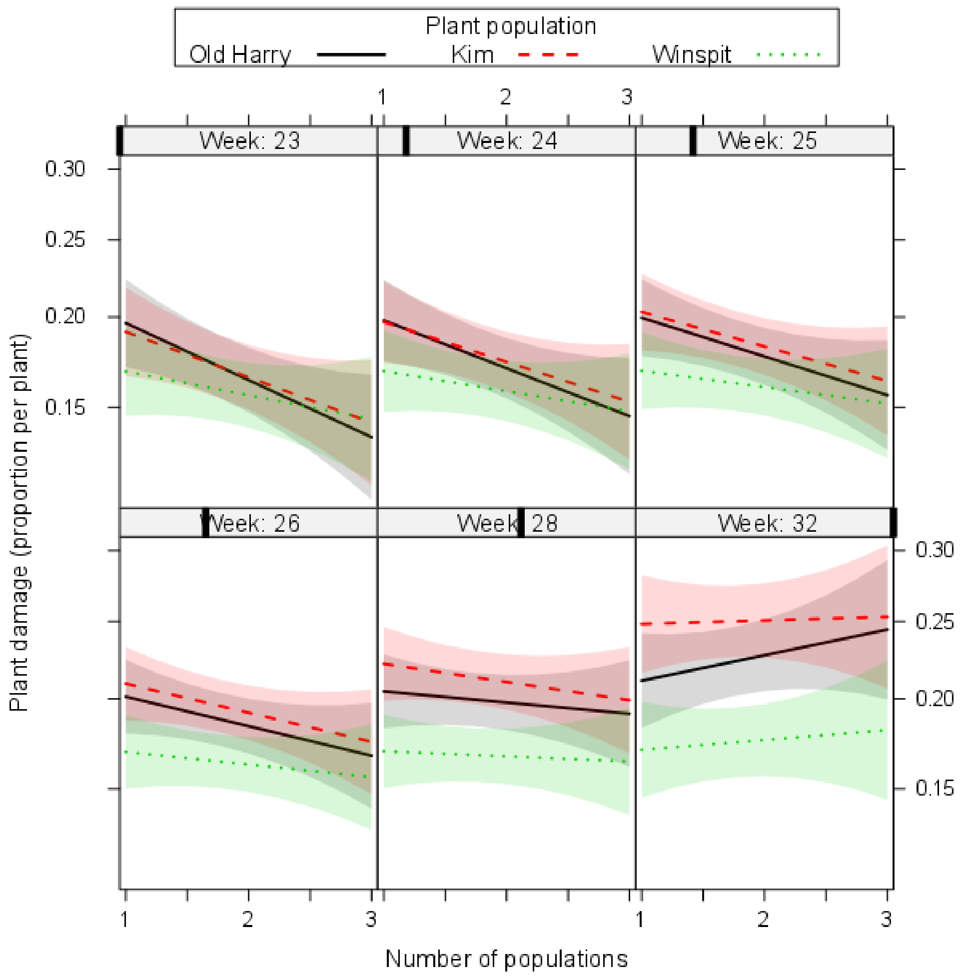

Figure S10. Predicted values of plant damage from the model analyzing the effects of plant diversity, plant population, time and their interactions. The relationships of plant damage with herbivore abundance, herbivore diversity and carnivore abundance were also considered in the model. The type II ANOVA table is shown above.

Generalized linear model (Poisson distribution):

Generalists abundance ~ Diversity treatment * (PLS-DA 1 + GS concentration + CV_{conc}) + Plant size

Response: Generalist herbivores abundance

	LR Chisq	Df	P	
PLS-DA 1	6.496	1	0.010814	*
GS GS concentration	0.854	1	0.355363	
CV(GS concentration)	1.371	1	0.241695	
Diversity treat	0.018	1	0.894011	
Plant size (log cm^3)	73.080	1	< 2.2e-16	***
PLS-DA 1×Diversity treat	4.684	1	0.030450	*
GS concentration×Diversity treat	10.235	1	0.001378	**
CV (GS conc)×Diversity treat	1.870	1	0.171430	

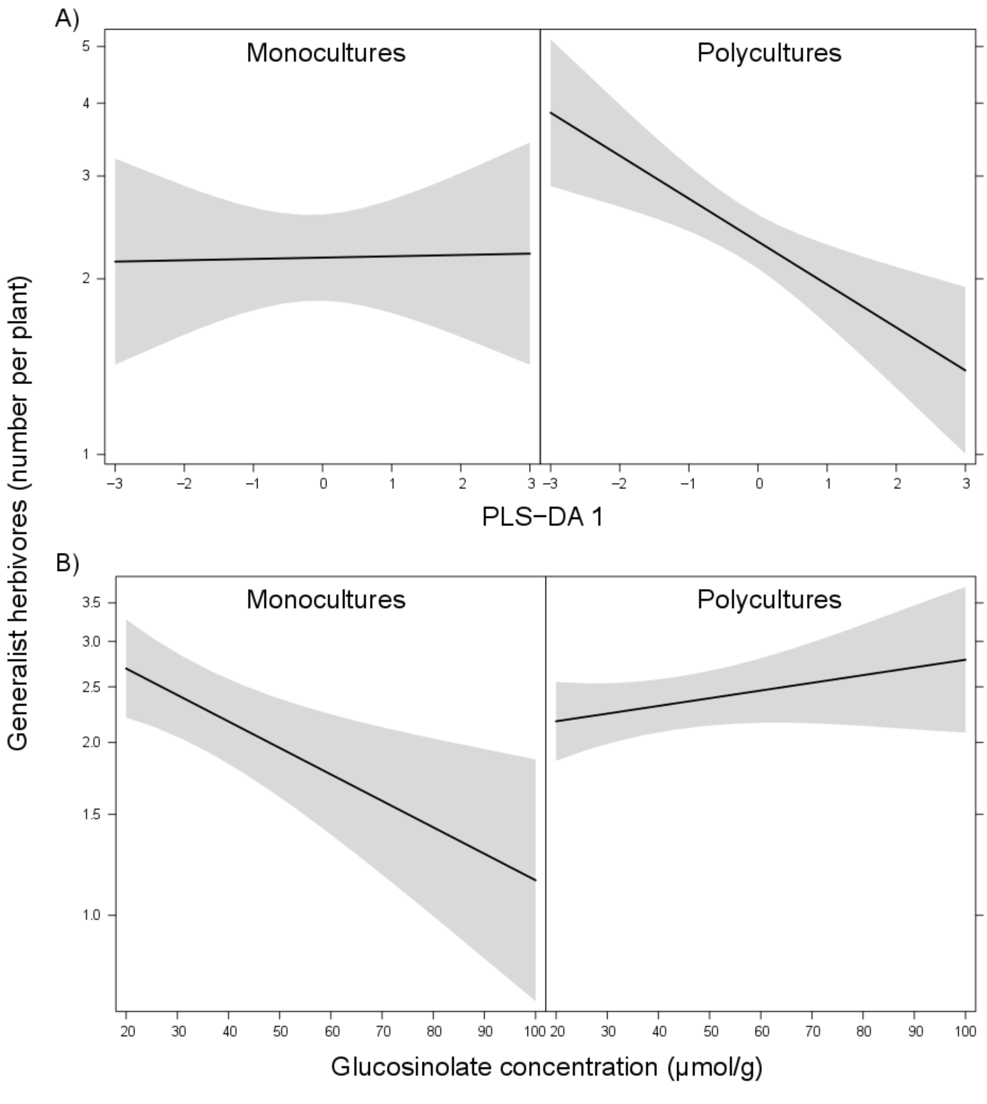

Figure S11. Predicted values of generalist herbivore abundance across the season from the model analyzing the effects of plant glucosinolates. Only the significant effects are plotted for glucosinolate composition using the first PLS-DA component (A) and total concentration (B), and their interaction with plant diversity treatment. The type II ANOVA table is shown above.

Generalized linear model (Poisson distribution):

Specialists abundance ~ Diversity treatment * (PLS-DA 1 + GS concentration + CV_{conc}) + Plant size

```
Response: Specialist herbivores abundance

                                  LR Chisq Df          P
PLS-DA 1                             2.620  1   0.105539
GS concentration                     8.050  1   0.004551 **
CV(GS concentration)                 0.310  1   0.577688
Diversity treat                      5.582  1   0.018142 *
Plant size (log cm³ )               53.055  1  3.243e-13 ***
PLS-DA 1×Diversity treat             0.291  1   0.589767
GS concentration×Diversity treat     0.440  1   0.506932
CV (GS conc)×Diversity treat         1.205  1   0.272342
```

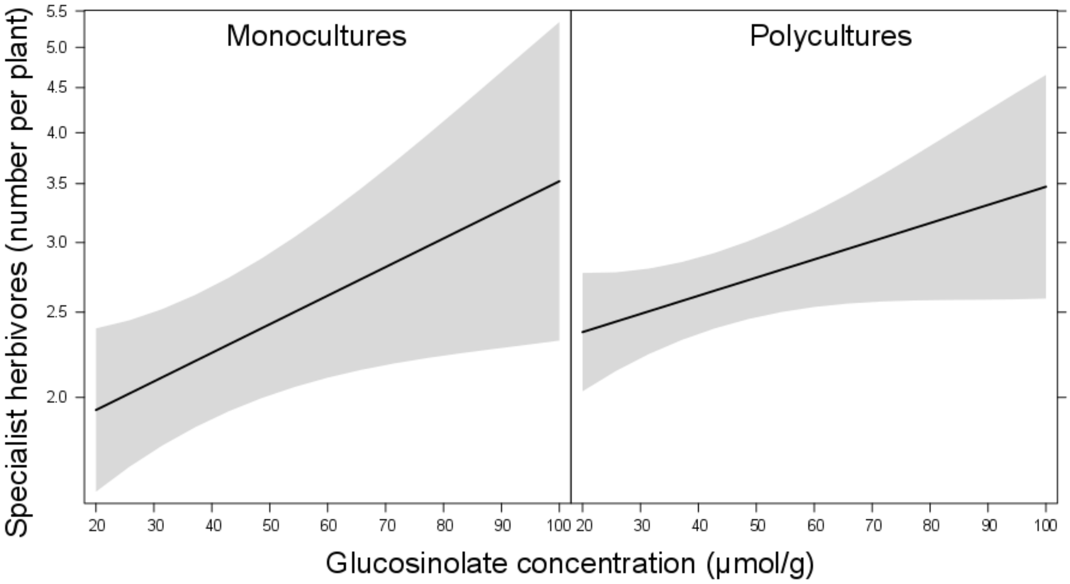

Figure S12. Predicted values of specialist herbivore abundance across the season from the model analyzing the effects of plant glucosinolates. Only the significant effect of glucosinolate concentration is plotted for mono and polycultures. The type II ANOVA table is shown above.

Generalized linear model (Poisson distribution):

B. brassicae abundance ~ Diversity treatment * (PLS-DA 1 + GS concentration + CV_{conc}) + Plant size

Response: *Brevicoryne brassicae* abundance

	Chisq	Df	P	
PLS-DA 1	57.16	1	4.021e-14	***
GS concentration	97.38	1	< 2.2e-16	***
CV (GS conc)	896.35	1	< 2.2e-16	***
Diversity treat	512.61	1	< 2.2e-16	***
Plant size (log cm^3)	505.58	1	< 2.2e-16	***
PLS-DA 1×Diversity treat	2.72	1	0.09903	
GS concentration×Diversity treat	2.89	1	0.08895	
CV (GS conc)×Diversity treat	94.57	1	< 2.2e-16	***

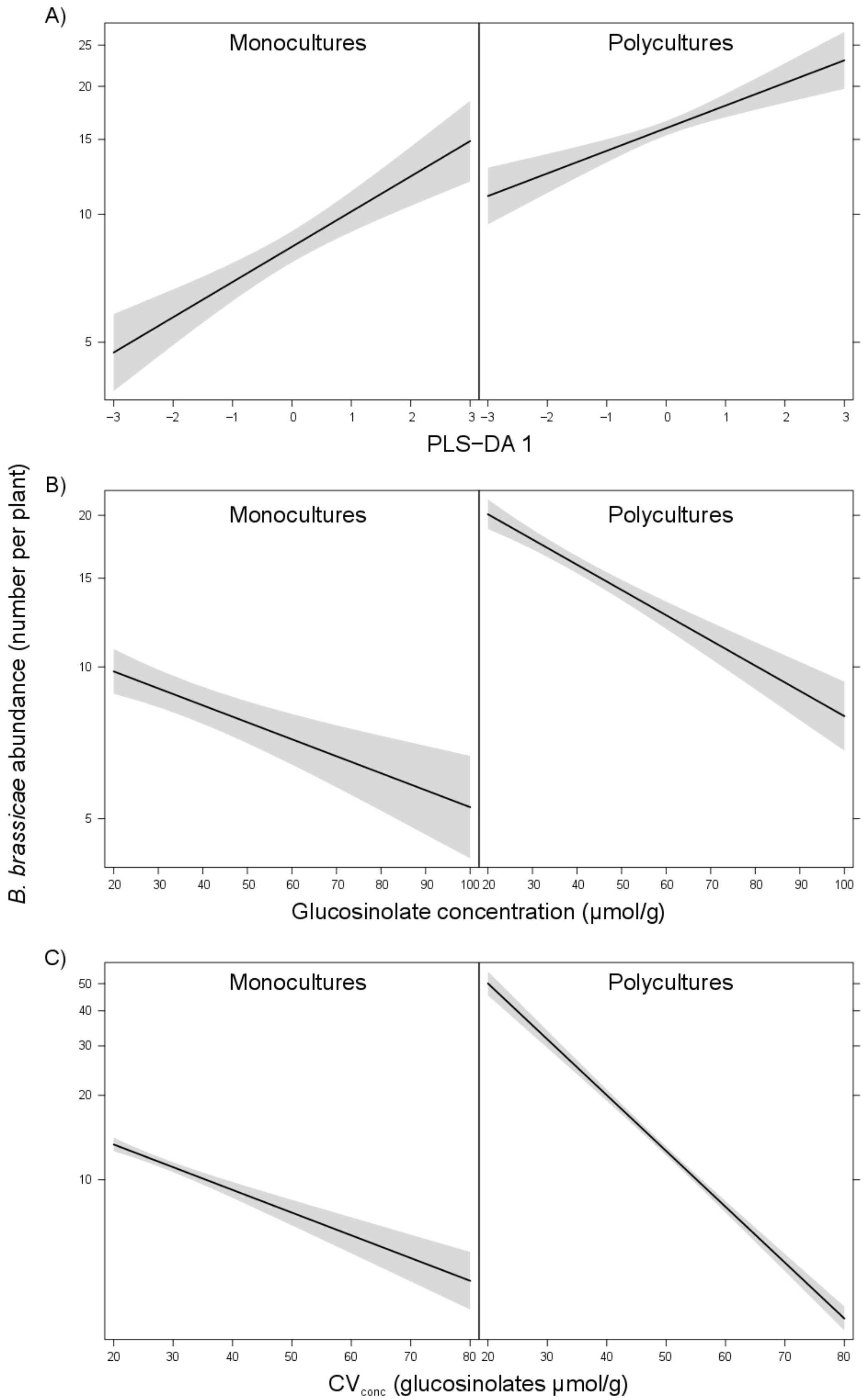

Figure S13. Predicted values of abundance of the cabbage aphid *B. brassicae* across the season from the model analyzing the effects of plant glucosinolate composition (A) and concentration (B), and glucosinolate variation among neighbour plants (C), and their interaction with plant diversity treatment. The type II ANOVA table is shown above.