

***New Phytologist* Supporting Information**

Article title:

Macroevolution of leaf defenses and secondary metabolites across the genus *Helianthus*

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The following Supporting Information is available for this article:

Notes S1 Population source localities and USDA accessions, population trait means, species trait means, and herbivore and disease resistance data (see separate file). (Data used in this study (Notes S1 and Notes S2) are available via Dryad: doi:10.5061/dryad.5hq56)

Notes S2 HeliaMet database: normalized peak data obtained by HPLC-MS (see separate file).

Methods S1 Additional methodological details.

Fig. S1 Principal components analysis of the 22 most common HeliaMet compounds.

Table S1 Putative identities and factor loadings for the 22 most common HeliaMet compounds.

Table S2 Macroevolutionary correlations between defenses and environmental characteristics.

Table S3 Macroevolutionary correlations between defenses and herbivores.

Table S4 Macroevolutionary correlations between defenses and pathogens/parasites.

Methods S1

Leaf-level physical and chemical defenses

Sampling for leaf defenses was standardized by ontogenetic stage, in order to account for large variation among species in growth form, growth rate, and whole-plant development (Mason *et al.*, 2013; Mason & Donovan, 2015a). As such, each species was considered separately, and all populations and replicates of each species were sampled on a single day, once all replicate plants had produced at least four fully-expanded leaf pairs but before the onset of reproduction (Mason & Donovan, 2015b). A single leaf was harvested from each replicate plant to assess leaf defense traits. The leaf was weighed for fresh mass with a digital scale, and leaf lamina thickness was then measured with digital calipers halfway down the length of the leaf. Leaf toughness was assessed with a penetrometer, using the average of three measurements of the force required to penetrate the leaf lamina with a millimeter-wide flat-tipped needle. The leaf was then dried at 60°C for at least 36 h and weighed for dry mass. Leaf dry matter content (LDMC) was calculated as the proportion of leaf dry mass to leaf fresh mass. Trichome density was then estimated on dried leaves with a dissecting microscope by counting the number of trichomes present in a 0.25 cm² region. It is important to note that while trichome density is normally assessed on fresh leaves, this was not feasible given the scale of this study. However, leaf shrinkage during the drying process should retain much of the relative ranking of trichome density among populations and species, especially given that these species range from completely glabrous to densely hirsute. The leaf was then ground into a fine homogenous powder with a ball mill for subsequent analyses. Tannin activity was assessed using the radial diffusion assay (Hagerman, 1987), which assesses one aspect of tannin activity (protein precipitation capacity) of leaf extracts relative to a tannic acid standard (C₇₆H₅₂O₄₆, CAS #1401-55-4). Leaf lipid content and leaf ash content were assessed using the protocols of Moles *et al.* (2011). Leaf lipid content was determined by extracting and discarding the lipid-soluble fraction of a sample of leaf powder with petroleum ether (b.p. 40–60°C), followed by drying the remainder at 60°C and calculating the proportion of mass lost. Leaf ash content was determined by combusting a sample of leaf powder in a muffle furnace at 600°C for 12 h, and calculating the proportion of mass remaining.

Environmental data

Soil and climate characteristics were obtained for the source site of each population, as reported previously in Mason & Donovan (2015b). In brief, five soil cores were collected at representative locations throughout each population to a depth of *c.* 20 cm, dried at 60°C and homogenized. Debris larger than 0.5 cm was removed to exclude root material and stones from samples before analyses. Samples were assessed for soil carbon content, nitrogen content, and C:N ratio with Micro-Dumas combustion (NA1500, Carlo Erba Strumentazione, Milan, Italy) at the University of Georgia Analytical Chemistry Laboratory. Samples were also submitted for standard bulk soil analysis with A&L Eastern Laboratories (Richmond, VA, USA), yielding soil organic matter content, cation exchange capacity, pH, available phosphorus, and exchangeable potassium, calcium, and magnesium. Estimates taken on each of the five soil cores per population were averaged to generate source site means for each soil characteristic. To characterize the climate of each population source site, estimates of altitude, mean annual temperature (MAT), mean diurnal range, temperature seasonality, mean annual precipitation (MAP), and precipitation seasonality were obtained from the WorldClim database (Hijmans *et al.*, 2005). Additionally, aridity index and potential evapotranspiration (PET) were extracted from the CGIAR Global Aridity and PET database (Zomer *et al.*, 2008).

Secondary metabolite variation via HPLC-MS

To characterize secondary metabolite profiles across species, a single leaf was collected from each replicate plant, snap-frozen in liquid nitrogen, and stored at -80°C. Samples were then ground in liquid nitrogen, and 10 mg of freeze-dried leaf powder was extracted in 400 µl of 1:1 methanol:chloroform (v/v) containing three internal standards (¹³C₆-cinnamic acid, D₅-benzoic acid, and resorcinol) by sonication in ice water for 30 min. After adding 200 µl of HPLC-grade water, samples were vortexed and centrifuged, and the aqueous layer was collected and re-centrifuged. Leaf compounds were analyzed by reverse phase high-performance liquid chromatography-mass spectrometry (HPLC-MS) as described in Xue *et al.* (2013).

In brief, samples (3 µl) were injected into an Agilent 1200 HPLC with a 6220 accurate time-of-flight mass spectrometer with dual electrospray ionization, and a diode array detector (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved using a ZORBAX Rapid Resolution Eclipse XDB-C18 column (4.6 × 50 mm 1.8 µm; Agilent), with mobile phase

solvents of water:acetonitrile:formic acid = 97:3:0.1 (A) and 3:97:0.1 (B). Flow rate was set to 1 ml min⁻¹, and the elution gradient was as follows: 3% B from 0 to 1 min, linear gradient to 17% B over 2 min, isocratic at 17% B for 2 min, linear gradient to 60% B over 4 min, then to 98% B over 2 min. Mass spectrometer acquisition was in negative ionization mode in m/z range of 100 to 1500 with the following settings: gas temperature, 350°C; drying gas flow, 13 l min⁻¹; nebulizer pressure, 60 psig; capillary voltage, 3500 V; and fragmentor voltage, 125 V. Diode array detection was set at 260, 270, 280, 310, and 350 nm.

Peak data were analyzed by MassHunter Qualitative Analysis, Mass Profiler, and MassHunter Quantitative analysis (Agilent Technologies, Santa Clara, CA, USA), then by manual curation. Peak areas for each sample were normalized by a normalization factor, which was calculated by dividing the mean peak area of the three internal standards within that sample by the overall mean across samples. Normalized peak areas were also standardized per unit dry mass. Individual samples from a target of 4 replicate plants per population were initially included for HPLC-MS analysis, though this was reduced to an average of 3.77 samples per population due to instrument breakdowns.

References

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Fig. S1 Species-level principal components analysis of the subset of 22 HeliaMet compounds present in at least 20 of the 28 study species (Table S1). Note that the first PC axis (explaining 41.2% of variation) loads positively with quantities of 19 out of the 22 compounds.

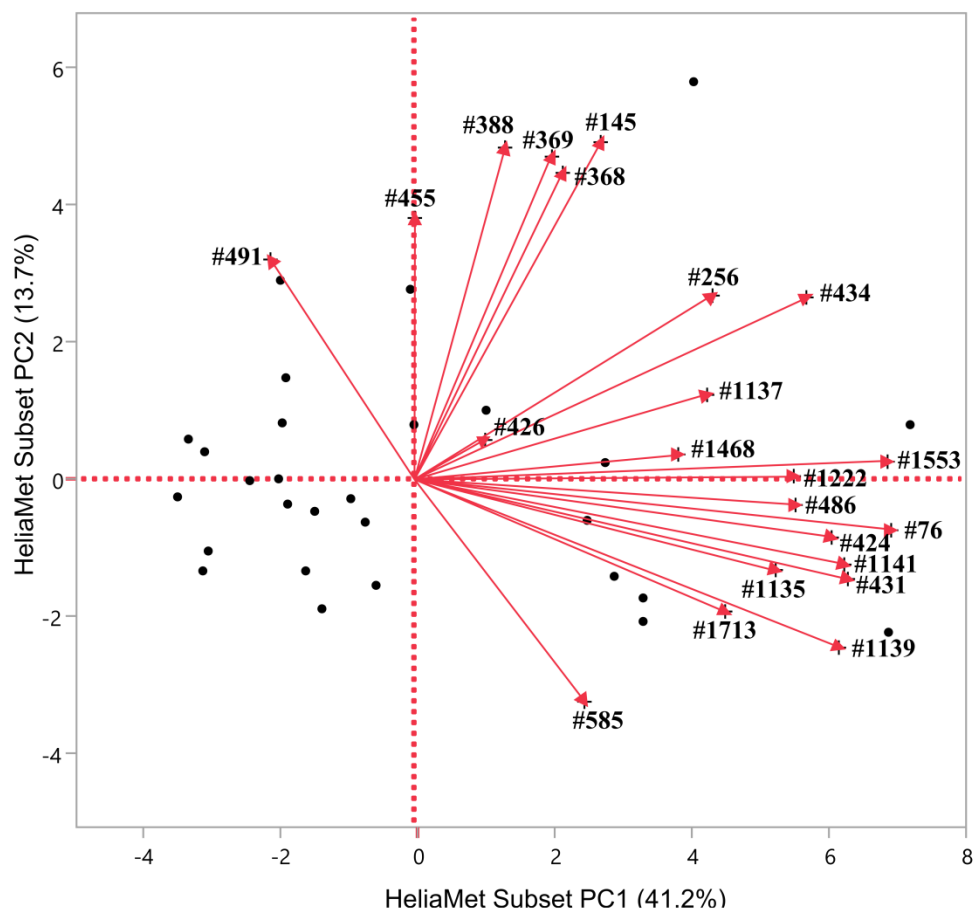


Table S1 Putative identity, mass-to-charge ratio (m/z), and factor loading of each trait in the species-level HeliaMet Subset PC1 axis. Putative identities were obtained by comparison of mass-to-charge ratios with either authentic standards (S), lookup in the METLIN database (D), or previous publications on sunflower phytochemistry (P).

#	Putative Identity	ID source	m/z	PCA 1 loading
76	Scopoletin* or Coumarin (formide adduct)*	P	191.0572475	0.96
145	Syringic acid (formide adduct)†	D	243.05036	0.38
256	Salicylic acid glucoside	S	299.07743	0.60
368	<i>p</i> -Coumaroylquinic acid (isomer)	S	337.0928625	0.30
369	<i>p</i> -Coumaroylquinic acid (isomer)	S	337.09296	0.28
388	Ciliarin¶	P	345.0838867	0.18
424	Scopolin* or Caffeoylequinic acid (isomer)†	D/P	353.0880125	0.84
426	3-O-Caffeoylequinic acid (chlorogenic acid)	S	353.0877933	0.14
431	4-O-Caffeoylequinic acid (cryptochlorogenic acid)	S	353.0884225	0.87
434	Scopolin* or Caffeoylequinic acid (isomer)†	D/P	353.085915	0.79
455	<i>no match</i>	D	357.1903367	0.00
486	Feruloylquinic acid (isomer)†	D	367.1035225	0.77
491	Methyl-(R)-9-hydroxy-10-undecene-5,7-diynoate glucoside†	D	367.139705	-0.29
585	Jasmolone glucoside (formide adduct)†	D	387.166315	0.34
1135	Di-O-caffeoylequinic acid (isomer)†	D	515.1208425	0.73
1137	Di-O-caffeoylequinic acid (isomer)†	D	515.1203375	0.59
1139	Di-O-caffeoylequinic acid (isomer)†	D	515.117135	0.86
1141	Di-O-caffeoylequinic acid (isomer)†	D	515.1153575	0.87
1222	<i>no match</i>	D	544.9621267	0.77
1468	<i>no match</i>	D	677.148355	0.53
1553	Isorientin 4'-O-glucoside 2"-O-p-hydroxybenzoate†	D	729.1638975	0.95
1713	<i>no match</i>	D	1053.21955	0.63

* matches Olson & Roseland (1991) or Tal & Robeson (1986).

¶ matches Chowdhury et al. (1980).

† one of several equivalent matches in the METLIN database (Smith et al., 2005).

References

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Table S2 Macroevolutionary correlations between environmental characteristics and leaf physical defenses, chemical defenses, C:N ratio, and HeliaMet metrics of secondary metabolite variation, as assessed by phylogenetic mixed model. R^2 and directionality of correlations are presented, with those found to be significant at $P < 0.05$ in blue, and those found to be also significant at the more stringent multiple comparisons cutoff (false discovery rate; Benjamini & Hochberg, 1995) in red. LDMC, leaf dry matter content; PET, potential evapotranspiration; CEC, cation exchange capacity.

	Trichome density	Thickness	Toughness	LDMC	Tannin activity	Lipid content	Ash content	C:N ratio	HeliaMet PCO1	HeliaMet PCO2	Avg. # Comp.	HeliaMet Subset PC1	HeliaMet Subset PC2
Latitude	(-)0.09	(-)0.13	(-)0.00	(+)0.04	(-)0.11	(+)0.26	(-)0.06	(-)0.16	(-)0.01	(-)0.00	(+)0.00	(+)0.00	(+)0.22
Altitude	(-)0.10	(-)0.08	(+)0.00	(+)0.00	(-)0.10	(+)0.24	(-)0.01	(-)0.16	(+)0.03	(+)0.06	(-)0.11	(-)0.04	(+)0.00
PET	(+)0.05	(+)0.13	(+)0.17	(-)0.03	(+)0.05	(-)0.16	(+)0.17	(+)0.09	(+)0.07	(-)0.01	(-)0.02	(-)0.00	(-)0.24
Aridity Index	(-)0.04	(-)0.10	(-)0.14	(+)0.01	(+)0.06	(-)0.07	(-)0.22	(+)0.02	(-)0.06	(-)0.22	(+)0.06	(+)0.15	(+)0.00
Mean Annual Temp.	(+)0.15	(+)0.20	(+)0.03	(-)0.03	(+)0.08	(-)0.23	(+)0.13	(+)0.16	(+)0.01	(+)0.00	(-)0.00	(-)0.01	(-)0.17
Mean Diurnal Range	(-)0.06	(-)0.00	(+)0.15	(-)0.00	(-)0.01	(+)0.02	(+)0.01	(-)0.03	(+)0.09	(-)0.01	(-)0.07	(+)0.00	(-)0.04
Temp. Seasonality	(-)0.05	(-)0.13	(-)0.04	(+)0.11	(-)0.13	(+)0.09	(-)0.09	(-)0.21	(-)0.07	(+)0.00	(+)0.05	(+)0.03	(+)0.44
Annual Precip.	(-)0.03	(-)0.04	(-)0.06	(+)0.00	(+)0.11	(-)0.12	(-)0.11	(+)0.08	(-)0.02	(-)0.21	(+)0.03	(+)0.11	(-)0.02
Precip. Seasonality	(+)0.05	(+)0.10	(+)0.13	(-)0.00	(+)0.00	(+)0.00	(+)0.43	(+)0.02	(+)0.01	(+)0.46	(-)0.01	(-)0.39	(-)0.00
Soil N	(-)0.06	(-)0.17	(-)0.07	(+)0.03	(-)0.01	(+)0.31	(-)0.58	(-)0.01	(-)0.23	(+)0.05	(+)0.48	(-)0.01	(+)0.00
Soil C	(-)0.05	(-)0.16	(-)0.08	(+)0.05	(-)0.01	(+)0.35	(-)0.73	(-)0.01	(-)0.31	(+)0.11	(+)0.64	(-)0.05	(+)0.01
Soil C:N ratio	(+)0.31	(+)0.08	(+)0.05	(+)0.01	(-)0.01	(-)0.17	(+)0.42	(-)0.02	(-)0.02	(+)0.25	(+)0.01	(-)0.18	(+)0.01
Organic Matter	(-)0.12	(-)0.25	(-)0.10	(+)0.06	(-)0.00	(+)0.29	(-)0.63	(-)0.00	(-)0.29	(+)0.03	(+)0.60	(-)0.01	(+)0.01
P	(-)0.00	(-)0.11	(-)0.08	(+)0.04	(-)0.09	(+)0.50	(-)0.26	(-)0.10	(-)0.03	(+)0.17	(+)0.09	(-)0.00	(+)0.42
K	(-)0.08	(-)0.00	(+)0.01	(+)0.00	(-)0.06	(+)0.36	(+)0.02	(-)0.04	(+)0.03	(+)0.16	(-)0.01	(-)0.11	(-)0.00
Mg	(-)0.04	(+)0.00	(+)0.04	(-)0.01	(-)0.07	(+)0.36	(+)0.06	(-)0.08	(+)0.04	(+)0.09	(-)0.03	(-)0.11	(-)0.01
Ca	(-)0.00	(-)0.01	(+)0.02	(+)0.03	(-)0.02	(+)0.09	(+)0.16	(-)0.01	(-)0.09	(+)0.56	(+)0.13	(-)0.26	(+)0.04
pH	(+)0.13	(+)0.01	(+)0.00	(+)0.04	(-)0.16	(+)0.16	(+)0.21	(-)0.17	(-)0.04	(+)0.41	(+)0.04	(-)0.13	(+)0.14
CEC	(-)0.01	(-)0.01	(+)0.02	(+)0.02	(-)0.03	(+)0.12	(+)0.15	(-)0.02	(-)0.05	(+)0.54	(+)0.08	(-)0.25	(+)0.03

Reference

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**(1): 289-300.

Table S3 Macroevolutionary correlations between leaf traits and metrics of herbivory, as assessed by the *phylopars* method (Bruggeman *et al.*, 2009) to incorporate missing data. R^2 and directionality of correlations are presented, with those found to be significant at $P < 0.05$ in blue, and those found to be also significant at the more stringent multiple comparisons cutoff (false discovery rate; Benjamini & Hochberg, 1995) in red. LDMC, leaf dry matter content; LES, leaf economic spectrum.

Reference	Rogers & Thompson (1978)		Rogers (1981)			E. W. Goolsby <i>et al.</i> (unpublished)	
Herbivore species	<i>Masonaphis masoni</i>		<i>Empoasca abrupta</i>			<i>Vanessa cardui</i>	
Order(Family)	Hemiptera (Aphididae)		Hemiptera (Cicadellidae)			Lepidoptera (Nymphalidae)	
Feeding guild	Piercing/sucking		Piercing/sucking			Leaf chewer	
Leaf Traits	# aphids at 1 wk*	# aphids at 1 month*	# leafhoppers at 1 wk†	Plant rating at 1 wk†	Plant rating at 1 month†	Fresh mass consumed (g)¶	Dry mass consumed (g)¶
Trichome density	(+)0.06	(+)0.24	(-)0.14	(+)0.04	(+)0.01	(+)0.04	(+)0.05
Thickness	(+)0.04	(+)0.09	(+)0.01	(-)0.00	(-)0.11	(+)0.02	(+)0.02
Toughness	(-)0.06	(-)0.01	(-)0.03	(+)0.00	(-)0.04	(-)0.37	(-)0.39
LDMC	(-)0.04	(-)0.02	(-)0.11	(+)0.13	(+)0.05	(+)0.94	(+)0.94
Tannin activity	(-)0.80	(-)0.92	(+)0.00	(+)0.01	(+)0.00	(-)0.02	(-)0.02
Lipid content	(-)0.10	(-)0.02	(-)0.13	(-)0.00	(+)0.29	(+)0.01	(+)0.00
Ash content	(+)0.25	(+)0.35	(-)0.00	(-)0.00	(-)0.07	(-)0.35	(-)0.35
C:N ratio	(+)0.05	(+)0.48	(-)0.02	(+)0.00	(-)0.00	(+)0.00	(+)0.00
LES PC 1	(+)0.00	(-)0.10	(+)0.08	(+)0.01	(+)0.02	(+)0.01	(+)0.15
HeliaMet PCO1	(+)0.32	(+)0.63	(-)0.10	(+)0.64	(-)0.00	(-)0.13	(-)0.15
HeliaMet PCO2	(+)0.58	(+)0.52	(+)0.00	(+)0.00	(+)0.29	(+)0.00	(+)0.00
Avg # compounds	(-)0.23	(-)0.32	(+)0.01	(-)0.23	(+)0.11	(+)0.08	(+)0.09
Compound 76	(-)0.82	(-)0.58	(-)0.03	(+)0.16	(-)0.53	(-)0.00	(-)0.00
Compound 145	(+)0.23	(+)0.20	(-)0.00	(-)0.03	(-)0.10	(+)0.94	(+)0.95
Compound 256	(+)0.67	(+)0.33	(-)0.02	(+)0.05	(-)0.07	(-)0.17	(-)0.14
Compound 368	(-)0.18	(-)0.18	(+)0.01	(-)0.05	(-)0.03	(-)0.96	(-)0.96
Compound 369	(-)0.16	(-)0.17	(+)0.04	(-)0.16	(-)0.04	(-)0.96	(-)0.96
Compound 388	(+)0.06	(+)0.13	(+)0.00	(+)0.01	(-)0.02	(+)0.02	(+)0.02
Compound 424	(-)0.50	(-)0.44	(-)0.02	(+)0.15	(-)0.47	(-)0.49	(-)0.50
Compound 426	(-)0.16	(+)0.03	(-)0.08	(+)0.27	(-)0.03	(+)0.15	(+)0.13
Compound 431	(-)0.73	(-)0.00	(-)0.08	(+)0.21	(-)0.41	(-)0.08	(-)0.08
Compound 434	(-)0.95	(-)0.89	(-)0.04	(+)0.02	(-)0.38	(-)0.11	(-)0.12
Compound 455	(+)0.10	(-)0.02	(-)0.01	(-)0.06	(-)0.08	(+)0.15	(+)0.11
Compound 486	(-)0.83	(-)0.71	(-)0.08	(+)0.18	(-)0.50	(-)0.09	(-)0.09
Compound 491	(+)0.37	(+)0.04	(+)0.08	(-)0.11	(+)0.00	(+)0.00	(+)0.00
Compound 585	(-)0.78	(-)0.86	(-)0.05	(-)0.00	(-)0.00	(-)0.04	(-)0.03
Compound 1135	(-)0.75	(-)0.62	(+)0.01	(+)0.03	(-)0.06	(-)0.01	(+)0.01
Compound 1137	(-)0.48	(-)0.12	(+)0.00	(-)0.00	(-)0.30	(+)0.66	(+)0.63
Compound 1139	(-)0.20	(+)0.32	(-)0.04	(+)0.20	(-)0.30	(-)0.03	(-)0.04
Compound 1141	(-)0.80	(-)0.85	(-)0.00	(+)0.03	(-)0.29	(-)0.06	(-)0.06
Compound 1222	(+)0.29	(+)0.52	(-)0.07	(+)0.14	(-)0.15	(-)0.17	(-)0.18
Compound 1468	(-)0.38	(-)0.90	(+)0.00	(+)0.00	(-)0.92	(+)0.79	(+)0.73
Compound 1553	(-)0.82	(-)0.76	(-)0.00	(+)0.06	(-)0.44	(+)0.00	(-)0.00
Compound 1713	(-)0.47	(-)0.39	(+)0.00	(+)0.16	(-)0.31	(+)0.28	(+)0.22
HeliaMet Subset PC1	(-)0.94	(-)0.83	(-)0.02	(+)0.11	(-)0.50	(-)0.01	(-)0.01
HeliaMet Subset PC2	(-)0.01	(-)0.01	(+)0.01	(-)0.07	(-)0.05	(+)0.23	(+)0.24

*Plants were grown under glasshouse conditions and infested with 10 aphids in laboratory enclosures, with the size of the colony recorded after 1 wk and 1 month in two separate tests.

†Plants were grown under glasshouse conditions and infested with 10 leafhoppers in laboratory enclosures, with the size of the colony recorded after 1 wk. Plant health was also rated at 1 wk and 1 month on a 0–5 scale, where 0=no visible injury, 1=slightly chlorotic, 2=moderately chlorotic, 3=severely chlorotic, 4=plant wilted, 5=plant dead.

¶Plants were grown under glasshouse conditions and individual leaves were fed to caterpillars under laboratory conditions, with mass consumed calculated.

References (Table S3)

- Benjamini Y, Hochberg Y. 1995.** Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**(1): 289-300.
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- Rogers CE, Thompson TE. 1978.** Resistance of wild *Helianthus* species to an aphid, *Masonaphis masoni*. *Journal of Economic Entomology* **71**(2): 221-222.

Table S4 Macroevolutionary correlations between leaf traits and metrics of disease and parasite resistance, as assessed by the *phylopars* method (Bruggeman *et al.*, 2009) to incorporate missing data. R^2 and directionality of correlations are presented, with those found to be significant at $P < 0.05$ in blue, and those found to be also significant at the more stringent multiple comparisons cutoff (false discovery rate; Benjamini & Hochberg, 1995) in red. LDMC, leaf dry matter content; LES, leaf economic spectrum.

Reference	Morris <i>et al.</i> (1983)	Yang <i>et al.</i> (1980)		Saliman <i>et al.</i> (1982)		Ruso <i>et al.</i> (1996)	
Pest species	<i>Alternaria helianthi</i>	<i>Rhizopus</i> spp.		<i>Erysiphe chicoracearum</i>		<i>Orobanche cernua</i>	
Taxonomy	Ascomycota	Zygomycota		Ascomycota		Orobanchaceae	
Description	Plant pathogen	Saprotrophic		Plant pathogen “powdery mildew”		Parasitic plant	
	Disease Index*	<i>R. arrhizus</i> Rot Index†	<i>R. oryzae</i> Rot Index†	Disease Index (Field)¶	Disease Index (Glasshouse)¶	Incidence§	Degree of Attack§
Leaf Traits							
Trichome density	(-)0.02	(-)0.23	(+)0.01	(-)0.36	(+)0.02	(+)0.09	(-)0.00
Thickness	(+)0.08	(+)0.02	(+)0.06	(-)0.03	(+)0.22	(+)0.15	(+)0.00
Toughness	(+)0.14	(-)0.01	(-)0.05	(-)0.00	(-)0.00	(-)0.05	(-)0.04
LDMC	(-)0.06	(-)0.06	(-)0.02	(+)0.01	(-)0.18	(-)0.03	(-)0.02
Tannin activity	(-)0.09	(+)0.04	(+)0.01	(+)0.01	(-)0.10	(-)0.28	(-)0.06
Lipid content	(+)0.00	(+)0.04	(+)0.04	(+)0.24	(+)0.01	(-)0.05	(-)0.01
Ash content	(+)0.03	(-)0.05	(+)0.01	(+)0.17	(+)0.17	(+)0.02	(-)0.05
C:N ratio	(-)0.02	(+)0.03	(+)0.01	(+)0.00	(-)0.16	(-)0.05	(-)0.02
LES PC 1	(+)0.00	(-)0.03	(-)0.00	(-)0.00	(+)0.06	(+)0.02	(+)0.03
HeliaMet PCO1	(-)0.01	(+)0.01	(+)0.03	(-)0.03	(-)0.00	(+)0.17	(+)0.05
HeliaMet PCO2	(+)0.13	(-)0.00	(+)0.10	(+)0.06	(+)0.13	(+)0.37	(+)0.14
Avg # compounds	(+)0.01	(+)0.00	(-)0.00	(+)0.02	(+)0.00	(-)0.07	(-)0.00
Compound 76	(-)0.06	(+)0.02	(-)0.03	(-)0.28	(-)0.53	(-)0.07	(+)0.00
Compound 145	(-)0.02	(-)0.38	(-)0.68	(-)0.05	(-)0.46	(-)0.01	(-)0.01
Compound 256	(-)0.04	(-)0.04	(-)0.20	(-)0.14	(-)0.49	(-)0.03	(-)0.00
Compound 368	(+)0.02	(-)0.06	(-)0.03	(+)0.01	(-)0.16	(-)0.14	(-)0.09
Compound 369	(-)0.00	(-)0.20	(-)0.10	(+)0.00	(-)0.40	(-)0.16	(-)0.10
Compound 388	(-)0.04	(-)0.01	(-)0.07	(+)0.04	(-)0.33	(+)0.01	(+)0.02
Compound 424	(+)0.03	(+)0.00	(-)0.10	(-)0.27	(-)0.56	(-)0.25	(-)0.11
Compound 426	(+)0.02	(-)0.40	(-)0.51	(-)0.16	(-)0.36	(+)0.01	(-)0.01
Compound 431	(-)0.05	(+)0.01	(-)0.02	(-)0.18	(-)0.58	(-)0.11	(-)0.01
Compound 434	(-)0.08	(-)0.21	(-)0.59	(-)0.01	(-)0.57	(-)0.04	(-)0.02
Compound 455	(+)0.03	(-)0.03	(-)0.14	(+)0.11	(+)0.00	(-)0.03	(-)0.00
Compound 486	(-)0.20	(+)0.10	(-)0.00	(-)0.13	(-)0.17	(-)0.36	(-)0.26
Compound 491	(-)0.01	(-)0.00	(-)0.12	(+)0.09	(+)0.01	(+)0.00	(+)0.08
Compound 585	(+)0.00	(+)0.10	(+)0.03	(+)0.16	(-)0.04	(-)0.34	(-)0.26
Compound 1135	(+)0.00	(+)0.12	(+)0.02	(-)0.20	(-)0.52	(-)0.05	(+)0.12
Compound 1137	(+)0.01	(-)0.29	(-)0.65	(-)0.26	(-)0.35	(-)0.01	(+)0.00
Compound 1139	(-)0.01	(+)0.04	(+)0.00	(-)0.28	(-)0.54	(+)0.33	(+)0.35
Compound 1141	(-)0.03	(+)0.07	(+)0.01	(-)0.11	(-)0.62	(-)0.61	(-)0.09
Compound 1222	(-)0.00	(+)0.00	(-)0.04	(-)0.19	(-)0.66	(+)0.01	(-)0.00
Compound 1468	(+)0.01	(+)0.06	(+)0.01	(+)0.76	(-)0.51	(+)0.27	(+)0.79
Compound 1553	(-)0.06	(-)0.00	(-)0.13	(-)0.20	(-)0.60	(-)0.07	(+)0.00
Compound 1713	(+)0.10	(+)0.19	(+)0.03	(-)0.35	(+)0.00	(+)0.01	(+)0.15
HeliaMet Subset PC1	(-)0.01	(-)0.00	(-)0.10	(-)0.29	(-)0.68	(-)0.08	(-)0.02
HeliaMet Subset PC2	(-)0.01	(-)0.31	(-)0.45	(+)0.00	(-)0.13	(-)0.03	(-)0.01

*Plants inoculated under glasshouse conditions. Degree of infection defined on a 0–5 scale, where 0=no infection, 1=0.1–5% leaf area infected, 2=6–25% leaf area infected, 3=26–50% leaf area infected, 4=51–75% leaf area infected, 5=75–100% leaf area infected.

†Plants inoculated under field conditions. Rot index defined on a 0–5 scale, where 0=no rot, 1=rot near inoculation site, 2=rot exceeding inoculation site but <25% leaf area, 3= 25–50% receptacle area, 4=50–75% receptacle area, 5= >75% receptacle.

¶Plants inoculated and observed in setting listed. Disease index defined on a 0–3 scale, where 0=no infection, 1=a few colonies present, 2=colonies on 11–50% leaf area, 3=colonies on >50% of leaf area.

§Plants grown in infested soil under glasshouse conditions, and transferred to field before assessment at blooming. Incidence defined as the proportion of infected plants. Degree of attack defined as the average number of broomrapes per sunflower plant.

References (Table S4)

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