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Limited evidence for allelopathic effects of Giant Hogweed on germination of native herbs

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Abstract Invasive alien plants often occur in monospecific stands with high density in the invaded range. Production of bioactive secondary metabolites in such stands could have allelopathic effects on germination of native species. We tested this component of the novel weapon hypothesis for *Heracleum mantegazzianum*, a prominent invader in Europe, using seeds of eleven native herbs exposed to soil or soil extracts from invaded stands, moist seeds or seed extracts of *Heracleum mantegazzianum*. There was no effect of the various treatments on germination of most species, while germination was reduced in *Urtica dioica* on invaded soil, in *Poa trivialis* with *Heracleum mantegazzianum* seed extract, and negative effects of the essential oil bergapten were found in three species. In *Poa trivialis* the results of the seed extract were not supported by the experiment with added seeds of the invasive plant. Thus, there is limited evidence for allelopathic effects of the invasive *Heracleum mantegazzianum* on germination of co-occurring native herbs.

Key words: Allelopathy; bergapten; *Heracleum mantegazzianum*; novel weapon; *Poa trivialis;* seed extract; *Urtica dioica*

Introduction

Invasive alien plant species have considerable effects on natural ecosystems and land use, because they displace native communities (e.g. Thiele *et al.*, 2010), and change ecosystem processes (e.g. McNeish *et al.*, 2012). One of the current explanations for the exceptional success of invasive plants is the 'novel weapon hypothesis' (Callaway and Ridenour, 2004). It predicts plant invasions based on the ability to release novel phytochemicals in the invaded ecosystem. These allelopathic compounds have phytotoxic or fitness-reducing effects on the susceptible non-coevolved competitors; here 'allelopathy' is used in a broad sense (Inderjit and Weiner, 2001). The novel weapon hypothesis has been introduced to understand the invasion success of *Centaurea diffusa* (Callaway and Aschehoug, 2000; but see Blair *et al.*, 2006), *Alliaria petiolata* (Prati and

Bossdorf, 2004), and *Solidago canadensis* (Abhilasha *et al.*, 2008). More recently, Yan *et al.* (2010) showed negative effects of phenolic compounds of the invasive alien *Merremia umbellata* on germination of *Arabidopsis thaliana*.

Thus, the novel weapon hypothesis might also help understanding the success of the Giant Hogweed (*Heracleum mantegazzianum*) in Europe. However, despite numerous studies on population dynamics and management of this problematic plant (cf. Pyšek *et al*., 2007), there are no published data on allelopathy. It is well known, that species within the Apiaceae family produce a multitude of secondary metabolites, such as coumarins, essential oils, flavones, terpenes and acetylenic compounds (Bohlmann, 1971), and furanocoumarins are characteristic for the Peucedaneae tribe to which the genus *Heracleum* belongs (Molho *et al.*, 1971). These enzyme-inhibiting substances support plant defence against herbivorous insects and pathogens (Murray *et al.*, 1982), as also described for *H. mantegazzianum* (Hattendorf *et al.*, 2007). Seed germination can be negatively affected by plant leachates (Ruprecht *et al*., 2008; Hassan *et al*., 2012), and already Baskin *et al*. (1967) showed that psoralen and furanocoumarins present in seeds of Apiaceae are responsible for inhibition of a competing species of *Psoralea subacaulis*. Junttila (1976) found inhibitory effects of the furanocoumarins of *Heracleum laciniatum* on the germiation of lettuce, as also supported by Reynolds (1989). A recent study on coumarins as allelopathic agents comes from Razavi (2011). However, our study is the first attempt to investigate whether or not the furanocoumarins produced by *H. mantegazzianum* have negative effects on the germination of native species, and thus may act as a novel weapon facilitating invasion. Allelopathy could be due to leaf litter, seeds or root exudates of the species acting directly or mediated through the soil (P. Dostal, pers. comm.).

The focus of this study is on soil and seed effects on germination of co-occurring native herbs. Thus, a series of experiments was performed to investigate whether or not germination of these species is negatively affected by soil or soil extracts from *H. mantegazzianum* stands, or by seeds or seed extracts of the invasive alien species.

Materials and methods

Study species

Heracleum mantegazzianum Sommier and Levier (Giant Hogweed, Apiaceae) is a monocarpic, perennial tall forb (Tiley *et al.*, 1996). It is native in the Western Greater Caucasus, where it occurs in tall-herb vegetation, abandoned grasslands, forest clearings and alluvial forests (Otte *et al.*, 2007). *Heracleum mantegazzianum* has invaded most temperate regions of Europe and North America. It often grows along roads, rivers and forest margins, on abandoned grasslands, rubbish dumps and other urban habitats (Pyšek and Pyšek, 1995; Thiele and Otte, 2006).

Like other members of the Apiaceae, *H. mantegazzianum* is an aromatic plant producing essential oils. The fruits (‘seeds’) of *H. mantegazzianum* are 6–18 mm long and 4–10 mm wide, with four oil ducts on the outer and two on the inner surface. *Heracleum mantegazzianum* is known to contain high concentrations of furanocoumarins in its roots, leaves and seeds (Molho *et al*., 1971). The following furanocoumarins occur in seeds (Herde, 2005; in descending concentrations): angelicin, imperatorin, bergapten, pimpinellin, unknown hydroxycoumarin, isopimpinellin, unknown furanocoumarin, sphondin, psoralen and xanthotoxol; Glowniak *et al.* (2000) also found limettin and a derivative of anisocoumarin.

To investigate whether or not the invasive plant has allelopathic effects on germination of other plants we selected eleven native species that co-occur with *H. mantegazzianum* in the invaded range in NW Europe (Thiele and Otte, 2006). *Heracleum mantegazzianum* seeds were collected in January and October 2008 from 25 plants within large populations on degraded peatlands near Hillerød (55.914943N, 12.3058E) and Ballerup, eastern Denmark (55.758017N, 12.282639E); seeds were stratified at 1–6 °C until late March. Seeds of the native species were obtained from the Botanical Gardens, University of Copenhagen, except *Calystegia sepium* (L.) R.Br. (Botanical Garden Graz). For logistic reasons not all experiments could be done with the full species set.

Germination experiments

Experiment 1 was done with seeds of *H. mantegazzianum*, *R. obtusifolius* L. and *Urtica dioica* L. on soil sampled from invaded and un-invaded sites with otherwise similar conditions. In early March 2008 the soil was collected from 19 locations near Copenhagen (Ballerup-Knadrup, Faxe Bay, Hillerød), eastern Denmark. The soil was sieved and placed as 2-cm layer in transparent plastic boxes with lid (11.5 cm x 7.7 cm x 4.5 cm). Within the boxes 40 seeds of one species were exposed on blotting paper (Munktell Filter Paper Grade 3 W) placed on top of ca. 200 ml moist soil. Sample size was 111 boxes [(19 invaded soil + 18 un-invaded soil) x 3 species]; one sample of un-invaded soil was lost. The design was completely randomised, and boxes were rearranged at each date of counting. The experiment started in late March 2008 in a climate cabinet set to 10/20 °C (12 hours light). Germination was recorded over 8 weeks; seeds were considered germinated when the radicle had emerged, and seedlings were removed.

Experiment 2 investigated effects of aqueous extract of soil from *H. mantegazzianum* stands on germination of *Lapsana communis* L. and *R. obtusifolius*. Peat soil was collected at the above location near Hillerød from invaded and un-invaded sites in early March 2007. The soil samples were pooled, sieved, homogenized and stored in the greenhouse. Soil extracts were prepared by adding 5 l of water to 5 l air-dried soil, stirring the mixture and letting it rest for 2 hours. The overstanding water was transferred to other containers; the extract of the invaded soil had pH 7.3 and a conductivity of 218 µS, compared with pH 6.5 and 174 µS for un-invaded soil. About 50 ml extract was filled into the plastic boxes, and seeds of the study species were placed on a plastic bridge inside the box covered with blotting paper. Samples comprised 20 seeds and were repeated eight times per species, invaded and un-invaded soil; as control, the set up was repeated with de-ionized water. The total number of samples was 48 (3 treatments x 2 species x 8 replications); one sample of *R. obtusifolius* with invaded soil became damaged and had to be excluded. Germination was recorded as above for 5 weeks.

Experiment 3 focussed on allelopathic effects of moist seeds of *H. mantegazzianum* on germination of *Brachypodium sylvaticum* (Huds.) P.B., *C. sepium*, *Euphorbia helioscopia* L., *Festuca gigantea* L., *Mentha arvensis* L., *Poa trivialis* L., *R. obtusifolius* L., *Vicia hirsuta* (L.) Grey, and *U. dioica*. The seeds of *C. sepium* and *V. hirsuta* were manually scarified by scratching the seed coat with sand paper as recommended by Baskin and Baskin (1998). The experiments were conducted in Petri dishes (BD Falcon Optilux™, 10 cm x 2 cm) on blotting paper (9 cm diameter), moistened with de-ionized water. Ten control dishes were prepared for each of the native species by placing 40 seeds per dish in a regular 8 mm x 8 mm grid pattern. In the mixed treatment 21 seeds of *H. mantegazzianum* were evenly distributed between the seeds of the native species. Sample size was 180 Petri dishes, i.e. 10 replications per treatment and species. The dishes were cold stratified in a refrigerator set to 4 °C for 3 weeks, after which they were transferred to a climate cabinet set to 10/20 °C and 12 hours light. Germination was recorded as above for 18 weeks.

Experiment 4 tested effects of *H. mantegazzianum* seed extracts on germination of *M. arvensis*, *P. trivialis*, *Sonchus oleraceus* L. and *U. dioica*. Seeds of these native species were exposed in five Petri dishes, respectively, to six treatments. In treatment 1, 40 seeds of each species were placed in a regular 8 mm x 8 mm grid pattern on blotting paper moistened with de-ionized water. In treatment 2, 21 seeds of *H. mantegazzianum* were added to the native seeds. For treatment 3 and 4, *H. mantegazzianum* seeds were frozen in liquid N, ground with pestle and mortar, and two concentrations of aqueous solution of ground seeds (0.02% and 0.2%; estimated after Herde, 2005) were used to moisturize the blotting papers with native seeds. In treatment 5, the blotting paper was moistened with a 0.2% bergapten solution (Sigma-Aldrich, 69664, Fluka, 484-20-8) in 5% DMSO, and treatment 6 was a control with aqueous 5% DMSO solution (Dimethyl sulfoxide; Sigma-Aldrich, CAS67-68-5). The furanocoumarin bergapten was chosen, because it is common in seeds of the study species and was readily available. All Petri dishes were placed in a climate cabinet at 10/20 °C and 12 hours light, and seed germination was recorded as above for 14 weeks.

Statistical analyses

We calculated mean proportions of germinated seeds as the sum of all germinated seeds divided by the total number of exposed seeds within each combination of treatment and species. Standard errors (SE) of the mean proportions were calculated using the equation

$$SE = \sqrt{\frac{p \times (1-p)}{n}}$$

where p is the proportion of germinated seeds and n is the number of exposed seeds (Crossley, 2008). Effects of treatments were assessed with tests of equal proportions ('prop.test' from the 'binom' package; Dorai-Raj, 2009) conducted on all pairwise comparisons of treatments within species. All statistical calculations were done in R 2.14.1 (R Development Core Team, 2011).

Results

Soil from stands of the invasive alien *H. mantegazzianum* significantly reduced germination in the co-occurring native herb *U. dioica* compared with similar soil from nearby vegetation (Table 1; test of equal proportions, $P < 0.001$). However, there was no significant difference in germination of *R. obtusifolius* and *H. mantegazzianum* on invaded and un-invaded soil (Experiment 1; $P > 0.05$). Soil extracts from stands of *H. mantegazzianum* had no significant effects on germination of *L. communis* and *R. obtusifolius* (Experiment 2; $P > 0.05$). There was also no significant difference between un-invaded soil extract and de-ionized water as a control ($P > 0.05$). Of the nine native herbaceous species tested in Experiment 3 only *C. sepium* showed reduced germination with seeds of *H. mantegazzianum* present ($P < 0.01$). Hogweed seeds and light seed extract had no negative effects on the four species tested in Experiment 4, while the stronger seed extract negatively affected germination of *P. trivialis* ($P < 0.001$) and *U. dioica* ($P < 0.05$) compared with germination on blotting paper with de-ionised water ('control'). Bergapten in DMSO solution affected germination of *S. oleraceus* ($P < 0.01$) and *U. dioica* ($P < 0.05$) more strongly than DMSO solution without bergapten.

Discussion

The germination experiments conducted with soil, soil extracts, seeds or seed extracts of *H. mantegazzianum* showed only limited and partly inconsistent negative effects on eleven native plant species. Germination of *U. dioica* was reduced by 11–33 % through strong seed extract, bergapten and soil from invaded stands (increasing order). *P. trivialis* was affected by strong seed extract, but not by bergapten, while *S. oleraceus* showed the opposite pattern (both were not tested in the soil experiment). *C. sepium* was the only species with reduced germination in presence of *H. mantegazzianum* seeds. Negative effects of root exudates of *H. mantegazzianum* on germination of *Dactylis glomerata* and *Plantago lanceolata* were found in a recent experiment conducted by P. Dostal *et al.* (pers. comm.). In their studies, soils from dominant stands of *H. mantegazzianum* showed variable patterns of allelopathic effects depending on target species and presence of soil biota. These findings underpin that allelopathic effects may be species-specific and depend on the source of the allelochemicals used in experiments.

The experiments with soil from *H. mantegazzianum* stands on *U. dioica* indicate that some compounds from this invasive species could have inhibitory effects on native plants from NW Europe. The apparent inconsistency with the results from the seed experiments could be due to indirect effects of these allelochemicals on native plants through changes in the chemical or microbial conditions of the soil (cf. Weir et al., 2004), or due to different concentrations of potential allelochemicals in soil, aqueous solutions and extracts from seeds.

Another possible explanation could be the enrichment and accumulation of inhibitory compounds in soil over time. Friedman et al. (1982) identified the coumarin xanthotoxin from the epicuticular waxes of the seeds of *Ammi majus* as a major compound in aqueous leachates inhibiting germination. Though Friedman *et al.* (1982) found a slow rate of efflux, with the inhibitory potential of the leachate increasing after 4 days, in many cases the presence of potential allelochemicals in the soil seems to be ephemeral (Weidenhamer and Callaway, 2010). While the identification of potential inhibitory compounds is relatively easy (e.g. Glowniak *et al*., 2000), it is a much more challenging task to measure the leaching and degradation of these compounds.

Table 1. Germination percentages (means ± SE) from four experiments on effects of the invasive alien *Heracleum mantegazzianum* on germination of native herbs in the invaded range. Treatments include soil and soil extracts from invaded sites vs. un-invaded sites, mixtures of native seeds with 21 seeds of *H. mantegazzianum* per Petri dish, extracts of ground seeds of *H. mantegazzianum* at two concentrations (light, strong), bergapten in DMSO solution, and DMSO solution without bergapten. In the control treatments, seeds were exposed to de-ionized water. Values without common superscript letters are significantly different (test of equal proportions; P < 0.05). In rows without letters there were no significant differences.

	Invaded soil	Un-invaded soil				Control
Heracleum mantegazzianum	17.9 ± 1.4	15.3 ± 1.3				
Rumex obtusifolius	5.4 ± 0.8	4.3 ± 0.8				
Urtica dioica	14.5 ± 1.3^{B}	21.7 ± 1.5^{A}				
	Invaded soil extract	Un-invaded soil extract				
Lapsana communis	18.1 ± 3.0	21.3 ± 3.2				13.1 ± 2.7
Rumex obtusifolius	77.5 ± 3.8	79.4 ± 3.2				76.9 ± 3.3
	Hogweed seeds					
Brachypodium sylvaticum	6.3 ± 1.2					8.5 ± 1.4
Calystegia sepium	39.0 ± 2.4^{B}					48.8 ± 2.5^{A}
Euphorbia helioscopia	31.0 ± 2.3					30.8 ± 2.3
Festuca gigantea	95.6 ± 1.1					95.3 ± 1.1
Mentha arvensis	47.0 ± 2.5					45.0 ± 2.5
Poa trivialis	69.8 ± 2.3					70.8 ± 2.3
Rumex obtusifolius	42.5 ± 2.5					47.0 ± 2.5
Urtica dioica	88.8 ± 1.6					87.8 ± 1.6
Vicia hirsuta	63.8.x ± 2.4					66.5 ± 2.4
	Hogweed seeds	Light seed extract	Strong seed extract	Bergapten (DMSO)	DMSO	
Mentha arvensis	31.0 ± 3.3^{BC}	28.5 ± 3.2^{BC}	38.0 ± 3.4^{AB}	44.0 ± 3.5^{A}	27.0 ± 3.1^{C}	34.0 ± 3.3^{ABC}
Poa trivialis	70.5 ± 3.2^{A}	64.0 ± 3.4^{A}	15.5 ± 2.6^{C}	50.5 ± 3.5^{B}	21.5 ± 2.9^{C}	73.0 ± 3.1^{A}
Sonchus oleraceus	98.0 ± 1.0^{A}	95.5 ± 1.5^{AB}	91.0 ± 2.0^{B}	3.0 ± 1.2^{D}	11.0 ± 2.2^{C}	92.0 ± 1.9^{B}
Urtica dioica	81.0 ± 2.8^{AB}	88.5 ± 2.3^{A}	76.0 ± 3.0^{B}	24.5 ± 3.0^{D}	35.5 ± 3.4^{C}	86.0 ± 2.5^{A}

The difficulty in using realistic concentrations of potential allelochemicals in germination experiments can be a reason for the incongruent results on *P. trivialis* treated with concentrated aqueous solutions from ground seeds of *H. mantegazzianum* compared with the moist seed mixtures with this species. While solutions of ground seeds may contain too high concentrations of compounds, mixtures of seeds often underestimate the microbial degradation of plant material and the chemical reactions with other compounds in soil. The use of Petri dishes distorts further the time these compounds remain in contact with the seeds, as they cannot leach out from the dishes, and using distilled water as a medium has limitations for poorly water soluble compounds. Finding natural or neutral solvents for such compounds is a methodological challenge, as many solvents have additional effects on the tested species. This can be seen in the overlapping results of the germination experiments conducted with bergapten and DMSO.

Furthermore, changes in soil pH or nutrient concentrations in stands invaded by *H. mantegazzianum* could explain differences in germination of other species. Rodgers *et al.* (2008) found that soils in North American temperate deciduous forest invaded by the European forb *Alliaria petiolata* were higher in nutrients and soil pH, in addition to the allelopathic effects observed by Prati and Bossdorf (2004). As seedling growth is often more sensitive to allelochemicals than germination (Araniti *et al*., 2012), seed extracts of *H. mantegazzianum* could also directly inhibit the growth of native plants (J. Thiele, unpubl. data). Should *H. mantegazzianum* contain compounds that have negative effects on the plant performance of native species in its invasive range, it still remains to be seen if allelopathy facilitates the invasion of this species, acting as a novel weapon, as shown for other species (Ridenour and Callaway, 2001; Inderjit *et al*., 2006).

We conclude that detection of allelopathic effects of invasive alien plant species depends on the experimental methods used and varies among the native species investigated. Despite high concentrations of potentially allelopathic furanocoumarins in the study species, there is only limited evidence that seeds or soil from *H. mantegazzianum* stands have negative effects on germination of co-occuring native herbs.

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Online Supplement

Limited evidence for allelopathic effects of Giant Hogweed on germination of native herbs
Wibke Wille, Jan Thiele, Emer Walker and Johannes Kollmann

Results of GLM analyses

Experiment 1: soil

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Call:
glm(formula = y.G08box ~ treatment * species, family = "quasibinomial",
    data = Total.G08box)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.6419  -0.9014  -0.1118   0.7682   3.9360

Coefficients:
                            Estimate Std. Error t value Pr(>|t|)
Intercept                   -1.71298    0.14347 -11.939  < 2e-16 ***
hogweed soil                 0.18948    0.19433   0.975  0.3318
R. obtusifolius             -1.38828    0.29198  -4.755  6.35e-06 ***
U. dioica                    0.42778    0.19048   2.246  0.0268 *
hogweed soil:R. obtusifolius 0.04748    0.38973   0.122  0.9033
hogweed soil:U. dioica      -0.68078    0.27176  -2.505  0.0138 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 1.918421)

    Null deviance: 395.24  on 110  degrees of freedom
Residual deviance: 223.96  on 105  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 4


Analysis of Deviance Table

Model: quasibinomial, link: logit

Response: y.G08box

Terms added sequentially (first to last)

                  Df Deviance Resid. Df Resid. Dev       F    Pr(>F)
NULL                                110     395.24
treatment          1    1.311       109     393.93  0.6835   0.41026
species            2  155.522       107     238.40 40.5339 9.009e-14 ***
treatment:species  2   14.441       105     223.96  3.7638   0.02638 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Experiment 2: soil extracts

```
Call:
glm(formula = y.G07 ~ treatment * species, family = "quasibinomial",
    data = Total.G07)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-4.0563  -1.1532   0.2436   1.1799   3.2434

Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
Intercept                      -1.8900     0.3999  -4.726  2.82e-05 ***
clean soil extract              0.5800     0.5185   1.119  0.270
hogweed soil extract            0.3821     0.5317   0.718  0.477
R. obtusifolius                 3.0912     0.5123   6.034  4.23e-07 ***
clean soil extract:R. obtusif. -0.4336     0.6948  -0.624  0.536
hogweed soil extract:R.obtusi. -0.3466     0.7244  -0.478  0.635
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 2.917029)

    Null deviance: 496.14  on 45  degrees of freedom
Residual deviance: 130.73  on 40  degrees of freedom
  (1 observation deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5


Analysis of Deviance Table

Model: quasibinomial, link: logit

Response: y.G07

Terms added sequentially (first to last)

                  Df Deviance Resid. Df Resid. Dev        F    Pr(>F)
NULL                                45     496.14
treatment          2     3.12         43     493.02   0.5349    0.5899
species            1   361.04         42     131.98 123.7690 8.171e-14 ***
treatment:species  2     1.25         40     130.73   0.2141    0.8082
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Experiment 3: hogweed seeds

```
Call:
glm(formula = y.G0809 ~ treatment * species, family = "quasibinomial",
    data = Total.G0809.subset)

Deviance Residuals:
     Min        1Q    Median        3Q       Max
-12.5415   -1.0109    0.0489    1.1678    7.8639

Coefficients:
                              Estimate Std. Error t value Pr(>|t|)
Intercept                     -2.37627    0.45210  -5.256  4.59e-07 ***
hogweed seeds                 -0.33178    0.68972  -0.481  0.63114
C. sepium                      2.32626    0.51771   4.493  1.33e-05 ***
E. helioscopia                 1.56444    0.52825   2.962  0.00352 **
F. gigantea                    5.37463    0.74550   7.209  2.03e-11 ***
M. arvensis                    2.17560    0.51829   4.198  4.44e-05 ***
P. trivialis                   3.25955    0.53030   6.147  5.93e-09 ***
R. obtusifolius                2.25613    0.51790   4.356  2.34e-05 ***
U. dioica                      4.34524    0.59354   7.321  1.09e-11 ***
V. hirsuta                     3.06193    0.52513   5.831  2.91e-08 ***
hogweed seeds:C. sepium       -0.06552    0.77856  -0.084  0.93303
hogweed seeds:E. helioscopia   0.34349    0.79037   0.435  0.66443
hogweed seeds:F. gigantea     -0.85129    0.97934  -0.869  0.38600
hogweed seeds:M. arvensis      0.41230    0.77702   0.531  0.59641
hogweed seeds:P. trivialis     0.28393    0.79238   0.358  0.72057
hogweed seeds:R. obtusifolius  0.14964    0.77755   0.192  0.84763
hogweed seeds:U. dioica        0.42827    0.88477   0.484  0.62901
hogweed seeds:V. hirsuta       0.21065    0.78477   0.268  0.78871
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 6.358839)

    Null deviance: 3191.9  on 179  degrees of freedom
Residual deviance: 1023.3  on 162  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 6


Analysis of Deviance Table

Model: quasibinomial, link: logit

Response: y.G0809

Terms added sequentially (first to last)

                  Df Deviance Resid. Df Resid. Dev       F Pr(>F)
NULL                                179     3191.9
treatment          1     6.17       178     3185.8  0.9700 0.3262
species            8  2137.71       170     1048.1 42.0224 <2e-16 ***
treatment:species  8    24.75       162     1023.3  0.4864 0.8646
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Experiment 4: hogweed seeds, seed extracts, DMSO and bergapten

```
Call:
glm(formula = y.GEmer ~ treatment * species, family = "quasibinomial",
    data = Total.GEmer)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.6706  -0.9167  -0.1703   0.6955   3.1359

Coefficients:
                                Estimate Std. Error t value Pr(>|t|)
Intercept                        -0.66329    0.22237  -2.983  0.00362 **
hogweed seeds                    -0.13683    0.31832  -0.430  0.66828
light seed extract               -0.25650    0.32234  -0.796  0.42815
strong seed extract               0.17375    0.31072   0.559  0.57735
DMSO                             -0.33133    0.32519  -1.019  0.31083
DMSO w/ Bergapten                 0.42213    0.30738   1.373  0.17286
P. trivialis                      1.65792    0.32519   5.098 1.72e-06 ***
S. oleraceus                      3.10564    0.44746   6.941 4.59e-10 ***
U. dioica                         2.47858    0.37632   6.586 2.40e-09 ***
hogweed seeds:P. trivialis        0.01342    0.45933   0.029  0.97674
light seed extract:P. trivialis  -0.16276    0.45647  -0.357  0.72221
strong seed extract:P. trivialis -2.86428    0.48741  -5.876 6.04e-08 ***
DMSO:P. trivialis                -1.95834    0.47728  -4.103 8.55e-05 ***
DMSO w/ Bergapten:P. trivialis   -1.39675    0.44179  -3.162  0.00210 **
hogweed seeds:S. oleraceus        1.58630    0.90457   1.754  0.08268 .
light seed extract:S. oleraceus   0.86920    0.71616   1.214  0.22784
strong seed extract:S. oleraceus -0.30246    0.61872  -0.489  0.62606
DMSO:S. oleraceus                -4.20176    0.60817  -6.909 5.33e-10 ***
DMSO w/ Bergapten:S. oleraceus   -6.34058    0.79157  -8.010 2.70e-12 ***
hogweed seeds:U. dioica          -0.22845    0.51536  -0.443  0.65855
light seed extract:U. dioica      0.48186    0.55236   0.872  0.38518
strong seed extract:U. dioica    -0.83636    0.49955  -1.674  0.09734 .
DMSO:U. dioica                   -2.08109    0.49636  -4.193 6.14e-05 ***
DMSO w/ Bergapten:U. dioica      -3.36288    0.49663  -6.771 1.02e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasibinomial family taken to be 2.21933)

    Null deviance: 2201.98  on 119  degrees of freedom
Residual deviance:  220.71  on  96  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 5

Analysis of Deviance Table

Model: quasibinomial, link: logit

Response: y.GEmer

Terms added sequentially (first to last)

                  Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                                119    2201.98
treatment          5   746.88       114    1455.10 67.307 < 2.2e-16 ***
species            3   394.92       111    1060.18 59.315 < 2.2e-16 ***
treatment:species 15   839.48        96     220.71 25.217 < 2.2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```