

Intergenerational assessment of biofumigation on fitness and phenology of *Ambrosia artemisiifolia* and *Abutilon theophrasti*

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ABSTRACT

The lethal and sublethal effects of Indian mustard (*Brassica juncea* L. cv. 'Caliente 199') biofumigation on common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.) fitness and phenology across generations were studied. First generation (G1) weed seeds were exposed in Petri dish germination test to allelochemicals produced after rehydrating the dried Indian mustard tissues at rates of 0 (control), 3.5 and 7.0 mg cm⁻² of dried tissues for *A. artemisiifolia* and 0 (control), 2.33 and 3.5 mg cm⁻² for *A. theophrasti*. Surviving germinated G1 seeds were sown in greenhouse for phenological surveys and reproduction component measurements. The same treatments were applied to G2 and G3 seeds. For each generation, seed parameters were measured. Biofumigation reduced the weed fitness by decreasing the seed germination and survival, increasing seedling mortality, delaying emergence and flowering for both species and decreasing the number of seeds produced of *A. artemisiifolia*. However, second and third generations may improve their tolerance to biofumigation by increased number of dormant seeds for *A. artemisiifolia*, seedling survival for both species, increased seed production for *A. theophrasti*, relative weight of embryo and testa thickness for *A. artemisiifolia*. Quantification of target weed fitness from biofumigation enables us to understand the weed population performance in the field.

Keywords: *Abutilon theophrasti*, allelochemicals, *Ambrosia artemisiifolia*, biofumigation, common ragweed, intergenerational, maternal effects, phenology, reproduction, seed response, seedling survival, velvetleaf.

INTRODUCTION

Allelopathy is defined as “any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals)” (34). Allelopathy is biotic environmental stress factor that reduces the competitive ability of target plant in numerous ways, including direct inhibition of plant functions (16,37). Phytotoxic allelochemicals can reduce photosynthesis, carbon acquisition, plant growth (23) and consequently reduce the plant fitness of target species. Plant fitness is a concept that assesses the relative ability of an individual or a population to survive and reproduce in its environment (25). Components of plant fitness are germination, establishment, survival and reproduction. Plant-produced

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allelochemicals are deleterious to target plant fitness mainly by inhibiting its establishment and growth (24). In some cases, phytotoxic allelochemicals affects the flower production, even causing complete reproductive failure (8). The lower fitness of target plant may be indirectly caused by the disturbed plant mutualism interactions (19).

Biofumigation is an agricultural practice, in which phytotoxic allelochemicals may reduce the weed seed viability in soil seed bank (31). Biofumigation occurs during the incorporation of *Brassica* cover crops containing high glucosinolate concentration in the soil. Volatile phytotoxic chemicals are released from the decomposing *Brassica* tissues and provide weed suppression. The most common volatile produced during the breakdown of *Brassica* are isothiocyanates (ITCs) which are released after the tissue disruption when myrosinase enzymes hydrolyze glucosinolates in presence of water (32,33). Isothiocyanates react easily with cellular thiols such as cysteines in proteins and low-molecular-weight thiols (especially glutathione), producing dithiocarbamate derivatives. These actions lead to loss of protein structure and function and decreased the enzymatic activity (14,50). In seeds, ITCs interact with glycolytic enzymes during the germination process and thereby prevent or delay the seed/tuber germination (13). The ITCs are strong germination inhibitors of many weed species (1,2,9,29,39). High concentrations of ITCs penetrate the seeds, irreversibly inhibit the protein synthesis and the seeds lose their viability (28). Biofumigation from *Brassica* cover crops reduces the weed seed germination, establishment and biomass production in field (9,26,47).



Photo 1. *Ambrosia artemisiifolia* (left) and *Abutilon theophrasti* (right).

Plants adapt quickly to chemical composition of neighbouring plants (17). Environmental conditions in which a plant grows, especially during the flowering stage, could influence the seed production (35) and fitness (40). Moreover, it also influences the offspring of these plants, a process called phenotypic maternal effect (43). Mother plants may affect their offsprings by one or more mechanisms; non-Mendelian genetic inheritance (e.g. extrachromosomal or cytoplasmic inheritance), through information which is passed from the mother to the offspring via chemicals produced by the mother or via epigenetic

modifications (22). As these maternal effects can have genetic and environmental components, they can be adaptive if they increase the offspring reproductive success (27). Few studies have evaluated the tolerance of species to allelopathic compounds. Callaway *et al.* (11) studied the tolerance of a native population exposed to *Centaurea maculosa* Lam. allelopathic root exudates and they observed that a new population exposed to this species was more affected than coexisting species. To the best of our knowledge, fitness response and possible adaptability to allelopathic compounds generated during biofumigation has not been studied.

While evaluating the potential of long-term use of allelochemical compounds in soil, released by biofumigation, it is important to assess the impact of biofumigation on fitness components and to evaluate the adaptive potential of weeds. It is necessary to know if a maternal plant in contact with ITCs, will influence the next-generation phenotypic response to biofumigation. If survival and reproduction of adult plants from seeds exposed to biofumigation are modified, fitness will also be changed and consequently their competitive ability. Furthermore, if the selection pressure of biofumigation was strong, it could lead to the development of tolerant weed populations. Generally, allelopathic studies focus on the seed germination and seedling growth (19). However, it is also important to assess the impact of allelopathy on plant fitness, not only on seeds and seedlings, but also on survival to flowering, adult plant growth and reproduction.

Two hypotheses were formulated: (i) Exposure of weed seeds to increasing concentration of allelochemicals generated during biofumigation will decrease survival and (ii) Sub-lethal concentrations of allelochemicals will negatively influence the growth and reproduction of plant and these effects will decrease when following generations are exposed to the same treatment. This study aimed to determine the common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.) fitness and phenological responses to biofumigation and to assess the potential changes in fitness and phenological responses of these weeds across generations.

MATERIALS AND METHODS

I. Biofumigation assay across generations

The study was conducted at the Research and Development Institute for Agri-environment (IRDA) Research Station, Saint-Bruno-de-Montarville (45.533°N, 73.35°W) on *A. artemisiifolia* and *A. theophrasti*. *Ambrosia artemisiifolia* seeds were collected in fall of 2012 from mature plants at IRDA, where seeds fell after vigorous plant shaking. *Abutilon theophrasti* seeds were collected from the Macdonald Campus Research Farm, McGill University (45.412°N, 73.944°W). *Ambrosia artemisiifolia* and *A. theophrasti* seeds had $60.3 \pm 2.9\%$ and $60.9 \pm 2.7\%$ germination in Petri dish germination test, respectively. Seeds from these initial seed lots were sown on May 26th in pots for both species (19 cm dia, 17.5 cm depth) containing 730 g commercial potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in greenhouse in 2014. Weed seedlings were thinned to 3 per pot. A total of 45 plants per weed species were grown to maturity until October 10, 2014 for *A. artemisiifolia* and November 11, 2014 for *A. theophrasti* and produced G1 (first generation) seeds. Primary dormancy of G1 seeds was broken to germinate these under biofumigation treatment. To break dormancy, *A. artemisiifolia*, seeds were kept in moist sand at 4 °C for 7 weeks and the *A. theophrasti*, seeds were immersed in water for 5 min at 70 °C. In 2015, 50-

G1 seeds were washed thoroughly with distilled water, placed in each Petri dish and exposed to two biofumigation treatments and control.

Petri dish biofumigation assays followed the methodology of Lefebvre *et al.* (29). Dry powder of Indian mustard (*Brassica juncea* L. cv. 'Caliente 199') was used as biofumigant material @ (control), 3.5 and 7.0 mg for *A. artemisiifolia* and 0, 2.33 and 3.5 mg for *A. theophrasti* per cm² exposed to the allelochemicals. Indian mustard plants were grown until flowering in pots (19 cm dia, 17.5 cm depth) containing 730 g commercial potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in growth chamber (Conviron E15, serial number 8D40801J) [26 ± 2 °C, 16/8 h light/darkness ($186 \mu\text{mol m}^{-2} \text{s}^{-1}$)]. At flowering, the above-ground plant parts were harvested, dried at 35 °C for 5 d and ground with laboratory mill grinder. Dried mustard powder was placed in a modified weighing dish, whose bottom was replaced by 5 μm pure nylon sieve membrane - CellmicroSieves™ (NSR, Biodesing, Carmel, NY). The sieve membrane served as a barrier to prevent potential fungal contamination and allowed both volatile and water-soluble components from the mustard to be released in to the Petri dish. A qualitative filter paper (Whatman, 90 mm dia, 190 μm thick, Fisher scientific, Whitby, ON) was placed in 91 mm dia. x 15 mm polystyrene disposable Petri dish (VWR International, Mississauga, ON) and the weighing dish was placed at the centre. Weed seeds were washed thoroughly with distilled water and then placed surrounding the weighing dish. Just before closing the Petri dish, 2 ml distilled water was added on the seeds and 4 to 6 ml of distilled water on the weighing dish to completely moisten the mustard tissues (more biomass requires more water). Water allowed the glucosinolate-myrosinase enzymatic reaction to occur. The Petri dish was closed with wrapping film (Parafilm, 5 cm width, Fisher) and immediately incubated in a growth chamber (MLR-350H, Sanyo) set to 22.5 ± 0.5 °C, 16/8 h light/darkness ($372 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Previous experiments had shown that these rates were not lethal and allowed germination (29). Prior to assays, the material was analysed by headspace (HS) (TurboMatrix™ HS 40 Trap, PerkinElmer, USA) and gas chromatography-mass spectrometry (GC-MS) [Clarus® 680 (GC) and Clarus® SQ8 (MS), PerkinElmer, USA] to determine the quantity of ITCs released during the biofumigation as described in Lefebvre *et al.* (29). Fifteen replications per treatment were arranged in a completely randomized design in growth chamber (MLR-350H, Sanyo) set to 22.5 ± 0.5 °C, 16/8 h light/darkness ($372 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Twenty surviving germinated G1 seeds with a radicle length of 2 mm long were removed from the Petri dishes and sown in pots (ITML Elite 4 L, HC Companies, cat.no. ITBM400) with potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in greenhouse without further exposure to allelochemicals. Environmental conditions in greenhouse were natural sunlight and without temperature control, however, air circulation was done by fans and vents. Temperature in greenhouse was recorded with data loggers (TIDBIT v1, TBI32-20+50, Onset Computer Corporation, MA, USA). Each Petri dish and its corresponding pot were considered as experimental units. For *Ambrosia artemisiifolia*, 15 replications were used for each biofumigation rate, whereas due to low germination of *A. theophrasti*, after biofumigation, the number of replicates was 15 for control and reduced to 13 and 7 for treatments with 2.33 and 3.5 mg cm⁻² of dry tissues, respectively.

After 3 weeks, seedlings were thinned to only 3 plants/pot except for G1 *A. theophrasti* which had one plant per pot due to its low emergence after sowing. The plants

were irrigated with water as needed. At the appearance of first inflorescence in *A. artemisiifolia* and *A. theophrasti*, soil was fertilized once at 1-2 cm depth with 50 and 30 g/pot of granulated chicken manure 5-3-2 (N-P-K, 80, 100 and 100% N-P-K efficacy, respectively) (Acti-Sol inc., Notre-Dame-du-Bon-Conseil, Quebec, Canada), respectively. These fertilisations correspond to 2, 1.5 and 1 g of nitrogen, phosphorus and potash nutrients, respectively, applied at 50 g/pot and 1.2, 0.9 and 0.6 g of nitrogen, phosphorus and potash nutrients, respectively, applied for the dose of 30 g/pot. Plants of both species have grown to maturity and produced G2 seeds in 2015. To avoid cross-pollination between experimental units each experimental unit of *A. artemisiifolia* was covered with transparent copolyester tube (VIVAK PETG, 0.02 mil., Plaskolite, Columbus, OH, USA) with air vents covered with white broadcloth during flowering. *Abutilon theophrasti* was assumed to be self-pollinated (48).

G2 (second generation) seeds were collected for each experimental unit, handled as described earlier and exposed to same biofumigation treatments to produce G3 (third generation) seeds in 2016. After-ripening, the treatments were also done for G2 and G3 seeds to allow germination. Finally, the collected seeds of G3 were also treated at the same rates in 2017 and seed germination, mortality and dormancy were evaluated without sowing in the greenhouse.

II. Data measurements

Germination of seeds in Petri dishes was recorded for 46 d for *A. artemisiifolia* and 25 d for *A. theophrasti* until no additional seeds germinated. The viability of non-germinated seeds was evaluated using a 1% solution of 2,3,5-Triphenyl-2H-Tetrazolium Chloride (Fisher scientific, Whitby, Ontario), in accordance with the Tetrazolium testing handbook for agricultural seeds (38) and the International Seed Testing Association (ISTA) working sheets on tetrazolium testing (30). Viable non-germinated seeds at end of assays were considered dormant. This laboratory procedure was repeated for G1, G2 and G3 seeds.

After sowing the germinated seeds into pots, seedling emergence was recorded for 3 weeks to assess the seedling survival. During G1 and G2 plant growth, number of reproductive structures, including the number of visible clusters of male flower heads for *A. artemisiifolia* and floral buds for *A. theophrasti* were recorded weekly. In the meantime, phenological stages of both species were monitored according to BBCH scale (21). The codes of the phenological stages are: 5 = Radicle begins to emerge from the seed; 10= Leaf development (main shoot); 20= Formation of side shoots/tillering; 30= Stem elongation/shoot development (main shoot); 40= Vegetative propagation/booting (main shoot); 50= Inflorescence emergence (main shoot)/heading; 60= Flowering (main shoot); 70= Development of fruit; 80= Ripening or maturity of fruit and seeds and 90= Senescence.

Falling seeds of *A. artemisiifolia* and *A. theophrasti* black mature fruits were collected until complete plant senescence. Seeds produced per replication were cleaned removing other plant material, counted and weighed. Reproductive effort was calculated using total seed weight per plant and plant biomass. To evaluate the plant biomass, relations between biomass, height and diameter were used. For both species, one of the three plants per pot at ripening stage was cut above ground and dried. Also, height and diameter of each plant was measured. For *A. theophrasti*, relation between G2 plant biomass, height and diameter was used to estimate the G1 plant biomass.

The following seed morphological parameters were measured in G1, G2 and G3 seeds: Seed length, width, thickness, calculated surface, thickness of testa, endosperm and pericarp and weight of the embryo and testa. Ratios of testa : seed weight and embryo : seed weight were also calculated. Following the methodology of Lefebvre *et al.* (29), those measurements were taken on 30 seeds/replications, with 5- replications per treatment.

III. Statistical analyses

Data analyses were performed using ‘Agricolea’, ‘ezANOVA’ and ‘Asbio’ packages of R software v.3.0.1 (42). Data were subjected to analyses of variance (ANOVAs) and Tukey’s honestly significant difference (HSD) were used to separate treatment means at 0.05 probability level for seed responses, seedling survival, phenological model parameters, seed number and weight, reproductive effort and each seed morphological parameter. Dependent variables were transformed whenever required to respect normality and homoscedasticity assumptions.

The phenological modelling of *A. artemisiifolia* and *A. theophrasti* were obtained using CIPRA computer software (Computer centre for agricultural pest forecasting, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec). General structure of phenological models included four specific phases: emergence, vegetative development, early and late reproductive development (Figure 1). *Ambrosia artemisiifolia* temperature response for each phase was based on the equation of Yan and Hunt (53) where the lower limit threshold temperature for development was 0.9 °C, the optimal temperature was 31.7 °C and the higher limit threshold temperature was 40 °C. *Abutilon theophrasti* temperature response for each phase was based on multi-linear model constructed from three linear components (12), where the lower limit threshold temperature for development was 4.1 °C, optimal temperatures range from 25.4 to 36.1 °C and the higher limit threshold temperature was 40 °C (36). Maximum emergence rate (E_{max}), maximum vegetative development rate (V_{max}), maximum early reproductive development rate (ER_{max}) and maximum late reproductive development rate (LR_{max}) were adjusted for each plant in every replicate.

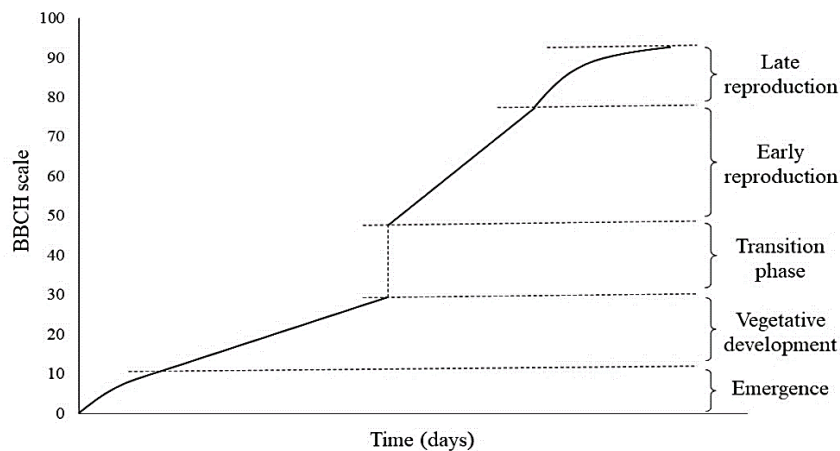


Figure 1. Typical phenological model generated by CIPRA computer software, where BBCH values are represented according to days and to four phases (right), where seeds and plants responded to temperature for development.

Non-linear curve fitting was performed to establish *A. artemisiifolia* and *A. theophrasti* flowering response using TableCurve 2D V.5.01 and SigmaPlot V.12.5 (Systat Software, San Jose, CA). Equation 1 represents a log normal function with three parameters.

$$y = a \exp[-0.5 (\ln(x/b)/c^2)] \quad [1]$$

Where, **a**: Amplitude, **b**: X axis value when Y corresponds to **a** and **c** is a shape parameter. Estimated coefficient parameters were considered statistically different according to the t-statistics for comparing means using SE at $P \leq 0.05$.

RESULTS AND DISCUSSION

The chemical analysis of dried tissues for each bioassay quantified the release of 1,116 μg of allyl-ITC g^{-1} of dry biomass to which G1 seeds were exposed, 1,577 μg g^{-1} of allyl-ITC for G2 seeds and 1,083 μg g^{-1} of allyl-ITC for G3 seeds. Analyses also revealed the traces of butyl-ITC. The quantities of allelochemicals generated after rehydrating dried Indian mustard biomass and released in the Petri dish are indicated in Table 1.

Seed responses

Exposure to Indian mustard tissues significantly decreased the seed germination and increased the seed mortality of *A. artemisiifolia* (Table 2) and *A. theophrasti* (Table 3). Seed germination and survival were always superior in control. Biofumigation at 7.0 mg cm^{-2} of dried mustard tissues caused greater impact on seed germination and mortality on each generation of *A. artemisiifolia* than 3.5 mg cm^{-2} rate. Only G2 *A. theophrasti* seeds were more affected by 3.5 mg cm^{-2} rate. Otherwise, the impact of 3.5 and 2.33 mg cm^{-2} of dried mustard tissues on *A. theophrasti* seed germination and mortality were similar. For both species, biofumigation rate and generation had significant effects on seed germination. The main factor biofumigation rate inhibited the seed germination. The biofumigation rates of 3.5 and 7.0 mg cm^{-2} , decreased the seed germination of *A. artemisiifolia* by 22.5 and 53.7%, respectively. The biofumigation rate of 3.5 mg cm^{-2} decreased the seed germination of *A. theophrasti* to 28.1%, and seed germination in control was 96.1%. Furthermore, there was significant interaction between the rate and generation. Seed germination in control increased over generations, while the germination decreased in biofumigation treatments,

Table 1. Quantities of allyl- isothiocyanate (ITC) released in the Petri dish assays according to rates of dry Indian mustard material for each generation*.

Biofumigation treatments (mg cm^{-2} of dried mustard biomass**)	G1	G2	G3
	($\mu\text{g cm}^{-2}$)		
0	0.00	0.00	0.00
2.33	2.60	3.63	2.53
3.5	3.90	5.45	3.79
7.0	7.81	10.89	7.58

*Analysis by headspace and gas chromatography-mass spectrometry of 0.2 g of dry Indian mustard. Analyses also revealed traces of butyl-ITC.

**Based on the calculation of allyl-isothiocyanate released from rehydration of corresponding amount of dry Indian mustard placed in the weighing dish, equivalent to 0.152, 0.228 and 0.456 g Petri dish⁻¹ (91.1 mm in diameter).

except the germination with 2.33 mg cm⁻² of dried mustard tissues for G2 *A. theophrasti*. Biofumigation rate effect and the interaction between rate and generation for *A. artemisiifolia* and *A. theophrasti* were significant for seed mortality. Generation had significant effects on *A. theophrasti* seed mortality, but not in *A. artemisiifolia* seeds.

Table 2. *Ambrosia artemisiifolia* seed responses to exposure of Indian mustard tissues and results of repeated measures analysis of variance for biofumigation rate and generation effects.

Generation	Rates (mg cm ⁻²)	Germination*	Mortality*	Dormancy*
G1	0	77.3 (1.9) a	17.1 (1.8) c	6.3 (0.8) b
	3.5	67.1 (2.0) b	27.8 (1.7) b	5.0 (1.1) b
	7.0	43.2 (2.0) c	41.9 (2.7) a	14.9 (1.9) a
G2	0	87.3 (1.4) a	12.0 (1.3) c	0.7 (0.3) b
	3.5	72.7 (4.7) b	25.5 (4.4) b	1.8 (0.7) ab
	7.0	42.3 (4.7) c**	54.5 (4.5) a	3.2 (1.4) a
G3	0	91.3 (2.0) a	5.5 (2.2) c	3.3 (0.8) b
	3.5	58.5 (3.9) b	33.7 (4.1) b	7.7 (1.7) ab
	7.0	33.1 (4.6) c**	55.3 (4.5) a**	11.5 (1.5) a**
Mean of main factors	G1	62.5 (2.0)	28.9 (2.1)	8.7 (1.3)
	G2	67.4 (3.6)	30.7 (3.4)	1.9 (0.8)
	G3	61.0 (3.5)	31.5 (3.6)	7.5 (1.3)
	0	85.3 (1.8)	11.5 (1.8)	3.4 (0.6)
	3.5	66.1 (3.5)	29.0 (3.4)	4.8 (1.2)
	7.0	39.5 (3.8)	50.6 (3.9)	9.9 (1.6)
Repeated measures ANOVA				
Rates	df***	2, 42	2, 42	2, 42
	F	69.4	119.3	23.8
	P	≤0.001	≤0.001	≤0.001
Generation	df	2, 84	2, 84	2, 84
	F	313.2	0.6	1462.5
	P	≤0.001	0.547	≤0.001
Rates*Generation	df	4, 84	4, 84	4, 84
	F	33.3	4.3	59.7
	P	≤0.001	0.004	≤0.001

*Means (± SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at P ≤ 0.05. P values in bold text indicate a significant factor.

**Mean separation based on non-transformed data, otherwise on log transformed data.

***Numbers separated by a comma represent degrees of freedom for rate and generation effects and degree of freedom for error, respectively.

The quantity of viable non-germinated *A. theophrasti* seeds at the end of Petri dish assays was not affected by biofumigation rates for each generation. However, *A. artemisiifolia* dormant seeds increased after exposure to allelochemicals released after rehydration of dry Indian mustard tissues. The 7.0 mg cm⁻² rate induced seed dormancy more than the 3.5 mg cm⁻² rate. Biofumigation rate and generation effects were significant factors for *A. artemisiifolia* dormant seeds, with 3.4% dormant seeds in control and 9.9% for the 7.0 mg cm⁻² rate. The interaction between rate and generation effects was also significant.

Table 3. *Abutilon theophrasti* seed responses to exposure of Indian mustard tissues and results of repeated measures analysis of variance for biofumigation rate and generation effects.

Generation	Rates (mg cm ⁻²)	Germination*	Mortality*	Dormancy*
G1	0	95.1 (0.7) a	2.0 (0.4) b	2.8 (0.5) a
	2.33	32.4 (3.2) b	64.8 (3.2) a	2.8 (0.8) a
	3.5	29.6 (3.3) b	67.3 (3.4) a	3.1 (0.6) a
G2	0	94.0 (1.2) a	5.8 (1.2) c	0.3 (0.2) a
	2.33	70.7 (4.6) b	29.3 (4.9) b	0.0 (0.0) a
	3.5	22.2 (6.7) c**	77.6 (7.2) a	0.1 (0.1) a
G3	0	99.2 (0.4) a	0.8 (0.4) b	0.0 (0.0)
	2.33	18.1 (4.7) b	80.8 (2.3) a	1.0 (1.0)
	3.5	13.5 (2.7) b**	85.5 (4.5) a**	1.1 (1.1)***
Mean of main factors	G1	52.4 (2.4)	44.7 (2.3)	2.9 (0.6)
	G2	62.3 (4.2)	37.6 (4.4)	0.1 (0.1)
	G3	43.6 (2.6)	55.7 (2.4)	0.7 (0.7)
	0	96.1 (0.8)	2.9 (0.7)	1.0 (0.2)
	2.33	40.4 (4.2)	58.3 (3.5)	1.3 (0.6)
	3.5	21.8 (4.2)	76.8 (5.0)	1.4 (0.6)
Repeated measures ANOVA				
Rates	df****	2, 33	2, 33	2, 33
	F	372.4	342.9	0.3
	P	≤0.001	≤0.001	0.737
Generation	df	2, 66	2, 66	2, 66
	F	22.8	23.6	12.9
	P	≤0.001	≤0.001	≤0.001
Rates*Generation	df	4, 66	4, 66	4, 66
	F	25.7	27.1	0.4
	P	≤0.001	≤0.001	0.752

*Means (\pm SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at $P \leq 0.05$. P values in bold text indicate a significant factor.

**Mean separation based on non-transformed data, otherwise on log transformed data.

***Not enough data to performed analysis.

****Numbers separated by a comma represent degrees of freedom for rate and generation effects and degree of freedom for error, respectively.

In a previous study, exposure of *A. artemisiifolia* seeds to similar quantities of allelochemicals released in Petri dish increased the mortality (%) and decreased the germination (%) (29). Moreover, the similar induction of seed dormancy was observed. *Ambrosia artemisiifolia* showed physiological dormancy and may return to the secondary dormancy stage, *A. theophrasti* seeds showed the physical dormancy (3). In the previous study, the *A. theophrasti* adjusted germination was not reduced at rates equivalent to 2.33 mg cm⁻² (29). The quantity of dormant seeds for both species differed considerably from those previously observed. *Ambrosia artemisiifolia* and *A. theophrasti* seeds exhibited < 2.8 and 6.3% of dormancy compared to 20 and 53%, respectively in previous study (29). After-ripening treatments reduced greatly the number of dormant seeds. The amount of dormancy in seed lot did affect the intraspecific seed response to biofumigation. Furthermore, biofumigant potential of Indian mustard used in the present experiment was lower than in

the previous experiment (2,455 μg of allyl-ITC g^{-1} of plants). Different seed responses observed between the experiments may be explained by relative importance of dormant seeds and the different amount of allelochemicals generated in the Petri dish experiments.

Variation may occur in dormant and non-dormant seeds found in same species. This variation is related to environmental conditions and genetic variability. Day light, light quality, mineral nutrition, age of mother plants, position on the mother plants, temperature, soil moisture and solar irradiance influence seed dormancy (6). Environmental factors including temperature, darkness, light, gases, water, inorganic and organic chemicals can change non-deep physiological dormancy (4). Moreover, preconditioning effects alter the germination and dormancy behaviour of seeds (5). Previous maternal environment (temperature, nitrate, light, water, oxygen and allelopathic compounds) will affect the sensitivity of seeds to environmental factors that influence the germination (18). Different seed responses observed between the generations in present experiment may be explained by previous maternal environment. However, maternal effects did not promote seed tolerance to biofumigation, as the biofumigation treatments were more detrimental to seed germination and survival for the second and the third generations.

Seedling survival

Ambrosia artemisiifolia seedling establishment was lower in the germinated seeds exposed to the allelochemicals released during biofumigation than control seeds for G1 (Table 4). *Ambrosia artemisiifolia* seedling survival was reduced by 16.8% with 7.0 mg cm^{-2} in G1 and the impact of 3.5 mg cm^{-2} was similar. Germinated seeds and seedlings exposed to biofumigation grew smaller, were light-coloured and had almost no root hairs based on visual observations. However, the impact of biofumigation on seedling survival was only significant on G1 *A. artemisiifolia* seeds. G2 seedling survival from germinated seeds that survived the biofumigation was not reduced compared to the control. Biofumigation rate and generation effects were significant factors for *A. artemisiifolia* seedling survival, like the interaction between those effects. The average seedling survival for G1 seeds was 82.7% and increased to 97.1% in G2 seeds.

Abutilon theophrasti seedling establishment at both rates of biofumigation was less than *A. artemisiifolia* (Table 5). For G1 *A. theophrasti* seedlings, biofumigation rates reduced survival by 88.4 and 71.7% with 3.5 and 2.33 mg cm^{-2} , respectively. *A. theophrasti* G2 seedling the survival was higher for germinated seeds exposed to biofumigation than G1 survival. Nevertheless, the rate of 3.5 mg cm^{-2} dried mustard tissues reduced G2 seedling survival by 25.3%, significantly different than control. There was significant interaction between the biofumigation rate and generation effect on *A. theophrasti* seedling survival.

Seed exposure to the allelochemicals generated during the rehydrating dried tissues of Indian mustard ultimately changed the offspring phenotypic response to the same allelochemicals. Indeed, G1 plants surviving biofumigation produced offspring with higher survival to the same allelochemical stress as they express themselves. Maternal effect studies focused on the induction of offspring phenotype by treatment or environment on seedlings or adult plants (22). Maternal effects on seed size and its impact on germination characteristics, seedling size and further competitive ability has been intensely studied (43). Variations in these processes result in differential plant fitness. However, it was unclear in the present study, if changes in offspring responses were caused by G1 seed exposure to biofumigation, or partly caused by delay in G1 seedling establishment and reduced plant

growth. Mechanisms involved in this transgenerational inheritance of allelochemical tolerance require further investigation.

Table 5. *Abutilon theophrasti* seedling survival from germinated seeds exposed to Indian mustard tissues and results of repeated measures analysis of variance for biofumigation rate and generation effects.

Generation	Rates mg cm ⁻²	Survival* (%)
G1	0	96.7 (1.1) a
	2.33	27.4 (4.9) b
	3.5	11.2 (5.0) c
G2	0	89.6 (3.0) a
	2.33	76.9 (6.1) ab
	3.5	66.9 (8.7) b
Mean of main factors	G1	45.1 (3.7)
	G2	77.8 (5.9)
	0	93.2 (2.1)
	2.33	52.2 (5.5)
	3.5	39.1 (6.9)
Repeated measures ANOVA		
Rates	df**	2, 42
	F	14.1
	P	≤0.001
Generation	df	1, 42
	F	94.5
	P	≤0.001
Rates*Generation	df	2, 42
	F	6.4
	P	0.003

*Means (\pm SE) within a column followed by the same letter within weed species are not significantly different according to Tukey's HSD test at $P \leq 0.05$. P values in bold text indicate a significant factor.

**Numbers separated by a comma represent degrees of freedom for rate and generation effects and degrees of freedom for error, respectively.

Seed exposure to allelochemicals released after rehydrating dry Indian mustard tissues proportionally slowed the seedling emergence of *A. artemisiifolia* for both generations (Table 6). The 7.0 mg cm⁻² biofumigation rate delayed the emergence by 27.9% compared to 3.5 mg cm⁻² rate. Biofumigation treatment did not impact the vegetative development of *A. artemisiifolia*. However, early reproductive development rate was higher with 7.0 mg cm⁻² and late reproductive development rate increased for both biofumigation treatments.

Abutilon theophrasti emergence was delayed by exposure to Indian mustard tissues compared to control and there was no difference between the rates 3.5 and 2.33 mg cm⁻². *Abutilon theophrasti* plants, from seeds that survived biofumigation treatments, had faster vegetative development than plants from control. G1 *A. theophrasti* emergence and vegetative development rates are similar to those of second generation. G1 early reproductive development was proportionally delayed by biofumigation treatments. However, late reproductive development rate was higher for surviving plants compared to control. There was no difference between the biofumigation treatments for G2 *A. theophrasti* for early or late reproductive development rates.

Table 6. Parameters of phenological models generated from CIPRA software for *Ambrosia artemisiifolia* and *Abutilon theophrasti* surviving plants after exposure to rates of Indian mustard tissues and to control for generations G1 and G2.

Species	Generation	Rates mg cm ⁻²	Model Parameters*			
			E _{max}	V _{max}	ER _{max}	LR _{max}
<i>Ambrosia artemisiifolia</i>	G1	0	0.200 (0.000) a	1.300 (0.012) a	1.211 (0.032) b	4.067 (0.067) b
		3.5	0.097 (0.005) b	1.282 (0.025) a	1.300 (0.023) b	4.733 (0.163) a
		7.0	0.066 (0.001) c	1.264 (0.056) a**	1.529 (0.048) a**	5.156 (0.276) a
	G2	0	0.130 (0.000) a	1.343 (0.019) a	0.473 (0.013) b	3.033 (0.091) b
		3.5	0.086 (0.002) b	1.346 (0.025) a	0.469 (0.011) b	3.728 (0.117) a
		7.0	0.066 (0.004) c**	1.371 (0.044) a	0.554 (0.023) a**	3.589 (0.143) a**
<i>Abutilon theophrasti</i>	G1	0	0.150 (0.000) a	0.635 (0.005) a	8.040 (0.105) a	2.06 (0.036) c
		2.33	0.092 (0.005) b	0.694 (0.012) b	6.969 (0.232) b	3.208 (0.359) b
		3.5	0.090 (0.000) b**	0.709 (0.014) b	5.971 (0.029) c	4.314 (0.130) a
	G2	0	0.169 (0.001) a	1.127 (0.012) a	7.500 (0.000) a	0.900 (0.000) a
		2.33	0.091 (0.007) b	1.336 (0.031) b	7.375 (0.259) ab	1.069 (0.033) b
		3.5	0.100 (0.017) b**	1.186 (0.071) b**	6.679 (0.242) b	1.257 (0.165) b

Abbreviations: E_{max}: Maximum emergence rate, V_{max}: Maximum vegetative development rate, ER_{max}: Maximum early reproductive development rate, LR_{max}: Maximum late reproductive development rate.

*Means (± SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at P ≤ 0.05.

**Mean separation based on log-transformed data, otherwise on reciprocal-transformed data.

Table 7. Analysis of coefficient parameters of *Ambrosia artemisiifolia* cluster of male flower head and *Abutilon theophrasti* floral bud production curves from surviving plants after exposure to rates of Indian mustard tissues and to control for G1 and G2 shown in Figures 2 and 3.

Species	Generation	Rates (mg cm ⁻²)	Coefficient parameters*		
			a	b	c
<i>Ambrosia artemisiifolia</i> male flower heads	G1	0	45.1 (1.2) a	86.8 (0.9) b	0.255 (0.011) a
		3.5	46.4 (1.6) a	88.8 (1.2) ab	0.252 (0.015) a
		7.0	40.6 (1.3) b	91.5 (1.3) a	0.252 (0.015) a
	G2	0	110.3 (3.6) a	89.6 (1.0) b	0.227 (0.011) a
		3.5	108.0 (3.3) ab	96.7 (1.3) a	0.227 (0.013) a
		7.0	98.4 (4.5) b	106.8 (5.0) a	0.251 (0.032) a
<i>Abutilon theophrasti</i> floral buds	G1	0	45.0 (1.3) b	57.4 (1.1) a	0.208 (0.012) a
		2.33	50.9 (1.6) a	59.1 (0.9) a	0.200 (0.009) a
		3.5	44.8 (3.2) ab	59.3 (2.1) a	0.191 (0.020) a
	G2	0	11.6 (0.4) a	50.0 (0.3) b	0.173 (0.005) a
		2.33	12.4 (0.5) a	52.7 (0.5) a	0.176 (0.006) a
		3.5	10.9 (1.0) a	54.4 (1.3) a	0.197 (0.019) a

*Values (± SE). Parameters for each treatment were compared to one another and significant groups were assigned according to the conclusions of the t-statistics for comparing means using SE at P ≤ 0.05. Coefficients within a column followed by the same letter within a generation are not significantly different.

Male flower head and floral bud production

The sublethal impact of biofumigation on *A. artemisiifolia* seeds influenced the amplitude and timing of flower head cluster production (Figure 2 and Table 7). The number of *A. artemisiifolia* male flower head clusters, fitted by log normal curves according to days

after the treatments in Petri dishes, are similar between the germinated seeds from control and 3.5 mg cm⁻² rate for G1. However, the amplitude of 7.0 mg cm⁻² rate was lower than other treatments. Moreover, the maximum production of *A. artemisiifolia* male flower clusters was delayed for this rate, approximately 5 days later than the control plants. Results of G1 curve fitting are similar to G2 *A. artemisiifolia* plants. Only the 7.0 mg cm⁻² rate significantly reduced the amplitude of male flowers produced and delayed the timing of maximum flower production. The shape parameter *c* was similar for all fitted curves for *A. artemisiifolia* (0.244 ± 0.016).

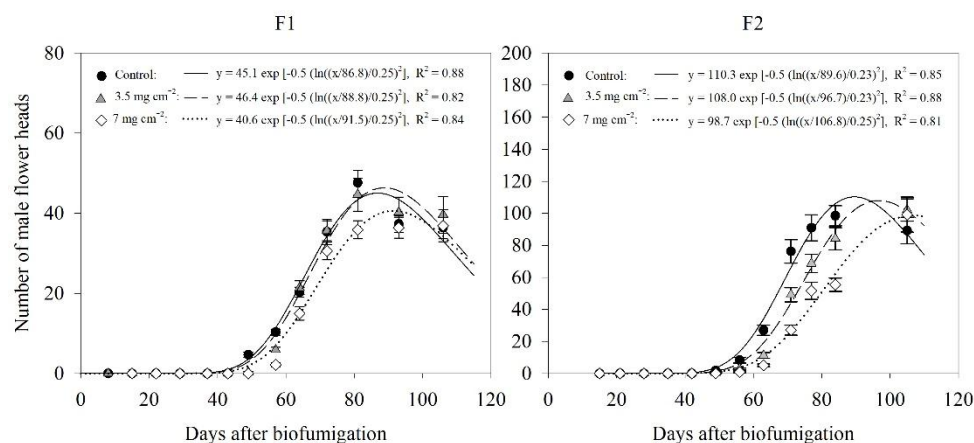


Figure 2. Number of clusters of male flower heads of *Ambrosia artemisiifolia* plants from germinated seeds exposed to Indian mustard tissues (3.5 and 7.0 mg cm⁻²) and to control for G1 and G2 according to the number of days after biofumigation treatment. Vertical bars indicate ± SE.

Biofumigation treatments influenced the amplitude and timing of floral bud production of *A. theophrasti* plants (Figure 3 and Table 7). The number of *A. theophrasti* floral buds produced reached higher peaks for G1 plants from seeds exposed to 2.33 mg cm⁻² rate compared to control plants. Timing of maximum floral bud production was similar in all G1 *A. theophrasti* plants. The G2 *A. theophrasti* floral bud production response differed from the G1 response. The amplitude of floral bud production was similar but was delayed by biofumigation treatments compared to control. The shape parameter *c* was similar for all fitted curves for *A. theophrasti* (0.191 ± 0.012).

Reproduction parameters

Seed number, total seed weight and reproductive effort from *A. artemisiifolia* plants exposed at the seed stage to allelochemicals changed between the generations (Table 8). In the first generation of *A. artemisiifolia*, seed number, total seed weight, weight of hundred seeds and plant biomass were all similar in all treatments.

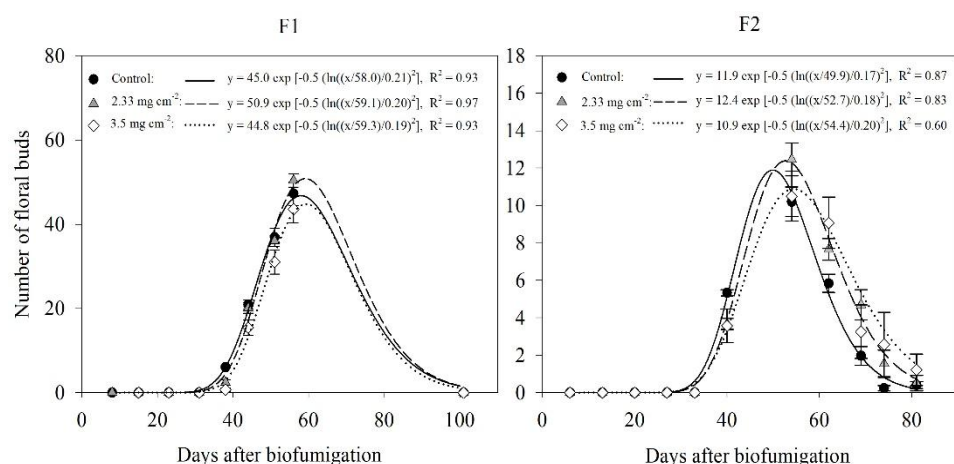


Figure 3. Number of floral buds of *Abutilon theophrasti* plants from germinated seeds exposed to Indian mustard tissues (2.33 and 3.5 mg cm⁻²) and to control for G1 and G2 according to the number of days after biofumigation treatment. Vertical bars indicate \pm SE.

Table 8. *Ambrosia artemisiifolia* reproduction parameters and seed number and weight from plants from germinated seeds exposed to rates of Indian mustard tissues and to control for generation G1 and G2 and results of the repeated measures analysis of variance for biofumigation rate and generation effects*.

Generation	Rates (mg cm ⁻²)	Seeds plant ⁻¹	Total seeds weight (g)	100 seeds weight (g)	Plant biomass (g)	Reproductive effort of seeds plant ⁻¹
G1	0	489 (35) a	2.0 (0.2) a	0.46 (0.07) a	15.0 (0.8) a	30.8 (1.5) b
	3.5	506 (41) a	2.2 (0.1) a	0.48 (0.07) a	12.7 (0.7) a	42.6 (3.7) a
	7.0	493 (35) a	2.0 (0.1) a	0.48 (0.06) a	13.5 (0.6) a	41.5 (3.4) ab
G2	0	1,200 (81) a	5.2 (0.4) a	0.45 (0.01) a	25.8 (1.2) a	47.6 (3.3) a
	3.5	983 (60) ab	4.4 (0.3) ab	0.47 (0.02) a	23.8 (1.8) a	43.8 (3.7) a
	7.0	817 (68) b	3.7 (0.3) b	0.47 (0.02) a	22.7 (1.8) a	39.2 (4.2) a
Mean of main factors	G1	496 (37)	2.1 (0.1)	0.47 (0.07)	13.7 (0.7)	38.3 (2.9)
	G2	1,000 (70)	4.4 (0.3)	0.46 (0.02)	24.1 (1.6)	43.5 (3.7)
	0	845 (58)	3.6 (0.3)	0.46 (0.04)	20.4 (1.0)	39.2 (2.4)
	3.5	745 (51)	3.3 (0.2)	0.48 (0.05)	18.3 (1.3)	43.2 (3.7)
	7.0	655 (52)	2.9 (0.2)	0.48 (0.04)	18.1 (1.2)	40.4 (3.8)
Repeated measures ANOVA**						
Rates	F	4.0	3.4	0.6	2.7	0.2
	P***	0.025	0.042	0.581	0.079	0.843
Generation	F	64.3	74.3	0.7	120.7	1.5
	P	≤0.001	≤0.001	0.421	≤0.001	0.231
Rates*Generation	F	3.8	2.8	0.0	0.5	2.1
	P	0.030	0.075	0.997	0.635	0.139

*Means (\pm SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at $P \leq 0.05$. The experiment had fifteen replicates for each treatment.

**Degrees of freedom for each factor and variables are 2 and 42 degrees of freedom for effects and error, respectively for rates and interaction rates*generation and 1 and 42 degrees of freedom for effects and error, respectively for generations.

***P values in bold text indicate a significant factor.

Reproductive effort of plants grown from treated seeds was higher than control plants. For G2 *A. artemisiifolia*, the 7.0 mg cm⁻² rate significantly reduced the number of seeds produced per plant and consequently the total seed weight per plant. Seed number and total seed weight changed between biofumigation rates and generations (Table 6). The reproductive effort was similar between all treatments of G2 *A. artemisiifolia* plants.

Reproduction parameters of first generation *A. theophrasti* plants were more affected than the second (Table 9). The 2.33 mg cm⁻² rate improved the number of fruits produced per plant, the number of seeds per plant and the total seed weight in G1 *A. theophrasti* plants. G1 *A. theophrasti* plants grown from treated seeds produced lighter seeds than control plants. Only the 3.5 mg cm⁻² rate significantly reduced G1 *A. theophrasti* plant biomass. Reproductive effort of treated *A. theophrasti* plants was higher than control plants. For all G2 *A. theophrasti* reproduction parameters, only reproductive effort was significantly higher for 3.5 mg cm⁻² rate than control. Generation was a significant factor for all variables analysed. Moreover, biofumigation rate was significant for seed number, total seed weight, plant biomass and reproductive effort. Significant interaction between the biofumigation rate and generation was found for seed number, total seed weight and weight of a hundred seeds.

Table 9. *Abutilon theophrasti* reproduction parameters and seed number and weight from plants from germinated seeds exposed to rates of Indian mustard tissues and to control for generation G1 and G2 and results of the repeated measures analysis of variance for biofumigation rate and generation effects*.

Generation	Rates (mg cm ⁻²)	Mature fruits plant ⁻¹	Seeds plant ⁻¹	Total seeds weight (g)	100 seeds weight (g)	Plant biomass (g)	Reproductive effort of seeds plant ⁻¹
G1**	0	34.6 (1.2) b	1,273 (34) b	11.3 (0.3) b	0.89 (0.01) a	32.8 (0.5) a	38.8 (0.9) b
	2.33	41.1 (1.3) a	1,537 (44) a	13.0 (0.4) a	0.85 (0.01) b	32.9 (0.5) a	46.8 (1.5) a
	3.5	34.8 (1.8) b	1,349 (84) b	11.5 (0.6) b	0.86 (0.02) b	30.1 (1.1) b	44.6 (1.4) a
G2	0	25.3 (0.9) a	470 (19) a	4.4 (0.2) a	0.94 (0.01) a	9.2 (0.3) a	51.7 (2.4) b
	2.33	30.5 (2.1) a	537 (29) a	4.9 (0.2) a	0.92 (0.01) a	8.7 (0.4) a	62.4 (2.8) ab
	3.5	27.9 (3.1) a	467 (37) a	4.3 (0.3) a	0.92 (0.02) a	6.7 (0.7) a	73.0 (6.9) a
Mean of main factors	G1	36.8 (1.4)	1,386 (54)	11.9 (0.4)	0.87 (0.01)	31.9 (0.7)	43.4 (1.3)
	G2	27.9 (2.0)	491 (28)	4.5 (0.2)	0.93 (0.01)	8.2 (0.5)	62.4 (4.0)
	0	30.0 (1.1)	236 (27)	7.9 (0.3)	0.92 (0.01)	21.0 (0.4)	45.3 (1.7)
	2.33	35.8 (1.7)	269 (37)	9.0 (0.3)	0.89 (0.01)	20.8 (0.5)	54.6 (2.2)
	3.5	31.4 (2.5)	234 (61)	7.9 (0.5)	0.89 (0.02)	18.4 (0.9)	58.8 (4.2)
Repeated measures ANOVA***							
Rates	F	3.9	10.7	8.2	1.0	8.2	10.1
	P****	0.031	≤0.001	0.001	0.372	0.001	≤0.001
Generation	F	74.1	1,005.4	1,210.9	33.6	3,797.9	77.9
	P	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Rates*Generation	F	1.8	7.1	4.8	2.4	1.3	2.8
	P	0.181	0.003	0.015	0.011	0.131	0.077

*Means (± SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at P ≤ 0.05. The number of replicates was 15 for the control and reduced to 13 and 7 for treatments with 2.33 and 3.5 mg cm⁻² of dry tissues, respectively.

**Means calculated using pots having 1 plant for *A. theophrasti*.

***Degrees of freedom for each factor and variables are 2 and 30 degrees of freedom effects and error, respectively for rates and interaction rates*generation and 1 and 30 degrees of freedom for effects and error, respectively for generations.

****P values in bold text indicate a significant factor.

Seed morphological parameters

Ambrosia artemisiifolia seed size (seed length, width, surface and weight) were not affected by seed exposure to Indian mustard biofumigation treatment nor generation (Table 10). Seed length ranged from $3,739 \pm 162 \mu\text{m}$ to $4,052 \pm 96 \mu\text{m}$, seed width ranged from $1,981 \pm 71 \mu\text{m}$ to $2,065 \pm 16 \mu\text{m}$ and seed surface ranged from $4.71 \pm 0.19 \text{ mm}^2$ to $5.18 \pm 0.37 \text{ mm}^2$. Neither embryo nor testa weights were influenced by treatment. However, biofumigation affected the *A. artemisiifolia* G1 embryo : seed weight ratio and the testa : seed weight ratio. Biofumigation at 7.0 mg cm^{-2} increased the relative weight of the testa and reduced the relative weight of the embryo. Whereas, the 3.5 mg cm^{-2} rate increased the relative weight of the embryo and reduced the relative weight of testa. Despite those significant differences, only generation was significant factor. *Ambrosia artemisiifolia* pericarp thickness decreased over G2 and G3, but more importantly in treated seeds compared to control seeds. Repeated measures ANOVA and main factor means of biofumigation rate showed that 3.5 mg cm^{-2} rate significantly decreased the pericarp thickness. The G3 seeds had the thin pericarp. The interaction between effects were also significant for pericarp thickness. Testa thickness was not affected by treatments in G2 but was significantly increased for G3 treated seeds compared to control. However, G3 testa thickness was generally lower than G2 testa thickness. Consequently, generation effects and the interaction between rate and generation effects were significant. Finally, seed testa weights of *A. artemisiifolia* were lower in G3 than in G2 seeds and treatment had no effect.

Abutilon theophrasti seed length, seed width and seed surface were not affected by seed exposure to Indian mustard biofumigation treatment nor across generations (Table 11). Seed length ranged from $2,706 \pm 29 \mu\text{m}$ to $2,927 \pm 114 \mu\text{m}$, width ranged from $3,265 \pm 28 \mu\text{m}$ to $3,357 \pm 19 \mu\text{m}$ and surface ranged from $6.37 \pm 0.13 \text{ mm}^2$ to $6.99 \pm 0.06 \text{ mm}^2$. G1 *A. theophrasti* plants exposed to the 2.33 mg cm^{-2} rate produced significantly lighter seeds. Embryo weight and embryo relative weight were also lower than in other treatments. Consequently, relative weight of the testa in G2 seed increased. However, differences in seed weight, relative weight of the embryo and relative weight of testa in G3 seeds were not significant. Embryo weight remained lowest in G3 seeds from plants exposed to 2.33 mg cm^{-2} . Significant differences were found for testa thickness of G3 seeds. However, for *A. artemisiifolia*, testa thickness of seeds from germinated G2 seeds exposed to biofumigation was lower than seeds in control treatment. Seedling survival increased significantly, but it could not be linked to a specific characteristic seed structure.

Plants tolerate allelochemicals by ability to reduce uptake of allelochemicals at root surface, compartmentalization of allelochemicals and detoxification of allelochemicals (15). Besides, seed tolerance to allelochemicals is related to seed size (39,51). We established that seed dormancy is interaction with seed morphological characteristics (seed surface, testa thickness), or the relative importance of testa in seed are better parameters to predict the weed tolerance to allelochemicals generated in biofumigation (29). These results agree with Waddington (46) and Weir *et al.* (50) regarding the assumption that seed morphology or other factors, such as biochemistry and detoxification potential, could be more related to seed emergence from allelopathic stress than seed size (46,50). For *A. artemisiifolia* and *A. theophrasti*, embryo : seed weight ratio, the testa : seed weight ratio and testa thickness are seed morphological parameters that changes across generations and could lead to offspring tolerance to biofumigation. The thin G3 seeds testa of *A. theophrasti* is not related to allelochemical tolerance.

Table 10. Seed morphological parameters of *Ambrosia artemisiifolia* plants from germinated seeds exposed to Indian mustard tissues and to control for generation G1 to G3 and results of the repeated measures analysis of variance for biofumigation rate and generation effects*.

Generation	Rates (mg cm ⁻²)		Seed		Pericarp		Embryo		Testa	
	Weight (100) (mg)	Thickness (µm)	Weight (mg)	Thickness (µm)	Weight: total seed weight ratio (mg:mg)	Weight (mg)	Thickness (µm)	Weight (mg)	Thickness (µm)	Weight: total seed weight ratio (mg:mg)
G1	0	504 (9)	2.49 (0.07)	22.9 (5.5)	0.49 (0.01)	2.55 (0.10)	149.0 (5.2)	2.55 (0.10)	149.0 (5.2)	0.51 (0.01)
G2	0	538 (54) a	2.37 (0.28) a	40.0 (1.5) a	0.44 (0.04) ab	3.01 (0.27) a	165.3 (3.6) a	3.01 (0.27) a	165.3 (3.6) a	0.56 (0.01) ab
	3.5	515 (34) a	2.41 (0.12) a	29.3 (1.3) b	0.47 (0.02) a	2.74 (0.26) a	155.7 (6.1) a	2.74 (0.26) a	155.7 (6.1) a	0.53 (0.02) b
	7.0	491 (36) a	1.98 (0.16) a	34.1 (1.3) b	0.40 (0.01) b	2.93 (0.20) a	159.1 (2.5) a	2.93 (0.20) a	159.1 (2.5) a	0.60 (0.01) a
G3	0	419 (18) a	2.19 (0.12) a	26.8 (1.5) a	0.52 (0.01) a	2.01 (0.07) a	127.7 (3.4) b	2.01 (0.07) a	127.7 (3.4) b	0.48 (0.01) a
	3.5	465 (27) a	2.36 (0.13) a	20.2 (1.2) b	0.51 (0.02) a	2.29 (0.19) a	141.5 (4.1) a	2.29 (0.19) a	141.5 (4.1) a	0.49 (0.02) a
	7.0	495 (34) a	2.53 (0.22) a	21.9 (0.8) b	0.51 (0.02) a	2.41 (0.16) a	147.6 (2.4) a	2.41 (0.16) a	147.6 (2.4) a	0.49 (0.02) a
Mean of main factors	G2	515 (41)	2.25 (0.19)	34.5 (1.4)	0.44 (0.02)	2.89 (0.24)	160.0 (4.1)	2.89 (0.24)	160.0 (4.1)	0.56 (0.01)
	G3	460 (26)	2.36 (0.16)	23.0 (1.2)	0.51 (0.02)	2.24 (0.14)	138.9 (3.3)	2.24 (0.14)	138.9 (3.3)	0.49 (0.02)
	0	479 (36)	2.28 (0.20)	33.4 (1.5)	0.48 (0.03)	2.51 (0.17)	146.5 (3.5)	2.51 (0.17)	146.5 (3.5)	0.52 (0.01)
	3.5	490 (31)	2.39 (0.13)	24.8 (1.3)	0.49 (0.02)	2.52 (0.23)	148.6 (5.1)	2.52 (0.23)	148.6 (5.1)	0.51 (0.02)
	7.0	493 (35)	2.26 (0.19)	28.0 (1.1)	0.46 (0.02)	2.67 (0.18)	153.4 (2.5)	2.67 (0.18)	153.4 (2.5)	0.55 (0.02)
Repeated measures ANOVA**										
Rates	F	0.1	19.7	0.3	2.8	0.3	1.3	0.3	2.8	2.8
	P***	0.928	≤0.001	0.771	0.103	0.719	0.299	0.719	0.103	0.103
Generation	F	4.6	134.7	0.6	38.6	21.7	58.6	21.7	38.7	38.7
	P	0.054	≤0.001	0.472	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Rates*Generation	F	1.9	1.6	2.5	2.7	1.5	9.1	1.5	2.7	2.7
	P	0.196	0.245	0.124	0.116	0.265	0.004	0.265	0.116	0.116

*Means (± SE) within a column followed by the same letter within species and generation are not significantly different according to Tukey's HSD test at P ≤ 0.05. Analyses were done on non-transformed data.

**Degrees of freedom for each factor and variables are 2 and 12 degrees of freedom for effects and error, respectively for rate and interaction rates*generation and 1 and 12 degrees of freedom for effects and error, respectively for generations.

***P values in bold text indicate a significant factor.

Table 11. Seed morphological parameters of *Abutilon theophrasti* plants from germinated seeds exposed to Indian mustard tissues and to control for generation G1 to G3 and results of the repeated measures analysis of variance for biofertilization rate and generation effects*.

Generation	Rates (mg cm ⁻²)	Seed		Endosperm		Embryo		Testa	
		Weight (100) (mg)	Thickness (µm)	Weight (mg)	Weight:total seed weight ratio (mg:mg)	Thickness (µm)	Weight (mg)	Weight:total seed weight ratio (mg:mg)	
G1	0	989 (7)	109.3 (5.6)	5.83 (0.14)	0.59 (0.01)	111.9 (3.7)	4.06 (0.14)	0.41 (0.01)	
	0	875 (9) a	103.6 (2.1) a	5.01 (0.04) a	0.57 (0.00) a	106.6 (1.5) a	3.74 (0.06) a	0.43 (0.00) b	
G2	2.33	824 (12) b	98.0 (2.8) a	4.53 (0.08) b	0.55 (0.01) b	104.3 (1.0) a	3.75 (0.08) a	0.45 (0.01) a	
	3.5	885 (12) a	103.6 (3.6) a	5.02 (0.11) a	0.57 (0.01) ab	107.4 (1.5) a	3.83 (0.05) a	0.43 (0.01) ab	
G3	0	949 (16) a	113.3 (3.9) a	5.50 (0.08) a	0.58 (0.00) a	111.2 (2.2) a	3.99 (0.10) a	0.42 (0.00) a	
	2.33	887 (21) a	109.8 (5.0) a	4.98 (0.14) b	0.57 (0.01) a	105.5 (1.3) b	3.78 (0.06) a	0.43 (0.01) a	
	3.5	903 (31) a	108.2 (5.0) a	5.08 (0.14) ab	0.58 (0.01) a	105.9 (0.9) b	3.76 (0.10) a	0.42 (0.01) a	
Mean of main factors	G2	861 (11)	101.7 (2.8)	4.85 (0.08)	0.56 (0.01)	106.1 (1.3)	3.77 (0.06)	0.44 (0.01)	
	G3	913 (23)	110.4 (4.6)	5.19 (0.12)	0.58 (0.01)	107.5 (1.5)	3.84 (0.09)	0.42 (0.01)	
	0	912 (13)	108.5 (3.0)	5.26 (0.06)	0.58 (0.00)	108.9 (1.9)	3.87 (0.08)	0.43 (0.00)	
	2.33	856 (17)	103.9 (3.9)	4.76 (0.11)	0.56 (0.01)	104.9 (1.2)	3.77 (0.07)	0.44 (0.01)	
	3.5	894 (22)	105.9 (4.3)	5.05 (0.13)	0.58 (0.01)	106.7 (1.2)	3.80 (0.08)	0.43 (0.01)	
Repeated measures ANOVA**									
Rates	F	2.2	0.5	2.5	1.8	1.8	0.8	1.9	
	P***	0.157	0.638	0.126	0.194	0.211	0.483	0.194	
Generation	F	12.2	16.0	18.4	15.4	1.5	1.7	15.4	
	P	0.004	0.002	0.001	0.002	0.241	0.219	0.002	
Rates*Generation	F	1.6	1.0	0.6	1.0	2.8	3.1	1.0	
	P	0.241	0.407	0.547	0.384	0.100	0.083	0.384	

*Means (± SE) within a column followed by the same letter within species and generation are not significantly different according to Tukey's HSD test at P ≤ 0.05.

**Analyses were done on non-transformed data.

***Degrees of freedom for each factor and variables are 2 and 12 degrees of freedom for effects and error, respectively for rate and interaction rates*generation and 1 and 12 degrees of freedom for effects and error, respectively for generations.

****P values in bold text indicate a significant factor.

Germination and establishment delays often lead to smaller adult plants (44). Moreover, large seedlings from large seeds have an increased growth rate and produced more flowers (45). Resource availability may influence the maternal seed production, as well as performance of seedlings (27).

Plant size, growth and seed number are pliable plant traits, but seed mass is relatively resistant to change (20). However, Callaway *et al.* (11) measured that population of species accustomed to *C. maculosa* allelopathic effect had smaller seeds than other populations. Also, Weiner *et al.* (49) found an environmental maternal effect, where initial seedling growth influences the seed weight. Whenever seed size varies, literature often reported a tradeoff between seed size and seed number (41,52). A tradeoff between seed size and seed number was observed for G1 *A. theophrasti* seeds, where plants grown from treated seeds produced more smaller seeds than control. In the present study, amplitude of flowering and flower bud production, seed production, reproductive effort, relative weight of the embryo and testa thickness are plant traits that were altered by biofumigation and where intergenerational responses vary.

Seed or plant priming is a condition whereby previous exposure to stress make future plants more resistant to the next exposure (10). Transgenerational induced stress tolerance may occur via epigenetic inheritance or maternal effect. The study provided the first detailed observations on the impact of biofumigation on weed fitness across generations. However, it was unknown if the observed response in adult plants (secondary expression of primary effects on metabolic processes) are due to stress signalling via metabolic response or mainly due to delay in germination and establishment. As *A. artemisiifolia* and *A. theophrasti* flowering is triggered by changes in photoperiods (7,48), we could hypothesize that other physiological processes are involved beside germination delay to explain our results. This assumption should be the subject of further transgenerational phenotype induction and epigenetic inheritance of allelochemical tolerance studies.

CONCLUSIONS

In laboratory bioassays, the exposure of test weed seeds (*A. artemisiifolia* and *A. theophrasti*) to phytotoxic allelochemicals from biofumigation decreased the seed germination and survival, increased seedling mortality, delayed flowering, decreased number of seeds produced and greatly reduced the weed fitness of both species. However, increased quantity of dormant seeds in *A. artemisiifolia*, seedling survival for both species, seed production for *A. theophrasti*, relative weight of embryo and testa thickness for *A. artemisiifolia* across generations changed the offspring fitness and increased the tolerance to allelochemicals generated during biofumigation. This study quantified the weed fitness and phenology of weeds exposed to sublethal biofumigant allelochemicals and helped in understanding the consequences of biofumigation on weeds population in field. Furthermore, evidence of modifications in seed and plant intergenerational responses underlined the importance to use the biofumigation technique rationally and advisedly and should be considered when biofumigation practice is part of weed management strategies.

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