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Supplemental Information

Evolutionary changes in an invasive plant support the defensive role of plant volatiles

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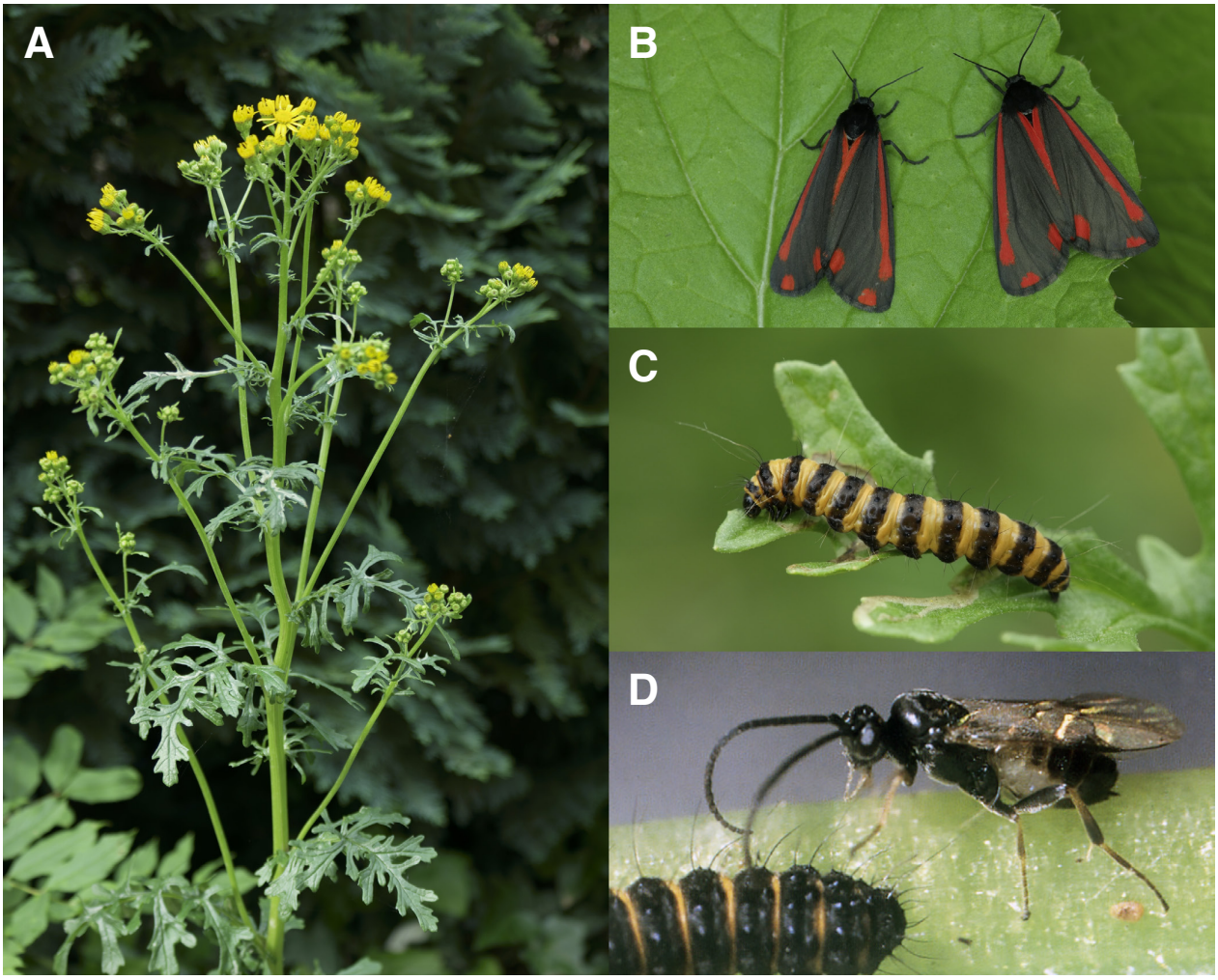

Figure S1. The tritrophic study system. Related to Figure 1-4. (A) *Jacobaea vulgaris* is the host plant of (B), (C) the specialist herbivore *Tyria jacobaeae*, which is parasitized by (D), the specialist parasitoid wasp *Cotesia popularis*. Pictures from Thomas Degen (A-C) and Eddy van der Meijden (D).

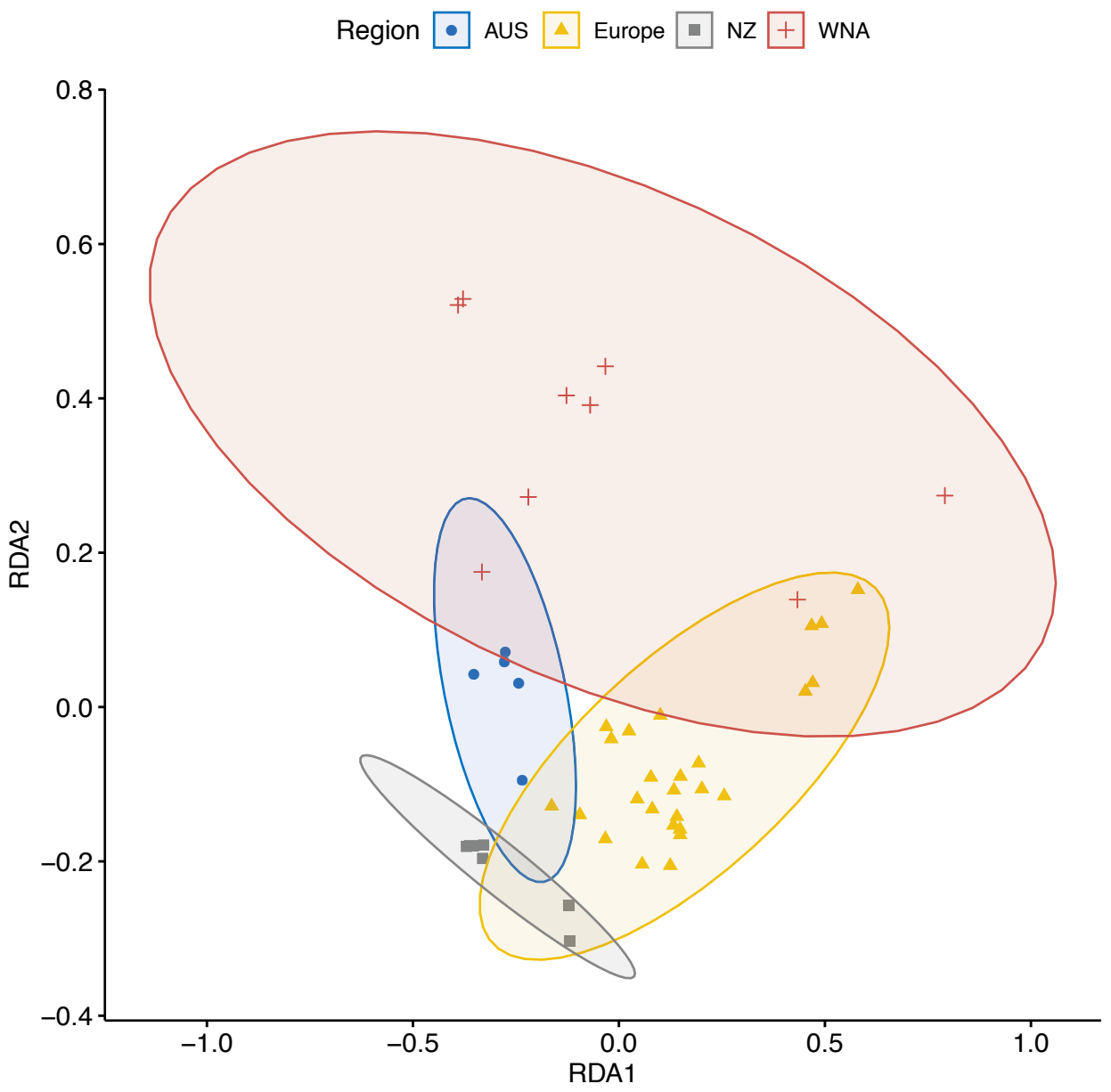

Figure S2. Redundancy discriminant analyses (RDA) of 19 bioclimatic variables from each of the sampled *J. vulgaris* populations. Related to STAR Methods and Table S2. *n* = 5 for Australia, *n* = 8 for New Zealand, *n* =10 for Western North America and *n* = 26 for Europe). Analysis of Deviance(type 2), Range: $\chi^2_{(3)} = 34.481$, $P < 0.001$.

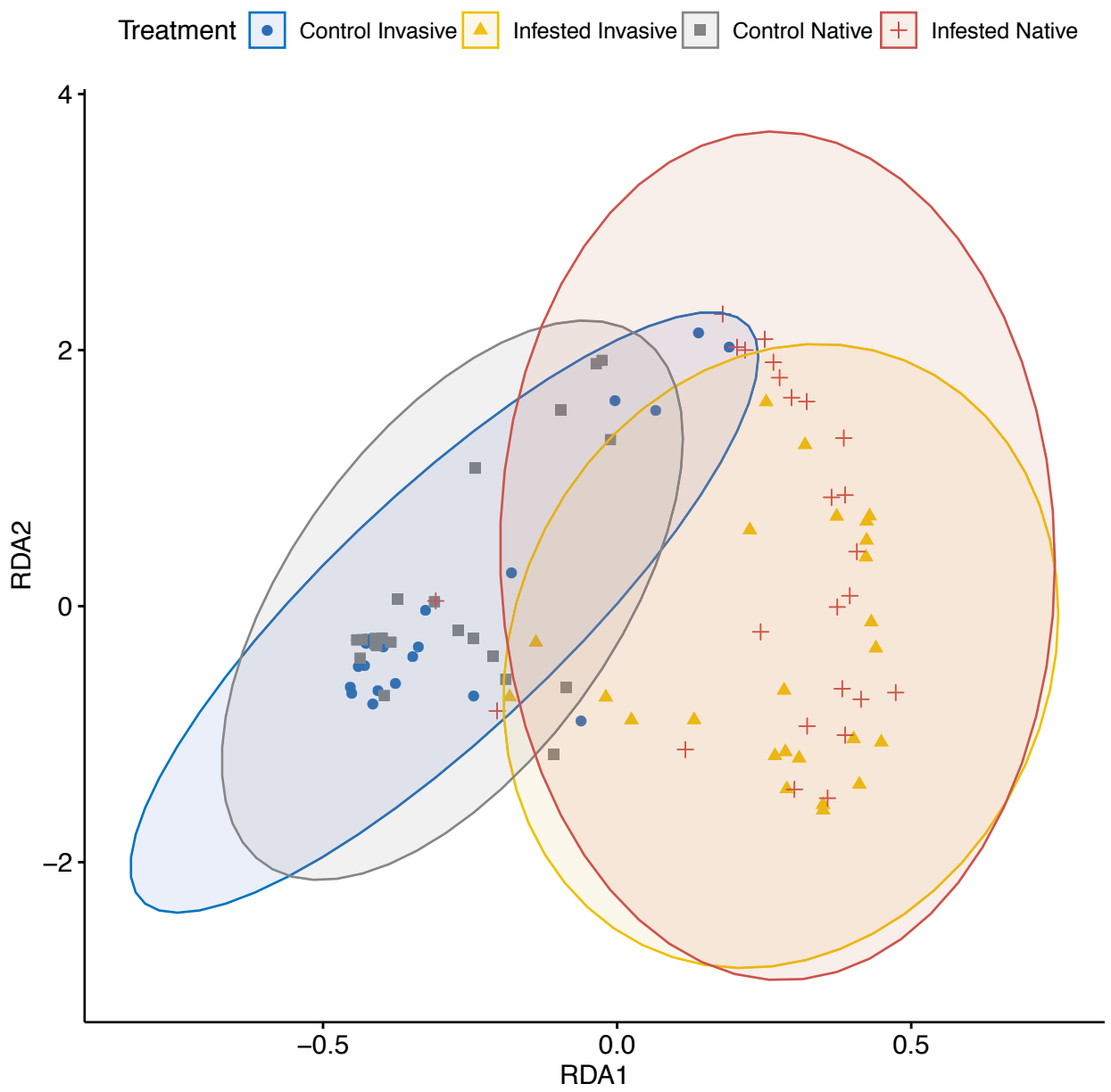

Figure S3 . Redundancy discriminant analyses (RDA) of total leaf volatile emissions (ng/24h) of native and invasive *J. vulgaris* genotypes before and after infestation with 15 first instar *T. jacobaeae* larvae for 24 hours. Related to Figure 4. Different treatments were marked with different symbols and colors. Control Native = native *J. vulgaris* genotypes before infestation, Infested Native = native *J. vulgaris* genotypes after infestation, Control Invasive = invasive *J. vulgaris* genotypes before infestation, Infested Invasive = *invasive J. vulgaris* genotypes after infestation. Analysis of Deviance(type 2), Timing: $\chi^2_{(1)}$ = 239.343, P < 0.001; origin: $\chi^2_{(1)}$ = 0.011, P = 0.92; timing x origin: $\chi^2_{(1)}$ = 0.071, P = 0.79.

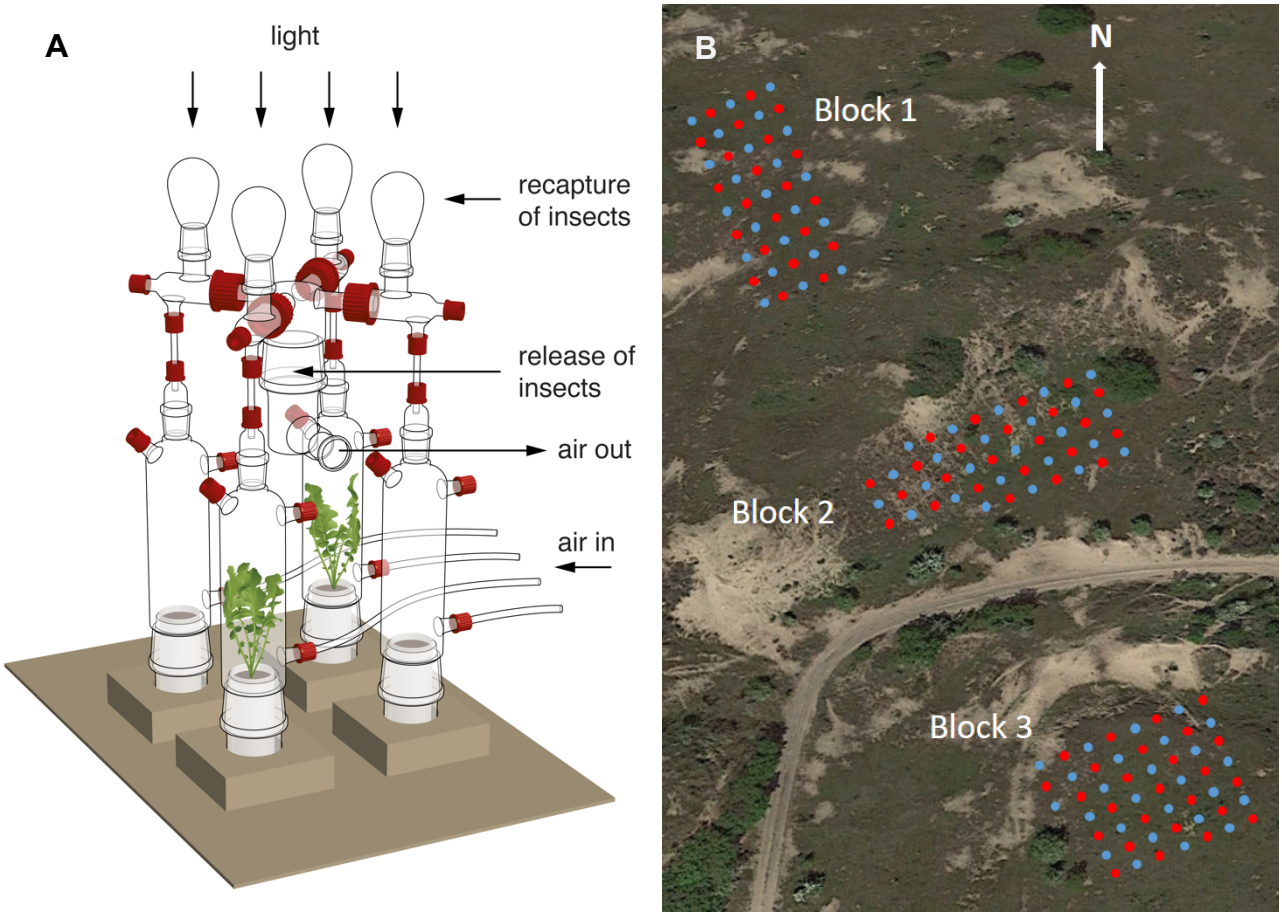

Figure S4. Four-arm olfactometer and layout of the field experiment. Related to Figure 1, 4 and STAR Methods. (A) Depiction of the four-arm olfactometer used to assess attraction of the study insects to plant volatiles. (B) Situation of the field experiment conducted in the dunes of Meijendel (near The Hague) in The Netherlands. Pairs of native and invasive *J. vulgaris* plants were placed in three blocks. Blue dots represent a pair of native plants that are 15 cm apart from each other, red dots represent a pair of invasive plants that are 15 cm apart from each other. The distance between pairs of plants was 5 m.

	Block 1			Block 2			Block 3		Total			Grand Total	
	native	invasive		native	invasive		native	invasive	native	invasive			
Number of plant pairs													
total	23	22		25	24		26	26	74	72		146	
with larvae retrieved	13	17	(18)*	21	22	(23)*	19	21	53	60	(62)*	113	(115)*
with larvae not parasitized	11	13	(14)*	14	11	(11)*	11	11	36	35	(36)*	71	(72)*
with larvae parasitized	1	4	(4)*	7	11	(12)*	8	7	16	22	(23)*	38	(39)*
where all larvae died during rearing	1	0	(0)*	0	0	(0)*	0	3	1	3	(3)*	4	(4)*
Number of larvae													
1st instar placed on pairs	920	880		1000	960		1040	1040	2960	2880		5840	
that stayed in the tube	15	13		28	23		21	24	64	60		124	
actual on pairs	905	867		972	937		1019	1016	2896	2820		5716	
retrieved after 12 days	98	154	(245)*	206	344	(414)*	118	116	422	614	(775)*	1036	(1197)*
died during rearing	25	52	(65)*	85	113	(124)*	39	37	149	202	(226)*	351	(375)*
pupated (not parasitized)	71	97	(175)*	96	205	(258)*	67	69	234	371	(502)*	605	(636)*
parasitized	2	5	(5)*	25	26	(32)*	12	10	39	41	(47)*	80	(86)*
Parasitism rate in %	**2.7**	**4.9**	**(2.8)***	**20.7**	**11.3**	**(11.0)***	**15.2**	**12.7**	**14.3**	**10.0**	**(8.6)***	**11.7**	**(11.9)***

*In two blocks one plant pair contained extra larvae due to a hatched egg batch from natural occurring *T. jacobaeae*. In parentheses the number of plant pairs and of larvae, respectively, including the hatched egg batch from natural occurring *T. jacobaeae*

Table S1. Parasitism rates and infestation of plants in the field. Related to STAR Methods. The table summarizes the number of native and invasive pairs of *J. vulgaris* plants and the number of *T. jacobaeae* larvae placed in the three blocks in the dunes of Meijendel (near The Hague) in The Netherlands.

Origin	Country	Location	Moths	Parasitoids	Longitude	Latitude
Invasive	Australia	Beech Forest, Victoria		+	143°33' E	38°38' S
		Dairy Plains, Tasmania	+	+	146°31' E	41°38' S
		Franklin 1, Tasmania,	+	+	146°59' E	43°05' S
		Franklin 2, Tasmania,	+		146°59' E	43°05' S
		Mole Creek, Tasmania	+	+	146°24' E	41°33' S
		Targa, Tasmania	+	+	147°23' E	41°18' S
	New Zealand	Burkes Pass, Canterbury		+	170°36' E	44°04' S
		Matapouri	+	+	174°30' E	35°33' S
		West dome, South island	+	+	168°12' E	45°35' S
		Whananaki S Rd (lower valley)		+	174°24' E	35°33' S
		Whananaki S Rd (upper valley)	+	+	174°26' E	35°32' S
		Whangarei, Pukeatua Rd 1	+	+	174°12' E	35°47' S
		Whangarei, Pukeatua Rd 2		+	174°12' E	35°46' S
		Whangarei, Whangarei Heads Rd	+	+	174°26' E	35°46' S
	Western North America	Cambell River, Vancouver Island	+	+	125°16' W	50°01' N
		Gabriola Island, Marvin road	+	+	123°42' W	49°09' N
		Gabriola Island, Westgyle road	+	+	123°42' W	49°09' N
		Kelowna, Chute Lake Rd	+	+	119°31' W	49°31' N
		Kootenai National Forest, Montana		+	114°53' W	48°17' N
		Nanaimo, Vancouver Island	+	+	123°56' W	49°09' N
		Orick 1, McDonald Creek Rd, California	+		124°05' W	41°12' N
		Orick 2, McDonald Creek Rd, California		+	124°03' W	41°17' N
		Orick 3, California	+	+	124°03' W	41°17' N
		Orick 4, California		+	124°03' W	41°17' N
		Port McNeill, Vancouver Island	+	+	127°05' W	50°35' N
		Victoria 1, Vancouver Island		+	123°21' W	48°25' N
		Victoria 2, Vancouver Island		+	123°21' W	48°25' N
Native	Belgium	Bertogne	+	+	05°40' E	50°05' N
	Finland	Inkoo, Kirkkonummi		+	24°00' E	60°02' N
	France	Brilllac Bretagne	+	+	02°48' W	47°32' N
		Les Essarts	+	+	01°10' W	46°07' N
		Le Sparre Medoc	+	+	00°54' W	45°17' N
		Lourdes	+	+	00°13' W	43°09' N
		Merpuis		+	05°26' W	46°07' N
		Pontorson Bretagne		+	01°36' W	48°33' N
	Germany	Nassenheide		+	13°13' E	52°49' N
		Niederwörresbach	+	+	07°20' E	49°46' N
		Pfingstberg	+	+	13°52' E	53°08' N
	Ireland	Dublin	+	+	06°15' W	53°20' N
	Netherlands	Bilthoven	+	+	05°12' E	52°08' N
		Den Helder	+	+	04°45' E	52°57' N
		Lelystad	+	+	05°27' E	52°29' N
		Meijendel	+		04°20' E	52°07' N
		Overveen		+	04°35' E	52°22' N
		Veluwe		+	05°49' E	52°14' N
		Wageningen	+	+	05°34' E	52°10' N
	Poland	Pulawy		+	21°59' E	51°24' N
	Sweden	Sit Olofsholm, Gotland	+	+	18°54' E	57°43' N
	UK	Corston	+		02°26' W	51°23' N
		Marshgreen	+	+	03°21' W	50°44' N
		Silwood park, Berks London	+	+	00°38' W	51°24' N
		Sinderhope	+	+	02°14' W	54°01' N
		Warcop		+	02°32' W	54°32' N
		Wytham		+	01°18' W	51°46' N

Table S2. Origin of the seeds of *Jacobaea vulgaris* used in the moth olfactometer and oviposition bioassays and the parasitoid olfactometer bioassays, respectively. Related to Figure 1, 2 and 4. Seeds from 18 native and 18 invasive populations were used in the moth assays, whereas seeds from 25 native and 25 invasive populations were used in the parasitoid olfactometer assay.

Origin	Country	Location	Longitude	Latitude	Number of plants
Invasive	Australia	Franklin	146°59' E	43°05' S	13
		Mole Creek	146°24' E	41°33' S	14
	New Zealand	Canterbury	170°36' E	44°04' S	5
		Otahuhu	174°50' E	36°56' S	5
		Otatara	168°17' E	46°25' S	6
		West Dome	168°12' E	45°35' S	12
		Whangarei	174°12' E	35°47' S	2
	Western North America	Chute, British Columbia	119°36' W	49°29' N	5
		Campbell river, British Columbia	125°16' W	50°01' N	2
		Gabriola Island, Marvin road	123°42' W	49°09' N	13
		Gabriola Island, Westgyle road	123°42' W	49°09' N	10
		Nanaimo, British Columbia	123°56' W	49°9' N	14
		Orick 1	124°03' W	41°17' N	7
		Orick 2	124°03' W	41°17' N	13
		Port McNeill, British Columbia	127°05' W	50°35' N	10
		Victoria	123°21' W	48°25' N	13
Native	Belgium	Filly	05°41' E	50°08' N	8
		Leut	05°44' E	50°59' N	12
	France	Jublains	00°29' E	48°15' N	7
		Lac de Lecy	06°26' E	45°59' N	5
		La Rochette	02°39' E	48°30' N	3
		Santenay	04°41' E	46°54' N	11
		Vavrette	05°17' E	46°07' N	10
		Villey St Etienne	05°58' E	48°43' N	10
	Germany	Idar Oberstein	07°18' E	49°42' N	3
	Ireland	Dublin	06°15' W	53°20' N	0
	Netherlands	Den Helder	04°45' E	52°57' N	10
		Zwanenwater	05°27' E	52°29' N	10
		Bilthoven	05°12' E	52°08' N	8
		Koningshof	04°35' E	52°22' N	9
		Meijdendel	04°20' E	52°07' N	12
	Poland	Mielenko	16°00' E	54°14' N	13
	Switzerland	St Imier	06°59' E	47°09' N	5
		Neuchâtel	07°01' E	47°01' N	11
	UK	Wytham	01°18' W	51°46' N	1

Table S3. Origin of the seeds used for the field experiment. Related to STAR Methods. We used seeds of 19 native and 16 invasive populations of *J. vulgaris* genotypes.

class	NIST ID	retention time (RT)	identification
aromatics	benzaldehyde	9.25	MS and RT
	unknown aromatic	26.56	MS
FAD GLVs	(*E*)-2-hexenal	6.78	MS and RT
	(*Z*)-3-hexen-1-ol	6.84	MS and RT
	(*Z*)-2-hexen-1-ol	7.11	MS and RT
	(*Z*)-3-hexenyl acetate	10.28	MS and RT
FAD other	4-methyl-octane	6.95	MS
	1-nonene	7.61	MS and RT
	(*E*)-2-decenal,	10.73	MS
	5-methyl-undecane	11.32	MS
	4,7-dimethyl-undecane	12.29	MS
	nonanal	12.35	MS and RT
	decanal	14.66	MS and RT
	1-pentadecene	21.43	MS and RT
terpenoids	trans-ocimene	11.14	MS and RT
	(*E*)-4,8-dimethyl-1,3,7-nonatriene	12.59	MS and RT
	β-copaene	19.16	MS
	β-caryophyllene	19.85	MS and RT
	β-bergamotene	20.07	MS and RT
	unknown terpene 1	20.19	MS
	(*E*)-β-farnesene	20.63	MS and RT
	(+)-Epi-bicyclosesquiphellandrene	20.88	MS
	germacrene D	21.30	MS
	unknown terpene 2	21.53	MS
	bicyclogermacrene	21.67	MS
	α-farnesene	21.84	MS
	β-cadinene	22.26	MS
	α-muurolene	22.60	MS
	unknown terpene 3	25.93	MS
	unknown terpene 4	29.02	MS
	farnesol	23.47	MS

Table S4. List of the 31 quantified volatile compounds, assigned to four chemical classes, aromatics, fatty acid derived (FAD) green leaf volatiles (GLV) and other compounds as well as terpenoids. Related to Figure 3. The identification of the compounds was either tentative and based only on the mass spectra (MS) or in addition proven by the injection of pure standards (MS and RT).