Impact of *Brassica juncea* L. biofumigation on annual weed ecology and population dynamic in organic soil

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To Emma and Benjamin

May you grow curious and passionate about the world,
loving and respectful of everything and everyone living in it.

I love you

Dad

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ABSTRACT

Critical processes, including seed bank persistence, seedling establishment, and interspecific interference govern agricultural annual weed population dynamics. Agronomic practices alter weed populations and limit the weed pressure via those key processes. Among them, biofumigation is an innovative technique to control soilborne pests and weeds. Biofumigation is a mass release of volatile chemicals following Brassicaceae tissue disruption when myrosinase enzymes hydrolyze glucosinolates (GSLs). The most common volatile produced following this reaction are isothiocyanates (ITCs). However, impacts of biofumigation on weeds are not all understood. The project focused on assessment of weed species susceptibility, surviving weed responses, and key population dynamic process changes in response to allelochemicals generated during biofumigation. To meet this objective, three studies were performed. First, a laboratory experiment examined relationships amongst seed dormancy, seed morphology, and weed seed susceptibility to biofumigation. A novel Petri dish biofumigation methodology exposed seeds of eight different weed species to allelochemicals released by increasing rates of Indian mustard (*Brassica juncea* L.) biomass. The first experiment demonstrated that the dormancy state was an important factor related to seed germination, seed mortality and changes in seed dormancy. Weed species expressed specific dose responses, estimated ED_{50}, LD_{50}, and maximal mortality. Among species in the experiment, hairy galinsoga (*Galinsoga quadriradiata* Cav.) and wild carrot (*Daucus carota* L.) were the most affected by biofumigation, where maximal mortality reached 97 and 95%, ED_{50} values for germination were 2.30 and 3.23 mg cm^{-2}, and LD_{50} were 3.99 and 4.44 mg cm^{-2} of mustard tissue, respectively. Second, laboratory and greenhouse experiments assessed the impact of biofumigation on common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon*

theophrasti Medik.) fitness components of surviving plants. Potential alterations of fitness components of the second and third generations were also examined. The second project research outlined that biofumigation modifies weed fitness components by reducing seed germination and survival, promoting seedling mortality, deferring emergence and flowering, and in some cases, decreasing number of seeds produced. However, following generations of surviving weeds may improve their tolerance to biofumigation by an increased number of dormant seeds, greater survival of seedlings, possible increased seed production, and increased relative weight of the embryo, and testa thickness. Survival and establishment of seedlings from the second generation exposed to the same treatment increased from 79.7 to 95.6% for *A. artemisiifolia* and from 11.2 to 66.9% for *A. theophrasti*. Finally, a three-year field experiment assessed seasonal variation and long-term impact of biofumigation on weed community and population dynamics. Indian mustard cover crops sown in the spring and fall, once or twice a year were compared to an oat (*Avena sativa* L.) cover crop and weedy check plots. At full flowering of mustard, all cover crops were finely chopped and incorporated into the soil. Weed emergence, establishment and weed community data were recorded during the cover crop growth and after biomass incorporation. Indian mustard cover crop impacts on weeds were associated to biofumigant potential. As the amount of ITC detected increased in 2015 and 2016, Indian mustard reduced weed establishment within the cover crop growth, and post incorporation weed spring emergence. Allelopathic interference of Indian mustard increased above the corresponding GSL level in tissues generating more than 600 µg of allyl-ITC g^{-1}. Biofumigation technique realized in good conditions may lead in lower weed species richness and diversity and contribute to altering community structure, according to biofumigant potential of mustard, weed community and sites. This project exposes the potential of biofumigation for weed control, allows a better recognition of allelopathy as a

defining factor of population dynamics, and defines the fitness of surviving plants to allelochemical pressure in agroecosystems. Overall, the project provides a comprehensive understanding of mechanisms of weed population and community responses to Indian mustard biofumigation.

RÉSUMÉ

La dynamique des populations des mauvaises herbes annuelles est régie par plusieurs processus importants, notamment la persistance des banques de graines dans le sol, la levée et l'établissement des plantules et l'interférence interspécifique. Les pratiques agricoles altèrent les populations de mauvaises herbes et limitent la pression de celles-ci via ces processus clés. Parmi ces pratiques, la biofumigation est une technique novatrice permettant de lutter contre les pathogènes du sol, les nématodes et les mauvaises herbes. La biofumigation est une diffusion dans le sol de molécules toxiques durant la décomposition des tissus végétaux de la famille des Brassicacées, notamment les isothiocyanates (ITCs) produits lorsque les glucosinolates (GSLs) sont hydrolysés par l'enzyme myrosinase. Néanmoins, les impacts de la biofumigation sur les mauvaises herbes ne sont pas tous bien compris. Le projet vise à approfondir les connaissances sur la sensibilité des mauvaises herbes à la biofumigation, sur les réponses des individus survivant à ce procédé et sur les changements des processus clés de la dynamique des populations suite à une exposition aux composés allélopathiques générés durant la biofumigation. Trois études ont été menées pour atteindre cet objectif. Une première expérience en laboratoire a été réalisée pour évaluer les relations entre la dormance, la morphologie et la sensibilité des graines de mauvaises herbes à la biofumigation. Une nouvelle méthode de biofumigation en vase de Pétri a été développée et a permis d'exposer les graines de huit espèces de mauvaises herbes aux composées allélochimiques libérés lors de l'utilisation de taux croissants de biomasse de moutarde brune (*Brassica juncea* L.). Cette première expérience a souligné l'importance de la dormance des graines pour expliquer la germination, la mortalité et les changements dans l'état de dormance. Les différentes espèces à l'étude ont montré une réponse spécifique à des taux croissants de biofumigation et avaient des valeurs spécifiques de

ED_{50}, LD_{50} et de mortalité maximale. Parmi ces espèces, le galinsoga cilié (*Galinsoga quadriradiata* Cav.) et la carotte sauvage (*Daucus carota* L.) étaient les plus affectés par la biofumigation, où les taux maximaux de mortalité des graines ont atteint 97 et 95%, les valeurs de ED_{50} pour la germination étaient de 2,30 et 3,23 mg cm^{-2} et les valeurs de LD_{50} étaient de 3,99 et 4,44 mg cm^{-2} de moutarde sèche broyée, respectivement. Une deuxième expérience, menée en laboratoire et en serre, a permis évaluer l'impact de la biofumigation sur les composantes de la valeur adaptative de la petite herbe à poux (*Ambrosia artemisiifolia* L.) et l'abutilon (*Abutilon theophrasti* Medik.). Les possibles altérations de la valeur adaptative ont aussi été examinées sur la deuxième et troisième génération de plantes ayant survécu à la biofumigation. Cette deuxième étude a permis d'établir que le biofumigation modifie la valeur adaptative des mauvaises herbes principalement en réduisant la germination et la survie des graines, en provoquant une mortalité accrue des plantules en émergence, en retardant la germination et la floraison, et dans certains cas, en réduisant le nombre de graines produites. Cependant, les générations subséquentes de mauvaises herbes exposées de nouveau à la biofumigation pourraient avoir une tolérance accrue avec un plus grand nombre de graines dormantes induit par leur exposition aux composés allélochimiques, un taux de survie de plantules plus élevé, une production de graines plus importante et une augmentation du poids relatif des embryons dans les graines et de l'épaisseur de la testa. La survie des plantules est passée de 79,7 à 95,6% et de 11,2 à 66,9% entre la première et deuxième génération de plantules de *A. artemisiifolia* et de *A. theophrasti*, respectivement. Finalement, une expérience en champ de trois ans a permis d'évaluer l'impact de la variation saisonnière et l'effet à long terme de la biofumigation sur la dynamique des populations et des communautés de mauvaises herbes. Une culture de couverture de moutarde brune semée au printemps et/ou à l'automne a été comparée à une culture de couverture d'avoine

(*Avena sativa* L.) et à un témoin enherbé. À la pleine floraison de la moutarde brune, toutes les cultures de couverture ont été fauchées finement et incorporées au sol. La levée des mauvaises herbes, l'établissement des plantules et les communautés de mauvaises herbes ont été évalués pendant la croissance des cultures de couverture et suite à leur incorporation au sol. Les impacts de la culture de couverture de moutarde brune ont été associés au potentiel biofumigant. Comme les quantités d'ITCs détectées étaient plus élevées en 2015 et 2016, la moutarde brune a permis de réduire l'établissement des mauvaises herbes et la levée printanière suite à son incorporation. La composante allélopathique de l'interférence de la moutarde brune était importante lorsque les plants contenaient plus de 600 µg d'allyl-ITC g^{-1} de plante. La biofumigation réalisée dans des conditions adéquates peut mener à une richesse spécifique et une biodiversité des mauvaises herbes plus faible et ainsi contribuer à changer la structure des communautés de mauvaises herbes, en fonction du potentiel biofumigant de la moutarde, des communautés de mauvaises herbes et des sites. Globalement, les observations réalisées dans ce projet nous permettent de croire qu'une utilisation efficace et raisonnée de la biofumigation peut dans certaines conditions réduire la pression des mauvaises herbes. Le projet a permis une meilleure reconnaissance de l'allélopathie en tant que facteur déterminant de la dynamique des populations, ainsi que le rôle des changements dans la valeur sélective des plantes survivantes à la pression allélopathique dans ces agroécosystèmes. De façon générale, le projet a permis de comprendre les mécanismes qui influencent la réponse des populations et des communautés de mauvaises herbes à la biofumigation.

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PREFACE AND CONTRIBUTIONS OF AUTHORS

This manuscript-based thesis was written according to the requirements and format of McGill University. In chapter 1, an introduction describes the context of the study, the rational, and general objectives of the thesis. Chapter 2 is a literature review of the important concepts involved in the dissertation. The body of the thesis includes three scholarly papers represented as individual chapters, which are chapters 3, 4, and 5, reformatted for inclusion in the present thesis. Connecting texts between articles provide logical links between chapters. Chapter 6 is the concluding chapter and includes an overall summary, a conclusion and some directions, and suggestions for future work.

Chapter 3 entitled "Seed dormancy and seed morphology related to weed susceptibility to biofumigation", written by Maxime Lefebvre, Maryse L. Leblanc and Alan K. was published in Weed Science in March 2018.

Chapter 4 entitled "Intergenerational assessment of biofumigation on fitness and phenology of *Ambrosia artemisiifolia* and *Abutilon theophrasti*" written by Maxime Lefebvre, Maryse L. Leblanc, Gaétan Bourgeois and Alan K. Watson will be submitted to Allelopathy Journal.

Chapter 5 entitled "Impact of Indian mustard growth and incorporation on annual weed population dynamics and communities" written by Maxime Lefebvre, Maryse L. Leblanc and Alan K. Watson will be submitted to Canadian Journal of Plant Science.

The candidate wrote the proposals and application forms for financial programs in collaboration with Maryse L. Leblanc, developed and designed the experiments; collected the data while supervising undergraduate students, performed the statistical analysis, interpreted the

results and wrote the first draft of the three papers as first authors. His supervisors Professor Alan K. Watson and Dr. Maryse L. Leblanc are co-authors for all the manuscripts. They critically reviewed the papers and provided guidance during all the project and supported statistical analysis and writing. Dr. Gaétan Bourgeois, Scientific researcher at Agriculture and Agri-Food Canada, co-authored the manuscript in chapter 5. He guided the candidate to perform phenological modelling. He provided the required computer software and critically revised the article.

CONTRIBUTIONS TO KNOWLEDGE

Biofumigation has been developed and studied as an alternative of methyl bromide, a noxious product now prohibited in different countries. Originally, the main targeted pests were soilborne pathogens and nematodes. However, the technique provided promising avenue for weed management as it releases allelochemicals in soil. Despite the extensive literature evaluating mustard cover crop impact on weeds, deep comprehension of weed susceptibility, fate of surviving plants, and how this process impact population dynamic and weed communities were lacking. This thesis was conceived to fill these gaps in the literature. The study provides specific contributions to weed science knowledge as listed below. Altogether, this new knowledge increases the comprehension of the impact of biofumigation on weeds and allows interpretation of variable results in the literature.

Original contributions to knowledge include:

1. In the first study, it was demonstrated that weed seed susceptibility to allelochemicals from biofumigation was linked to seed dormancy and seed morphological characteristics.

2. The study provided first evidence of the intraspecific weed response to biofumigation according to seed dormancy.

3. A novel methodology was developed to perform biofumigation in laboratory assays, which is efficient, rapid, and easily repeatable.

4. The study demonstrated intraspecific variations of Indian mustard production of ITCs and evaluated the impact of *Brassica* cover crop biofumigation related to quantified biofumigant potential.

5. The study was the first to perform quantification of *Ambrosia artemisiifolia* and *Abutilon theophrasti* fitness under specific allelopathic pressure from *Brassica juncea* biofumigation.

6. The study was the first to achieve detailed phenological modelling of weeds exposed to sublethal biofumigant allelochemicals.

7. The experiment on intergenerational assessment of biofumigation on seed and plant responses is unique. The study was also the first to provide evidence of biofumigation exposure improving seedling survival, altering reproduction components, and seed structure of surviving weeds.

CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

Agroecosystems are highly disturbed environments where human activities tend to promote one or few species; the crops (Gliessman 2007). However, weeds are well adapted to this type of system and may compete with cultivated crops, causing yield losses or crop failure. Weed traits give huge advantages to colonize and compete with other species (Basu et al. 2004). Seed dormancy allows some species to create persistent seed banks. The weed seed bank is a reserve of viable weed seeds present in the soil (Menalled 2008). Because of the dynamic characteristics of seed dormancy, seed banks will change during the season, mostly according to environmental conditions (Benech-Arnold et al. 2000; Gardarin et al. 2012; Murdoch 1998). Seed bank persistence is a key process for plant population dynamics. Seed bank inputs and outputs control seed density and species composition, and changes in their relative importance may reshape the weed community (Simpson et al. 1989). Seedling establishment and interspecific interference are other important processes of annual weed population dynamics (Gallandt et al. 1999). Seedling emergence from the soil seed bank is an important factor that dictates the need to apply weed control measures repeatedly (Foley 2001). Interspecific interference refers to plant-to-plant competition. A goal in weed management is to reduce the interference of weeds to promote the crop (Liebman and Gallandt 1997). To suppress weeds, organic growers must use numerous tools, strategies and practices. However, success of those practices depends on ecologically based exhaustive knowledge of their impact on processes of population dynamics (Coleman and Hendrix 1988).

An emerging method to control soilborne pests and weeds is biofumigation (Lopez-Martinez et al. 2006; Matthiessen and Kirkegaard 2006). Biofumigation is defined by the release

of toxic molecules, mainly isothiocyanate (ITCs) after enzymatic reaction during Brassicaceae decomposition (Morra and Kirkegaard 2002). This process occurs in the soil, consequently, ITCs affect essentially the seed bank. Biofumigation crops interfere and control weeds by vigorous early season growth, leaching of glucosinolates (GSLs) into the soil during plant growth, and release of allelochemicals at incorporation of plant residues (Jabran 2017; Narwal and Haouala 2013).

Isothiocyanates decrease germination of many weed species (Al-Khatib et al. 1997; Al-Sherif 2013; Boydston et al. 2004; Petersen et al. 2001). High concentrations of ITCs diffuse into the seeds, irreversibly inhibit protein synthesis, and reduce seed viability (Leblova-Svobodova and Kostir 1962). Biofumigation can reduce weed germination, establishment, and weed biomass in the field (Boydston et al. 2004; Kumar et al. 2009; Walker and Kremmydas 2010).

Impact of biofumigation on weeds requires more investigations. Impacts of this process on soilborne pathogens are documented, but this project attempts to understand how this method acts on specific key points of weed population dynamics. The susceptibility of dormant and quiescent seeds in the seed bank to biofumigation performed at different moments during the season is unknown. This strategy could lead to efficient weed management in the field. Assessment of seed mortality to biofumigant allelochemicals was required to know if this method could deplete the seed bank. It is necessary to know if maternal plants in contact with ITCs influence the next generations of seeds that survived the treatment. If biofumigation impacts the reproduction parameters (flowers, buds, seeds), the fitness will also be modified, consequently their competitive ability. Furthermore, if the selection pressure brought by biofumigation is strong, it could lead to resistance development for weed species. Production of glucosinolates could vary between plants that grew at different times during the year (Inderjit et

al. 2011). Timing of Brassicaceae seeding could lead to different amounts of ITC released into the soil. Moreover, knowing that the seed bank changes during the season, it is possible that time became a factor with strong impact on the repressive potential of biofumigation on weeds. To our knowledge, this strategy has not been previously studied for weed management.

The proposed project promotes the understanding of the population dynamics mechanisms to this allelopathic pressure. Exploring the susceptibility of types of seed dormancy and weed fitness to allelochemicals is a novel contribution to knowledge and allows deeper comprehension of field results. The thesis highlights allelopathy as a defining factor of population dynamics and the fitness of surviving plants to allelochemical pressure. The Brassica biofumigation technique can be efficiently and wisely used in an ecologically based weed management.

1.2 Objectives of the thesis

The global objective of the thesis was to assess and understand weed susceptibility, responses of surviving weeds and impacts on key processes of population dynamics to allelochemicals generated during biofumigation. To achieve this goal, the dissertation includes three main studies to reach specific objectives and to verify different hypotheses. Indian mustard (*Brassica juncea* L. 'Caliente 199') served as the biofumigant crop and the biological model to test those hypotheses.

The objectives of the study in chapter 3 were to determine weed seed responses to allelopathic compounds generated during biofumigation and to assess species susceptibility related to seed dormancy and seed morphological parameters. This study was designed to explain susceptibility of a weed species to biofumigation, including clarification of weed responses to

biofumigation and underline key morphological variables, such as testa thickness, seed size, or proportion of dormant seeds.

The objectives in chapter 4 were to determine *Ambrosia artemisiifolia* and *Abutilon theophrasti* fitness and phenological responses to biofumigation, and to assess potential changes in fitness and phenology of those weeds across generations.

In chapter 5, the objectives were to determine, in a controlled environment, the impact of increasing rates of Indian mustard tissues incorporated in soil on seed mortality and seedling establishment. Furthermore, this study aimed to assess the susceptibility of field weed populations to biofumigation throughout the season, to assess the effect of repeated biofumigation treatments within the same year on weed populations, and to assess the cumulative effects of biofumigation on the weed biodiversity and community.

1.3 Hypotheses

The following hypotheses were tested in the thesis according to each study presented:

Chapter 3: 1) seeds with physical dormancy (PY) are less affected by ITCs than seeds with physiological dormancy (PD) because greater protection was provided by the testa; 2) a greater proportion of dormant seeds in a seed lot (i.e. seeds collected from several individuals of one population) decreases the impact of biofumigation on seed germination and survival response (intra and interspecific); and 3) physical parameters of the seed are related to species germination and survival from biofumigation.

Chapter 4: 1) exposure of weed seeds to increasing concentrations of allelochemicals generated during biofumigation decreases survival; and 2) seed exposure to sublethal

concentrations of allelochemicals negatively influences the growth and reproduction of plants, and these effects decrease when next generations are exposed to the same treatment.

Chapter 5: 1) incorporation of Indian mustard tissues in soil reduces buried seed survival and reduces seedling establishment; 2) repressive effect of biofumigation on a weed population changes through the year; 3) repeated biofumigation reduces weed emergence at each operation; and 4) specific response of weeds to biofumigation changes the abundance, the richness, and the diversity over a season and through the season.

CHAPTER 2. LITERATURE REVIEW

2.1. Weed population dynamics and weed control

Agroecosystems undergo numerous perturbations, mainly man-made. Even if those perturbations are done to favour the crop, weeds may find their way to compete and overcome the crop. Weed management strategies attempt to minimize the impact of weeds on crops. Those tactics can be preventive, physical, managerial, chemical, and biological or the integration of the above (Zimdahl 2016). Weed control techniques act on one or more key processes of population dynamics to reduce weed infestations.

Population dynamics of agricultural annual weeds include three key processes; seed bank persistence, seedling establishment and interspecific interference. Those are critical processes having a major role in annual weed population dynamics (Swanton et al. 1993).

The weed seed bank is a reserve of thousands of viable weed seeds present on the soil surface and scattered in the soil profile, where they can persist over several years (Menalled 2008). Weed populations successfully establish from persistent seeds in the seed bank. Soil environment is associated with the fate of seeds from the seed bank. Germination, predation, decay, and embryo death are mechanisms that could decrease the seeds viability in soil (Gallandt et al. 1999). Several defining factors are related to those mechanisms, for example, dormancy reduces germination, predator abundance represents predation and time passed in soil is related to embryo death (Gallandt et al. 1999). Effect of phytochemicals in soil is more related in the literature to seedling establishment and interspecific interference than losses of seeds from the seed bank (Liebman and Gallandt 1997). Seed banks change in time, mostly in function of the dynamic characteristic seed dormancy to environmental conditions (Benech-Arnold et al. 2000; Gardarin et al. 2012; Murdoch 1998). Annual emergence patterns and variations in the state of

dormancy are central factors of seed bank dynamics and regulate weed pressure in time (Cavers and Benoit 1989). Agronomic practices could modify seed dormancy in the seed bank (Dyer 1995). Few agronomic strategies target the seed bank to reduce weed emergence even if there is a strong correlation between future population size and seed mortality (Gonzalez-Andujar and Fernandez-Quintanilla 1991; Jordan 1993; Jordan et al. 1995).

Success of seedling establishment relies on environmental conditions of the germination site. Seeds should germinate in an environmentally safe site (appropriate depth, light, temperature, water, free of predators, and disease) (Harper 1977). Germination, emergence, and survival are processes related to the seedling establishment. Defining factors like resources and edaphic conditions, seed size, surface residues, pathogens, or allelochemicals regulate those processes (Naylor 1985). Seed abundance and availability of safe-sites are directly related to weed invasion. Weed control techniques should aim to reduce these safe-sites or fill them with crops or other desired plants. Nonetheless, emergence from the soil seed bank is an important factor that dictates the need to apply weed control measure repeatedly (Foley 2001).

Interspecific interference is defined as direct and indirect plant-to-plant competition. Performance of weeds or crop is determined by species-specific resource capture, conversion efficiency of resources, biomass allocation, and response to allelochemicals (Gallandt et al. 1999). Relative time of emergence, resource quality, quantity, placement, and allelopathy may increase crop interference to the detriment of weeds (Liebman and Gallandt 1997).

2.2. Weed community and agronomic practices

In agroecosystems, weed management strategies are obligatory filters to weed communities causing strong effects on weed ecology. According to the community assembly

theory, management practices may filter specific plant traits, such as emergence periodicity, growth habit or susceptibility to phytochemicals (Storkey et al. 2010). Biotic and abiotic filters may reduce the establishment, growth and reproduction of weeds, resulting in a specific weed community (Booth and Swanton 2002). Susceptibility of plant communities to environmental filters varies in time. Weed control strategies may be more effective when weed communities are vulnerable to disturbance (Booth and Swanton 2002). Resulting weed populations are directly linked to population dynamic processes, and variations in the relative importance of those processes will restructure weed communities (Simpson et al. 1989; Swanton et al. 1993). Weed life history and emergence periodicity are also important functional traits related to weed community assembly in organic systems (Ryan et al. 2010).

2.3. Environment and maternal effect

Environmental conditions in which a plant grows, especially during sensitive growth stages, could influence seed production (Parrish and Bazzaz 1985) and its fitness (Platenkamp and Shaw 1993). Moreover, it could influence the offspring of those plants, known as the phenotypic maternal effect (Roach and Wulff 1987). Seed or plant priming refers to previous exposure to stress making future plants more tolerant to this stress (Bruce et al. 2007). Maternal effect studies focus on the offspring phenotypic induction by treatment or environmental exposure on seedlings or adult plants (Holeski and al. 2012). Mother plants may affect their seeds by one or more mechanisms: genetics non-Mendelian inheritance (e.g. extrachromosomal or cytoplasmic inheritance), through information passed from the mother to the offspring via chemicals produced by the mother. Transgenerational induced stress tolerance may also happen via epigenetic inheritance (Holeski and al. 2012). Preconditioning effects are recognized to

modify germination and dormancy behaviour of seeds (reviewed in Baskin and Baskin 1998a). Indeed, previous maternal environment may change seed sensitivity to environmental factors related to germination (temperature, nitrate, light, water, oxygen, and allelopathic compounds) (Finch-Savage and Leugner-Metzger 2006). Maternal effects may have genetic and environmental components and will be adaptive if offspring reproductive success increases (Lacey 1998). Some components of seed dormancy are heritable in several species, including dormancy breaking and germination requirements.

Plant size, growth, and seed number are plastic traits, but seed mass was relatively resistant to modification (Harper et al. 1970). Whenever seed size varies, a trade-off between seed size and seed number may be observed (Wilbur 1977; Primack 1978). Nonetheless, Callaway et al. (2005) recorded smaller seeds in species exposed to *Centaurea maculosa* Lam. allelopathic compounds than in un-exposed populations of the same species.

2.4. Seed dormancy

Dormancy is an adaptive trait that promotes the survival and distribution over time in an ever-changing environment of many organisms (Foley 2001). Dormancy in plants occurs mainly in vegetative propagules and seeds. Seed dormancy remains misunderstood regardless of considerable research during past decades (Hilhorst 2011). Seed dormancy is a state where viable seeds fail to germinate under a specific set of environmental conditions required for germination (Baskin and Baskin 2004, Simpson 1990). Nevertheless, these conditions will allow quiescent seeds to germinate. Classification systems and nomenclature exist for improved description and understanding of seed dormancy. Primary and secondary dormancy indicate the moment when dormancy happens (Foley 2001). Primary dormancy refers to freshly mature seeds that fail to

germinate when seeds are removed from the mother plant, or just after dispersal. Secondary dormancy generally occurs after dispersed seeds come across environmental conditions for a lengthy period to induce a quiescent state, known as after-ripening (Bewley and Black 1994). The classification system used by Baskin and Baskin (1998b, 2004) includes two main categories of dormancy, attributed to the location of the mechanisms or restrictions to germination. Exogenous or seed coat-imposed dormancy implies characteristics of covering structures that avert germination. Whereas, endogenous dormancy indicates that some characteristics of the embryo prevent germination. This classification system examines the mechanism involved and includes five classes of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY + PD). PD is a physiological inhibiting mechanism in the embryo that prevents radicle emergence and growth. However, structures adjacent to the embryo may be involved in PD. Covering structures could restrict oxygen availability or could control the release of growth inhibitors. PD can be divided into three levels; non-deep, intermediate and deep. Non-deep PD is the common type of dormancy among weeds in arable fields, explaining why most studies have focused on it. Common lambsquarters (*Chenopodium album* L.), common ragweed (*A. artemisiifolia)*, and green foxtail (*Setaria viridis* (L.) Beauv.) are examples of species exhibiting PD. When *A. artemisiifolia* seeds mature, they are initially primary dormant. Moist chilling is required to release primary dormancy. The seeds can go in and out of the secondary dormancy cycle, but require stratification to come out of secondary dormancy (Baskin and Baskin 1980). This species begins to emerge in southern Quebec at the beginning of May with a maximum occurrence between mid-July and mid-August (Bassett and Crompton 1975). Mature seeds occur on plants in early September to the end of November. *Ambrosia artemisiifolia* is wind-pollinated. About

95% of *A. artemisiifolia* plants are monoecious, a few are completely pistillate, and some plants exhibit an intermediate phenotype, being predominantly staminate or pistillate (Bassett and Crompton 1975). *Chenopodium album* is an important summer annual weed. It requires cold stratification to release seed dormancy, but in some situations, elevated temperatures may act as after-ripening treatment. Germination is superior in light than dark conditions and in temperatures between 15 C to 30 C (Baskin and Baskin 1977). *Setaria viridis*, a summer annual, also requires cold stratification to release dormancy. The optimum temperature for germination is around 25 C (Vanden-Born 1971). Morphological dormancy refers to an underdeveloped embryo. Only time will allow the embryo to mature and germinate. Wild carrot (*Daucus carota* L.) was a biennial weed with morphological seed dormancy. Seeds can after-ripen in dry laboratory storage at 15-30 C and light increases germination (Baskin and Baskin 1980). A seed coat impermeable to water causes physical dormancy. Seeds exhibit heavily lignified palisade cells impregnated with various water-repellent compounds (Baskin and Baskin 1998b; 2004). A specialized "water-gap" on the impermeable layer must be open to release dormancy in response to proper environmental cues. Stratification could also release dormancy, as for bird vetch (*Vicia cracca* L.), clovers (*Trifolium spp.*), and *A. theophrasti*. *Vicia cracca* and *Trifolium spp.* are perennial weeds and seed germination occurs from 15-20 C (Grime et al. 1981). *Abutilon theophrasti*, is a self-compatible, autogamous species, flowers are fertilized the day they open and seeds mature 17 to 22 days after pollination. Primary dormancy can be released by stratification after one year of storage in moist conditions, and 5-10 minutes in hot water (60-70 C) also releases dormancy. In eastern Canada, *A. theophrasti* starts to flower in late August to September, setting seeds from September to October, and continues producing flowers

on axillary branches until the first frost (Horowitz and Taylorson 1983; Warwick and Black 1988).

2.5. Allelopathy and weeds

Allelopathy is known 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)” (Narwal and Haouala 2013). Allelopathy is a biotic environmental stress factor reducing the competitive ability of a target plant in many ways, including direct inhibition of plant functions (Duke and Dayan 2006; Pedrol et al. 2006). Phytotoxic allelochemicals can decrease photosynthesis rate, carbon acquisition, and plant growth (Hussain and Reigosa 2011).

Allelopathy plays a significant role for pest management in agronomical ecosystems, particularly in organic and sustainable plant production (Jabran et al. 2015). Allelopathy has been widely studied and its potential for weed management is recognized. In numerous agronomic practices, allelopathy is a defining factor reducing weed pressure (Gallandt et al. 1999; Qasem 2010, 2013). It perturbs key processes of population dynamics, especially germination, and growth of weeds (Qasem 2010, 2013). Focusing on the seed bank, allelopathy can inhibit or stimulate germination of some weeds (Netzly et al. 1988). Allelopathy may be used in fields as: crop accessions, varieties or cultivars, bark mulch, bioherbicides, cover crops or living mulch, dead mulch, dead woodchips mulch, decomposed straw, extracts, formulations, green manures, intercropping, relay crops, plant residues as a soil cover or soil incorporation, root layer, crop rotation, seed meal, etc. (Qasem 2010; Qasem 2013). Allelopathic crop residues can reduce germinable seeds in soil, and cover crops contribute to weed management by

interference and seedling establishment (Kohli et al. 2006; Weston and Duke 2003). However, suppression varies by crops and by weed species (Qasem 2010; Qasem 2013). Phytotoxic allelochemicals are released by crops, cover crops or their residues and may be water soluble, insoluble or volatile (Kohli et al. 2006). Furthermore, a phytochemical may act as an allelochemical in one circumstance, but not in another situation because concentration, residence time, and the fate of a chemical are controlled by substratum factors (Inderjit 2001).

2.6. Allelopathy and weed fitness

Weeds express weediness traits that increase their competitive ability in agroecosystems, such as rapid seedling growth, high seed output, environmental plasticity or discontinuous dormancy (Zimdahl 2013). Plant fitness is a notion that evaluates the relative ability of an individual or a population to survive and reproduce in its environment (Krebs 2001). Plant fitness components are germination, establishment, survival, and reproduction. Plant-produced allelochemicals have harmful consequences on target plant fitness mostly by inhibiting the establishment and/or growth (Inderjit et al. 2011). Some authors have also related lower fitness of the target plant to indirect disturbance in plant mutualism interactions (Reviewed in Hale and Kalisz 2012). In some situations, allelochemicals could influence flower production, and may even completely prevent reproduction (Batlang and Shushu 2007). Germination and establishment delays frequently lead to smaller plants (Ross and Harper 1972). Additionally, large seedlings from large seeds have improved growth rates and produced more flowers (Stanton 1984). Resource availability may affect maternal seed production, along with performance of seedlings (Lacey 1998).

2.7. Allelopathy and seed dormancy

Variation may occur in dormant and non-dormant seeds found in the same species. This variation is related with the 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 (Baskin and Baskin 2006). Environmental factors can cause changes in the dormancy state of non-deep PD; temperature, darkness, light, gases, water, inorganic and organic chemicals. Although, no allelochemicals have been shown to cause direct changes in dormancy state (Baskin and Baskin 1998a). Allelochemicals could constrain germination until further environmental factors induce dormancy (Baskin and Baskin 1998a), for example, by altering the optimal temperature for seed germination or light responses of seeds (Batak et al. 2002; Finch-Savage and Leubner-Metzger 2006).

2.8. Biofumigation

Biofumigation has been developed mainly as an alternative of methyl bromide. This extremely noxious product is prohibited in different countries (Australia, Italy, USA) since January 1st 2005 (Michel 2008). Biofumigation is performed in the field with a Brassicaceae cover crop, aiming to control soilborne pests, nematodes, and weeds (Matthiessen and Kirkegaard 2006). The toxic potential of biofumigation comes from the enzymatic reaction of myrosinase found in the cytoplasm of special idioblasts with glucosinolates (GSL) found in the vacuole of other cells. Brassicaceae plants are known to contain high concentrations of GSL (Yoneyama and Natsume 2010). When plant tissues decompose, cell walls breakdown and myrosinase comes in contact with GSL. Combined with water, the chemical pathway leads to

production of isothio- and thiocyanates (Michel 2008; Matthiessen and Kirkegaard 2006). *Brassica juncea* (L.) Czern. is one of the main species used as a biofumigant plant. Several varieties are selected for their glucosinolate concentration, including *Brassica juncea* v. Caliente 20 and Caliente 199 (Langlois et al. 2010). Volatile phytotoxic chemicals are generated from decomposing *Brassica* tissues and offer weed control.

The general structure of ITCs is R-N=C=S, with a highly electrophilic carbon atom, that easily reacts with cellular thiols, such as cysteines in proteins and low-molecular-weight thiols (especially glutathione), resulting in dithiocarbamate derivatives. These modification cause losses of protein structure and function, and thus decreases enzymatic activity (Dufour et al. 2013). Benzyl-ITC causes intracellular protein aggregation in *Campylobacter jejuni* by targeting proteins, resulting in the disruption of major metabolic processes and eventually cell death. In seeds, the mode of action of ITCs involves interaction with glycolytic enzymes in the germination progression and so prevents or defers seed/tuber germination (Drobnica et al. 1977).

There are some other mechanisms during *Brassica* decomposition that can influence seed germination dynamics in lower extant. Biofumigation does not generate only ITCs during decomposition. In addition to ITCs, breakdown of GSLs also creates a cocktail of other compounds. Low pH could result in generation of other molecules, such as nitriles, epinitriles, oxazolidinethiones, and ionic thiocyanates during GSL hydrolysis, that could influence targeted pests (Brown and Morra 1997; Parvatha 2013). Furthermore, high levels of residues reduce and delay emergence, mainly by decreasing soil thermal amplitude and preventing light penetration (Dyer 1995). Finally, it should be considered that decaying residues can immobilize large amounts of N; resulting in low soil nitrate levels preventing termination of dormancy in some species. Mechanism for releasing dormancy by low nitrate concentrations is unknown, but may

act at the membrane level (Karssen and Hilhorst 1992). Altogether, these other processes during biofumigation could influence weed populations.

The biofumigation technique involves some actions to expand effectiveness. In the field, cover crops must be finely cut and incorporated into the soil at full flowering, when the GSL concentrations are maximum (Taylor et al. 2014). Sufficient soil moisture is essential for the chemical reaction between myrosinase and GSL and the soil temperature must be more than 10 C (Matthiessen et al. 2004; Michel 2008). After incorporation, the soil must be sealed (ex. with plastic mulch) (Kumar 2005; Langlois et al. 2010; Michel 2008). Maximum concentration of ITC in soil from rapeseed (*Brassica napus* L.) and Indian mustard degradation are observed just after incorporation and very little is detected after five days (Gimsing and Kirkegaard 2006; Morra and Kirkegaard 2002).

2.9. Environment and biofumigation

Environment greatly influences plant physiology and growth. Production of secondary metabolites like glucosinolate are also affected by the environment, such as the occurrence of other species or by seasonal variation (Brown and Morra 1997; Inderjit et al. 2011). Glucosinolate concentrations in plants are associated with sulfur bioavailability in soils (Ciska et al. 2000; Falk et al. 2007). Glucosinolates are sulfur-containing organic molecules (Halkier and Gershenzon 2006). Sulfur supply increases GSLs in some *Brassica* species (Kim et al. 2002; Schonhof et al. 2007) as well as Indian mustard (Tong et al. 2014). Additionally, *in situ* factors may disturb the activity of biofumigants including soil colloidal adsorption and microbial alteration of allelochemicals (Price et al. 2005).

In field conditions, plants may not generate their total potential of ITCs (Morra and Kirkegaard 2002). Unhydrolyzed GSLs in soil are caused by imperfect tissue pulverization or other restrictions to GSL hydrolysis (soil condition, water availability, or crop variety). Morra and Kirkegaard (2002) measured around 1% of ITC release efficiency in a field experiment, which increased to 26% using frozen plant tissues. In that context, *Brassica* plants were not chopped nor irrigated when incorporated. Under ideal situations, ITC release efficiency ranged from 9.7% to 18.5%, with a peak at 56% when high GSLs mustard was incorporated (Bangarwa et al. 2011, Gimsing and Kirkegaard 2006).

2.10. Weeds and biofumigation

Processes of weed population dynamics recognized to be influenced by biofumigation in the field are seedling establishment and interference. Biofumigation crops interfere and reduce weed infestation by strong early season growth, leaching of GSLs into the soil during plant growth, and production of allelochemicals at incorporation of cover crops (Jabran 2017; Narwal and Haouala 2013). Allelopathy and direct competition are together important contributors of *Brassica* cover crops interference to weeds (Kunz et al. 2016). Incorporation of *Brassica* cover crops could reduce weed biomass in following crops, also reported by Boydston and Hang (1995). Incorporation of rapeseed decreased weed biomass by 50 to 96% in following potato crop, compared to bare fallow treatment. In the field, white mustard decreased *Galinsoga ciliata* (Raf.) Blake. growth and seed production during cover crop growth and after incorporation (Kumar et al. 2009).

Weed responses to phytotoxic allelochemicals from biofumigation are known to be species-specific, positives, or negatives. Norsworthy (2003) reported the aqueous extract of wild

radish (*Raphanus raphanistrum* L.) decreased germination of dicotyledons more than monocotyledonous species. Exposure to ITCs in a greenhouse experiment decreased emergence for Palmer amaranth (*Amaranthus palmeri* S. Watson) more than pitted morning glory (*Ipomoea Lacunosa* L.) and yellow nutsedge (*Cyperus esculentus* L.) (Norsworthy and Meehan 2005a). In a greenhouse experiment, 20 g of white mustard (*Sinapsis alba* L.) or rapeseed were incorporated in 400 g of dry soil, shepherd's-purse (*Capsella bursa-pastoris* (L.) Medicus.) emergence decreased by 97 and 76%, kochia (*Kochia scoparia* L. Schrad.) by 54 and 25%, and *S. viridis* by 49 and 25% (Al-Kathib et al. 1997). During Brassicaceae growth (Indian and white mustard), *Setaria* spp., *Trifolium spp*. and *Poa* spp. had lower germination rates in greenhouse tests. Incorporation of Brassicaceae cover crops could also cause a global reduction of weed density (Walker and Kremmydas 2010). A biofumigation mix decreased the dry weight of scarlet pimpernel (*Anagallis arvensis* L.) by 40% and its density, but not the density of annual bluegrass (*Poa annua* L.) in field trials (Mattner et al. 2008). Seed size was not significantly correlated with reduction in emergence of weeds caused by cover crops, including Brassicaceae (Haramoto and Gallandt 2005). In greenhouse experiments, when white mustard (*Brassica hirta* L.), Indian mustard, and canola (*Brassica napus* L.) were cut and incorporated into soil, weed emergence was reduced by 20 to 95% and weed biomass by 8 to 90% (Boydston et al. 2004). Hormetic dose response is a stimulatory response at low concentration of a chemical that is detrimental at higher concentration. Germination of Texas millet (*Urochloa texanum* (Buckl.) R. Webster), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and sicklepod (*Senna obtusifolia* (L.) H.S. Irwin & Barneby) increased at low levels of allyl-ITC (Norsworthy and Mehan 2005b).

A promising characteristic of biofumigation for weed control in agroecosystems may be the potential to increase losses from the seed bank. Like other toxic molecules, high

concentrations and increased exposure time of ITCs increase inhibition of seed and tuber germination, leading to mortality (Aliki et al. 2014). When the ITCs were in sufficient amounts to penetrate the seed, reactions with enzymes are irreversible and seeds lose their viability (Bangarwa and Norsworthy 2015, Leblova-Svobodova and Kostir 1962). Rapeseed crop killed 98, 68 and 51% of milkweeds (*Asclepias* spp.), *Setaria* spp. and prairie dock (*Silphium terebinthinaceum* Jacq.), respectively. Covering the soil with plastic mulch, after incorporation, increased suppression of *S. terebinthinaceum* to 94 and 100% for *Asclepias* spp., whereas *Setaria* spp. germination increased instead of being reduced (Parvatha 2013). Benzyl-ITCs from decomposition of white mustard was phytotoxic to *A. theophrasti* and *S. obtusifolia* (Dharamraj et al. 1994). Mustard seed meal application at 1.0 t ha^{-1} decreased the densities of *C. album* and scentless mayweed (*Matricaria inodora* L.) by 56 to 100% and completely controlled *C. bursa-pastoris* (Ascard and Johanson 1991; Nilsson and Halgren 1992). Higher rates of biofumigant from turnip rape (*Brassica rapa* L.) and rapeseed material in bioassays caused greater reduction of germination in seven clover species, and seed size did not influence susceptibility (Mattner et al. 2008). Seed germination was delayed with increasing concentration from 1 to 4% (w/v) of *Brassica* powder in distilled water and at 4%, no germination was observed in laboratory experiments (Al-sherif 2013). Petersen et al. (2001) observed that ITCs act like strong suppressants of the germination of spiny sowthistle (*Sonchus aper* (L.) Hill), *M. inodora*, smooth pigweed (*Amaranthus hybridus* L.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), black grass (*Alopecturus mysuroides* Huds), and wheat (*Triticum aestivum L.*). Low concentrations delayed germination and ungerminated seeds remained viable.

Petersen et al. (2001) hypothesized that all ITCs have the same mode of action in weed seeds, targeting the enzymes of glycolysis and respiration, possibly due to the same general

structure of ITCs. However, some authors found contrasting results, where different ITCs released during biofumigation did not have the same allelopathic effect on different weed species. Al-Kathib et al. (1997) reported that methyl, ethyl, allyl, and phenyl-ITC suppressed germination and growth of E. *crus-galli*, redroot pigweed (*Amaranthus retroflexus* L.), and common pea (*Pisum sativum* L.) better than other ITCs. According to Norsworthy and Meehan (2005a), weed responses are different when expose to different ITCs, and phenyl-ITC and 3-methylthiopropyl were the most effective in inhibiting germination of *A. palmeri*, *I. Lacunosa*, and *C. esculentus*.

In other contexts, incorporation of Brassicaceae green manure did not perform better than other green manures to control weeds. Yellow mustard 'Idagold' cover crop was not more efficient in repressing weeds than buckwheat (*Fagopyrum esculentum* Moench), oat, crimson clover (*Trifolium incarnatum* L.), canola 'Hyola', or rapeseed 'Dwarf Essex' in the Haramoto and Gallandt's (2005) experiment. Björkman et al. (2015) detected no relation between weed control and incorporated mustard biomass, nor GSL content in the cover crops. Biofumigation decreased the early emergence of weeds compared to wheat by 30%, but differences according to weed density fade before harvest, later in the summer (Al-Kathib et al. 1997).

Regarding the fate of dormant seeds exposed to these treatments, Teasdale and Taylorson (1986) reported methyl-ITC (MIT) delayed germination of *D. sanguinalis* at 0.6 to 1.0 mM concentration and stimulated germination of dormant seeds. However, at 4.0 mM, non-dormant and dormant seeds were killed. The product tested was not from Brassicaceae tissues, but was the chemical fumigant, Metham. However, MIT could be one of the products generated during the biofumigation.

2.11. Tolerance to allelochemicals

Plants can stand allelochemicals by a reduced uptake of allelochemicals at the root surface, compartmentalization, and detoxification of allelochemicals (Duke 2003). Small seeds are frequently reported as susceptible to allelopathy (Petersen et al. 2001; Westoby et al. 1996), but not in other situations (Haramoto and Gallandt 2005; Mattner et al. 2008). Embryo and endosperm are seed structures at the core of the germination process. They may act as possible sites for allelochemical detoxification (Bailly 2004). Seed morphology or seed biochemistry could be more linked to seed emergence from allelopathic pressure than seed size (Waddington 1978, Weir et al. 2004).

Resistance in plants evolves rapidly in response to man-made chemical herbicides. Plants can also adapt to the soil specific chemical composition of neighbouring plants community (Ehlers and Thompson 2004). Few studies evaluated the resistance of species to allelopathic compounds. Callaway et al. (2005) studied the resistance of a native population exposed to *C. maculosa* allelopathic root exudates and they observed that new populations exposed to this species were more affected than coexisting species. Possible resistance to ITCs from biofumigation has not been studied.

CONNECTING STATEMENT BETWEEN CHAPTERS 2 AND 3

Important concepts and information about weed ecology, annual weed population dynamics, seed dormancy, allelopathy, and biofumigation were reported in chapter 2. However, there was a lack of knowledge about weed seed and plant susceptibility to biofumigation. A first step toward an increased comprehension of seed and individual weed responses as driving mechanisms of biofumigation influence on weed ecology, laboratory experiments were conducted and reported in chapter 3. This study assesses the weed seed responses to biofumigation and investigates the relations amongst seed response, seed morphological characteristic, and seed dormancy. Moreover, intraspecific weed response was evaluated according to seed dormancy.

CHAPTER 3. Seed dormancy and seed morphology related to weed susceptibility to biofumigation

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3.1 Abstract

Biofumigation is practiced for control of soilborne pests and weeds in agronomic fields. The objectives of this research were to assess the dose response of weed seeds to Indian mustard biofumigation and associate responses to seed dormancy state, initial dormancy and seed parameters. A Petri dish biofumigation methodology was developed to expose seeds of common lambsquarters (*Chenopodium album* L.), bird vetch (*Vicia cracca* L.), wild carrot (*Daucus carota* L.), common ragweed (*Ambrosia artemisiifolia* L.), green foxtail (*Setaria viridis* (L.) Beauv.), velvetleaf (*Abutilon theophrasti* Medik.), hairy galinsoga (*Galinsoga quadriradiata* Cav.), and red clover (*Trifolium pratense* L.) to allelochemicals produced after rehydrating 0 (control), 2.33, 3.50, 7.00, 14.00, and 21.00 mg cm^{-2} of dried mustard powder. Weed species expressed specific dose responses, estimated ED_{50}, LD_{50}, and maximal mortality. *Galinsoga quadriradiata* and *Daucus carota* were consistently the most affected by biofumigation, where maximal mortality reached 97 and 95%, ED_{50} values for germination were 2.36 and 3.23 mg cm^{-2} and LD_{50} were 4.03 and 4.38 mg cm^{-2} of dried mustard tissue respectively. Initial dormancy was assessed by germination and tetrazolium tests. Seed parameters such as testa thickness, relative weight of the testa, and seed size were measured directly by manual dissection, weighing seed structures, and stereomicroscope imaging software measurements. Linear regression analyses revealed initial dormancy to be positively related to ED_{50} and LD_{50} values with a significant interaction with seed surface and seed width respectively. Exposure to 7.00 mg cm^{-2} of dried mustard powder increased *A. artemisiifolia* seed mortality for after-ripened seeds by 293% and by 58% for primary dormant seeds compared to untreated seeds. Mortality of *C. album* secondary and primary dormant seeds increased by 730 and 106%, respectively and for *D. carota* by 1,193 and

156%, respectively. Results underline the potential to incorporate biofumigation into weed management programs for repression of susceptible weed species.

Nomenclature: Velvetleaf, *Abutilon theophrasti* Medik. ABUTH; common ragweed, *Ambrosia artemisiifolia* L. AMBEL; Indian mustard, *Brassica juncea* L. 'cv. Caliente 199'; common lambsquarters, *Chenopodium album* L. CHEAL; wild carrot, *Daucus carota* L. DAUCA; hairy galinsoga, *Galinsoga quadriradiata* Cav. GASCI; green foxtail, *Setaria viridis* (L.) Beauv. SETVI; red clover, *Trifolium pratense* L. TRFPR; bird vetch, *Vicia cracca* L. VICCR; ED_{50}: estimated half maximal effective dose of dry mustard biomass that decrease germination; LD_{50}: lethal dose of dry mustard biomass that kills 50% of viable seeds.

Keywords: Allelochemical tolerance, allelopathy, embryo, isothiocyanates (ITCs), non-linear response, seed size, testa.

3.2 Introduction

Biofumigation is an agronomic practice that could be used in organic farming to reduce the weed seed bank (Matthiessen and Kirkegaard 2006). Volatile phytotoxic chemicals are released from decomposing *Brassica* tissues and provide weed suppression. Biofumigation crops compete and suppress weeds by vigorous early season growth, leaching of GSLs into the soil during plant growth, and release of allelochemicals at incorporation of plant residues (Narwal and Haouala 2013). The most common volatile produced during the breakdown of *Brassica* are isothiocyanates (ITCs) which are released following tissue disruption when myrosinase enzymes

hydrolyze glucosinolates (GSLs) in presence of water (Michel 2008; Morra and Kirkegaard 2002). However, low pH could lead to glucosinolate hydrolysis to generate other compounds, such as nitriles, epinitriles, oxazolidinethiones, and ionic thiocyanates that could impact the targeted pest response (Brown and Morra 1997; Parvatha 2013). 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 a loss of protein structure and function and decrease enzymatic activity (Dufour et al. 2013; Weir et al. 2004). ITCs react with glycolytic enzymes during the germination process and thereby prevent or delay seed/tuber germination (Drobinca et al. 1977).

ITCs are strong germination suppressant of many different weed species (Al-Khatib et al. 1997; Al-Sherif 2013; Boydston et al. 2004; Petersen et al. 2001). Weed responses to biofumigation or exposure to ITCs are known to be species-specific (Al-Khatib et al. 1997; Björkman et al. 2015; Norsworthy and Meehan 2005a, b; Mattner et al. 2008; Parvatha 2013). As with other toxic molecules, higher concentrations of ITCs and increasing exposure time increase inhibition of seed and tuber germination, leading to mortality (Al-Sherif 2013; Aliki et al. 2014; Bangarwa and Norsworthy 2015; Mattner et al. 2008; Petersen et al. 2001). High concentrations of ITCs penetrate the seeds and irreversibly inhibit protein synthesis and the seeds lose their viability (Leblova-Svobodova and Kostir 1962).

Weed management practices act on key processes of population dynamics to reduce weed infestations (Gallandt et al. 1999), including seed dormancy (Dyer 1995). Dormancy is an adaptive trait that promotes the survival and distribution over time in an ever-changing environment of many organisms (Foley 2001). Seed dormancy is still a misunderstood seed trait (Hilhorst 2011). Seed dormancy is a state where viable seeds fail to germinate under a specific

set of environmental conditions that are normally favourable for its germination (Baskin and Baskin 2004; Simpson 1990). Alternatively, quiescent ungerminated seeds will germinate under those required conditions.

Several classification systems and nomenclature exist for seed dormancy. Primary and secondary dormancy refers to the period when dormancy occurs (Foley 2001). Primary dormancy denotes freshly mature seeds that fail to germinate removed from the mother plant before or collected right after dispersal. Secondary dormancy generally occurs after seeds encounter environmental conditions for a certain period that induce the quiescent state (after-ripening) (Bewley and Black 1994). The classification system used by Baskin and Baskin (1998a, 2004) includes two main categories of dormancy referring to the location of the mechanisms or constraints to germination. Exogenous or seed coat-imposed dormancy refers to characteristics of covering structures that prevent germination, whereas endogenous dormancy refers to characteristics of the embryo that prevent germination. This classification system examines the mechanism involved and includes five classes of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY), and combinational (PY + PD). PD is caused by physiological inhibiting mechanisms in the embryo that prevent radicle growth. However, structures surrounding the embryo may play a role in PD. Covering structures could restrict oxygen availability for embryos or they could regulate the release of growth inhibitors to the embryo. *Chenopodium album*, *A. artemisiifolia*, and *S. viridis* exhibit PD. Morphological dormancy refers to an underdeveloped and differentiated embryo. Time will allow embryos to grow and germinate, and so release the dormancy. *Daucus carota* is a biennial weed with this class of seed dormancy. Physical dormancy is generally caused by the testa being impermeable to water. These seeds contain heavily lignified palisade cells impregnated with

various water-repellent compounds (Baskin and Baskin 1998a; 2004). To release dormancy, a specialized “water gap” on the impermeable layer must be opened in response to appropriate environmental signals. Stratification could also release dormancy, as for *V. cracca*, *T. pratense*, and *A. theophrasti*.

The susceptibility of dormant and quiescent seeds in the soil seed bank to biofumigation is important information that could lead to efficient weed management in crop fields. Biofumigation could promote seed mortality and reduce weed germination and establishment after incorporation of mustard biomass. To know if this method can be used to reduce weed seed banks, it is essential to clearly understand how biofumigation affects seeds. Mechanisms responsible for different classes of seed dormancy or seed characteristics could reflect the susceptibility of various weed species to biofumigation. Furthermore, how dormancy state influences seed susceptibility to this type of allelochemical pressure is not reported in the literature. Our study differs from previous work by investigating relationships among seed responses, seed dormancy, and seed morphological parameters for a better understanding of weed responses reported in the literature. Annual emergence pattern and seasonal changes in states of dormancy are important factors of seed bank dynamics and govern weed pressure over seasons (Cavers and Benoit 1989). Accurate comprehension of the tolerance of dormant seeds to biofumigation is necessary to understand seed bank response to field biofumigation.

Three hypotheses were formulated: 1) seeds with PY are less affected by ITCs than seeds with PD because greater protection is provided by the testa; 2) the proportion of dormant seeds in a seed lot (i.e. seeds collected from several individuals of one population) decreases the impact of biofumigation on seed germination and survival response (intra and interspecific); 3) physical parameters of the seed are related to species germination and survival to biofumigation. The

objectives of this study were to determine weed seed responses to allelopathic compounds generated during biofumigation and to assess species susceptibility related to seed dormancy and seed morphological parameters. The aims of the study were to clarify weed responses to biofumigation and underline which morphological variable, such as testa thickness, seed size or proportion of dormant seeds, explains susceptibility of a weed species to biofumigation.

3.3 Materials and methods

3.3.1 Dose-response experiment

3.3.1.1 Species and seed collection

Germination tests were conducted to evaluate the herbicidal activity of different rates of dry mustard tissues on germination and seed viability of common weeds in southern Quebec that exhibit various forms of dormancy: *C. album* (PD), *V. cracca* (PY), *D. carota* (MD), *A. artemisiifolia* (PD), *S. viridis* (PD), *A. theophrasti* (PY), *G. quadriradiata* (no dormancy) and *T. pratense* (PY). Dormancy classes of each species are reported in Baskin & Baskin (1998b). Mature weed seed lots were randomly collected in the fall of 2012 from mature plants at the former Research and Development Institute for the Agri-environment (IRDA) research station fields (St-Hyacinthe, Canada, 45.619°N, 72.958°W), where seeds fell after a vigorous plant shaking. Seeds overwintered in a non-heated storage facility under natural field temperature to release primary dormancy prior to conducting experiments. Natural viabilities (± SE) measured for each seed lot (dormant and germinated seeds) were 82.8 ± 2.8% for *A. artemisiifolia*, 94.8 ± 1.7% for *C. album*, 93.6 ± 1.1% for *D. carota*, 75.6 ± 2.8% for *S. viridis*, 60.4 ± 3.8% for *V. cracca*, 98.0 ± 1.0% for *A. theophrasti*, 97.3 ± 1.2% for *G. quadriradiata* and 80.2 ± 3.0% for *T. pratense*.

3.3.1.2 Bioassays methodology

A procedure was developed to expose seeds to the compounds generated during the biofumigation in Petri dish germination tests while avoiding direct contact of plant material with the seeds. The study was carried out at the Organic Agriculture Innovation Platform (OAIP), IRDA, St-Bruno-de-Montarville, QC, Canada. Indian mustard selected for its purported high levels of GSL sinigrin (Taylor et al. 2014), was used as biofumigant material. Plants were grown to until flowering in 19 cm diameter by 17.5 cm depth pots (ITML Elite 4 L, HC Companies, Middlefield, OH) containing a commercial potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in a growth chamber (Conviron E15, serial number 8D40801J) set at 26 ± 2 C, 16/8 h light/darkness (186 μmol m^{-2} s^{-1}). At flower, the above-ground plant parts were harvested, dried at 35 C for 5 d and ground with a laboratory mill grinder (unknown model, Arthur H. Thomas Company). The detail of the assembly of an experimental unit is shown in Figure 3.1. (1) Dried mustard powder was placed within a modified weighing dish, where the bottom was replaced by a 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 the Petri dish. (2) A qualitative filter paper (Whatman, diameter 90 mm, thickness 190 μm, Fisher scientific, Whitby, ON) was placed in a 91 x 15 mm polystyrene disposable Petri dish (VWR International, Mississauga, ON) and the weighing dish was placed at the centre. (3) Fifty weed seeds were washed thoroughly with distilled water and then placed surrounding the weighing dish. Seeds were exposed to six treatments in this experiment: 0 (control), 2.33, 3.50, 7.00, 14.00 and 21.00 mg cm^{-2} of dry tissues of Indian mustard. (4) Just before closing the Petri dish, 2 ml of distilled water were added on the seeds and 4 to 6 ml of distilled water on the

weighing dish to humidify completely the mustard tissues (more biomass required more water). Water allowed the glucosinolate-myrosinase enzymatic reaction to occur. (5) 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 μmol m^{-2} s^{-1}). For all test species, the temperature setting allowed seeds to germinate. Petri dishes were first open after 4 d to water the seeds, measure germination, and record further observations. After 8 d, the weighing dishes with the biofumigant material were removed from the Petri dishes. Under field situations, volatile ITCs mostly disappear 4 d after soil incorporation (Bangarwa and Norsworthy 2015). Seed germination was recorded every 2-3 d for one month, until germination stopped in every treatment. Five replicates per treatment were arranged in a completely randomized design. The experiment was replicated twice. 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 (Peters 2000) and the International Seed Testing Association (ISTA) working sheets on tetrazolium testing (Leist et al. 2003). Viable non-germinated seeds at the end of the assays were considered dormant. Initial dormancy of the seed lots (proportion of dormant seeds in the control) from the sampled population was also determined with tetrazolium tests. Changes in the proportion of dormant seeds in treatments compared with the control reflected how biofumigation could influence the relative proportion of dormant seeds.

3.3.2 Seed morphological parameters

Seed morphology of each test species in the dose-response experiment was characterized to establish relations between responses and physical parameters. Imaging software [Motic

Image Plus 2.0 (Motic, Hong Kong)] was used to calculate seed length, seed width, seed thickness (height), thickness of the testa, and seed surface. Pictures were taken with a Moticam 580 (Motic, Hong Kong) camera mounted on a Meiji Techno RZ (Japan) stereomicroscope. For seed length, width, and surface, a single picture was taken from a seed placed longitudinally. Software allows measurement of straight lines that were used for length, width and thickness and area automatic calculation was used for seed surface. Seed weight, embryo weight and testa weight were measured with a PR2003 Delta Range® (Mettler Toledo, Switzerland, SNR: 1116291931) analytic scale. Seeds were manually dissected by splitting the seed evenly with one longitudinal cut prior to evaluation of thickness of testa, embryo weight and testa weight. Following the dissection, one half of the seed was placed directly under the stereomicroscope permitting a perpendicular view of the cut. A picture was taken and testa thickness measurement was determined using the software straight line calculation mode. The embryo was then manually removed from the two halves of the seed. Embryo and testa were weighed separately. Except for seed weight, all measurements were done on 30 seeds. Seed weight was measured on eight replicates of 100 seeds. These measurements were used to establish the testa : seed weight ratio and embryo : seed weight ratio and to estimate seed volume ((4π(length)/2(width)/2(thickness)/2)/3 = volume).

3.3.3 Intraspecific dormancy experiment

Germination tests following the same methodology described in the dose-response experiment section were realized to compare intraspecific seed responses to biofumigation with respect to different dormancy states (primary and secondary). Mature weed seed lots of *C. album*, *A. artemisiifolia*, *D. carota*, *V. cracca* and *S. viridis* were randomly collected during

fall 2012 (assessed as secondary dormancy) and fall 2013 (assessed as primary dormancy) at OAIP in St-Bruno (45° 32’ 00’’N, 73° 21’ 00’’ W). Seeds collected in 2012 overwintered outside in a non-heated storage facility for after-ripening treatment. Seeds collected in 2013 were stored in the dark at room temperature until the beginning of the laboratory experiments and were assumed to be in a primary dormancy state. For each species, four treatments were compared: seeds with or without after-ripening treatment that were exposed or not to 7.00 mg cm^{-2} of dry tissues of Indian mustard. Since the previous experiments displayed high tolerance of *V. cracca* and *S. viridis* to biofumigation, rates were increased to 21.00 and 14.00 mg cm^{-2} of dry tissues, respectively. Percentages of germination, mortality and dormant seeds were measured as in the dose-response experiment. Four replicates per treatment were arranged in a completely randomized design. The experiment was replicated twice.

3.3.4 Isothiocyanate analysis

Analysis 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) were performed on dried Indian mustard powder material prior to experiments to identify and quantify ITCs and other allelopathic compounds released during the biofumigation. Samples were prepared for HS GC by adding 5 ml of water to 0.2 g of dried mustard directly in the HS vials and incubated for 5 h. The HS operation conditions were: vial equilibration at 70 C for 20 min, needle temperature at 105 C, transfer line at 140 C, carrier helium pressure at 177.2 kPa, vial pressurization time of 2 min, injection time of 0.02 min and withdraw time of 0.4 min. HS analysis revealed mainly the production of allyl-ITC, allyl-thiocyanate, and butyl-ITC, so these specific external standards were used for quantitative

measurements. Samples were analyzed on GC-MS with a capillary column (Elite 5MS, 30 m x 0.25 mm x 0.5 µm). The temperature program was 40 C for 1 min followed by 10 C min^{-1} to 130 C. Mass spectrometry was run at 200 C over a scan range of 35 to 350 Da, with scan time of 0.1 s and interscan delay of 0.06 s. To ensure the biofumigant potential of the dried material used in the Petri dish methodology, comparative analyses were completed with fresh material and dried ground material at a low temperature. Quantities of ITCs measured from different sampling conditions were similar (296.7 µg g^{-1} of allyl-ITC in fresh material *vs* 406.1 µg g^{-1} of allyl-ITC in dried material, 2 h of incubation). The melting temperature varies for different proteins, but temperature less than 40 C should not denature them. Activity of myrosinase was still unaffected at 40 C (Van Eylen et al. 2007). Furthermore, without water, the glucosinolate-myrosinase chemical reaction leading to ITC production cannot occur during grinding. Keeping intact the glucosinolates and myrosinase by drying the biomass preserved the biofumigant potential in dried mustard tissues.

3.3.5 Statistical analysis

3.3.5.1 Dose-response experiment

Linear and non-linear curve fittings were performed to establish the germination, mortality and dormancy responses to mustard biomass quantities using TableCurve 2D V.5.01 and SigmaPlot V.12.5 (Systat Software, San Jose, CA). Seed germination (Equation 1) and mortality (Equation 2) were adjusted to the response observed in the control treatment as follows:

Adjusted Germination = (G_T / G_C) 100 [1]

With G_T corresponding to the germination percentage observed in a treatment, G_C to the mean percentage of germination observed in the control.

Adjusted Mortality = $M_T - M_C$ [2]

With M_T corresponding to the mortality observed in the treatment and M_C to the mean percentage of mortality observed in the control. Means of adjusted germination, adjusted mortality and percentage of dormancy are the average of two replicated assays. Percentages of dormancy were not adjusted prior to analysis. Adjusted germination and mortality were used independently to establish lethal doses of dry mustard biomass that kill 50% of the total viable seeds (LD_{50}) and estimated half maximal effective doses of dry mustard biomass that decrease germination (ED_{50}) and maximal mortality for each species. ED_{50}, LD_{50}, and maximal mortality were calculated using the estimated parameters of the fitted curves. For the logistic dose-response curve with three parameters, ED_{50} and LD_{50} correspond to parameter b and maximal mortality to parameter a; c is a shape parameters logistics curve. For the logistic curves with three parameters, maximal mortality corresponds to parameter a, ED_{50} and LD_{50} correspond to equation 3:

ED_{50} or $LD_{50} = b + (3.52\ c) / 2$ [3]

Those values served as comparative responses of interspecific susceptibility to biofumigation suitable for further regression analysis. The percentage of dormancy was subjected to ANOVA and Tukey's honestly significant difference (HSD) was used to separate treatment means at 0.05 probability level. Furthermore, ANOVAs were performed on LD_{50}, ED_{50} or maximal mortality per seed dormancy classes.

Species were classified based on their relative susceptibility to biofumigation according to ED_{50}, LD_{50}, and maximal mortality as a first assessment of interspecific similarity. Similar

estimated parameters without overlapping their 95% confidence limits were classified together, not according to a specific value of the estimated parameter. Consequently, it was possible to observe which species or group of species were more affected by biofumigation in terms of germination and survival.

3.3.5.2 Seed morphological parameters and relations with seed responses

Seed responses in the dose-response experiment (LD_{50}, ED_{50} and maximal adjusted mortality) were related to all seed morphological parameters measured. Seed parameters, initial dormancy in seed lots and seed responses were first analyzed by principal component analysis (PCA) with R software v.3.0.1 (R development core team, 2008) and the library 'vegan'. PCA was calculated with redundancy analysis function. Scaling preserved the correlation between descriptors.

To confirm correlations underlined by the PCA, each seed response was analyzed with regression analyses. One or two seed parameters served as explanatory variables. The significance of interaction between explanatory variables was assessed. All possible combinations of seed parameters were analyzed for LD_{50}, ED_{50} or maximal adjusted mortality in order to find unrevealed relations by PCA. Regression analyses were performed using the 'lm' function of R software. Those analyses were performed on dicotyledons species only or for all species to assess if the monocotyledon species affected the conclusion of the tests.

3.3.5.3 Intraspecific dormancy experiment

Analyses of variance and Tukey's HSD were used for mean comparison of seed responses (percentage of germination, mortality and dormancy). Dependent variables were log-

transformed (log_{10} [x+1]) whenever required to respect normality and homoscedasticity assumptions. For each dormancy state germination, mortality, and dormancy were adjusted using the equation 4 to reflect only the relative impact of biofumigation to the control:

Impact of biofumigation = $((R_T - R_C) / R_C)$ 100 [4]

With R_T corresponding to the percent response value observed in the treatment and R_C to the mean percent response value observed in the corresponding control. The R_C term was used to correct seed germination, mortality and dormancy not caused by biofumigation treatments. A negative value of the impact of biofumigation indicates that the response decreased and at the opposite, positive value indicates that the response increased after treatment. Statistical differences of the impact of biofumigation between dormancy states for each species were assessed by ANOVAs at 0.05 probability level.

3.4 Results and discussion

3.4.1 Isothiocyanate analysis

The chemical analysis and detection from the headspace and GC-MS demonstrated that the three main compounds released were allyl-isothiocyanate (2,455 ± 53 µg g^{-1} of dried biomass), allyl-thiocyanate (1,431 ± 140 µg g^{-1}) and butyl-isothiocyanate (131± 31 µg g^{-1}). Analysis also revealed traces of butenyl-ITC, isopropyl-ITC and butane 1-ITC. Accordingly, the amount of allelochemicals released from each rate of mustard tissues in Petri dish was calculated (Table 3.1).

In fields, plants do not release their total potential of ITCs (Morra and Kirkegaard 2002). Unhydrolyzed GSLs in soil are mainly due to incomplete tissue pulverization or other limitations to GSL hydrolysis (soil condition, water availability, crop variety). In a field experiment, Morra

and Kirkegaard (2002) measured around 1% of ITC release efficiency and succeeded in increasing to 26% using frozen plant material. In their experiment, *Brassica* plants were not chopped and there was no post incorporation irrigation. When biofumigation was carried out under optimum conditions, ITC release efficiency increased to 9.7% - 18.5%, with a peak at 56% when mustard with high GSLs concentration was incorporated (Bangarwa et al. 2011, Gimsing and Kirkegaard 2006). The mid-rate 7.00 mg cm^{-2} of dried mustard powder was equivalent to 10% of a field dry mustard biomass of 7,000 kg ha^{-1} based on a conversion of GSLs in *Brassica* tissues into ITCs of 10%. The results of the present experiment were presented via rates of dry plant tissues, but should also be considered as the corresponding quantity of allelochemicals released.

3.4.2 Dose-response experiment

Curve fitting of seed responses following increasing biofumigation rates are presented in Figure 3.2, details of equations and statistical parameters are in Table 3.2. Except for *T. pratense*, increasing biofumigation rates increased mortality following a logistic dose response (Figure 3.2A). Response of *T. pratense* fitted a logistic curve with three parameters, where higher rates caused almost no mortality. Biofumigation had the most lethal effect on *G. quadriradiata*, *D. carota*, and *C. album* where mortality reached 97, 95, and 93%, respectively.

Biofumigation decreased cumulative germination of all species (Figure 3.2B). Rates 2.33 and 3.50 mg cm^{-2} of mustard dry tissues stimulated germination of *V. cracca*, following a logistic curve with three parameters. Germination responses of *C. album*, *D. carota*, *A. artemisiifolia*, *S. viridis*, *A. theophrasti*, *G. quadriradiata* and *T. pratense* fitted a logistic dose-response curve. *Trifolium pratense*, *G. quadriradiata* and *D. carota* had the lowest ED_{50} (0.96, 2.36 and 3.23 mg

cm^{-2} respectively) (Table 3.2). Being non-dormant (De Cauwer et al. 2014), *G. quadriradiata* seeds can only germinate or die under the treatments. For this species, all non-germinated seeds were dead.

Percentage of dormant seed responses were hormetic (*C. album*, *A. artemisiifolia* and *D. carota*), positive (*T. pratense*), negative (*A. theophrasti* and *V. cracca*) or without impact (*S. viridis*) (Figure 3.2). Non-germinated seeds of *T. pratense* were not dead but became dormant (Figure 3.2C). The numbers of dormant seeds were significantly reduced at 14.00 mg cm^{-2} of dry mustard material for *A. artemisiifolia*, *C. album*, *V. cracca*, *D. carota* and at 21.00 mg cm^{-2} of dry mustard material for *S. viridis* and *A. theophrasti* ($P \leq 0.05$). The percentage of *T. pratense* dormant seeds was positively related to biofumigation rates. Percentages of dormancy of all species did not fit a logistic dose-response curve. For *D. carota*, *A. artemisiifolia* and *C. album*, treatments induced dormancy for some of the rates, where curve fitting was either a logistic curve or a logistic dose-response curve with a peak.

Hormetic dose response, which is a stimulatory response to low levels of stress, observed in the dose-response experiment with *V. cracca* concurs with the results of Teasdale and Taylorson (1986). Germination of Texas millet [*Urochloa texanum* (Buckl.) R. Webster], large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] were stimulated at low concentrations of allyl-ITC (Norsworthy and Mehan 2005a). However, in our study hormetic responses for *D. carota*, *C. album* and *A. artemisiifolia* percentage of dormant seeds did not fit that conclusion. For these species and in the conditions of the experiment, lower rates seem to enforce dormancy or induce a secondary dormancy state instead of releasing dormancy and promoting germination. Furthermore, hormetic response of *C. album* was observed at a mid-rate. Perhaps the hormetic response could be triggered, not only

at lower rates, but by a specific concentration of inhibitory compound. Results highlight that a second mechanism of dormancy could be at issue for *D. carota*, known to have a morphological type of seed dormancy (Baskin and Baskin 1998b). Since this mechanism permits germination after adequate time for the embryo to mature, seeds would not return to a dormant state without other mechanisms. From our understanding, a physiological mechanism of dormancy for *D. carota* is unreported and thus requires further investigation.

Various environmental factors including temperature, darkness, light, gases, water, inorganic and organic chemicals can cause changes in the dormancy state of non-deep PD (Baskin and Baskin 1998c). Allelochemical compounds are abundant and present in a wide variety of habitats, although so far none of them have been suggested to cause changes in dormancy states. Allelochemicals could inhibit germination until other environmental factors induced dormancy (Baskin and Baskin 1998d), for example by changing the optimal temperature for seed germination or light responses of seeds (Batak et al. 2002; Finch-Savage and Leubner-Metzger 2006). The methodology used in the Petri dish assays provided adequate and uniform temperature, light and moisture conditions for seed germination after exposure to allelochemicals released by biofumigation. Therefore, this evidence suggests that the induced dormancy observed for *D. carota*, *C. album* and *A. artemisiifolia* was caused by a specific quantity of compounds generated during biofumigation, directly or indirectly.

Dose-response experiment results provided a detailed response of weed seeds to allelochemicals released by biofumigation. Various weed species responses to biofumigation or exposure to ITCs were reported in the literature. Norsworthy and Meehan (2005b) observed interspecific susceptibility of weeds to biofumigation, where emergence inhibition for Palmer amaranth (*Amaranthus palmeri* S. Wats.) was more pronounced than for pitted morning glory

(*Ipomoea lacunosa* L.) and yellow nutsedge (*Cyperus esculentus* L.). Germination, mortality and dormant seed responses to biofumigation in the experimental conditions were species specific, where various types of curves and different shapes of the same type of curve were also observed.

Class of seed dormancy was not a significant variable explaining LD_{50}, ED_{50} or maximal mortality values (Figure 3.3). Maximal mortality in physiological seed dormancy was slightly different from physical dormancy, but not statistically ($P > 0.05$).

Species relative susceptibility to biofumigation according to each estimated parameter are presented in Table 3.3. For LD_{50} of viable seeds, *G. quadriradiata*, *D. carota* and *A. theophrasti* (group 1, most affected) are followed by *C. album* (group 2), *A. artemisiifolia* (group 3) and *T. pratense* (group 4, least affected). *Vicia cracca* and *S. viridis* confidence limits overlapped every group. For maximal mortality, *G. quadriradiata*, *D. carota* and *C. album* (group 1) are followed by *A. artemisiifolia* and *A. theophrasti* (group 2), and then by *V. cracca* and *T. pratense* (group 3). Once more, *S. viridis* confidence limits overlapped other groups. For ED_{50}, *T. pratense* (group 1) and *G. quadriradiata* (group 1 and 2) are followed by *D. carota* (group 2), *A. theophrasti* (group 3), *C. album*, *A. artemisiifolia* and *V. cracca* (group 4) and finally, *S. viridis* (group 5). *Galinsoga quadriradiata* and *D. carota* were constantly in the most susceptible groups. Groups do not appear to be related to seed dormancy classes per this criterion.

3.4.3 Seed morphological parameters and relations with seed responses

Galinsoga quadriradiata and *C. album* had the smallest seeds regarding length, width, volume and weight (Table 3.4). Embryo : seed weight and testa : seed weight ratios represent the proportion of the embryo, or the testa in the seed. For the embryo : seed weight ratio, a value close to one indicates that the embryo within the seed was large compared to the covering

structure. For testa : seed weight ratio, a value close to one indicates that the testa was heavier than other structures including the embryo. Seed parameter measurements indicated that *A. artemisiifolia* had the highest embryo: seed weight ratio (0.903:1 mg) while *V. cracca* had the lowest testa: seed weight ratio (0.251:1 mg).

All seed physical parameters measured and calculated were analyzed in relation to initial dormancy, germination and survival responses by a principal component analysis. The two axes shown in Figure 3.4 explained 73.1% of the variation. *D. carota, C. album* and *G. quadriradiata* were grouped together, as were *V. cracca* and *A. theophrasti*. Testa weight and thickness, seed weight, and embryo weight were correlated seed parameters. The analysis revealed that ED_{50} was negatively correlated with testa : seed weight ratio. Linear regression analyses confirmed PCA results (Table 3.5). For a similar seed weight, ED_{50} was greater in dicotyledonous seeds with a lighter testa than seeds with a heavier testa. Therefore, the proportion of testa in seed weight could reflect seed germination vulnerability. The rest of the seed components, mainly embryo and endosperm, are the core of the germination process. Those seed components could act as potential sites for allelochemical detoxification (Bailly 2004). Seed morphology or other factors, such as biochemistry and detoxification potential, could be more related to seed emergence from allelopathic stress than seed size (Waddington 1978, Weir et al. 2004). Moreover, initial dormancy of the seed lots and seed surface were related to ED_{50} values for all species (Table 3.5). Seed surface alone did not explain the response. There was a significant interaction between seed surface and initial dormancy. In seed lots with small seeds (up to 4 mm^2), initial dormancy was positively related to ED_{50} values. However, in seed lots in which seed surface was more than 4 mm^2, initial dormancy was negatively related to ED_{50} values.

The relations established between ED_{50}, seed parameters and seed dormancy could help to understand variable results reported in the literature. Small seeds are often mentioned as more susceptible to allelopathy (Petersen et al. 2001; Westoby et al. 1996), but this was not always confirmed (Haramoto and Gallandt 2005; Mattner et al. 2008). Al-Khatib et al. (1997) suspected that seed size was negatively correlated with weed suppression following mustard incorporation in greenhouse experiments. Nevertheless, seed size was not significantly correlated with reduction of weed emergence following incorporated residues of short-season cover crops, including *Brassica* (Haramoto and Gallandt 2005).

Principal component analysis did not show a clear relation between LD_{50} and physical parameters. However, regression analysis presented in Table 3.5 showed the initial dormancy in the seed lots was positively related to LD_{50}. There was a significant interaction between testa thickness and initial dormancy for dicotyledon species and a significant interaction between seed width and initial dormancy for all assessed species. Proportion of dormant seeds was a more important factor related to LD_{50} values in small seeds and seed with thin testa (width lesser than 2000 µm and testa thickness lesser than 60 µm) than in big seeds and seeds with thick testa.

Correlation underlined by PCA between maximal mortality reached in treatments and initial dormancy in seed lots was confirmed (Table 3.5). For all species, initial dormancy was negatively related to maximal mortality caused by biofumigation treatments. Maximal mortality could also be explained by seed length and embryo weight (Table 3.5). Exposure to dry mustard biomass allowed the highest mortality in small seeds with light embryos (seed length lesser than 2000 µm and embryo weight lesser than 0.001 g) or in large seeds with big embryos (seed length more than 3500 µm and embryo weight more than 0.003 g). Embryo : seed weight ratio was not correlated to maximal mortality observed under treatment. Instead, embryo weight : seed length

ratio would become more relevant to predict maximal mortality. All other relations not presented between seed responses and seed parameters were not significant ($P > 0.05$).

3.4.4 Intraspecific dormancy experiment

Generally, intraspecific seed dormancy status influenced seed responses observed in the experiment. However, the relative impact of biofumigation between dormancy states was not always significantly different from the control (Table 3.6). For *A. artemisiifolia*, almost no seed in primary dormancy germinated compared to 62.9% of natural germination for seeds in secondary dormancy state. Consequently, biofumigation had greater impact on seeds with secondary dormancy than primary dormant seeds ($P \leq 0.001$). Biofumigation at 7.00 mg cm^{-2} of dried mustard powder increased mortality of *A. artemisiifolia* seeds in secondary dormancy state by 293% and primary dormancy state by 58%. Proportion of dormant seeds was reduced by biofumigation, but the HSD test failed to find statistical difference between dormancy states. Impact of biofumigation on both states of dormancy was not different for this species ($P = 0.458$).

For *C. album*, biofumigation treatments reduced germination for both seed dormancy status by 81% ($P = 0.947$). Nevertheless, biofumigation increased mortality more in seeds in a secondary dormancy state than seeds in a primary dormancy state. Biofumigation increased the proportion of dormant seeds for *C. album* after-ripened seeds by 417%. Induction of dormancy was also measured in the dose-response experiment under the exposure of 7.00 mg cm^{-2} of dry tissues of Indian mustard.

For *D. carota*, germination was highly reduced, but by similar extent between dormancy states (95 and 97%). Like *A. artemisiifolia* and *C. album* seeds, biofumigation killed more *D.*

carota seeds exhibiting secondary than primary dormancy (1,196 and 156% respectively). The amount of primary and secondary dormant seeds increased by 50 and 222% respectively. The dose-response experiment results underlined an induced dormancy state at a low biofumigation level.

For *S. viridis*, germination was completely prevented by biofumigation for both dormancy states. At the opposite of previous species, seeds that have primary dormancy were the most susceptible to biofumigation. Mortality of primary and secondary dormant seeds increased by 353 and 104% respectively. Proportion of dormant seeds in primary and secondary dormancy states decreased by 89 and 54% respectively. Referring to the second regression analysis of LD_{50} in Table 3.5, estimates of LD_{50} for a species with seed width of 1,338 mm are 1.56 and 0.92 mg cm^{-2} of dried mustard powder for initial dormancy of 48.4 and 24.2%, respectively. According to this estimation, mortality of seeds that have entered secondary dormancy was expected to be higher than seeds in a primary dormancy state. However, those relations did not account for other parameters in the equation of dose response to biofumigation, such as the slope. If indeed the initial proportion of dormancy changes LD_{50} value and slope parameter in response to biofumigation, it would be possible that the response to a specific rate in a regression with a high inflection point and smooth slope would be lower than a response to the same rate, but on a regression with a lower inflection point with a steeper slope.

For *V. cracca*, germination, survival and proportion of dormant seeds decreased under biofumigation, and the seeds were no less susceptible in the primary dormancy. The last assumption about possible changes in the slope of dose response holds. However, the estimated LD_{50} according to second regression analysis in table 3.5 for a species with seed width of 2,691 mm were 0.60 and 0.41 mg cm^{-2} of dried mustard powder for initial dormancy of 51.32 and

22.18% respectively. The significant interaction between explanatory variables would possibly explain the fact that the initial dormancy did not influence seed responses. Another explanation for *S. viridis* and *V. cracca* responses would be that the seed lots came from the same species but several individuals, leading to possible variability. Further dose-response assays with the same individuals with different proportion of dormant seeds would be required to test those assumptions.

Allelopathy is recognized to play an important role for pest management in agroecosystems, particularly in organic or low input agriculture (Jabran et al. 2015). In many agronomic practices, allelopathy is a defining factor leading to lower weed pressure (Gallandt et al. 1999; Qasem 2010, 2013). Agronomic practices could influence seed dormancy (Dyer 1995) and the present study showed that allelochemicals released during biofumigation technique are no exception. The experiments described in this manuscript demonstrate the herbicidal activity of ITCs released in *Brassica* tissues, specifically by reducing the viability of dormant and non-dormant seeds. Inherent seed dormancy and characteristic are related to seed responses to biofumigation, which provide a better understanding of inter- and intraspecific response to biofumigation. Those relations could help to anticipate seed susceptibility to this technique. Biofumigation may well be used to control specific vulnerable species, such as *G. quadriradiata*, which has small seeds and no dormancy (Kumar et al. 2009).

Selected rates in the experiments, expressing 3% to 30% of the ITC release efficiency rate in the field, showed a significant impact of biofumigation on seeds germination and survival. Consequently, biofumigation realized in good conditions (10% of ITC conversion efficiency with similar GSLs content) in a field could alter the seed bank of vulnerable species (Bangarwa et al. 2011). However, to confirm this assumption, investigation under natural condition should

be realized where other factors are involved. In situ factors may affect the activity of biofumigants including soil colloidal adsorption and possible microbial transformation of allelochemicals (Price et al. 2005). Seed bank persistence is one of the major processes involved in annual weed dynamics, and seed mortality has a strong impact on future weed population size (Gallandt et al. 1999).

To improve the impact of ITCs on soil seed bank, it will be necessary to use the results of the study regarding weed biology. Seed characteristics and annual dormancy cycles should be considered prior to biofumigation in fields to improve weed control. For example, *A. artemisiifolia*, a summer annual species, has seeds with physiological dormancy where the seeds are dormant in the fall, non-dormant in the summer and where winter serve as after-ripening conditions (Baskin and Baskin 1980). To control this weed, it would not be wise to perform biofumigation in fall, where the seeds are more tolerant per our conclusions. Growers should wait to perform biofumigation in late-spring, where *A. artemisiifolia* seeds are non-dormant and thus more vulnerable.

In addition to weed specific biological information, the biofumigation technique itself requires some considerations to improve efficiency. Biofumigation mode of action is the release of large quantities of ITCs onto target species (Morra and Kirkegaard 2002). However, production of secondary metabolites such as GSLs is known to be influenced by the environment, such as the presence of other species or by seasonal variation (Brown and Morra 1997; Inderjit et al. 2011). Glucosinolate content in plants is highly correlated to sulfur availability in soils (Ciska et al. 2000; Falk et al. 2007). To be successful in the field, plants must be finely chopped and incorporated at full flowering stage, where the glucosinolate concentrations are at the highest (Taylor et al. 2014). Adequate soil moisture is required to allow

the chemical reaction and the soil temperature must be at least 10 C (Matthiessen et al. 2004; Michel 2008). More importantly, the time to reach flowering stage by *Brassica* cover crops is non-negligible. This delay could prevent growers to performed biofumigation at the appropriate time to control a specific weed species. However, whenever this technique could be included in crop rotation systems, the use of *Brassica* cover crops in organic cropping systems could provide increased weed management options and facilitate weed seed bank depletion.

3.5 Acknowledgments

The authors would like to thank Laurence Jochems-Tanguay and Patrick Dubé for their technical assistance as well as many summer students who worked on this project. This research was funded by the Agriculture and Agri-Food Canada (AAFC)'s Canadian Agricultural Adaptation Program (CAAP) managed by the Conseil pour le Développement de l'Agriculture du Québec – CDAQ. This study was carried out in partial fulfillment of the requirements for a Ph.D. degree at McGill University.

Table 3.1: Quantities of allelochemicals released in the Petri dish assays according to rates of dry Indian mustard material[a].

Biofumigation treatments	Allyl-ITC	Butyl-ITC	Allyl-thiocyanate
mg cm^{-2} of dried mustard biomass		µg cm^{-2}	
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.33	5.73 ± 0.12	0.31 ± 0.07	3.34 ± 0.33
3.50	8.59 ± 0.19	0.46 ± 0.11	5.01 ± 0.49
7.00	17.18 ± 0.37	0.92 ± 0.22	10.01 ± 0.98
14.00	34.31 ± 0.74	1.83 ± 0.43	20.00 ± 1.96
21.00	51.49 ± 1.11	2.75 ± 0.65	30.01 ± 2.94

[a]Abbreviation: ITC, isothiocyanate. Analysis by headspace and gas chromatography-mass spectrometry of 0.2 g of dry Indian mustard. Based on the calculation of allyl-isothiocyanate detected at 2,455 ± 53 µg g^{-1} of dried biomass, allyl-thiocyanate at 1,431 ± 140 µg g^{-1} and butyl-isothiocyanate at 131± 31 µg g^{-1}. Analysis also revealed traces of butenyl-ITC, isopropyl-ITC and butane 1-ITC.

Table 3.2. Parameters and curves of the linear and non-linear responses of the weed seeds to biofumigation represented in Figure. 3.1.

Species	Curve fitted	Coefficient parameters[a]			Regression		
		a	b	c	Adj. R^2	F value	P
Adjusted Mortality							
Ambrosia artemisiifolia	LDR abc[b]	73.84 ± 8.89	8.8 ± 1.75	-5.29 ± 3.06	0.86	161.64	<0.0001
Chenopodium album	LDR abc	93.19 ± 4.02	6.81 ± 0.38	-4.43 ± 1.29	0.96	703.86	<0.0001
Daucus carota	LDR abc	95.26 ± 4.19	4.38 ± 0.39	-3.36 ± 0.63	0.95	482.26	<0.0001
Setaria viridis	LDR abc	58.62 ± 56.38	14.10 ± 12.46	-2.75 ± 2.92	0.63	41.31	<0.0001
Vicia cracca	LDR abc	19.66 ± 15.63	8.12 ± 7.87	-3.48 ± 10.30 [e]	0.28	8.50	0.0008
Abutilon theophrasti	LDR abc	62.75 ± 10.02	4.58 ± 1.26	-2.48 ± 1.34	0.89	83.37	<0.0001
Galinsoga quadriradiata	LDR abc	97.28 ± 1.18	4.03 ± 0.53	-10.58 ± 9.59	0.99	6,798.33	<0.0001
Trifolium pratense	Logistic abc[c]	12.80 ± 6.50	8.79 ± 2.41	3.41 ± 1.86	0.16	5.20	0.0009
Adjusted Germination							
Ambrosia artemisiifolia	LDR abc	100	6.63 ± 0.93	2.15 ± 0.46	0.84	286.40	<0.0001
Chenopodium album	LDR abc	100	6.10 ± 0.6	6.83 ± 3.78	0.96	979.80	<0.0001
Daucus carota	LDR abc	100	3.23 ± 0.27	2.49 ± 0.51	0.92	652.20	<0.0001
Setaria viridis	LDR abc	100	11.81 ± 3.08	2.75 ± 1.48	0.50	57.11	<0.0001
Vicia cracca	Logistic abc	135.81 ± 29.20	2.57 ± 1.85	2.63 ± 1.61	0.24	9.98	0.0002
Abutilon theophrasti	LDR abc	100	4.10 ± 0.54	7.36 ± 5.39	0.98	873.11	<0.0001
Galinsoga quadriradiata	LDR abc	100	2.36 ± 0.71	2.21 ± 1.85	0.64	94.24	<0.0001
Trifolium pratense	LDR abc	100	0.96 ± 0.87	1.68 ± 1.35	0.93	788.80	<0.0001
Dormancy							
Ambrosia artemisiifolia	Logistic abc	30.95 ± 4.67	5.71 ± 1.3	3.51 ± 1.04	0.44	23.17	<0.0001
Chenopodium album	Piecewise						
Rates 0-0.228	$Y=a+be^{-x}$	5.34 ± 1.97	5.94 ± 3.37	---	0.34	13.13	0.0013
Rates 0.228-1.367	LDR Peak[d]	15.93 ± 1.86	6.85 ± 0.79	4.93 ± 0.82	0.79	61.28	<0.0001
Daucus carota	Logistic abc	22.30 ± 2.92	3.24 ± 0.6	1.24 ± 0.34	0.68	55.29	<0.0001
Setaria viridis	Logistic abc	33.93 ± 7.78	7.86 ± 8.06[e]	10.92 ± 12.65[e]	0.03	0.71	0.5000
Vicia cracca	Y=a+bx	55.96 ± 5.24	-1.41 ± 0.84	---	0.17	11.31	0.0010
Abutilon theophrasti	Y=a+bx	49.51 ± 3.98	-1.03 ± 0.52	---	0.46	17.33	0.0005
Trifolium pratense	Y=a+bx	66.54 ± 2.91	0.73 ± 0.41	---	0.18	12.63	0.0008

[a]± confidence limits at 95% of each parameter estimate.

[b]LDR abc: Logistic dose response with three parameters: $y = a/(1+(x/b)^c)$; where a = maximal adjusted mortality; b = ED_{50} for germination and LD_{50} for adjusted mortality. For adjusted germination curves, the parameter a was fixed to 100.

[c]Logistic abc: $y = 4a \exp[-((x-b)/c)]/[1+\exp[-((x-b)/c)]]^2$; a = maximal adjusted mortality; b + (3.52c)/2 = ED_{50} and LD_{50}.

[d]LDR peak: $y = (4ax^{(-c-1)} b^{(c+1)} c^2)/((c-1+cx^{(-c)} b^c+x^{(-c)} b^c)^2)$.

[e]Parameter non-significant (P > 0.05).

Table 3.3. Groups of relative susceptibility of weed species to biofumigation according to germination and mortality responses.

Species	Groups by estimated parameters			Classes of seed dormancy
	ED_{50}	LD_{50}	Maximal mortality	
Galinsoga quadriradiata	1[a], 2	1	1	No dormancy
Daucus carota	2	1	1	Morphological
Abutilon theophrasti	3	1	2	Physical
Chenopodium album	4	2	1	Physiological
Ambrosia artemisiifolia	4	3	2	Physiological
Trifolium pratense	1	4	3	Physical
Vicia cracca	4	---[b]	3	Physical
Setaria viridis	5	---	---	Physiological

[a]Each number represents a susceptibility to biofumigation group, ranging from 1 (highly susceptible) to 5 (slightly affected). Species with the same number for each parameter are similar per overlapping of the confidence limits (95%).

[b]Confidence limits overlap other groups, classification of the species by the estimated parameters not possible per this criterion.

Table 3.4. Seed morphological parameters of weed species (Mean ± SE).

Species	Seed						Embryo		Testa		
	Length	Width	Thickness	Volume[b]	Surface	Weight	Weight	Weight: total seed weight ratio	Thickness	Weight	Weight: total seed weight ratio
	µm	µm	µm	mm^3	mm^2	mg	mg	mg:mg	µm	mg	mg:mg
Ambrosia artemisiifolia	3,922 ± 93	1,996 ± 39	1,749 ± 40	7.162 ± 0.185	5.07 ± 0.06	427 ± 2	3.9 ± 1.1	0.903 ± 0.045	63.7 ± 2.4	1.4 ± 0.7	0.334 ± 0.029
Chenopodium album	1,278 ± 13	1,160 ± 17	660 ± 9	0.512 ± 0.014	1.14 ± 0.09	67 ± 1	0.2 ± 0.1	0.275 ± 0.004	52.6 ± 2.3	0.2 ± 0.1	0.321 ± 0.016
Daucus carota	2,587 ± 54	1,487 ± 34	580 ± 18	1.169 ± 0.072	3.31 ± 0.17	100 ± 1	____[c]	_____	55.9 ± 2.2	_____	_____
Setaria viridis	2,488 ± 22	1,338 ± 19	906 ± 12	1.580 ± 0.049	2.32 ± 0.04	170 ± 1	1.4 ± 0.1	0.806 ± 0.009	55.3 ± 2.1	$0.8 \pm 3.3 \times 10^{-2}$	0.452 ± 0.004
Vicia cracca	2,651 ± 45	2,691 ± 48	2,736 ± 38	10.219 ± 0.277	5.63 ± 0.17	1,130 ± 100	9.4 ± 0.3	0.833 ± 0.006	92.1 ± 2.5	2.8 ± 0.1	0.251 ± 0.002
Abutilon theophrasti	3,684 ± 30	2,891 ± 20	1,689 ± 14	9.453 ± 0.194	6.88 ± 0.24	988 ± 4	6.0 ± 0.1	0.594 ± 0.012	118.9 ± 1.2	4.0 ± 0.1	0.406 ± 0.012
Trifolium pratense	4,006 ± 39	2,256 ± 23	2,019 ± 29	9.576 ± 0.227	8.01 ± 0.14	172 ± 2	$1.1 \pm 3.8 \times 10^{-5}$	0.378 ± 0.008	46.4 ± 1.9	$0.6 \pm 2.1 \times 10^{-5}$	0.622 ± 0.008
Galinsoga quadriradiata	1,305 ± 18	531 ± 10	532 ± 10	0.098 ± 0.004	0.55 ± 0.01	17 ± 0.1	$0.1 \pm 7.4 \times 10^{-6}$	0.514 ± 0.023	19.2 ± 0.9	$0.1 \pm 6.5 \times 10^{-6}$	0.486 ± 0.023

[b]Volume = (4π(width)/2(length)/2(thickness)/2)/3 except for *G. quadriradiata* where volume = $(1/3\pi((\text{width})/2)^2(\text{length})$ for *G. quadriradiata*.

[c]Separation of the embryo and testa were not feasible for *D. carota* seeds.

Table 3.5. Linear regression analyses of seed parameters, initial dormancy of seed lots and seed responses from the dose-response experiment[a].

Seed responses[b]	Seed parameters[c]	Coefficient	P	Adj R^2	F ratio	P of the model
ED_{50}	Intercept	11.96	0.005	0.94	83.18	0.0008
	Testa : seed weight ratio	-18.352	0.001			
	Intercept	0.11	0.268	0.88	18.75	0.0080
	Surface	0.039	0.281			
	Initial dormancy	0.030	0.002			
	Surface * Initial dormancy	-0.005	0.003			
LD_{50}	Intercept	0.25	0.158	0.92	23.34	0.0140
	Testa thickness	$2.829x10^{-3}$	0.389			
	Initial dormancy	$2.352x10^{-2}$	0.006			
	Testa thickness * Initial dormancy	$2.320x10^{-4}$	0.029			
	Intercept	0.29	0.101	0.92	27.76	0.0040
	Width	$-7.142x10^{-6}$	0.948			
	Initial dormancy	$4.618x10^{-2}$	0.001			
	Width * Initial dormancy	$-1.475x10^{-5}$	0.005			
Maximal mortality	Intercept	100.86	≤0.001	0.86	42.32	0.0006
	Initial dormancy	-1.2310	0.001			
	Intercept	$1.43x10^{2}$	0.003	0.84	11.16	0.0390
	Length	$-3.368x10^{-2}$	0.017			
	Embryo weight	$-3.624x10^{4}$	0.021			
	Length * Embryo weight	$1.229x10^{1}$	0.025			

[a]Linear regression analyses were done according to R's lm(y~a+b+a*b) function.

[b]ED_{50}, half maximal effective doses of dry mustard biomass that decrease germination; LD_{50}, lethal doses of dry mustard biomass that kill 50%.

[c]Analysis on dicotyledon species only for analysis of ED_{50} and testa : seed weight ratio and the analysis LD_{50} according to testa thickness and initial dormancy.

Table 3.6. Intraspecific responses to exposure of Indian mustard tissues according to dormancy state and relative impact of biofumigation.

Seed species	Treatment[a]	Dormancy state	Seed responses[b,c]					
			Germination		Mortality		Dormancy	
			%					
Ambrosia artemisiifolia	Treated	Primary	1.43 ± 0.90	c	42.97 ± 8.17	ab	55.60 ± 8.25	a
		Secondary	22.96 ± 4.79	b	60.73 ± 6.34	a	16.31 ± 3.50	b
	Control	Primary	0.52 ± 0.34	c	27.64 ± 5.73	ab	71.84 ± 5.72	a
		Secondary	62.88 ± 3.97	a	19.21 ± 4.11	b	17.91 ± 2.08	b
Impact of biofumigation[d]		Primary	48.48 ± 92.12		57.59 ± 35.36		-23.64 ± 8.60	
		Secondary	-60.16 ± 9.67		293.25 ± 84.09		-8.08 ± 18.49	
		P value[e]	≤ 0.0001		0.0217		0.458	
Chenopodium album	Treated	Primary	8.96 ± 3.46	c	54.37 ± 3.73	a	36.67 ± 5.77	a
		Secondary	16.47 ± 4.22	c	38.84 ± 5.01	ab	44.68 ± 4.59	a
	Control	Primary	45.92 ± 3.84	b	26.79 ± 4.71	b	27.29 ± 3.94	a
		Secondary	86.66 ± 0.92	a	4.82 ± 0.78	c	8.51 ± 1.35	b
Impact of biofumigation		Primary	-81.60 ± 7.12		106.29 ± 16.82		32.54 ± 14.22	
		Secondary	-81.02 ± 4.85		730.19 ± 117.55		416.95 ± 36.63	
		P value	0.9471		0.0001		≤ 0.0001	
Daucus carota	Treated	Primary	0.78 ± 0.78	c	90.01 ± 4.09	a	9.21 ± 3.55	ab
		Secondary	4.67 ± 2.00	c	87.25 ± 4.31	a	8.09 ± 2.46	ab
	Control	Primary	26.72 ± 2.10	b	45.36 ± 7.79	b	27.92 ± 8.45	a
		Secondary	90.47 ± 1.86	a	7.03 ± 1.69	c	2.50 ± 0.62	b
Impact of biofumigation		Primary	-97.33 ± 2.67		156.53 ± 49.89		49.70 ± 63.12	
		Secondary	-94.87 ± 2.19		1,196.00 ± 123.49		221.89 ± 98.35	
		P value	0.4870		≤ 0.0001		≤ 0.0001	

Table 3.6 (continued): Intraspecific responses to exposure of Indian mustard tissues according to dormancy state and relative impact of biofumigation.

Setaria viridis	Treated	Primary	0.00 ± 0.00	b	93.98 ± 2.67	a	6.02 ± 2.67	c
		Secondary	0.00 ± 0.00	b	88.80 ± 1.07	a	10.95 ± 1.08	bc
	Control	Primary	30.26 ± 2.54	a	21.38 ± 3.97	c	48.35 ± 5.62	a
		Secondary	32.27 ± 2.20	a	43.50 ± 2.88	b	24.23 ± 4.48	b
Impact of biofumigation		Primary	-100.00 ± 0.00		353.27 ± 24.88		-88.83 ± 4.57	
		Secondary	-99.25 ± 0.75		104.07 ± 3.71		-54.20 ± 4.47	
		P value	0.3020		≤ 0.0001		0.0014	
Vicia cracca	Treated	Primary	0.50 ± 0.33	c	56.26 ± 2.07	b	43.24 ± 2.12	a
		Secondary	2.24 ± 0.91	bc	78.69 ± 3.20	a	19.07 ± 3.63	b
	Control	Primary	5.13 ± 1.71	b	43.55 ± 3.70	c	51.32 ± 3.25	a
		Secondary	16.38 ± 2.23	a	61.44 ± 2.64	b	22.18 ± 2.78	b
Impact of biofumigation		Primary	-87.29 ± 4.70		28.15 ± 5.81		-14.52 ± 15.29	
		Secondary	-89.11 ± 7.15		29.13 ± 3.23		-15.94 ± 3.36	
		P value	0.8350		0.8860		0.9290	

[a]Treated weed seeds were exposed to 7.00 mg cm^{-2} of *Brassica juncea* tissues except for *S. viridis* and *V. cracca* where the amount of mustard biomass was respectively 21.00 and 14.00 mg cm^{-2}.

[b]± SE.

[c]Means within a column followed by the same letter within weed species are not significantly different according to Tukey-Kramer HSD test at $P \leq 0.05$. Data of germination of *A. artemisiifolia* and *V. cracca*, of mortality of *A. artemisiifolia*, *C. album* and *V. cracca* and of dormancy of *C. album* and *D. carota* are presented with mean separation on the basis of log-transformed data.

[d]Impact of biofumigation = $((R_T - R_C) / R_C)$ 100 with R_T corresponding to the percent response value observed in the treatment and R_C to the mean percent response value observed in the corresponding control.

[e]P value according to ANOVAs, where relative impact of primary or secondary dormant seeds are compared.

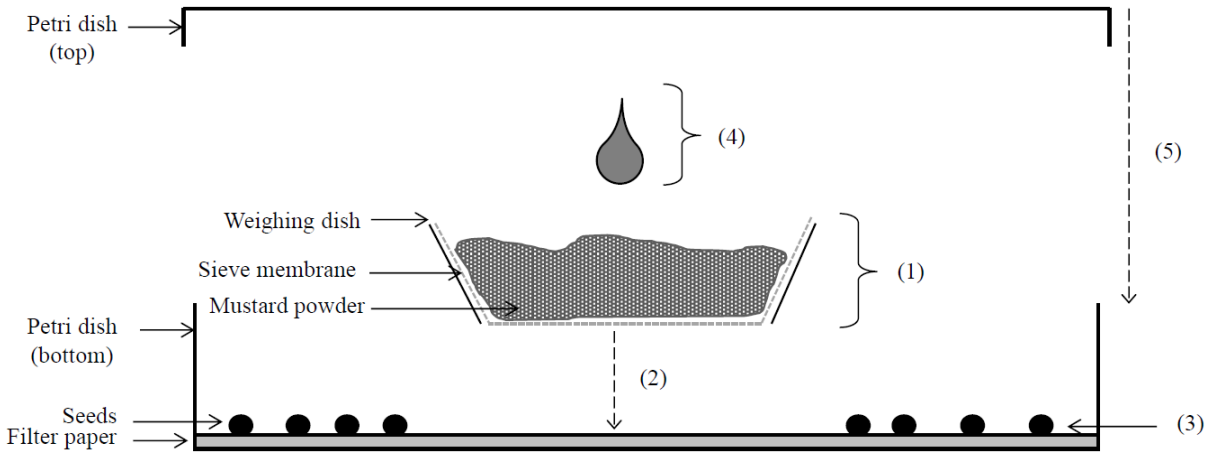

Figure 3.1. Diagram of an experimental unit and step-by-step methodology used in dose-response and intraspecific dormancy experiments. (1) Dried Indian mustard powder was weighed per treatment and placed within a weighing dish. The bottom of the weighing dish was replaced by a sieve membrane. (2) A filter paper was placed in a polystyrene disposable Petri and the weighing dish deposed at the centre. (3) Seeds were washed with distilled water and placed surrounding the weighing dish. (4) Water was added on the seeds and on the mustard tissues. (5) The Petri dish was sealed with Parafilm® and immediately incubated.

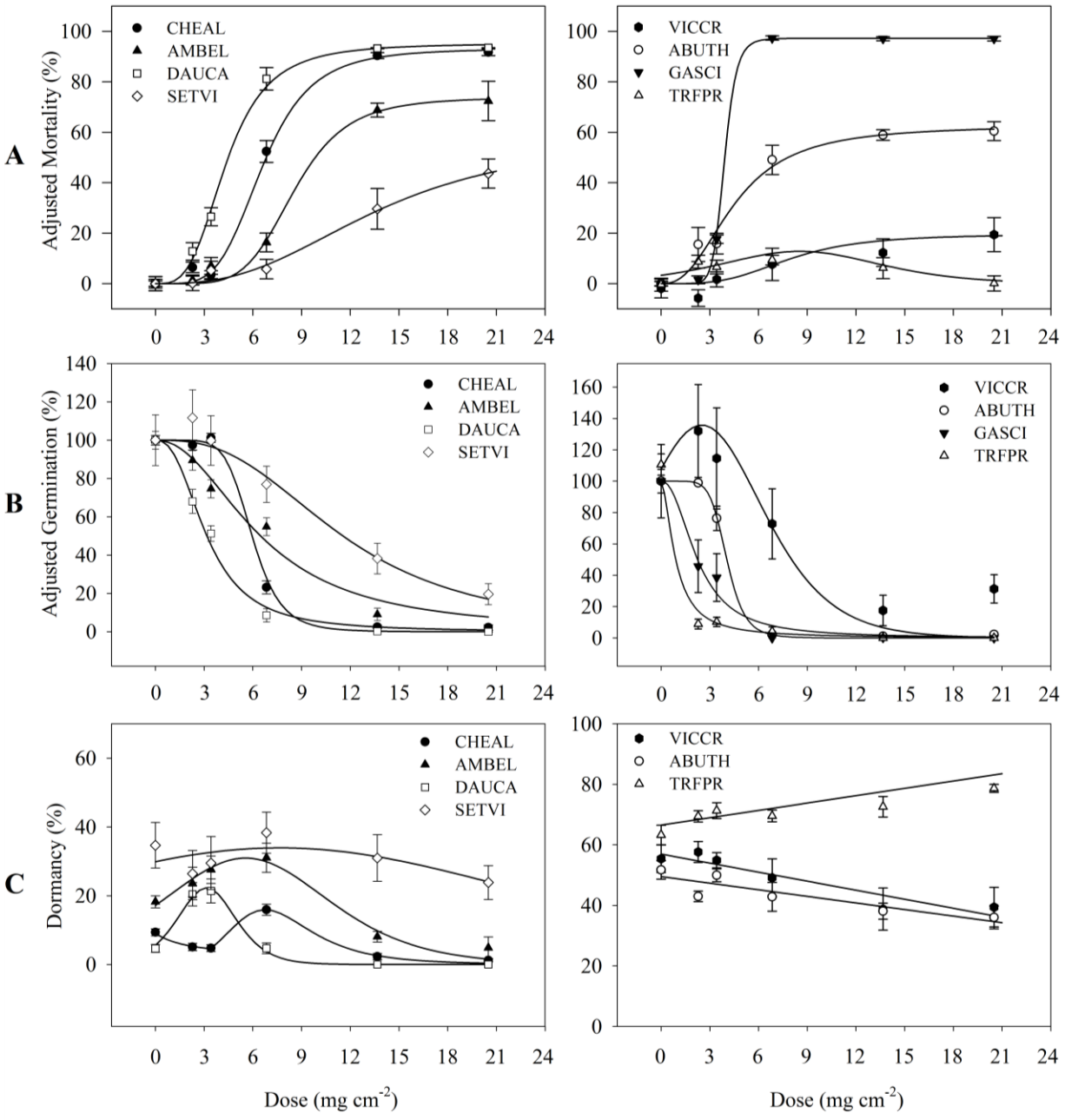

Figure 3.2. Weed seed responses to growing rates of Indian mustard dried biomass in a Petri dish experiment: A) percentage of adjusted mortality, B) percentage of adjusted germination and C) percentage of dormant viable seeds. Abbreviations: CHEAL, *C. album*; VICCR, *V. cracca*; DAUCA, *D. carota*; AMBEL, *A. artemisiifolia*; SETVI, *S. viridis*; ABUTH, *A. theophrasti*; GASCI, *G. quadriradiata*; TRFPR, *T. pratense*. Vertical bars indicate ± SE. Equations and regression coefficients are presented in Table 3.2.

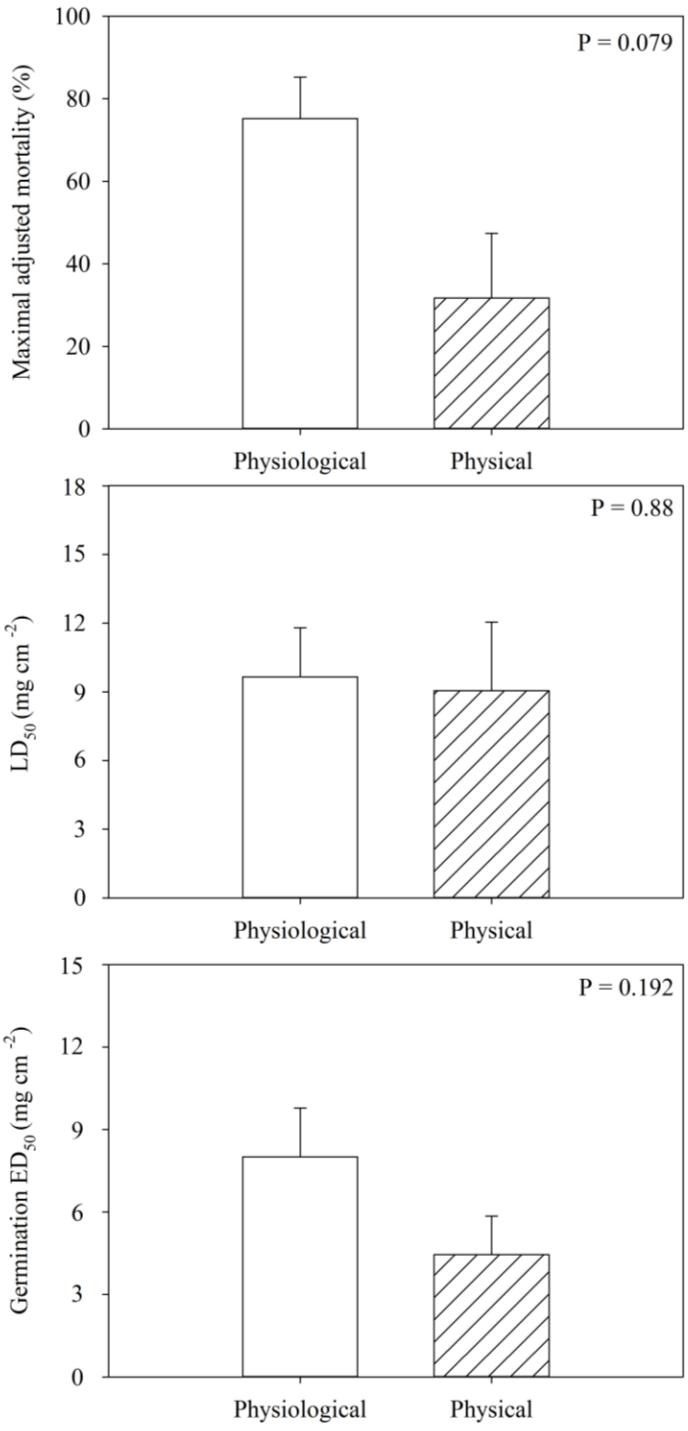

Figure 3.3. Percentages of maximal adjusted mortality, lethal doses of dry mustard biomass that kill 50% (LD_{50}) and estimated half maximal effective doses of dry mustard biomass that decrease germination (ED_{50}) according to the classes of seed dormancy. Vertical bars indicate ± SE.

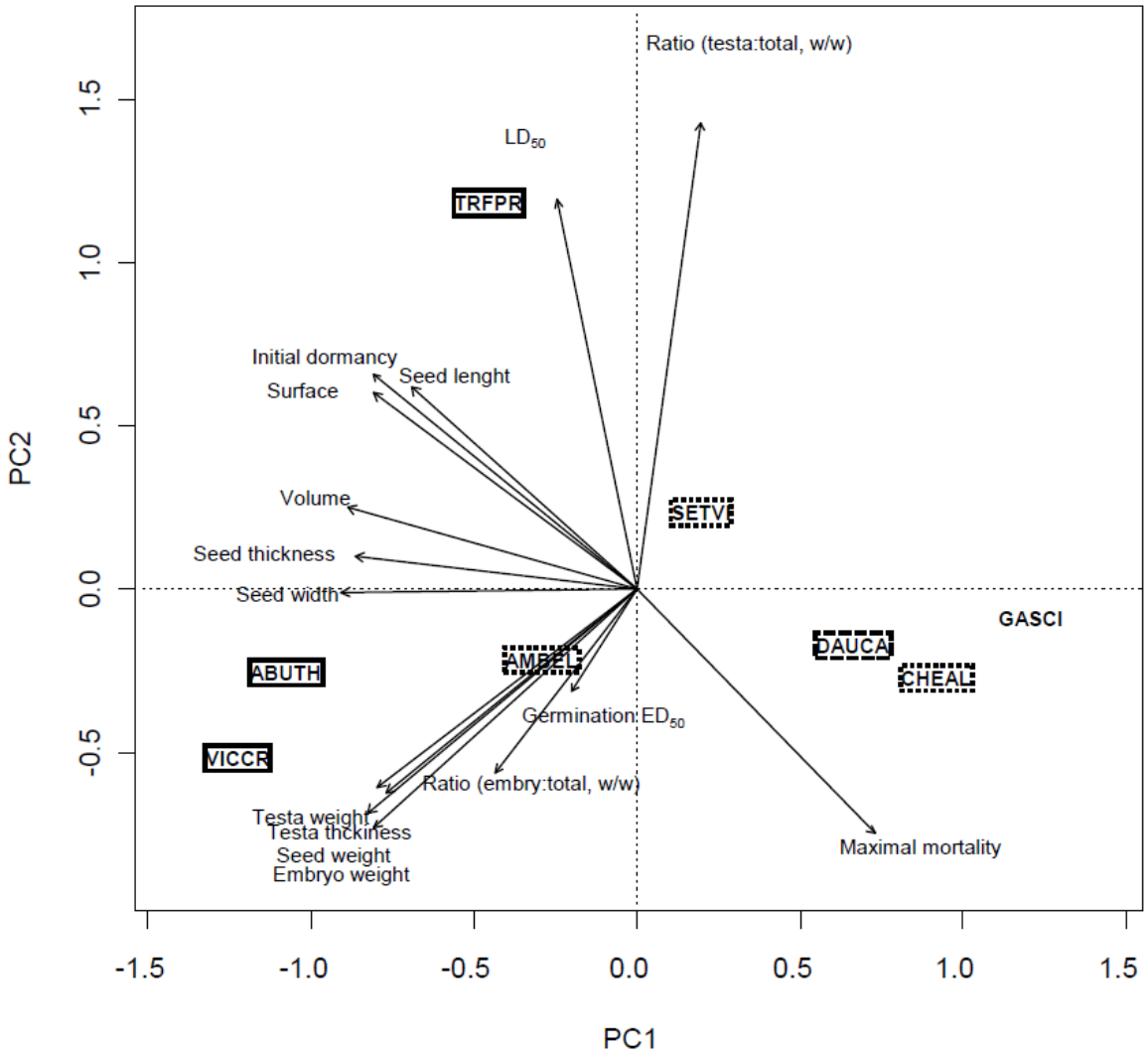

Figure 3.4. Principal component analysis of seed parameters and seed responses. Species in bold line text box have physical dormancy, species in dotted line text box have physiological dormancy, species in dashed line text box have morphological dormancy and species without text box are non-dormant. 73.1% of the variation is explained by the axes. PCA performed with vegan library. Species are scaled proportional to eigenvalues. Scaling preserves correlation between variables. General scaling constant of scores is 3.25. Abbreviations: CHEAL, *C. album*; VICCR, *V. cracca*; DAUCA, *D. carota*; AMBEL, *A. artemisiifolia*; SETVI, *S. viridis*; ABUTH, *A. theophrasti*; GASCI, *G. quadriradiata*; TRFPR, *T. pratense*; LD_{50}, lethal doses of dry mustard biomass that kill 50%; ED_{50}, half maximal effective doses of dry mustard biomass that decrease germination.

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CONNECTING STATEMENT BETWEEN CHAPTERS 3 AND 4

Experiments performed in chapter 3 shown that weed species expressed specific dose responses, estimated ED_{50}, LD_{50}, and maximal mortality. Initial dormancy was found to be positively related to ED_{50} and LD_{50} values with a significant interaction with seed surface and seed width, respectively for interspecific response. Number of dormant seeds also changed intraspecific response of weed species in the study. Weed responses to biofumigation were clarified and underlined which morphological parameters, such as testa thickness, seed size, or proportion of dormant seeds, explained susceptibility of a weed species to biofumigation. The experiment conducted in chapter 4 also evaluated the impact of allelochemicals released in Petri dishes on seed germination, mortality, and dormancy, but investigated on how those responses changed over generations. Moreover, seedling survival, growth and reproduction parameters of surviving plants were assessed, allowing a better understanding of individual responses across generations. Those laboratory and greenhouse studies allowed evaluation of different parameters of weed biology, hardly possible *in situ*.

CHAPTER 4. Intergenerational assessment of biofumigation on fitness and phenology of *Ambrosia artemisiifolia* L. and *Abutilon theophrasti* Medik.

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4.1 Abstract

Biofumigation is an agronomic practice known to generate phytotoxic allelochemicals detrimental to seed germination and survival. The objective of the study was to assess lethal and sublethal effects of Indian mustard (*Brassica juncea* L. 'Caliente 199') biofumigation on weed fitness and phenology across generations. Laboratory and greenhouse experiments were performed on common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.). Weed seeds were exposed in a Petri dish germination test to the allelochemicals produced after rehydrating dried Indian mustard tissues at the 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 a greenhouse for phenological surveys and reproduction component measurements. The same treatments were applied to G2 and G3 seeds. For each generation, seed parameters such as testa thickness, relative weight of the testa, and seed size were measured directly by manual dissection, weighing seed structures, and stereomicroscope imaging software measurements. Biofumigation reduced weed fitness by decreasing seed germination and survival, increasing seedling mortality, delaying emergence and flowering for both species, and decreasing number of seeds produced for *A. artemisiifolia*. However, second and third generations may improve their tolerance to biofumigation by an increased number of dormant seeds for *A. artemisiifolia*, seedling survival for both species, increased seed production for *A. theophrasti*, relative weight of the embryo, and testa thickness for *A. artemisiifolia*. Quantification of target weed fitness from *Brassica* cover crop biofumigation enables us to understand the consequences for weed population performance in the field and should be considered when this practice is used for weed management.

Keywords: Maternal effect, phytotoxic allelochemicals, reproduction, seed response, seedling survival.

4.2 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)" (Narwal and Haouala 2013). Allelopathy is a biotic environmental stress factor that reduces the competitive ability of a target plant in numerous ways, including direct inhibition of plant functions (Duke and Dayan 2006; Pedrol et al. 2006). Phytotoxic allelochemicals can reduce photosynthesis, carbon acquisition, plant growth (Hussain and Reigosa 2011), and consequently reduce plant fitness of the 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 (Krebs 2001). Components of plant fitness are germination, establishment, survival, and reproduction. Plant-produced allelochemicals have deleterious effects on target plant fitness mainly by inhibiting the establishment and/or growth (Inderjit et al. 2011). In some cases, phytotoxic allelochemicals could affect flower production, even causing complete reproductive failure (Batlang and Shushu 2007). Some authors also established that lower fitness of the target plant may be indirectly caused by disturbed plant mutualism interactions (Reviewed in Hale and Kalisz 2012).

Allelopathy is recognized to play a significant role for pest management in agricultural ecosystems, particularly in organic or low input agriculture (Jabran et al. 2015). In many agricultural practices, allelopathy is a defining factor leading to lower weed pressure (Gallandt et

al. 1999). Those practices include crop accessions, variety or cultivar selection, bioherbicide or plant extract application, dead mulch, and green manure (Qasem 2010; 2013). Phytotoxic allelochemicals are water soluble, insoluble, or volatile, and are released by crops, cover crops, or their residues (Kohli et al. 2006).

Weeds are well adapted to agricultural ecosystems by expressing traits that increase their competitive ability, such as rapid seedling growth, high-seed output, environmental plasticity, and discontinuous dormancy (Basu et al. 2004; Zimdahl 2013). Allelopathic cover crop residues may decrease weed fitness by reducing germination, establishment, and growth (Kohli et al. 2006; Qasem 2010; 2013; Weston and Duke 2003).

Biofumigation is an agricultural practice in which phytotoxic allelochemicals may reduce weed seed viability in the soil seed bank (Matthiessen and Kirkegaard 2006). Biofumigation occurs during the incorporation of *Brassica* cover crops containing high glucosinolate concentration in the soil. Volatile phytotoxic chemicals are released from decomposing *Brassica* tissues and provide weed suppression. The most common volatile produced during the breakdown of *Brassica* are isothiocyanates (ITCs) which are released following tissue disruption when myrosinase enzymes hydrolyze glucosinolates in the presence of water (Michel 2008; Morra and Kirkegaard 2002). 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 a loss of protein structure and function and decreased enzymatic activity (Dufour et al. 2013; Weir et al. 2004). In seeds, ITCs interact with glycolytic enzymes during the germination process and thereby prevent or delay seed/tuber germination (Drobinca et al. 1977). The ITCs are strong germination suppressants of many different weed species (Al-Khatib et al. 1997; Al-Sherif 2013; Boydston et al. 2004; Lefebvre et al. 2018;

Petersen et al. 2001). High concentrations of ITCs penetrate the seeds, irreversibly inhibit protein synthesis, and the seeds lose viability (Leblova-Svobodova and Kostir 1962). Biofumigation from *Brassica* cover crops is recognized to reduce weed germination, establishment, and biomass production in the field (Boydston et al. 2004; Kumar et al. 2009; Walker and Kremmydas 2010).

Plants can adapt rapidly to the precise chemical composition of neighbouring plants (Ehlers and Thompson 2004). Environmental conditions in which a plant grows, especially during sensitive growth stages, could influence seed production (Parrish and Bazzaz 1985) and fitness (Platenkamp and Shaw 1993). Moreover, it could influence the offspring of those plants, a process known as the phenotypic maternal effect (Roach and Wulff 1987). Mother plants may affect their offspring 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 (Holeski and al. 2012). Because these maternal effects can have genetic and environmental components, they can be adaptive if they increase offspring reproductive success (Lacey 1998). Few studies have evaluated the tolerance of species to allelopathic compounds. Callaway et al. (2005) 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 the authors' knowledge, fitness response and possible adaptability to allelopathic compounds generated during biofumigation have not been studied.

While evaluating the potential of long-term utilization 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 altered, fitness will also be modified, and consequently their competitive ability. Furthermore, if the selection pressure brought by biofumigation was strong, it could lead to the development of tolerant weed populations. Generally, allelopathic studies focus on seed germination and seedling growth (Hale and Kalisz 2012). However, it was important to assess the impact of allelopathy on plant fitness, not only on seeds and seedlings, but including survival to flowering, adult plant growth, and reproduction.

Two hypotheses were formulated: 1) exposure of weed seeds to increasing concentration of allelochemicals generated during biofumigation will decrease survival and 2) sublethal concentrations of allelochemicals will negatively influence the growth and reproduction of the plant, and these effects will decrease when following generations are exposed to the same treatment. The objectives of the study were to determine common ragweed (*Ambrosia artemisiifolia* L.) and velvetleaf (*Abutilon theophrasti* Medik.) fitness and phenological responses to biofumigation and to assess potential changes in fitness and phenological responses of those weeds across generations.

4.3 Materials and methods

4.3.1 Biofumigation assay across generations

The study was conducted at the Research and Development Institute for the Agri-environment (IRDA) research station in Saint-Bruno-de-Montarville (45.533°N, 73.35°W) on *A. artemisiifolia* and *A. theophrasti*. *Ambrosia artemisiifolia* seeds were haphazardly collected in the fall of 2012 from mature plants at IRDA, where seeds fell after a vigorous plant shaking.

Abutilon theophrasti seeds came from a seed collection at Macdonald campus research farm, McGill University (45.412°N, 73.944°W). *Ambrosia artemisiifolia* and *A. theophrasti* had 60.3 ± 2.9% and 60.9 ± 2.7% seed germination in a Petri dish germination test, respectively. Seeds from those initial seed lots were sown in pots in a greenhouse in 2014. Weed seedlings were thinned to 3 per pot. A total of 45 plants per species grew to maturity and produced G1 seeds (1st generation). Primary dormancy of G1 seeds was released to allow seeds to germinate under biofumigation treatment. For *A. artemisiifolia*, seeds were kept in moist sand at 4 C for 7 weeks and for *A. theophrasti*, seeds were immersed for 5 min in water at 70 C. Fifty G1 seeds were washed thoroughly with distilled water, placed in each Petri dish and exposed to two biofumigation treatments and a control. Dry powder of Indian mustard (*Brassica juncea* L. 'Caliente 199') was used as biofumigant material at rates of 0 (control), 3.5, and 7.0 mg for *A. artemisiifolia* and 0, 2.33, and 3.5 mg for *A. theophrasti* per cm^2 exposed to the allelochemicals. Indian mustard plants were grown to until flowering in 19 cm diameter by 17.5 cm depth pots containing a commercial potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in a growth chamber (Conviron E15, serial number 8D40801J) set at 26 ± 2 C, 16/8 h light/darkness (186 µmol m^{-2} s^{-1}). At flower, the above-ground plant parts were harvested, dried at 35 C for 5 d and ground with a laboratory mill grinder.

Previous experiments showed those rates were not lethal and allowed germination. The rates were calculated according to amount of dry tissues placed in the weighing dish and releasing compounds in a 91.1 mm diameter Petri dish. This corresponds to a treated surface of 65.18 cm^2. Prior to the assays, the material was analyzed 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. (2018). Petri dish biofumigation followed the methodology developed in this previous study. Fifteen replicates per treatment were arranged in a completely randomized design in a growth chamber (MLR-350H, Sanyo) set to 22.5 ± 0.5 C, 16/8 h light/darkness (372 μmol m^{-2} s^{-1}).

Twenty surviving germinated G1 seeds with a radicle of 2 mm long were removed from the Petri dish 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 a greenhouse without further exposition to allelochemicals. Environmental conditions in the greenhouse included a natural photoperiod and no control on temperature was done except for air circulation by fans and vents. Temperature in the 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,* fifteen replicates were conducted for each biofumigation rate, whereas due to low germination of *A. theophrasti* after biofumigation, 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.

After three weeks, seedlings were thinned to only three plants per pot except for G1 *A. theophrasti* which had one plant per pot due to its low emergence after sowing. The plants had been supplied with water as needed. When the first inflorescence appeared for *A. artemisiifolia* and *A. theophrasti*, soil was fertilized once at 1-2 cm depth with 50 and 30 g per pot of granulated chicken manure 5-3-2 (Acti-Sol inc., Notre-Dame-du-Bon-Conseil, Quebec, Canada), respectively. Plants of both species have grown to maturity and produced G2 seeds in 2015. Each experimental unit of *A. artemisiifolia* was covered with a transparent copolyester tube (VIVAK PETG, 0.02 mil., Plaskolite, Columbus, OH, USA) with air vents covered with white broadcloth

during flowering to avoid cross-pollination between experimental units. *Abutilon theophrasti* was assumed to be self-pollinated (Warwick and Black 1988).

G2 seeds were collected for each experimental unit and handled in the previously described manner and exposed to the same biofumigation treatments to produce G3 seeds in 2016. After-ripening treatment 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 being sown in the greenhouse.

4.3.2 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 in any treatment. 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 (Peters 2000) and the International Seed Testing Association (ISTA) working sheets on tetrazolium testing (Leist et al. 2003). Viable non-germinated seeds at the end of the assays were considered dormant. This laboratory procedure was repeated for G1, G2, and G3 seeds.

After planting the germinated seeds into growing pots, seedling emergence was recorded for three weeks to assess seedling survival for each treatment. During G1 and G2 plant growth, number of reproductive structures, including number of visible clusters of male flower heads for *A. artemisiifolia* and floral buds for *A. theophrasti* were recorded weekly during the season. In the meantime, phenological stages of both species were monitored according to the BBCH scale (Hess et al. 1997). The general 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 replicate were cleaned from other plant material, counted, and weighed. Reproductive effort was calculated using total number of seeds per plant and plant biomass. To evaluate the plant biomass, relations between biomass, height and diameter were used. One of the three plants per pot at ripening stage was cut above ground and dried for both species. Also, height and diameter of each the plants were measured. For *A. theophrasti*, relation between G2 plant biomass, height and diameter was used to estimate G1 plant biomass.

The following seed morphological parameters were measured in G1, G2, and G3 seeds: length, width, thickness (height), 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 described in Lefebvre et al. (2018), those measurements were taken on 30 seeds per replicate and five replicates per treatment randomly selected per generation of each species.

4.3.3 Statistical analyses

Data analyses were performed using ‘Agricolea’ and ‘Asbio’ packages of R software v.3.0.1 (R Development Core Team 2008). 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 (percentage of germination, mortality, and dormancy), seedling survival (percentage of survival), seed number and weight, reproductive effort, and each seed morphological parameter. Dependent variables were log-transformed (log_{10} [x+1]) whenever required to respect normality and homoscedasticity assumptions. Furthermore, repeated measures analyses of variance for biofumigation rate and generation effect were performed using 'ezANOVA' package of R software.

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). CIPRA uses bioclimatic models and weather data to generate forecast graphics for the incidence of diseases, population development of insect pests, and crop development (phenology). This software allows users to develop specific bioclimatic phenological models of crops and weeds based on the BBCH scale. General structure of phenological models included four specific phases: emergence, vegetative development, early, and late reproductive development (Figure 4.1). For each phase, BBCH values will increase according to specific temperature response. *Ambrosia artemisiifolia* temperature response for each phase was based on the equation of Yan and Hunt (1999) 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 (based on previous experiments). *Abutilon theophrasti* temperature response for each phase was based on multi-linear model constructed from three linear components (Coelho and Dale 1980), 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 (Patterson 1992). 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, using BBCH measurements and air temperatures recorded by data loggers in the growth chamber and the greenhouse. ANOVAs and Tukey's HSD tests were performed to assess significant difference between model parameters of each treatment. Phenological parameters were log-transformed ($\log_{10}$ [x+1]) or reciprocal-transformed (1/x) whenever required to respect normality and homoscedasticity assumptions.

Non-linear curve fitting was performed to establish the responses of *A. artemisiifolia* cluster of male flower heads and *A. theophrasti* floral bud production to treatments 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 used to describe the number of *A. artemisiifolia* male flower head clusters and the number of *A. theophrasti* floral buds as functions of days after the biofumigation treatment.

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

where **a** refers to the amplitude, **b** represents 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 $P \leq 0.05$. Parameters for each treatment were compared to one another and significant groups were assigned according to conclusions of those analyses. Finally, estimation of uncut plant biomass was done with Table Curve 3D V.4.0 (Systat Software, San Jose, CA).

4.4 Results and discussion

The chemical analysis performed on the 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 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 4.1.

4.4.1 Seed responses

Exposure to Indian mustard tissues significantly decreased *A. artemisiifolia* (Table 4.2) and *A. theophrasti* (Table 4.3) seed germination and increased seed mortality. Seed germination and survival were always superior in the control treatment. Biofumigation rate of 7.0 mg cm^{-2} of dried mustard tissues caused greater impact on seed germination and mortality on each generation of *A. artemisiifolia* than the 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 a significant effect on seed germination. Furthermore, there was a significant interaction between rate and generation. Seed germination in the control treatments increased over generations, while the germination decreased in the biofumigation treatments, except for 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 was a significant effect for *A. theophrasti* seed mortality, but not for *A. artemisiifolia* seed mortality.

The quantity of viable non-germinated *A. theophrasti* seeds at the end of the Petri dish assays was not affected by biofumigation rates for each generation. However, *A. artemisiifolia* dormant seeds increased after exposure to allelochemicals released following rehydration of Indian mustard dry 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, and the interaction between rate and generation effects was also significant.

In a previous study, exposure of *A. artemisiifolia* seeds to similar quantities of allelochemicals released in Petri dish increased the percentage of adjusted mortality and decreased percentage of adjusted germination (Lefebvre et al. 2018). Moreover, the same induction of seed dormancy was observed. *Ambrosia artemisiifolia* exhibits physiological dormancy and may return to the secondary dormancy stage, *A. theophrasti* seeds exhibiting physical dormancy (Baskin and Baskin 1998a). In the same previous study, the *A. theophrasti* adjusted germination was not reduced at the rates equivalent to 2.33 mg cm^{-2} (Lefebvre et al. 2018). The quantity of dormant seeds for both species differed considerably from those previously observed. *Ambrosia artemisiifolia* and *A. theophrasti* seeds exhibited less than 2.8 and 6.3% of dormancy compared to 20 and 53%, respectively in the previous study. After-ripening treatments reduced greatly the number of dormant seeds. The amount of dormancy in a seed lot did affect intraspecific seed response to biofumigation. Furthermore, biofumigant potential of the 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 were likely to be explained by the 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 the 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 (Baskin and Baskin 2006). Environmental factors including temperature, darkness, light, gases, water, inorganic, and organic chemicals can cause changes in nondeep physiological dormancy (Baskin and Baskin 1998b). Moreover, preconditioning effects are known to alter germination and dormancy behaviour of seeds (reviewed in Baskin and Baskin 1998c). Previous maternal environment will affect the sensitivity of seeds to environmental factors that influence germination (temperature, nitrate, light, water, oxygen, and allelopathic compounds) (Finch-Savage and Leugner-Metzger 2006). Different seed responses observed between generations in the present experiment may be explained by previous maternal environment. However, maternal effect 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.

4.4.2 Seedling survival

Ambrosia artemisiifolia seedling establishment was lower for germinated seeds exposed to the allelochemicals released during biofumigation than the control seeds for generation G1 and G2 (Table 4.4). *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.

Abutilon theophrasti seedling establishment at both rates of biofumigation was less than *A. artemisiifolia* establishment (Table 4.5). 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 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 seedling survival by 25.3%, significantly different from the control. There was a significant interaction between biofumigation rate and generation effect on *A. theophrasti* seedling survival.

Seed exposure to the allelochemicals generated during rehydrating dried tissues of Indian mustard had ultimately changed 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 (Holeski and al. 2012). Maternal effects on seed size and its impact on germination characteristics, seedling size and further competitive ability has been intensely studied (Roach and Wulff 1987). Variation in those processes results in differential plant fitness. However, it was unclear in the present study if changes in offspring responses are caused by G1 seed exposure to biofumigation, or partly caused by a delay in G1 seedling establishment and a reduced plant growth. Mechanisms involved in this transgenerational inheritance of allelochemical tolerance require further investigation.

4.4.3 Phenological surveys

4.4.3.1 Phenological analyses

Seed exposure to allelochemicals released after rehydrating dry Indian mustard tissues proportionally slowed *A. artemisiifolia* emergence for both generations (Table 4.6). The 7.0 mg cm^{-2} biofumigation rate delayed the emergence by 27.9% compared to the 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 the 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 a faster vegetative development than the plants from the control. G1 *A. theophrasti* emergence and vegetative development rates are similar to those of the second generation. G1 early reproductive development was proportionally delayed by biofumigation treatments. However, late reproductive development rate was higher for surviving plants compared to the control. There was no difference between biofumigation treatments for G2 *A. theophrasti* for early or late reproductive development rates.

4.4.3.2 Male flower head and floral bud production

The sublethal impact of biofumigation on *A. artemisiifolia* seeds influenced amplitude and timing of flower head cluster production (Figure 4.2 and Table 4.7). 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 germinated seeds from the control and 3.5 mg

cm^{-2} rate for G1. However, the amplitude of the 7.0 mg cm^{-2} rate was lower than the 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 reduced significantly 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).

Biofumigation treatments influenced amplitude and timing of floral bud production of *A. theophrasti* plants (Figure 4.3 and Table 4.7). The number of *A. theophrasti* floral buds produced reached higher peaks for G1 plants from seeds exposed to the 2.33 mg cm^{-2} rate compared to the control plants. Timing of maximum floral bud production was similar for all G1 *A. theophrasti* plants. The G2 *A. theophrasti* floral bud production response differed from G1 response. The amplitude of floral bud production was similar, but was delayed by biofumigation treatments compared to the control. The shape parameter c was similar for all fitted curves for *A. theophrasti* (0.191 ± 0.012).

4.4.4 Reproduction parameters

Seed number, total seed weight, and reproductive effort from *A. artemisiifolia* plants exposed at the seed stage to allelochemicals changed between generations (Table 4.8). In the first generation of *A. artemisiifolia*, seed number, total seed weight, weight of a hundred seeds, and plant biomass were all similar in all treatments. For *A. artemisiifolia* plants, relations established between height, diameter, and biomass for G1 were:

Biomass = 5.0 – 112.4 ln(Height) / Height + 12.44 ln(Diameter), R^2= 0.53 [2]

and for G2:

Biomass = 2.03 – 9,759 / (Height$^{1.5}$) + 20.2 * ln(Diameter), R^2 = 0.46 [3]

For G1 and G2 *A. theophrasti* plants, relation established was:

Biomass = 10.21 + 0.09 (Height) + 1.88 (Diameter), R^2= 0.89 [4]

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 4.6). 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 4.9). 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 the control plants. Only the 3.5 mg cm^{-2} rate reduced significantly G1 *A. theophrasti* plant biomass. Reproductive effort of treated *A. theophrasti* plants was higher than the control plants. For all G2 *A. theophrasti* reproduction parameters, only reproductive effort was significantly higher for the 3.5 mg cm^{-2} rate than the control. Generation was a significant factor for all variables analyzed. Moreover, biofumigation rate was significant for seed number, total seed weight, plant biomass, and reproductive effort. Significant interaction between biofumigation rate and generation was found for seed number, total seed weight, and weight of a hundred seeds.

4.4.5 Seed morphological parameters

Ambrosia artemisiifolia seed size, represented by the seed length, width, surface, and weight, were not affected by seed exposure to Indian mustard biofumigation treatment nor

generation (Table 4.10). Seed length ranged from 3,739 ± 162 µm to 4,052 ± 96 µm, seed width ranged from 1,981 ± 71 µm to 2,065 ± 16 µm and seed surface ranged from 4.71 ± 0.19 mm^2 to 5.18 ± 0.37 mm^2. Neither embryo nor testa weights were influenced by treatment. However, biofumigation affected *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 the testa. Despite those significant differences, only generation was a significant factor. *Ambrosia artemisiifolia* pericarp thickness decreased over G2 and G3, but more importantly in treated seeds compared to control seeds. Biofumigation rate and generation effects, and the interaction between effects were significant for pericarp thickness. Testa thickness was not affected by treatments in G2 but was significantly increased for G3 treated seeds compared to the 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 impact.

Abutilon theophrasti seed length, seed width, and seed surface were not affected by seed exposure to Indian mustard biofumigation treatment nor across generations (Table 4.11). Seed length ranged from 2,706 ± 29 µm to 2,927 ± 114 µm, width ranged from 3,265 ± 28 µm to 3,357 ± 19 µm, and surface ranged from 6.37 ± 0.13 mm^2 to 6.99 ± 0.06 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 the 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 the seeds in the control treatment. Seedling survival increased significantly, but it could not be linked to a specific characteristic seed structure.

Plants can tolerate allelochemicals through the ability to reduced uptake of allelochemicals at the root surface, compartmentalization of allelochemicals, and detoxification of allelochemicals (Duke 2003). Additionally, seed tolerance to allelochemicals is often related to seed size (Petersen et al. 2001; Westoby et al. 1996). In a previous study, we established that seed dormancy in interaction with seed morphological characteristics, such as seed surface, testa thickness, or the relative importance of testa in seed are better parameters to predict weed tolerance to allelochemicals generated from a biofumigation process (Lefebvre et al. 2018). Those results agree with the observations of Waddington (1978) and Weir et al. (2004) 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 (Waddington 1978, Weir et al. 2004). 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. However, the thinner testa of *A. theophrasti* G3 seeds would be unlikely to be associated with improved allelochemical tolerance.

Germination and establishment delays often lead to smaller adult plants (Ross and Harper 1972). Moreover, large seedlings from large seeds have an increased growth rate and produced

more flowers (Stanton 1984). Resource availability may influence maternal seed production, as well as performance of seedlings (Lacey 1998).

Plant size, growth and seed number are pliable plant traits, but seed mass is relatively resistant to change (Harper et al. 1970). However, Callaway et al. (2005) measured that population of species accustomed to *C. maculosa* allelopathic effect had smaller seeds than other populations. Also, Weiner et al. (1997) found an environmental maternal effect where initial seedling growth influences seed weight. Whenever seed size varies, literature often reported a tradeoff between seed size and seed number (Wilbur 1977; Primack 1978). 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 the 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 (Bruce et al. 2007). 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 (Basset and Crompton 1975; Warwick and Black 1988), 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.

Lower seed germination and survival, increased seedling mortality, delay in flowering, emergence rates deferred for both species, and lower number of seeds produced for *A. artemisiifolia* are responses that greatly reduced weed fitness when exposed to phytotoxic allelochemicals released during the laboratory procedures of the experiment. However, increased quantity of dormant seed for *A. artemisiifolia*, seedling survival for both species, seed production for *A. theophrasti*, relative weight of the embryo, and testa thickness for *A. artemisiifolia* across generations changed offspring fitness, and may lead to tolerance to this process. The detailed quantification of target plant fitness from *Brassica* cover crops allelopathy enables us to understand the consequences for weed population performance in the field. This new information should be considered when biofumigation practice is part of weed management strategies.

4.5 Acknowledgments

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Table 4.1. Quantities of allyl- isothiocyanate (ITC) released in the Petri dish assays according to rates of dry Indian mustard material for each generation[a].

Biofumigation treatments	G1	G2	G3
mg cm^{-2} of dried mustard biomass[b]	µg 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

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

[b]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).

Table 4.2. *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	Germination[a]	Mortality[a]	Dormancy[a]
	mg cm^{-2}		%	
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[b]	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[b]	55.3 (4.5) a[b]	11.5 (1.5) a[b]
Repeated measures ANOVA				
Rates	df[c]	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**

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

[b]Mean separation based on non-transformed data, otherwise on log transformed data.

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

Table 4.3. *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	Germination[a]	Mortality[a]	Dormancy[a]
	mg cm^{-2}		%	
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[b]	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[b]	85.5 (4.5) a[b]	1.1 (1.1)[c]
Repeated measures ANOVA				
Rates	df[d]	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

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

[b]Mean separation based on non-transformed data, otherwise on log transformed data.

[c]Not enough data to performed analysis.

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

Table 4.4. *Ambrosia artemisiifolia* 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	Survival[a]
	mg cm^{-2}	%
G1	0	92.0 (1.8) a
	3.5	79.7 (2.0) b
	7.0	76.5 (2.4) b
G2	0	99.0 (0.5) a
	3.5	96.7 (1.3) a
	7.0	95.6 (2.2) a
Repeated measures ANOVA		
Rates	df[b]	2, 36
	F	58.1
	P	**≤0.001**
Generation	df	1, 36
	F	49.3
	P	**≤0.001**
Rates*Generation	df	2, 36
	F	24.5
	P	**≤0.001**

[a]Means (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.

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

Table 4.5. *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	Survival[a]
	mg cm^{-2}	%
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
Repeated measures ANOVA		
Rates	df[b]	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**

[a]Means (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.

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

Table 4.6. 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	Model Parameters[a]			
			E_{max}[b]	V_{max}[c]	ER_{max}[d]	LR_{max}[e]
		mg cm^{-2}				
Ambrosia artemisiifolia	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[f]	1.529 (0.048) a[f]	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[f]	1.371 (0.044) a	0.554 (0.023) a[f]	3.589 (0.143) a[f]
Abutilon theophrasti	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[f]	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) b
		2.33	0.091 (0.007) b	1.336 (0.031) b	7.375 (0.259) ab	1.069 (0.033) a
		3.5	0.100 (0.017) b[f]	1.186 (0.071) b[f]	6.679 (0.242) b	1.257 (0.165) a

[a]Means (SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

[b]E_{max}: Maximum emergence rate.

[c]V_{max}: Maximum vegetative development rate.

[d]ER_{max}: Maximum early reproductive development rate.

[e]LR_{max-2}: Maximum late reproductive development rate.

[f]Mean separation based on log-transformed data, otherwise on reciprocal-transformed data.

Table 4.7. 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 4.2 and 4.3.

Species	Generation	Rates	Coefficient parameters[a]		
			a	b	c
		mg cm^{-2}			
Ambrosia	G1	0	45.1 (1.2) a	86.8 (0.9) b	0.255 (0.011) a
artemisiifolia		3.5	46.4 (1.6) a	88.8 (1.2) ab	0.252 (0.015) a
male flower		7.0	40.6 (1.3) b	91.5 (1.3) a	0.252 (0.015) a
heads	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
Abutilon	G1	0	45.0 (1.3) b	57.4 (1.1) a	0.208 (0.012) a
theophrasti		2.33	50.9 (1.6) a	59.1 (0.9) a	0.200 (0.009) a
floral buds		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

[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 $P \leq 0.05$. Coefficients within a column followed by the same letter within a generation are not significantly different.

Table 4.8. *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.[a]

Generation	Rates	Seeds	Total seed weight	Weight of 100 seeds	Plant biomass	Reproductive effort
	mg cm^{-2}	nb $plant^{-1}$	g $plant^{-1}$		g	nb of seeds g of $plant^{-1}$
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
Repeated measures ANOVA[b]						
Rates	F	4.0	3.4	0.6	2.7	0.2
	P[c]	**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

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

[b]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.

[c]P values in bold text indicate a significant factor.

Table 4.9. *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.[a]

Generation	Rates	Mature fruits	Seeds	Total seed weight	Weight of 100 seeds	Plant biomass	Reproductive effort
	mg cm^{-2}	nb plant^{-1}		g plant^{-1}		g	nb of seeds g of plant^{-1}
G1[b]	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
Repeated measures ANOVA[c]							
Rates	F	3.9	10.7	8.2	1.0	8.2	10.1
	P[d]	**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

[a]Means (SE) within a column followed by the same letter within a generation are not significantly different according to Tukey's HSD test at $P \leq 0.05$. 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.

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

[c]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.

[d]P values in bold text indicate a significant factor.

Table 4.10. 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.[a]

		Seed	Pericarp	Embryo		Testa		
Generation	Rates	Weight (100)	Thickness	Weight	Weight : total seed weight ratio	Thickness	Weight	Weight : total seed weight ratio
	mg cm^{-2}	mg	µm	mg	mg : mg	µm	mg	mg : mg
G1	0	504 (9)	22.9 (5.5)	2.49 (0.07)	0.49 (0.01)	149.0 (5.2)	2.55 (0.10)	0.51 (0.01)
G2	0	538 (54) a	40.0 (1.5) a	2.37 (0.28) a	0.44 (0.04) ab	165.3 (3.6) a	3.01 (0.27) a	0.56 (0.01) ab
	3.5	515 (34) a	29.3 (1.3) b	2.41 (0.12) a	0.47 (0.02) a	155.7 (6.1) a	2.74 (0.26) a	0.53 (0.02) b
	7.0	491 (36) a	34.1 (1.3) b	1.98 (0.16) a	0.40 (0.01) b	159.1 (2.5) a	2.93 (0.20) a	0.60 (0.01) a
G3	0	419 (18) a	26.8 (1.5) a	2.19 (0.12) a	0.52 (0.01) a	127.7 (3.4) b	2.01 (0.07) a	0.48 (0.01) a
	3.5	465 (27) a	20.2 (1.2) b	2.36 (0.13) a	0.51 (0.02) a	141.5 (4.1) a	2.29 (0.19) a	0.49 (0.02) a
	7.0	495 (34) a	21.9 (0.8) b	2.53 (0.22) a	0.51 (0.02) a	147.6 (2.4) a	2.41 (0.16) a	0.49 (0.02) a
Repeated measures ANOVA[b]								
Rates	F	0.1	19.7	0.3	2.8	1.3	0.3	2.8
	P[c]	0.928	**≤0.001**	0.771	0.103	0.299	0.719	0.103
Generation	F	4.6	134.7	0.6	38.6	58.6	21.7	38.7
	P	0.054	**≤0.001**	0.472	**≤0.001**	**≤0.001**	**≤0.001**	**≤0.001**
Rates*Generation	F	1.9	1.6	2.5	2.7	9.1	1.5	2.7
	P	0.196	0.245	0.124	0.116	**0.004**	0.265	0.116

[a]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 $P \leq 0.05$. Analyses were done on non-transformed data.
[b]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.
[c]P values in bold text indicate a significant factor.

Table 4.11. 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 biofumigation rate and generation effects.[a]

		Seed	Endosperm	Embryo		Testa		
Generation	Rates	Weight (100)	Thickness	Weight	Weight : total seed weight ratio	Thickness	Weight	Weight : total seed weight ratio
	mg cm^{-2}	mg	µm	mg	mg : mg	µm	mg	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)
G2	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
	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
Repeated measures ANOVA[b]								
Rates	F	2.2	0.5	2.5	1.8	1.8	0.8	1.9
	P[c]	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

[a]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 $P \leq 0.05$. Analyses were done on non-transformed data.

[b]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.

[c]P values in bold text indicate a significant factor.

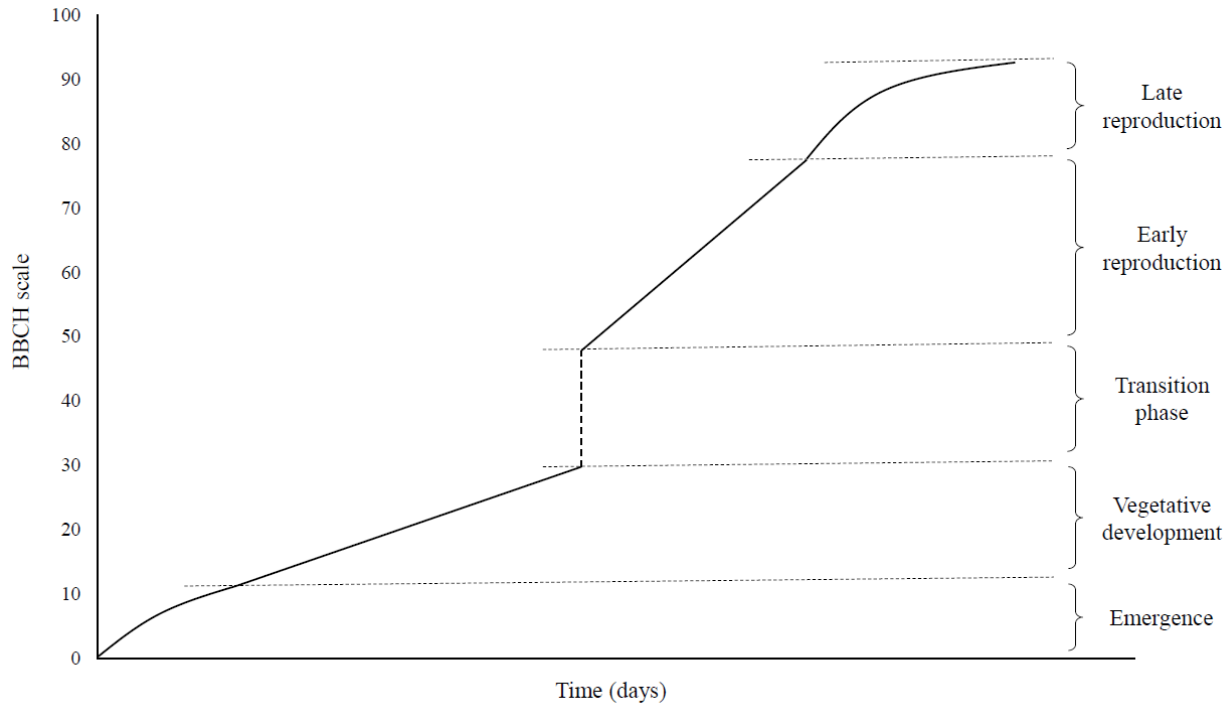

Figure 4.1. Typical phenological model generated by CIPRA computer software, where BBCH values are represented according to days and to four phases (right), where seeds and plants responded to temperature for development.

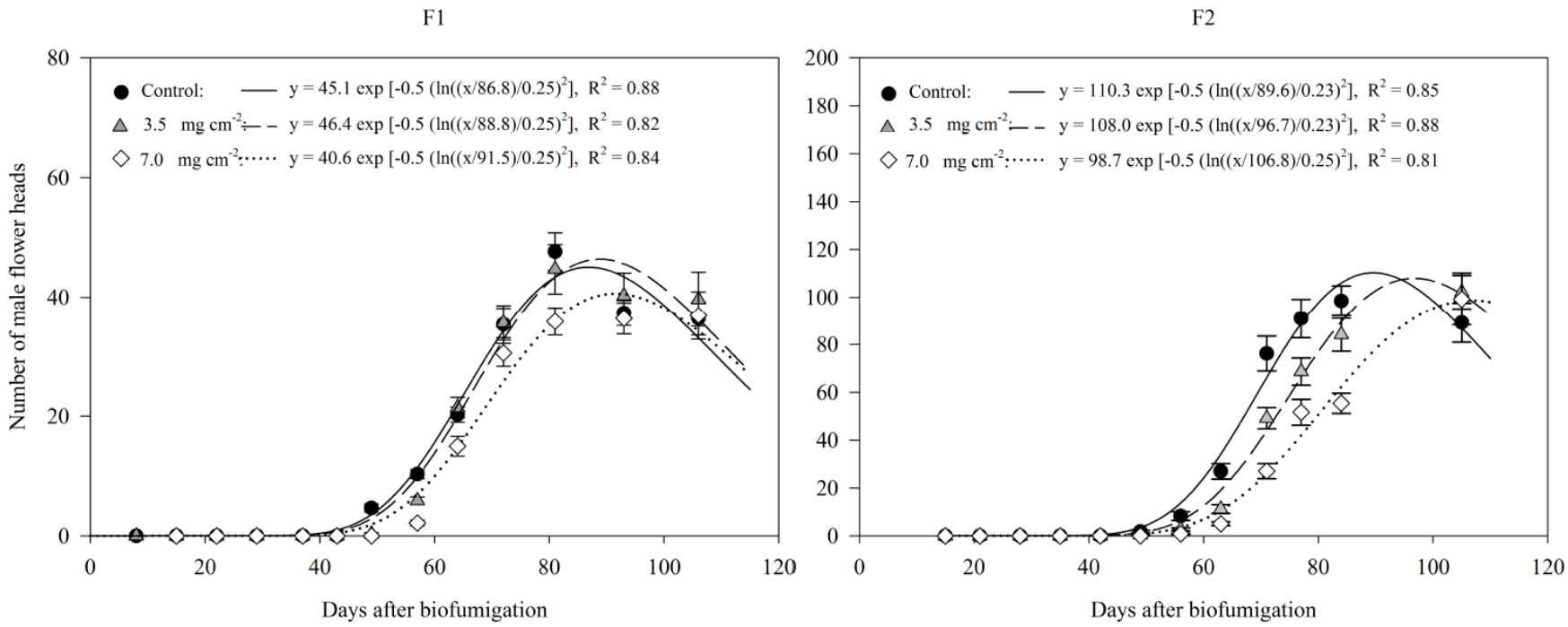

Figure 4.2. Number of clusters of male flower heads of *Ambrosia artemisiifolia* plants from germinated seeds exposed to Indian mustard tissues (3.5 and 7.0 mg cm^{-2}) and to control for G1 and G2 according to the number of days after biofumigation treatment. Vertical bars indicate ± SE.

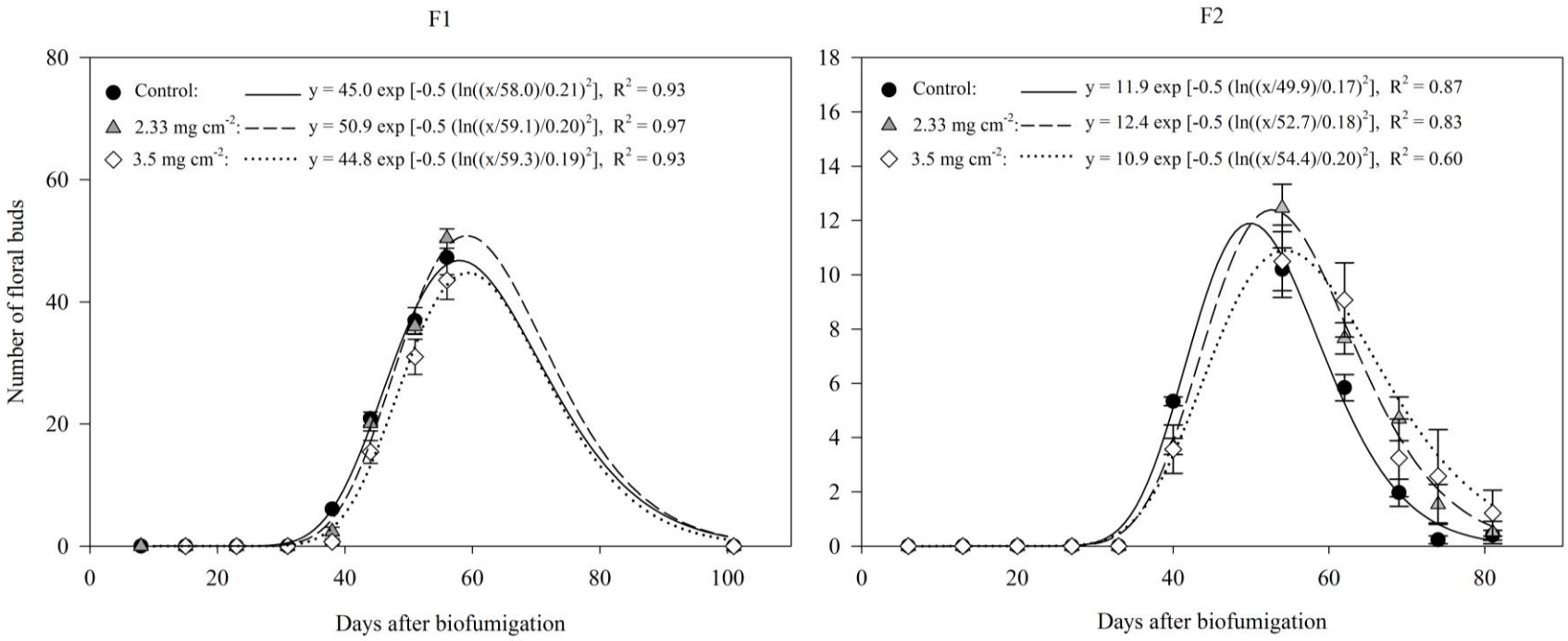

Figure 4.3. Number of floral buds of *Abutilon theophrasti* plants from germinated seeds exposed to Indian mustard tissues (2.33 and 3.5 mg cm^{-2}) and to control for G1 and G2 according to the number of days after biofumigation treatment. Vertical bars indicate ± SE.

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CONNECTING STATEMENT BETWEEN CHAPTERS 4 AND 5

Results in chapter 4 indicate that biofumigation modifies weed fitness components by reducing seed germination and survival, promoting seedling mortality, deferring emergence and flowering, and in some cases decreasing number of seeds produced. However, following generations of surviving weeds may improve their tolerance to biofumigation by an increased number of dormant seeds, greater survival of seedlings, increased of seed production, increased relative weight of the embryo, and testa thickness. With this new knowledge, it was now possible to have a better understanding of the weed population responses in the field. The study in chapter 5 evaluated the impact of repeated biofumigation on natural weed populations. Pot and field experiments were conducted to investigate the impact of biofumigation on key processes of weed population dynamics and to evaluate how the allelochemicals released can contribute to reshaping weed communities.

CHAPTER 5. Impact of Indian mustard growth and incorporation on annual weed population dynamics and communities

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5.1 Abstract

Biofumigation from *Brassica* cover crops may be used to control soilborne pests and weeds. Two experiments were conducted to understand the influence of biofumigation on key processes of annual weed population dynamics. In the first experiment, *Ambrosia artemisiifolia* L. and hairy galinsoga (*Galinsoga quadriradiata Cav.*) seeds were exposed in pots at biofumigation rates equivalent to the incorporation of 1,667, 2,500, 5,000, 7,500, and 10,000 kg ha^{-1} of Indian mustard (*Brassica juncea* L. 'Caliente 199') dry biomass in a soil with high organic matter. *Ambrosia artemisiifolia* seed mortality increased significantly by 75.9% at a biofumigation rate of 2,500 kg ha^{-1}, while 7,500 kg ha^{-1} was required to reduce significantly *G. quadriradiata* emergence by 32.4%. In the second experiment, five combinations of Indian mustard (M) and oat (*Avena sativa* L.) (O) cover crop treatments were assessed in a field trial at two locations. Treatments included four spring/fall cover crop combinations (M/M, M/O, O/M, O/O) and a weedy check control with no cover crop. Prior to mowing and incorporation of cover crops, weed identification, count and biomass measurements were recorded to evaluate the total weed density, to calculate the relative neighbour effect (RNE), weed diversity indexes, and to perform indicator species and principal coordinates analyses. Indian mustard cover crops had no impact on weed emergence in 2014 due to low biofumigant potential compared to oat cover crop. In 2015 and 2016, isothiocyanate (ITC) amounts increased, weed establishments within the Indian mustard cover crop, the post cover crop incorporation, and the spring weed emergence decreased. Allelopathic interference of Indian mustard was significant when plant tissues contained more than 600 µg of allyl-ITC g^{-1}. It is now possible to rationalize the use of *Brassica* cover crops and biofumigation for weed control with an enhanced understanding of the impact of biofumigation on key processes of weed population dynamics.

Keywords: Allelopathy, biofumigation, interference, seed bank persistence, seedling establishment.

5.2 Introduction

Weed communities are affected by agronomic practices performed on farms. According to the community assembly theory, management practices work as filters for selection of plant traits, such as emergence periodicity, growth habit or susceptibility to phytotoxins (Storkey et al. 2010). Biotic and abiotic filters may negatively affect the establishment, growth and reproduction of weed species, and lead to a specific resultant weed community (Booth and Swanton 2002). In agroecosystems, weed management strategies are imposed filters on weed communities having strong influence on weed ecology. They target one or more key processes of population dynamics to reduce weed infestations (Gallandt et al. 1999). However, success of those strategies relies greatly on an ecologically-based exhaustive understanding of their impact on processes of population dynamics (Coleman and Hendrix 1988).

Population dynamics of agricultural annual weeds depend mainly on three critical processes related to soil quality: seed bank persistence, seedling establishment, and interspecific interference (Gallandt et al. 1999). Resultant weed populations are greatly affected by these processes, and changes in their relative importance will ultimately reshape the weed community (Simpson et al. 1989; Swanton et al. 1993).

Success of weed population establishment begins with seed bank persistence. Loss of seeds from the seed bank is closely related to the soil environment. However, effect of allelochemicals present in soil is more related in the literature to seedling establishment and

interspecific interference than on seed bank persistence (Gallandt et al. 1999). Emergence from the soil seed bank is an important factor that dictates the need to apply weed control measures repeatedly (Foley 2001). Future population size and seed mortality are strongly correlated (Gonzalez-Andujar and Fernandez-Quintanilla 1991; Jordan 1993; Jordan et al. 1995). The weed seed bank should be targeted by management tactics.

Success of seedling establishment depends on several environmental factors of the germination site, including adequate seed depth, light, temperature, water, and the absence of predators and disease (Harper 1977). Potential of weed invasion is correlated with abundance of available safe-sites (Gallandt et al. 1999). Being able to exploit these empty niches is imperative in weed management. Crop residues can reduce germinable seeds in the soil, and cover crops contribute to weed management by interference and decreasing seedling establishment. However, suppression varies by crops and by weed species (Qasem 2010, 2013).

Interspecific interference refers to plant-to-plant competition. Performance of weeds or crops is dependent of species-specific resource capture, conversion efficiency of those resources, biomass allocation, and response to allelochemicals (Gallandt et al. 1999). Weed management strategies aim to reduce the interference of weeds to the crop. In many agronomic practices, allelopathy is a defining factor leading to lower weed pressure (Gallandt et al. 1999). Those practices include crop accessions, variety or cultivar selection, bioherbicide or plant extract application, dead mulch, and cover crops (Qasem 2010, 2013).

Brassica cover crops are sometimes used for biofumigation and can function to control soilborne pests, nematodes, and weeds (Matthiessen and Kirkegaard 2006). Biofumigation crops compete and suppress weeds by vigorous early season growth, leaching of glucosinolates (GSLs) into the soil during plant growth, and release of allelochemicals at incorporation of plant residues

(Jabran 2017; Narwal and Haouala 2013). The toxic potential of biofumigation occurs during the breakdown of *Brassica* where isothiocyanates (ITCs) are produced following tissue disruption when myrosinase enzymes hydrolyze glucosinolates (GSLs) in presence of water (Morra and Kirkegaard 2002; Yoneyama and Natsume 2010). In seeds, the mode of action of ITCs involves interaction with glycolytic enzymes in the germination progression and so prevents or defers seed/tuber germination (Drobnica et al. 1977).

Annual weed population dynamic processes known to be affected by biofumigation are seedling establishment and interference from the cover crop. In a field trial, white mustard (*Sinapis alba* L.) reduced *G. quadriradiata* biomass and seed production during cover crop growth. *Sinapis alba* biomass incorporation also reduced *G. quadriradiata* emergence and biomass (Kumar et al. 2009). A global reduction of 69% to 83% of weed density was observed after incorporation of *Brassica* tissues (Walker and Kremmydas 2010). Promising aspect of biofumigation for weed control in agroecosystems is the potential to reduce seed bank persistence. Indeed, high concentration of ITCs and increased exposure time may cause a lethal effect on weed seeds (Bangarwa and Norsworthy 2015; Lefebvre et al. 2018; Petersen et al. 2001).

The biofumigation technique is achieved in the field according to several steps and requires some considerations to improve efficiency. To be successful in the field, *Brassica* plants must be finely chopped and quickly incorporated at full flowering, where GSL concentrations in plants are at the highest (Taylor et al. 2014). Adequate soil moisture is required to allow the chemical reaction between GSLs and myrosinase. The soil temperature must be at least 10 C and after incorporation the soil may be sealed to increase exposure of the targeted pest to ITCs (Kumar 2005; Michel 2008). Maximum concentration of ITC in soil from rapeseed (*Brassica*

napus L.) and Indian mustard degradation are observed just after incorporation and very little is detected after five days (Gimsing and Kirkegaard 2006; Morra and Kirkegaard 2002). Furthermore, in the field, plants may not release their total potential of ITCs mainly because of incomplete tissue pulverization or other limitations to GSL hydrolysis (soil condition, water availability, variety used) (Morra and Kirkegaard 2002). It is well established that environment influences plant physiology and growth. Production of secondary metabolites such as GSL are known to be influenced by the environment, like in the presence of other species or by seasonal variation (Inderjit et al. 2011). According to this information, we could expect that production of glucosinolate will vary between plants that grow at different times of the year. All those considerations may not reach optimum conditions in field situations and may lead to variable results on the targeted pest.

Susceptibility of plant communities to environmental filters changes through time. Weed control measures may be more effective when weed communities are more susceptible to disturbance (Booth and Swanton 2002). Seed banks will evolve during the season, mostly according to dynamic characteristics of seed dormancy to environmental conditions (Benech-Arnold et al. 2000; Gardarin et al. 2012; Murdoch 1998). It is unknown from the literature if weed community susceptibility to biofumigation is related to emergence periodicity. The number of dormant seeds in a seed lot affects weed seed response to biofumigation (Lefebvre et al. 2018). Therefore, it is pertinent to assess in field conditions, if biofumigation timing could be a key factor increasing the repressive effect of biofumigation on weeds.

Our study was designed to assess the variability of the biofumigation technique, its impact on key processes of annual weed population dynamics, and how these impacts contribute to altering weed biodiversity and community structure. Four hypotheses were formulated: 1)

incorporation of Indian mustard tissues in the soil reduced buried seed survival and reduced seedling establishment; 2) repressive effect of biofumigation on weed population will change through the year; 3) repeated biofumigation reduce weed emergence at each operation; and 4) specific response of weeds to biofumigation will change the abundance, the richness and diversity through years. The aim of the first experiment was to determine in pots, in growth chamber conditions, the impact of rates of *Brassica* tissue incorporated in the soil on the seed mortality and seedling establishment. The second experiment was a three years cropping systems field experiment that investigated the impact of biofumigation on weed community and population dynamics. The objectives of the second experiment were to assess (1) the susceptibility of the weed populations to biofumigation throughout the season, (2) the effect of repeated biofumigation treatments within the same year on weed populations, and (3) the cumulative effects of biofumigation on the weed biodiversity and community.

5.3 Materials and methods

5.3.1 Growth chamber experiment

5.3.1.1 Plant material

The study was carried out at the Organic Agriculture Innovation Platform (OAIP), Research and Development Institute for the Agri-environment (IRDA), St-Bruno-de-Montarville, QC, Canada (45.533°N, 73.35°W). Indian mustard, *B. juncea* 'Caliente 199' (Agrocentre Fertibec inc., Seminova, Saint-Rémi, Canada) was used as the biofumigant material. Plants were grown to flowering in 19 cm diameter by 17.5 cm depth pots (ITML Elite 4 L, HC Companies, cat.no. ITBM400) containing a commercial potting mix (Agro mix O2, Fafard Inc., Saint-Bonaventure, Canada) in a growth chamber (Conviron E15, serial number 8D40801J) set at 26 ±

2 C, 16/8 h light/darkness (186 μmol m^{-2} s^{-1}). At flowering, corresponding to 65 on the BBCH scale, the above-ground plant parts were harvested, dried at 35 C for 5 d and ground with a laboratory mill grinder (unknown model, Arthur H. Thomas Company). Drying at low temperature prevent denaturation of glucosinolate and myrosinase. The ground dried material was analyzed to quantify ITCs and then used in the experiment. In the meantime, additional Indian mustard were seeded 5 days later than the previous cohort to be used as fresh material in the experiment. Fresh material was cut in 2 cm pieces prior to incorporation.

5.3.1.2 Bioassay methodology

A dose response pot experiment was conducted in a growth chamber to evaluate seed mortality in the soil and weed establishment in response to increasing rates of Indian mustard tissues incorporated in the soil. Verchères organic soil from a field at OAIP was used in a 9.4 cm width by 9.4 cm long and 15 cm deep pots (Pure-Pak® cartons, Elopak, Oslo, Norway). This soil was collected where the field experiment occurred in order to assess the impact of biofumigation in the same media. The pots were filled with 280 g of dry soil. Soil had 78.1% of organic matter, pH of 5.5, 79.4 mg kg^{-1} P and 393 mg kg^{-1} K. Fresh and dried Indian mustard biomass were incorporated into the soil according to corresponding treatments: 0 (control), 1.47, 2.21, 4.42, 6.63 and 8.84 g based on dry matter, where plant water content was 90.3%. Fresh and dried biomass were used to assess the impact of drying at low temperature on the allelochemical potential of the dried Indian mustard biomass. Those treatments are equivalent to 0, 1,667, 2,500, 5,000, 7,500, and 10,000 kg ha^{-1} of biomass per dry matter basis in the field, incorporated into the top 5-cm soil layer, respectively. Five replicates per treatment were arranged in a completely randomized design. Immediately after biomass incorporation, 100 common ragweed

(*Ambrosia artemisiifolia* L.) seeds in a 0.5 mm nylon mesh bag were placed at 3 cm deep in each pot to evaluate seed mortality. Also, 50 *G. quadriradiata* seeds were scattered on the soil surface and slightly incorporated by scratching the soil to 1-2 mm deep to evaluate weed emergence. *Ambrosia artemisiifolia* and *G. quadriradiata* seeds were randomly collected in the summer and fall of 2015 at OAIP on mature plants, where seeds fell after a vigorous plant shaking. *Galinsoga. quadriradiata* seeds has no dormancy and were stored in dark at room temperature until the beginning of the laboratory experiment (Baskin and Baskin 1998). Primary dormancy of *A. artemisiifolia* seeds was released by keeping the seeds in moist sand at 4 C for 7 weeks. *Ambrosia artemisiifolia* and *G. quadriradiata* seed germination (± SE) were 84.3 ± 1.4% and 90.0 ± 4.2%, respectively. One hundred ml of water were added in each pot to allow chemical reaction leading to ITC production. Plastic mulch (polyethylene 0.9 mil., Dubois Agrinovation Inc. Quebec, Canada) was laid on the soil surface and pots were placed in the growth chamber (Conviron E15, serial number 8D40801J) set at 26 ± 2 C 16/8 h light/darkness (186 μmol m^{-2} s^{-1}). Four days after incorporation, the plastic coverings were removed and weed emergence was recorded every 2-3 days for a month, until weed emergence ceased. At the end of the assay, mesh bags were removed from the pots and seeds were cleaned with distilled water. The viability of *A. artemisiifolia* 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 (Peters 2000) and the International Seed Testing Association (ISTA) working sheets on tetrazolium testing (Leist et al. 2003).

5.3.2 Field experiment

5.3.2.1 Site description

A three-year experiment was conducted at two sites at OAIP, Saint-Bruno-de-Montarville on Verchères muck soil (Table 5.1). Monthly precipitations and temperature through the experiment were recorded (Table 5.2). For several years, previous site management was meadows cut twice a year. Most abundant species (> 5% of relative density) found at site 1 were shepherd's-purse [*Capsella bursa-pastoris* (L.) Medicus.], field pennycress (*Thlaspi arvense* L.), redroot pigweed (*Amaranthus retroflexus* L.) and common purslane (*Portulaca oleracea* L.), with a relative abundance of 31.8, 19.6, 15.5, and 6.2%, respectively, at the beginning of the experiment. Site 2 was dominated by monocotyledons with yellow foxtail [*Setaria glauca* (L.) P. Beauv.], giant foxtail (*Setaria faberi* Herm.), rhombic copperleaf (*Acalypha rhomboidea* Raf.), green smartweed (*Polygonum scabrum* Moench.), and witchgrass (*Panicum capillare* L.) with a relative abundance of 28.6, 19.3, 13.8, 8.8, and 14.9%, respectively.

5.3.2.2 Treatments and cover crop management

At each site, the experimental design was a randomized complete block design with four replicates. Permanent plots were 15 m long by 3.7 m wide and 2.35 m apart from each other. Each plot had 2 beds of 1.2 m wide and 65 cm apart. Treatments consisted of cover crops established in the spring and the fall, with or without biofumigation. The biofumigant crop was Indian mustard (M), and whenever biofumigation did not occur, oat (*Avena sativa* L.) (ordinary #1 non-treated) (O) was used as a cover crop (Semences Crépeau inc., Sainte-Hugues, Canada) . Between spring and fall cover crops, horticultural crops were grown for about 6 weeks. In 2014, mini carrots (*Daucus carota* L. 'Little Finger') was seeded at 172 seeds m^{-1} in 2 rows 0,76 m

apart on July 24th, flamed preemergence on July 30th, and weeded mechanically between rows and manually on the row on August 7th and 21st, respectively. In 2015, a short growing summer squash (*Cucurbita pepo* L. 'Spineless Beauty') was seeded at 2.2 seeds m^{-1} in one row on July 14th and mechanically weeded on July 28th. Finally, in 2016, baby green romaine lettuce (*Lactuca sativa* L. 'Aerostar') were seeded at 150 seeds m^{-1} in 2 rows 0,76 cm apart on July 20th and no weeding was done. Horticultural crop fertilization followed provincial recommendations (CRAAQ 2010).

Five treatments were assessed where four of them were spring/fall cover crop combination: (1) M/M, (2) M/O, (3) O/M, (4) O/O and (5) a weedy check control with no cover crop. The oat was used as a common cover crop species, representing the impact of cover crop interference and biomass incorporation. It also has allelopathic properties from the release of L-tryptophan (Kato-Noguchi et al. 1994; Sánchez-Moreiras et al. 2003). Seeding rates were 12 and 120 kg ha^{-1} for Indian mustard and oat, respectively with a spacing of 19 cm between rows. When Indian mustard reached full flowering stage (BBCH 65), cover crop biomass was harvested in a 0.285 m^2 quadrat (0.5 m x 0.57 m) per plot, dry weight was measured after 7 days at 70 C, and plant samples were taken for HS GC-MS analyses. However, in fall of 2014, Indian mustard did not reach flowering. Biofumigation was done while soil temperature was above 10 C. The same day of sampling, the biofumigation operation occurred. Plants in all treatment were chopped as finely as possible with a flail mower (model 390, John Deere), immediately incorporated with a rotovator (model b55s 185, Breviglieri), irrigated to 100,000 L ha^{-1} and beds were covered with black embossed plastic mulch film (polyethylene 0.9 mil., Dubois Agrinovation Inc. Quebec, Canada) for 7 days to maximize ITCs exposure. These mechanical operations were also done in the weedy check treatment, preventing weeds to set seeds and

providing a uniform distribution of weed seeds in the soil into germination zone. Immediately after biomass incorporation, volatile organic probes (VOC-MOLE, Markes International, Inc., Cal. USA) were inserted into the soil to 7 cm depth under the plastic mulch for 7 days to record the total amount of ITC released in the soil. Operational dates and details are provided in Table 5.3. Cover crops were neither weeded nor irrigated.

5.3.2.3 Weed sampling

Just before incorporation of the spring and fall cover crops and before the harvest of the horticultural crop in the summer, weeds were identified, counted and dry shoot biomass was measured in three 0.5 m long by 0.2 m wide permanent quadrats per plot. Dry biomass was measured after 7 days at 70 C. In 2015, one of the quadrats was used to assess the impact of 2014 treatments on weed spring emergence in a soil with no cover crop. This plot section was not cultivated or seeded. In 2016, another permanent quadrat was used to assess the impact of 2014-2015 treatments on weed spring emergence with no cover crop. Finally, in 2017, the remaining quadrat was used to evaluate the weed spring emergence with no cover crop following three years of treatment. Weed sampling dates are presented in Table 5.3. Weed identification, count and biomass measurement were used to evaluate the total weed density, weed density according to functional types, to calculate to relative neighbour effect (RNE), weed diversity indexes and to perform indicator species analysis and weed community ordination analysis.

5.3.3 Isothiocyanate analysis

Analysis by headspace (TurboMatrix™ HS 40 Trap, PerkinElmer, USA) and gas chromatography-mass spectrometry (GC-MS) [Clarus® 680 (GC), and Clarus® SQ8 (MS),

PerkinElmer, USA)] were performed on Indian mustard material to identify and quantify ITCs and other allelopathic compounds that were released during biofumigation. Samples were prepared for HS GC as described in Lefebvre et al. (2018). Probes used in the second experiment were also analyzed via thermal desorption-gas chromatography.

In the field experiment, equation 1 was used to calculate the ITC release efficiency rate which was the percentage of ITC measured in plants that was released in soil and likely in contact with seeds.

Percentage of ITC release efficiency = (((*a b* (1000)) / *c*) / (*x* / *d*)) 100 [1]

where *a* corresponds to ITCs measured from plant analysis (µg g^{-1}), *b* was the plant biomass (kg ha^{-1}), *c* was the volume of treated soil reported by hectare, in this case 7 x 10^8 cm^3, *x* corresponds to ITCs measured from the probe (µg), and *d* was the effective volume detected by the probe, i.e. 0.117 cm^3 according to previous assessments.

5.3.4 Statistical analysis

5.3.4.1 Growth chamber experiment

Data analyses were performed with 'Agricolea' and 'Asbio' packages of R software V.3.0.1 (R Development Core Team 2008). Tukey's honestly significant difference (HSD) was used to separate treatment means at 0.05 probability level for percentage of *A. artemisiifolia* seed mortality and percentage of *G. quadriradiata* emergence. Dependent variables were log-transformed (log_{10} [x+1]) to meet normality and homoscedasticity assumptions. The results are presented as untransformed means. For both weeds, incorporation of fresh or dry Indian mustard tissues did not change seed responses (P>0.05) and were combined.

5.3.4.2 Field experiment

5.3.4.2.1 Weed density, emergence and establishment

ANOVAs and Tukey's HSD tests were used for mean comparison of the total weed density and weed density according to functional type. Dependent variables were log-transformed (log_{10} [x+1]) whenever required to meet normality and homoscedasticity assumptions.

5.3.4.2.2 Relative neighbour effect (RNE).

Relative neighbour effect was used to quantify the competition of cover crops to weeds (Markham and Chanway 1996). Relative neighbour effect compares growth of weeds in presence or not of cover crop species following equation 2:

$$RNE = (P_{control} - P_{treatment}) / P_{control} \quad [2]$$

where $P_{control}$ is the biomass of weeds in the control treatment, with no cover crop and $P_{treatment}$ is the biomass of the weeds in the presence of the cover crop. ANOVAs and Tukey's HSD tests were used for mean separation of RNE based on the log-transformed data (log_{10} [x+1]) whenever required to respect normality and homoscedasticity assumptions.

5.3.4.2.3 Diversity indexes

To investigate impact of biofumigation on weed community richness and diversity, three diversity indexes were used. Species richness (SR) was the total number of species per plot. Shannon-Weiner diversity index (H'), represented by equation 3, is sensitive to difference in abundance of rare species and will increase with richness and evenness of weed communities:

$H' = \sum [-p_i (\ln p_i)]$ [3]

where p_i is the proportional abundance of the i^{th} species. Simpson's dominance index (D^{-1}) also represents diversity and follows equation 4:

$D^{-1} = (\sum \{[n_i (n_i-1)]/[N(N-1)]\})^{-1}$ [4]

where n_i is the density or number of the i^{th} species, and N is the total number of individuals of all species (Nkoa et al. 2015). Simpson's index ranges from 0 to 1, where high values reflect high diversity. ANOVAs and Tukey's HSD tests were used for mean separation of diversity indexes based on non-transformed data.

5.3.4.2.4 Indicator species analysis

Dufresne-Legendre indicator species analyses were performed using 'Labdsv' package of R software. The 'indval' function calculates the indicator value, representing fidelity and relative abundance of species to a cover crop treatment. Calculations were done based on 9999 number of randomized iterations to calculate probabilities at 0.05 probability level.

5.3.4.2.5 Weed community analysis

Weed communities were analyzed by Principal coordinates analysis (PCoA) with a Bray-Curtis metric distance and following Hellinger data transformation. PCoA analyzes were done using 'Vegan', 'MASS' and 'BiodiversityR' packages of R software. To complete PCoA analyses, permutational multivariate analysis of variance (PerMANOVA) were done to determine significance of cover crop treatment and time effects on weed communities. PerMANOVA were done with the 'Adonis' R package, conducted on matrices of Euclidian

distance coefficients and P values were based on 999 permutations assuming a randomized complete block design.

5.4 Results and discussion

5.4.1 Growth chamber experiment

Analysis of the plant material by headspace and gas chromatography-mass spectrometry measured the production of 1,083 µg of allyl-ITC g^{-1} of Indian mustard. Incorporation of Indian mustard tissues in the soil significantly reduced *A. artemisiifolia* seed survival and *G. quadriradiata* germination and establishment (Table 5.4). *Ambrosia artemisiifolia* seed mortality increased significantly by 75.9% at biofumigation rate corresponding to 2,500 kg ha^{-1} compared to the control. Beyond this rate, increasing biofumigant biomass in pots did not result in increased seed mortality. A biofumigation rate of 7,500 kg ha^{-1} was required to reduce significantly *G. quadriradiata* emergence by 32.4%, and the rate 10,000 kg ha^{-1} had the same effect. These results contrast with the general assumption that exposure to phytochemicals generated during biofumigation reduced germination and survival in a more linear dose-dependent manner (Al-sherif 2013; Lefebvre et al. 2018; Mattner et al. 2008). Soil organic matter or other edaphic conditions may have prevented complete exposure of seeds to the allelochemicals (Price et al. 2005). Nevertheless, the aim of the first experiment was to confirm that biofumigation, as performed in the field experiment, may increase seedbank mortality and reduced the establishment of weeds in the field.

5.4.2 Field experiment

Indian mustard cover crop above ground biomass ranged from 1,853 to 5,058 kg ha^{-1} and oat cover crop biomass, from 1,376 to 6,477 kg ha^{-1} (Table 5.5). Average temperature was similar between spring of 2014 to 2016 (Table 5.2). Furthermore, precipitation was greater in the spring of 2014 than in 2015 and 2016. The improvement of Indian mustard biomass in the second year was likely due to addition of potassium sulfate and dolomitic lime at the experimental sites (Table 5.3). The chemical analysis and detection from the headspace and GC-MS demonstrated that the main compound released was allyl-ITC. Analysis also revealed traces of butyl-ITC, butenyl-ITC, isopropyl-ITC and butane 1-ITC. The biofumigant potential of Indian mustard was low in 2014 (Table 5.5). Indian mustard plants benefited from potassium sulfate supply and the GSL concentration increased in 2015 to generate up to 1,155 to 2,299 µg of allyl-ITC g^{-1} of Indian mustard in the spring of 2016. Glucosinolates are sulfur-containing organic molecules (Halkier and Gershenzon 2006), and high S supply increased GSL in several *Brassica* species (Kim et al. 2002; Schonhof et al. 2007) including Indian mustard (Tong et al. 2014). Indian mustard plants in the fall of 2015 did not reach full flowering stage, probably leading to a low level of ITC detected (Taylor et al. 2014).

Unhydrolyzed GSLs in soil are mainly due to incomplete tissue pulverization or other limitations to GSL hydrolysis such as soil condition, water availability, and crop variety (Morra and Kirkegaard 2002). Isothiocyanate release efficiency rates calculated in this study varied from 1.02% to 21.64% and were similar to those reported in the literature, ranging from 1% to 26%, with some exceptional measurement up to 56% (Bangarwa et al. 2011; Gimsing and Kirkegaard 2006; Morra and Kirkegaard 2002). The best biofumigation conditions realized in the field experiment occurred at site 1 in the spring and the fall of 2016 and in the fall of 2016 at site 2,

where Indian mustard biomass exceeded 3,000 kg ha^{-1}, the headspace and GC-MS measurements were higher than 1,000 µg of allyl-ITC g^{-1}, and the ITC release efficiency rate was higher than 2.5%.

5.4.2.1 Weed establishment in spring cover crop

In 2014, total weed density in all spring cover crops at site 1 were not significantly different from the weedy check (Figure 5.1). Indian mustard cover crops did not reduce the number of weeds of a functional group compared to the weedy check. At site 2, total weed density in the Indian mustard cover crop was lower than the control, but not different from the oat cover crops. Monocotyledon and dicotyledon responses to treatments were different. However, all the cover crops had similar impact on weed densities of all the dicotyledon functional types. The results of the analyses were either non-significant for all treatments or all cover crop treatments were part of the same statistical group.

In 2015 at site 1, Indian mustard cover crops reduced total weed density more than the oat cover crops. Indian mustard tended to reduce weed establishment more compared to the oat cover crops for all functional groups, but without significant difference. At site 2, Indian mustard cover crops did not perform better than oat to reduce total weed density. Also, all the cover crops had the same impact on the number of dicotyledonous weeds. The treatment O/M decreased significantly monocotyledon density compared to the control.

In 2016 at site 1, total weed density in spring cover crops was reduced by the Indian mustard cover crop. Indeed, the treatment where biofumigation was done twice or in the spring had significantly lower total weed density than the oat cover crops. However, all functional group responses were similar. Total weed density at site 2 was similar throughout all treatments.

The number of dicotyledon weeds in the spring of 2016 was not reduced by the treatment. Only biofumigation in the spring reduced the monocotyledon density compared to the weedy check.

5.4.2.2 Weed emergence in spring without cover crop

In 2015, total weed spring emergence without a cover crop was not different between cover crop treatments at site 1 but significantly lower than the control (Figure 5.2). Analyses according to functional types were the same for *Brassica* dicot and winter annual dicotyledons, and not significant for the other types. At site 2, differences in total densities of all treatment were not significant.

Total weed spring density in 2016 at site 1 was significantly lower in the treatment M/O than the O/O treatment. The statistical groups generated from Tukey's HSD test were the same as those in the spring cover crop in 2015 at site 1. All functional group responses were similar. However, the statistical difference between M/O and O/O was lost. At site 2, total weed spring emergence was lower for the three biofumigation treatments, but only the treatments M/M or O/M were significantly lower than the O/O treatment. Non-*Brassica* and summer annual dicotyledon weed emergence was significantly reduced by the biofumigation done twice a year compared to the oat treatment. However, monocotyledon emergence was not reduced by the three biofumigation treatments compared to the weedy check.

In 2017 at site 1, all the cover crop treatments had similarly reduced total weed emergence compared to the weedy check. Non-*Brassica* and summer annual weed spring emergence was slightly increased by biofumigation treatments. Those treatments had the same impact on *Brassica* and winter annual dicotyledons as the oat treatment. The impact of cover crops on monocotyledonous weeds was not significant. At site 2, total weed density was more

reduced by previous biofumigation done twice a year or in the fall compared to the weedy check, but not from the treatment O/O. *Brassica* dicotyledon, winter annual dicotyledon and monocotyledon densities were not reduced by any treatment. Spring biofumigation slightly increased non-*Brassica* and summer annual dicotyledons compared to other cover crops. However, the difference was not significant.

5.4.2.3 Weed density in the horticultural crop

In 2014, Indian mustard cover crop incorporation did not reduce the total weed density compared to the oat cover crops in the summer at both sites, nor the density of any functional groups (Figure 5.3). In 2015 at site 1, Indian mustard cover crop incorporation did not reduce post incorporation total weed density compared to oat cover crops. Moreover, incorporation of Indian mustard cover crop increased density of *Brassica* and winter annual dicotyledons compared to the treatments O/O and O/M. However, the impact was not statistically different than the oat cover crops. At site 2, cover crops had similar impact on total weed emergence and on dicotyledon density. However, M/O and O/M treatments had greater monocotyledon density compared to O/O or M/M.

Total weed density in 2016 was not different between any treatment at site 1. Fall biofumigation had increased weed density of non-*Brassica* weeds compared to spring biofumigation. At the opposite, it has decreased the weed density of *Brassica* and winter annual weeds. At site 2, total weed density was not different between biofumigation treatments and oat cover crop. Also, weed density of any functional group was affected by treatments.

5.4.2.4 Weed establishment in the fall cover crop

At site 1 in all 2014 fall cover crops, total weed establishment was not significantly different from the weedy check, nor weed density according to functional types (Figure 5.4). At site 2, total weed density was not significantly different from the weedy check. Also, all the cover crops had similar impact on weed density of *Brassica*, summer and winter annual dicotyledon functional groups and monocotyledonous species. However, the treatment M/O increased the density of non-*Brassica* more than the M/M treatment.

In the fall of 2015 at site 1, total weed density was lower in the three biofumigation treatments than in the O/O treatment, but they were not statistically different. There was no significant difference between treatments for non-*Brassica* and summer annual dicotyledons. Like the total weed density, *Brassica* and winter annual weed densities were lower in the three biofumigation treatments than the number of weeds in the O/O treatment. All five treatments were not different in the fall of 2015 at site 2.

Total weed density in 2016 at sites 1 was slightly reduced in biofumigation treatments compared to the oat cover crop. *Brassica* dicotyledon and winter annual responses were similar to the total weed density. At site 2, the impact of the biofumigation done twice or in the fall on total weed density were greater than the treatment where biofumigation was done in the spring. M/M or O/M treatments significantly reduced non-*Brassica* and summer annual dicotyledon spring emergence compared to the oat treatment. *Brassica* dicotyledon, winter annual dicotyledon and monocotyledon densities were not reduced by any treatment.

According to Figures 5.1 to 5.4, monocotyledons were barely affected by biofumigation. Norsworthy (2003) assessed that aqueous extract of wild radish (*Raphanus raphanistrum* L.) reduced germination of dicotyledons more than monocotyledonous species. In field trials, a

biofumigation mix reduced the dry weight by 40% and the density of scarlet pimpernel (*Anagallis arvensis* L.), but not the density of annual bluegrass (*Poa annua* L.) (Mattner et al. 2008). Incorporated rapeseed biomass killed 98, 68, and 51% of buried seeds of milkweeds (*Asclepias* spp.), foxtails (*Setaria* spp.), and prairie dock (*Silphium terebinthinaceum* Jacq.), respectively. When plastic mulch was laid immediately after incorporation, suppression increased to 94% for *S. terebinthinaceum* and 100% for *Asclepias* spp., whereas *Setaria* spp. germination was stimulated instead of being reduced (Reddy 2013).

When biofumigant potential was strong enough to impact weed establishment or emergence, dicotyledonous groups had similar densities in the spring in both sites. In fall at site 2, *Brassica* or winter annuals were less affected by treatments than non-*Brassica* or summer annuals. Contrarily, *Brassica* or winter annuals were more affected by treatments than non-*Brassica* or summer annuals at site 1 in the fall. Moreover, biofumigation slightly increased weed emergence in the spring of 2017 at site 1 for non-*Brassica* or summer annuals dicotyledons compared to the oat treatment.

Summer weed emergence results in our study contrast with previous work on biofumigation, where it was assessed that incorporation *Brassica* cover crops containing high glucosinolate concentration can reduce post-incorporation weed emergence (Boydston et al. 2004; Kumar et al. 2009; Walker and Kremmydas 2010). However, spring biofumigation at site 1 and biofumigation done twice a year or in the fall of 2016 did reduce spring weed emergence and concord with previous work. Our results also underlined the highly variable impact of the biofumigation technique on weed density. Several studies from the literature also reported that incorporation of *Brassica* residues did not perform better than other green manure plants to control weeds in some situations. *Sinapis alba* cover crop was not more efficient in weed

suppression than buckwheat (*Fagopyrum esculentum* Moench), oat, crimson clover (*Trifolium incarnatum* L.), or rapeseed (*B. napus* 'Hyola' or *B. napus* 'Dwarf Essex') (Haramoto and Gallandt 2005). Björkman et al. (2015) observed no relation between weed suppression and incorporated mustard biomass, nor GSL content in the cover crops. Biofumigation reduced the early emergence of weeds relative to wheat by 30%, but there was no difference in the weed density before harvest, later in the summer (Al-Khatib et al. 1997). However, those studies did not report the biofumigant potential of the *Brassica* cover crop used.

Our study shows that Indian mustard cover crop had no impact on weed emergence when the biofumigant potential was low. As the quantities of ITCs detected increased, Indian mustard had greater impact on weed establishment within the cover crop growth, especially at site 1 in the spring of 2015 and at site 2 in the fall of 2016. When biofumigation was performed in those situations, weed spring emergence without the presence of a cover crop was also reduced, as expected at site 1 in 2017. Two dicotyledonous species had greater seedling survival from exposure to allelochemicals generated during biofumigation when mother plants also germinated under biofumigation stress (data not shown). This result may partly explain why weed emergence at site 1 in 2017 was not more reduced by biofumigation. Also, a chemical may act as an allelochemical in one situation, but not in another. This may occur because the concentration, residence time, and fate of a chemical are largely controlled by substratum factors (Inderjit 2001). Furthermore, *in situ* factors may affect the activity of biofumigants including soil colloidal adsorption and possible microbial transformation of allelochemicals (Price et al. 2005).

Weed life history and emergence periodicity were among other important functional traits determining how weed community assembles in organic management systems (Ryan et al. 2010). Our functional classification underlined some difference between groups, but may have

been too broad, as phylogenetic relatedness was not a good predictor of competition between plant species (Cahill et al. 2008; Smith et al. 2015). In general, we found inconsistent evidence for the hypothesis about the relation between emergence periodicity and susceptibility to biofumigation. An analysis based on seed traits (morphology and dormancy) and other traits related to phytotoxic tolerance should be investigated and could lead to different results and better predictability for susceptibility to biofumigation. However, it should be noted that the species present at the experimental site and biological information available for those species prevent us from creating such classification.

5.4.2.5 Relative neighbour effect

The relative neighbour effect represents the relative reduction of weed growth in an environment with competition compared to the weed growth in absence of competition (Markham and Chanway 1996). A positive value of RNE means weed biomass was reduced by the cover crop. In 2014, RNE was not different between cover crop treatments, except at site 2 where the O/O treatment had greater impact on weed biomass than the M/O treatment (Figure 5.5). In the spring of 2015 at site 1, Indian mustard decreased weed biomass significantly more than the oat cover crop. However, at site 2, only M/O was different than the O/O. In the spring of 2016 at both sites, weed biomass was reduced more importantly in the three biofumigation treatments, even if oat was present in the treatment O/M. In the fall of 2014, there was no significant difference between treatments at both sites. In the fall of 2015, the RNEs from the biofumigation treatments (M/M and O/M) was higher than the oat cover crop (M/O). In the fall of 2016 at site 1, weed biomass was reduced more importantly in the three biofumigation

combination treatments than the only oat treatment (O/O). In the fall of 2016 at site 2, RNE of M/O were statistically lower than the M/M.

RNE results showed that a previous exposure of biofumigation can reduce weed biomass in future populations. Indeed, the weed biomass in the oat cover crop in 2015, in the treatment where biofumigation was done in the fall of 2015, was decreased more than the weed biomass in oat with previous oat cover crops. In a previous experiment, we found that sublethal impact of seeds exposed to phytotoxic allelochemicals generated during biofumigation may reduce adult plant biomass (data not shown). Boydston and Hang (1995) also reported that incorporation of *Brassica* cover crops could reduce weed growth in subsequent crops. Incorporation of rapeseed green manure reduced weed biomass by 50% to 96% in a following potato crop, compared to bare fallow treatment. Species specific response to biofumigation may explain why this secondary effect on weed growth was not observed in the fall of 2016 at site 2.

5.4.2.6 Diversity indexes

Despite the impact of Indian mustard cover crops on weed establishment when biofumigant potential increased, species richness, Shannon-Weiner diversity index (H') and Simpson's dominance index (D^{-1}) were scarcely significantly affected throughout the study, except for site 1 in the spring of 2015 and 2016, and species richness at site 2 in the fall of 2016. In 2015, the D^{-1} value for weedy check at site 1 was 0.53 ± 0.06 (± SE), significantly lower than 0.77 ± 0.10 for the treatment M/M and 0.75 ± 0.03 for the treatment M/O ($P>0.05$). However, in the spring of 2016 at site 1, the M/M treatment where biofumigation was done twice a year reduced significantly species richness from 5.8 ± 0.5 to 3.3 ± 0.6, D^{-1} from 0.73 ± 0.03 to 0.60 ± 0.11 and H' from 1.48 ± 0.11 to 0.88 ± 0.21 compared to the O/O treatment ($P>0.05$). In

2016 at site 2, only species richness was reduced significantly by the M/M treatment 2.3 ± 0.5 compared to the weedy check 5.5 ± 0.6 ($P>0.05$). In all other situations, biodiversity indexes were not significantly different between treatments.

5.4.2.7 Indicator species analysis

Indicator species analysis did not underline any significant species associated with treatments for weed establishment in a cover crop at site 1 in the spring of 2014 and 2016 and the fall of 2014 and 2015, nor at site 2 in the spring of 2016, and in the fall of 2014 to 2016 (Table 5.6). The only species significantly associated with a biofumigation treatment was *A. retroflexus* associated with the biofumigation treatment O/M in the fall of 2016 at site 1 during cover crop growth. However, spring emergence of mouseear chickweed (*Cerastium fontanum* Baumg.) was associated with the biofumigation treatment O/M in the spring of 2017 at site 1. At site 2, *C. bursa-pastoris* and rough cinquefoil (*Potentilla norvegica* L.) were significant species associated with the biofumigation treatment M/M in the spring of 2015 and 2017, respectively. The other listed species were associated either with the weedy check or the oat cover crop treatment.

5.4.2.8 Weed community analysis

Principal coordinates analysis (PCoA) and PerMANOVA underlined a significant change in the weed community at the site 1 in the spring of 2016 (Figure 5.6 and Table 5.7). The ellipses were framed according to the standard error of each treatment. As the distance between ellipses increased, structure of weed communities differed. There was a clear distinction between the

weed community in the weedy check and the weed communities in the treatments where biofumigation was done twice a year or in spring according to the separation of the ellipses. The weed communities for the treatment where biofumigation occurred in fall or the oat treatment were similar and located in between. At site 1, there were minor separations of weed communities in the fall of 2016 or in the spring of 2016 without the presence of cover crops, but PerMANOVA failed to find significant difference. Minor ellipse separations were also observed at site 2 in the spring of 2016 for weed communities in the cover crops or without the presence of a cover crop (Figure 5.7). Similarly, PerMANOVA did not underline significant difference between those communities. PerMANOVA realized according to treatment, year and block effects for each weed community always underlined significance of year and block effects (Table 5.8). Fluctuation in weed community can be seasonal or yearly changes (Swanton et al. 1993) as observed throughout our study. The treatment effect and the interaction between treatment and year were only significant for the site 1 in spring.

Outcomes of the first experiment underlined detrimental impact of phytotoxic allelochemicals generated during biofumigation on seed bank persistence and seedling establishment, but not in a linear dose-response manner. Soil organic matter and other soil properties are likely responsible for this conclusion. However, first experiment observations support and explain, in part, the field results. Low biofumigant potential had no significant impact on seed viability in soil and seedling establishment. Moreover, interference of *Brassica* increased when biofumigant potential was high. Allelopathy and competition are both significant contributors of *Brassica* cover crops interference to weeds (Kunz et al. 2016). Our study provided evidence that allelopathic interference of Indian mustard increased above the corresponding GSL level in tissues generating more than 600 µg of allyl-ITC g^{-1}. Allelopathy

affects key processes of population dynamics, especially germination and growth of weeds (Qasem 2010, 2013).

Our study provided evidence of intraspecific variability in weed suppression ability of a *Brassica* cover crop. Field biomass and GLS concentration or ITCs production should always be information available in *Brassica* cover crop studies to avoid misleading conclusions about weed suppression ability. The study provided essential information about biofumigation negative impacts on key defining processes of weed population dynamics. Whenever the impact of *Brassica* cover crops on key processes are high enough, significant changes in weed diversity and in-community structure may occur. However, monocotyledon weed communities were more tolerant than dicotyledons to biofumigation. The results highlight situations where it would be beneficial to performed biofumigation in the spring, as at site 1, or in the fall, as at site 2. Overall, there was no advantage to realize biofumigation twice a year, nor to repeat biofumigation over three years. It is now possible to rationalize the utilization of *Brassica* cover crops and biofumigation for weed control. This study provided necessary information that enhanced our understanding of *Brassica* cover crops used for biofumigation as a biotic weed management filter, and stated the importance of allelochemicals for weed control via its impacts on key processes of population dynamics.

5.5 Acknowledgments

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No conflicts of interest have been declared.

Table 5.1. Description of the Verchères muck soil of the field experiment occurred.

Year	Site	pH	Organic matter	P	K
			%	mg kg^{-1}	mg kg^{-1}
2014	1	5.2	78.9	51.9	201
	2	6.2	30.2	19.5	75.7
2015	1	5.4	72.6	95	352
	2	5.6	28.4	25.6	151
2016	1	5.5	78.1	79.4	393
	2	5.8	28.5	27.8	175

Table 5.2. Monthly precipitation and temperature from May to June (Spring) and July to October (Summer/fall) during the field experiment.

Year	Season	Precipitation		Temperature		
		min	max	min	max	mean
		mm			C	
2014	Spring	108.4	168.3	13.9	19.4	16.7
	Summer/fall	57.6	80.7	10.6	20.8	15.7
2015	Spring	63.9	101.5	16.5	17.8	17.2
	Summer/fall	88.1	96.4	7.2	20.9	14.1
2016	Spring	55.1	85.3	14.2	18.8	16.5
	Summer/fall	22.6	124.9	11.9	21.6	16.8
2017	Spring	81.5	120.4	11.7	18.0	14.9

Table 5.3. Summary of seeding dates and operation details of the field experiment in 2014 to 2016.

Operations	Details	2014		2015		2016	
		Spring	Fall	Spring	Fall	Spring	Fall
Fertilisation	Date	May 14th	September 2nd	May 6th	August 27th (site 1) August 31st (site 2)	May 4th	August 16th
	Potassium sulfate (kg ha^{-1})[a]	---	---	278	278	278	278
	Chicken manure[b] (kg ha^{-1})	1,500	1,500	1,500	1,500	1,500	1,500
	Dolomitic lime (kg ha^{-1})	---	---	2,000	---	---	---
Seedbed preparation	1.5 m wide cultivator	May 14th	September 2nd	May 6th	August 27th (site 1) August 31st (site 2)	May 4th	August 16th
Cover crop seeding	Date	May 15th (site 1) May 16th (site 2)	September 4th	May 6th	August 27th (site 1) August 31st (site 2)	May 5th (site 1) May 6th (site 2)	August 16th
Biofumigation operation		July 4th (site 1) July 9th (site 2)	October 28th	June 25th (site 1) June 27th (site 2)	October 26-27th	June 28th	October 12th
Weed sampling[c]	Date	May 30th to July 2nd (site 1) July 3rd to July 8th (site 2)	October 14th to 17th (site 1) October 20th (site 2)	June 22nd	September 21st to 23rd	June 23rd	September 29th and 30th

[a]Corresponding rate of Sul-po-mag (0-0-22, Les Engrais Naturels McInnes, Stanstead Quebec, Canada).

[b]Granulated chicken manure 5-3-2 (Acti-Sol Inc., Notre-Dame-du-Bon-Conseil, Quebec, Canada).

[c]In 2014, summer evaluations occurred on August 26th at site 1 and August 29th at site 2. In 2015, weeds were sampled in summer on August 19th and 20th at both sites. In 2016, summer evaluations were done on August 15th for the summer at both sites.

Table 5.4. *Ambrosia artemisiifolia* seed mortality and *Galinsoga quadriradiata* seedling emergence according to different rates of Indian mustard (*Brassica juncea* L. 'Caliente 199') biomass incorporated in pots with organic soil.

Biomass	*Ambrosia artemisiifolia* seed mortality[a]	*Galinsoga quadriradiata* seedling emergence[a]
kg ha^{-1}	(%)	nb pot^{-1}
0 (control)	13.1 (1.5) b	27.2 (2.3) a
1,667	18.4 (2.1) ab	24.4 (2.0) ab
2,500	23.0 (1.5) a	23.7 (1.1) ab
5,000	23.9 (2.4) a	27.0 (1.3) a
7,500	23.4 (3.0) a	18.4 (1.4) b
10,000	21.9 (2.5) a	18.4 (2.2) b

[a]Within each column, means (SE) followed by the same letter are not significantly different according to Tukey-Kramer HSD test at $P \leq 0.05$. Mean separation based on log-transformed data.

Table 5.5. Cover crop biomass and biofumigant potential of Indian mustard (*Brassica juncea* L. 'Caliente 199') at each experimental site from 2014 to 2016[a].

	Spring		Fall	
Spring/Fall cover crops	Biomass	Allyl-ITC	Biomass	Allyl-ITC
	kg ha^{-1}	μg g^{-1}	kg ha^{-1}	μg g^{-1}
Site 1 Year 2014				
Mustard/Mustard	2,525 (530)	69 (4)	1,944 (356)	10 (2)
Mustard/Oat	2,947 (587)	76 (14)	2,214 (92)	
Oat/Mustard	4,331 (202)		2,203 (133)	9 (2)
Oat/Oat	3,998 (141)		1,520 (361)	
ITC release efficiency (%)		na		21.6
Site 1 Year 2015				
Mustard/Mustard	4,025 (194)	644 (123)	3,161 (184)	0 (0)
Mustard/Oat	4,230 (549)	857 (157)	1,868 (178)	
Oat/Mustard	5,587 (224)		2,800 (248)	6 (11)
Oat/Oat	4,762 (152)		1,618 (88)	
ITC release efficiency (%)		2.3		na
Site 1 Year 2016				
Mustard/Mustard	3,861 (280)	1,155 (106)	3,190 (281)	1,161 (234)
Mustard/Oat	4,170 (227)	1,284 (119)	3,761 (196)	
Oat/Mustard	5,240 (470)		4,282 (357)	1,248 (534)
Oat/Oat	4,975 (271)		3,550 (400)	
ITC release efficiency (%)		2.6		3.4
Site 2 Year 2014				
Mustard/Mustard	3,840 (536)	57 (6)	1,943 (139)	9 (4)
Mustard/Oat	4,173 (514)	74 (11)	1,565 (128)	
Oat/Mustard	6,477 (437)		1,853 (219)	8 (2)
Oat/Oat	5,853 (468)		1,376 (120)	
ITC release efficiency (%)		15.2		17.3
Site 2 Year 2015				
Mustard/Mustard	4,421 (404)	663 (130)	3,071 (580)	11 (11)
Mustard/Oat	5,022 (536)	546 (137)	2,080 (75)	
Oat/Mustard	3,689 (118)		3,572 (233)	13 (8)
Oat/Oat	3,991 (372)		1,832 (161)	
ITC release efficiency (%)		4.2		18.7
Site 2 Year 2016				
Mustard/Mustard	3,741 (405)	2,299 (157)	4,367.5 (268)	1,249 (184)
Mustard/Oat	4,136 (387)	1,647 (157)	2,726.3 (275)	
Oat/Mustard	4,727 (346)		5,057.9 (289)	628 (228)
Oat/Oat	4,787 (517)		2,940.4 (133)	
ITC release efficiency (%)		1.0		8.3

[a]Means (SE).

Table 5.6. Indicator species analysis conducted on weed communities established in cover crops or in spring with no cover crop at each experimental site.

Site	Season	Year	Scientific name[a]	Bayer code	Cover crop[b]	Indicator value[c]	Prob
1	Spring	2014	---[d]				
		2015	*Potentilla norvegica* L.	PTLNO	O/O	66.70	0.039
		2016	---				
2	Spring	2014	*Potentilla norvegica* L.	PTLNO	O/O	53.14	0.004
			Polygonum scabrum Moench.	POLSC	Weed	50.00	0.057
			Chenopodium album L.	CHEAL	O/O	27.80	0.060
		2015	*Capsella bursa-pastoris* (L.) Medik.	CAPBP	Weed	48.60	0.003
		2016	---				
1	Fall	2014	---				
		2015	---				
		2016	*Amaranthus retroflexus* L.	AMARE	O/M	46.15	0.066
			Capsella bursa-pastoris (L.) Medik.	CAPBP	Weed	40.46	0.053
			Potentilla norvegica L.	PTLNO	O/O	46.15	0.087
2	Fall	2014	---				
		2015	---				
		2016	---				
1	Spring no cover crop	2015	*Polygonum scabrum* Moench.	POLSC	Weed	56.25	0.067
			Ambrosia artemisiifolia L.	AMBEL	Weed	55.56	0.018
		2016	*Ambrosia artemisiifolia* L.	AMBEL	Weed	47.62	0.063
		2017	*Cerastium fontanum* Baumg.	CERFO	O/M	53.85	0.037
			Ambrosia artemisiifolia L.	AMBEL	Weed	46.15	0.072
			Chenopodium album L.	CHEAL	Weed	42.86	0.025
			Capsella bursa-pastoris (L.) Medik.	CAPBP	Weed	38.55	0.038
2	Spring no cover crop	2015	*Capsella bursa-pastoris* (L.) Medik.	CAPBP	M/M	54.55	0.037
		2016	*Solanum ptychanthum* Dunal.	SOLPT	O/O	45.00	0.070
			Setaria glauca (L.) Beauv.	SETLU	O/O	31.69	0.085
		2017	*Potentilla norvegica* L.	PTLNO	M/M	53.57	0.099
			Polygonum scabrum Moench.	POLSC	Weed	67.19	0.013

[a]Shows significant species at 0.1 probability level.

[b] Abbreviations: M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weed, no cover crop in spring nor fall.

[c]Indicator value = proportion value of specificity and fidelity. Ranges from 0 (no association) to 100 (perfect association).

[d]No significant species.

Table 5.7. Principal coordinates analysis (PCoA) results for each weed communities represented in Figures 5.5 and 5.6 and PerMANOVA assessing difference in weed communities between treatment.

			PCoA			PerMANOVA
Site	Community	Year	Axes	R^2	P values[a]	P values[b]
1	Spring	2014	83.7	0.98	≤ **0.001**	0.372
		2015	72.8	0.92	≤ **0.001**	0.340
		2016	84.2	0.97	≤ **0.001**	**0.049**
1	Fall	2014	90.0	0.95	≤ **0.001**	0.844
		2015	85.3	0.97	≤ **0.001**	0.113
		2016	78.3	0.96	≤ **0.001**	0.541
1	Spring no cover crop	2015	80.7	0.96	≤ **0.001**	0.303
		2016	82.7	0.97	≤ **0.001**	0.518
		2017	64.2	0.85	≤ **0.001**	0.676
2	Spring	2014	76.3	0.98	≤ **0.001**	0.542
		2015	84.8	0.98	≤ **0.001**	0.390
		2016	82.5	0.95	≤ **0.001**	0.615
2	Fall	2014	75.8	0.91	≤ **0.001**	0.416
		2015	76.5	0.92	≤ **0.001**	0.891
		2016	64.4	0.67	≤ **0.001**	0.622
2	Spring no cover crop	2015	67.7	0.91	≤ **0.001**	0.969
		2016	77.7	0.94	≤ **0.001**	0.474
		2017	63.5	0.88	≤ **0.001**	0.370

[a]Represent significance of ordinations.

[b]P values in bold text indicate a significant difference between treatments.

Table 5.8. Results of the PerMANOVA for treatment and year effects for each weed communities.

Site	Community	Factors	F. Model	R^2	P values[a]
1	Spring	Treatment	1.674	0.081	**0.018**
		Year	14.693	0.178	≤ **0.001**
		Block	5.919	0.071	≤ **0.001**
		Treat*Year	1.674	0.081	**0.024**
1	Fall	Treatment	1.218	0.062	0.209
		Year	15.248	0.193	≤ **0.001**
		Block	6.526	0.083	≤ **0.001**
		Treat*Year	0.822	0.042	0.692
1	Spring emergence	Treatment	0.833	0.048	0.736
		Year	9.179	0.132	≤ **0.001**
		Block	5.225	0.075	≤ **0.001**
		Treat*Year	0.741	0.043	0.856
2	Spring	Treatment	0.976	0.053	0.511
		Year	12.603	0.171	≤ **0.001**
		Block	4.254	0.058	≤ **0.001**
		Treat*Year	1.012	0.055	0.424
2	Fall	Treatment	0.976	0.053	0.525
		Year	12.603	0.171	≤ **0.001**
		Block	4.254	0.058	≤ **0.001**
		Treat*Year	1.012	0.055	0.436
2	Spring emergence	Treatment	0.976	0.053	0.500
		Year	12.603	0.171	≤ **0.001**
		Block	4.254	0.058	≤ **0.001**
		Treat*Year	1.012	0.055	0.436

[a]P values in bold text indicate a significant factor.

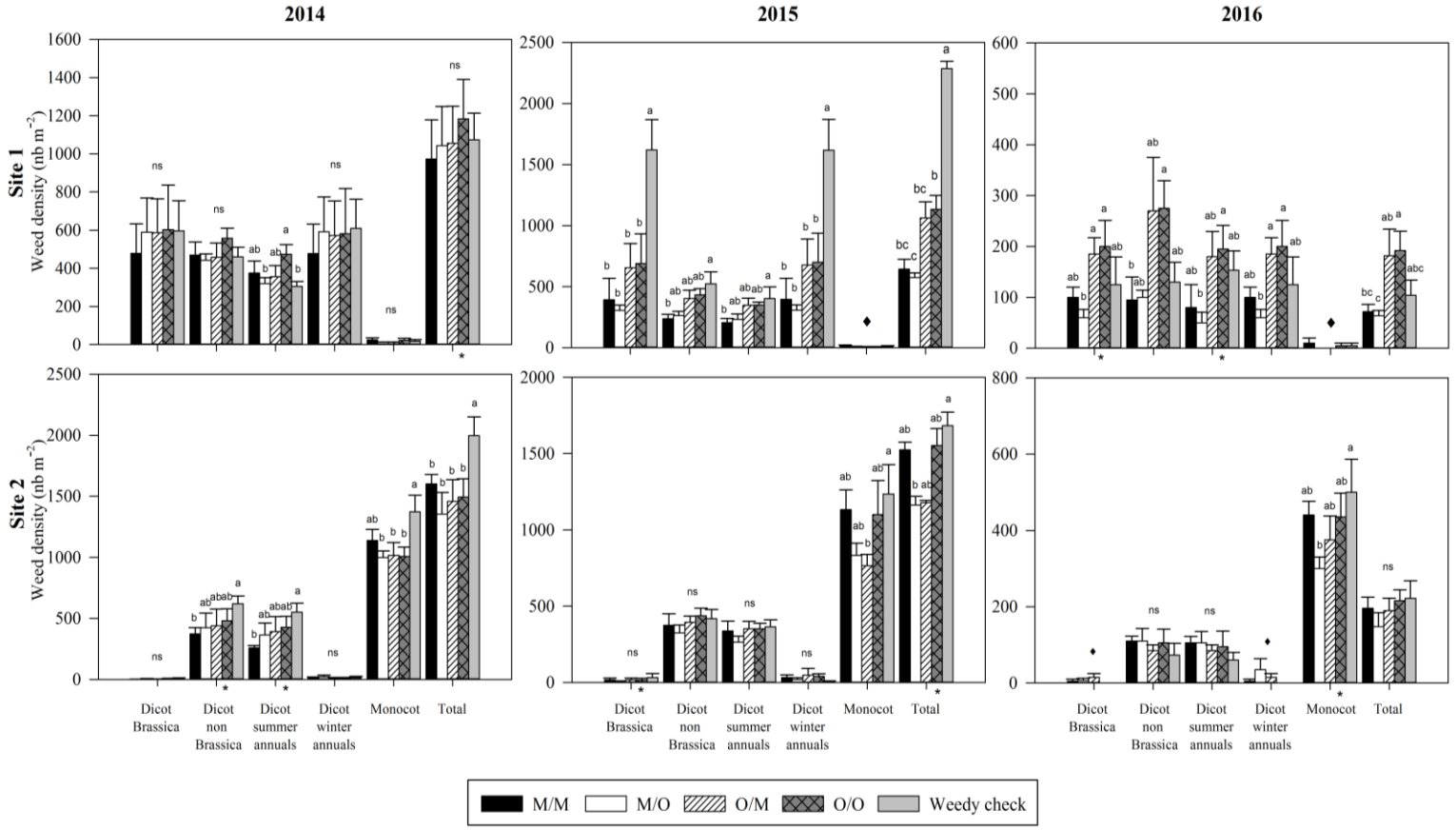

Figure 5.1. Weed establishment in spring cover crop at each experimental site according to treatments from 2014 to 2016. For each year within total density and each weed functional types, means followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate ± SE. * = Mean separation based on log-transformed data, otherwise on raw data. ♦ = Not enough data to performed analysis. Abbreviations: ns, non-significant; M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weedy check, no cover crop in spring nor fall.

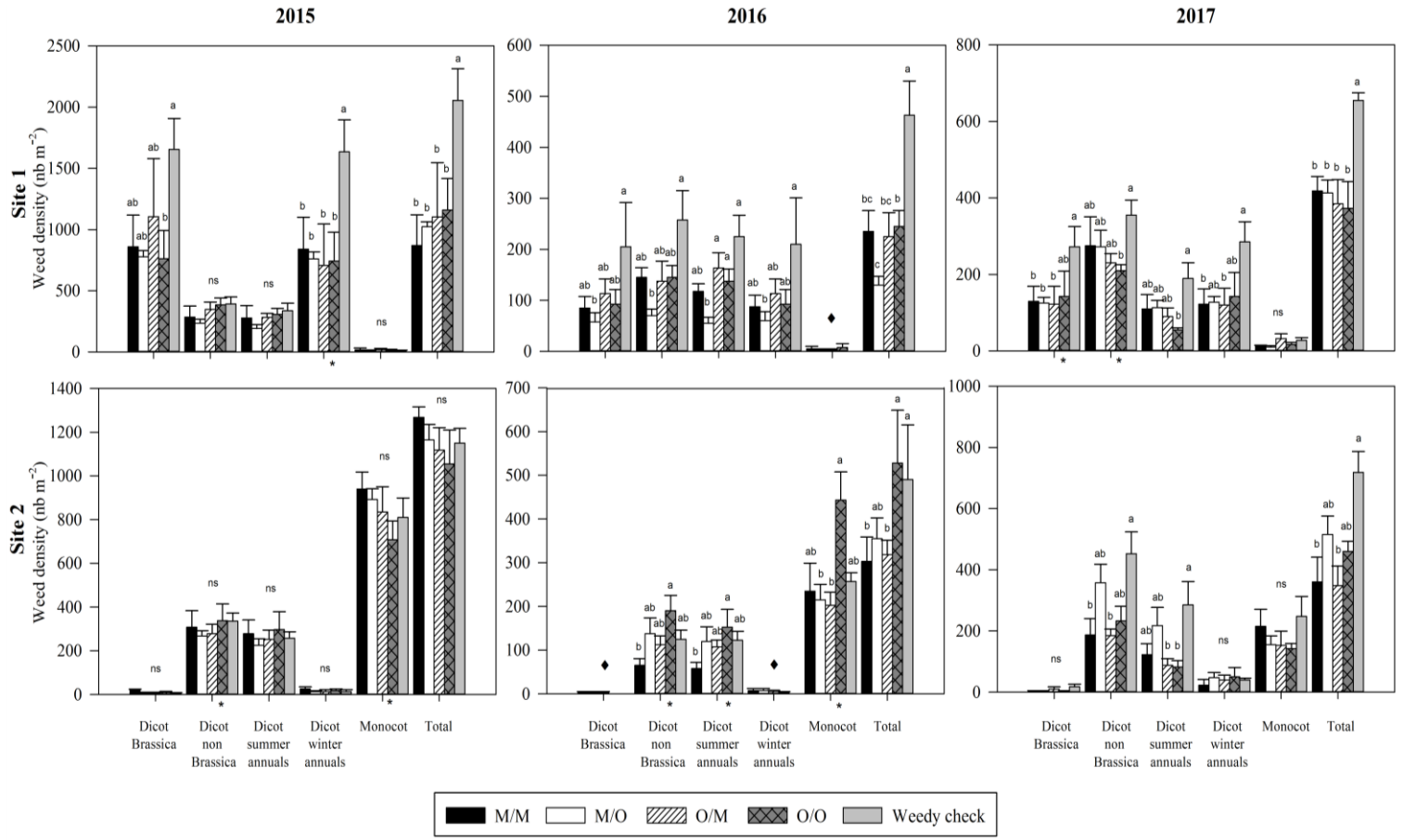

Figure 5.2. Weed emergence in spring without cover crop at each experimental site according to treatments from 2015 to 2017. For each year within total density and each weed functional types, means followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate ± SE. * = Mean separation based on log-transformed data, otherwise on raw data. ♦ = Not enough data to performed analysis. Abbreviations: ns, non-significant; M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weedy check, no cover crop in spring nor fall.

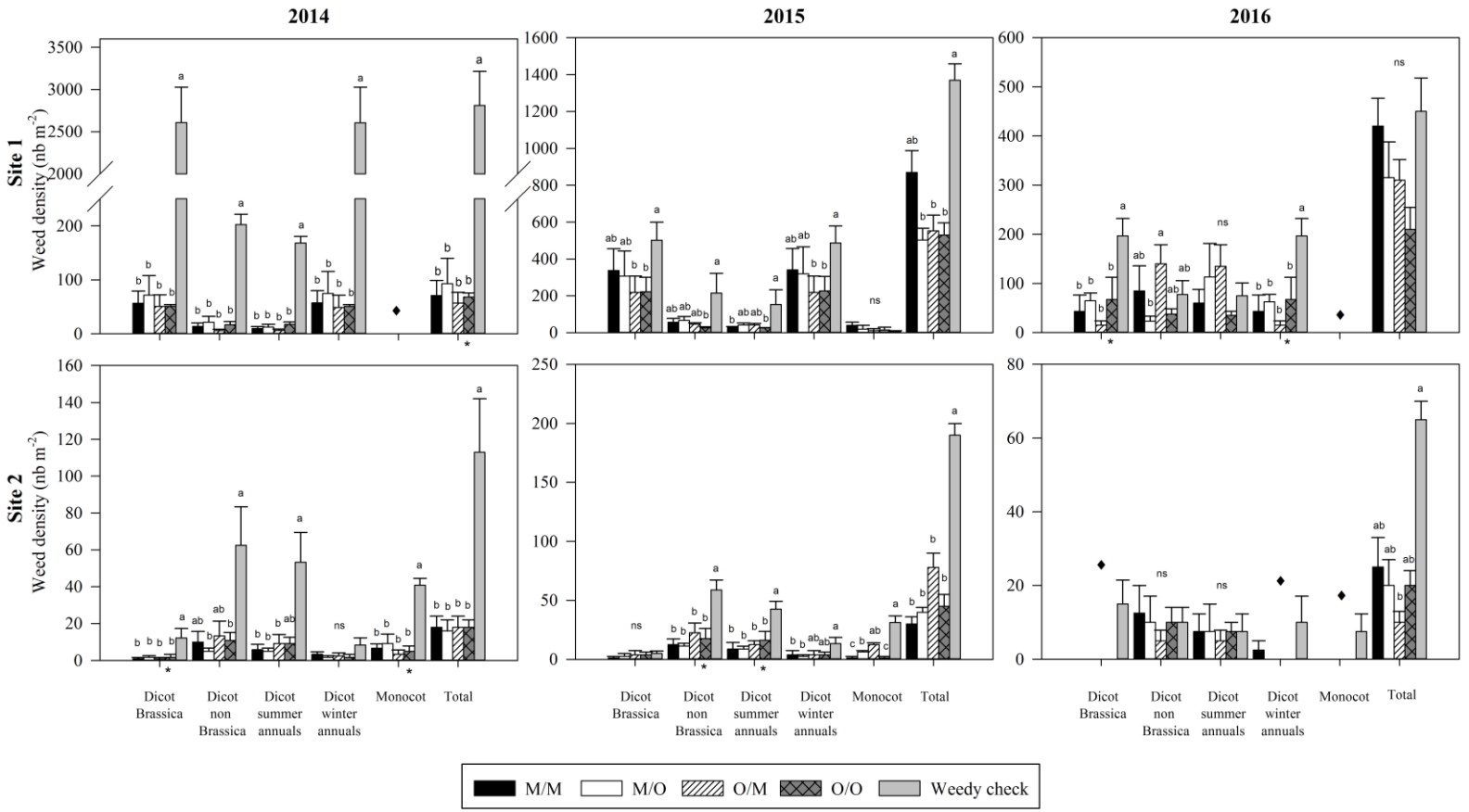

Figure 5.3. Weed density in horticultural crop at each experimental site according to treatments from 2014 to 2016. For each year within total density and each weed functional types, means followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate ± SE. * = Mean separation based on log-transformed data, otherwise on raw data. ♦ = Not enough data to performed analysis. Abbreviations: ns, non-significant; M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weedy check, no cover crop in spring nor fall.

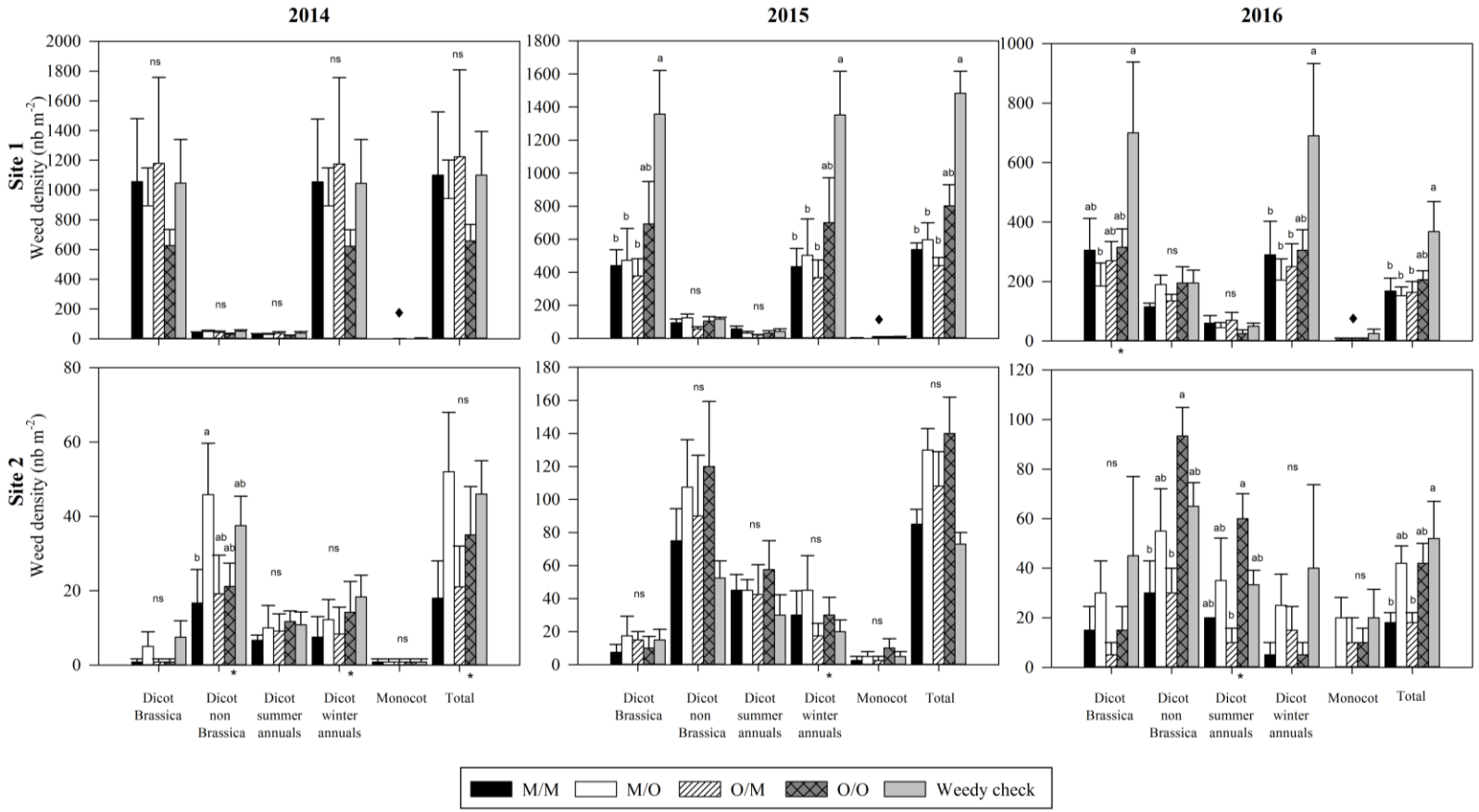

Figure 5.4. Weed establishment in fall cover crop at each experimental site according to treatments from 2014 to 2016. For each year within total density and each weed functional types, means followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. Vertical bars indicate ± SE. * = Mean separation based on log-transformed data, otherwise on raw data. ♦ = Not enough data to performed analysis. Abbreviations: ns, non-significant; M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weedy check, no cover crop in spring nor fall.

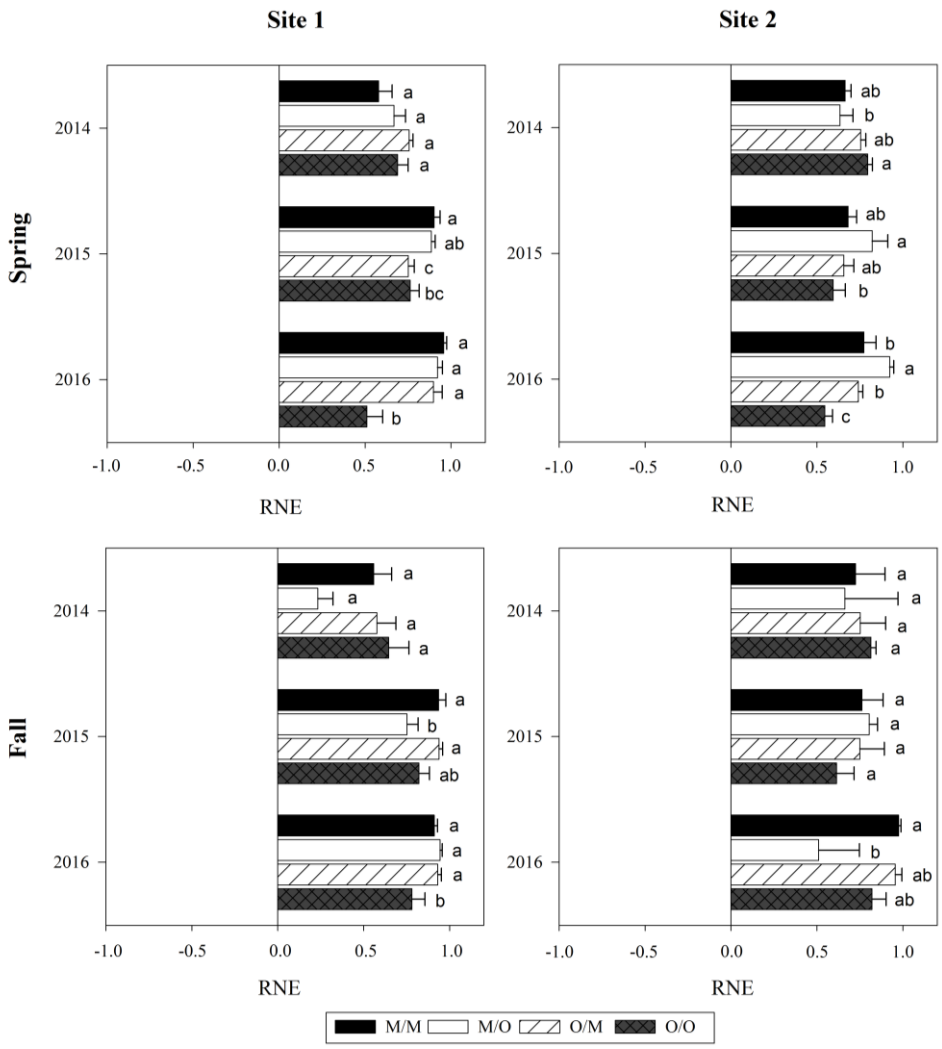

Figure 5.5. Relative neighbour effect (RNE) of the cover crops according to treatments reflecting cover crop competition when seeded in spring or fall at two experimental sites from 2014 to 2016. Vertical bars indicate ± SE. Within each year, means followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. Mean separation based on raw data, excepted for sites 1 and 2 in the spring of 2015 (log-transformed data), for site 1 in the fall of 2014 and for site 2 in the fall of 2016 (arcsine-transformed data). Abbreviations: M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weedy check, no cover crop in spring nor fall.

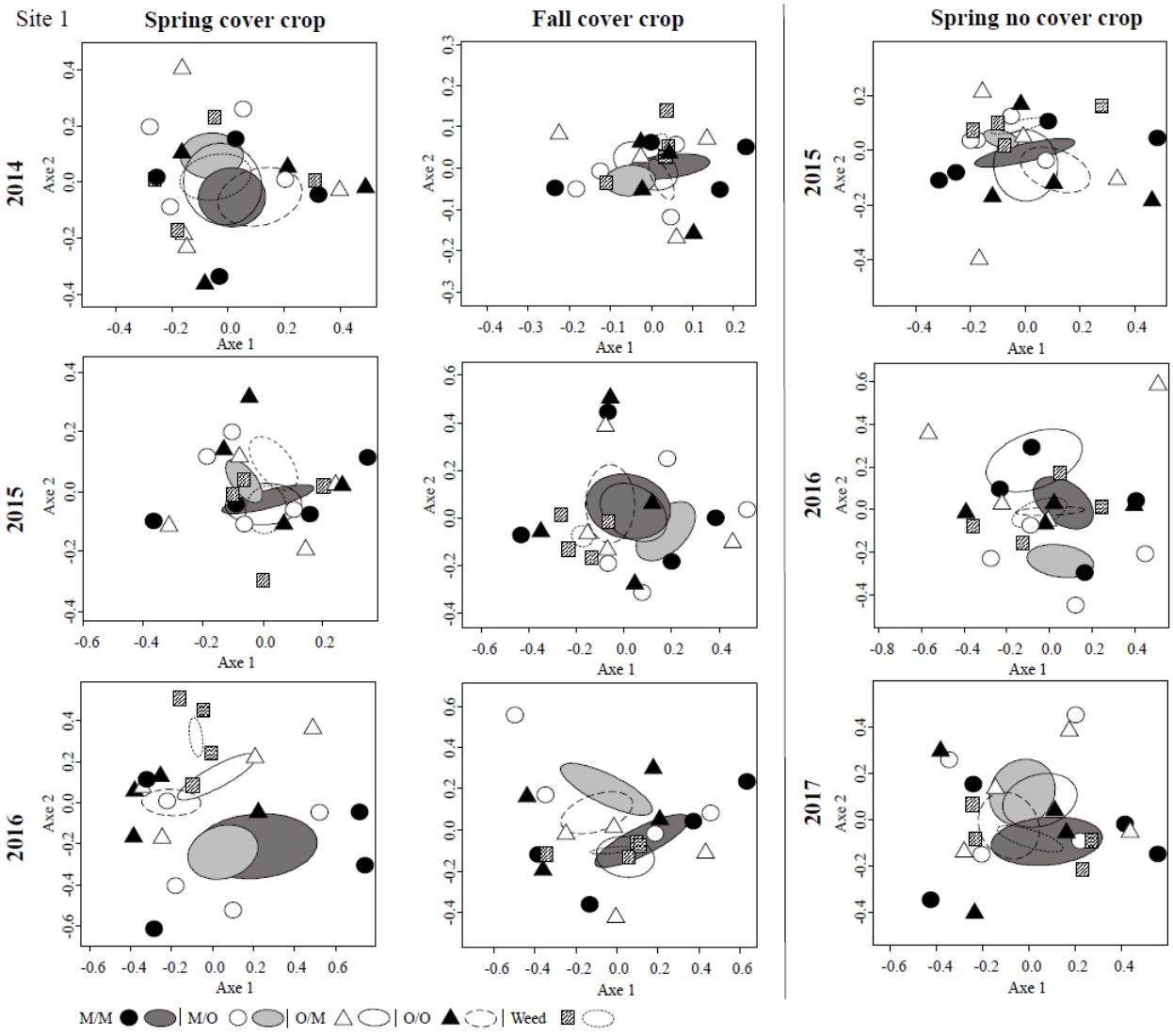

Figure 5.6. Principal coordinates analysis (PCoA) showing the weed communities according to treatments at site 1. Calculations of ellipses based on SE. Species are not represented in the graph. Spring and fall PCoA represent weed communities established in cover crops. Spring no cover crop (right) shows weed communities that established in undisturbed soil. The variance explained by axes, R^2 and significance of ordinations are described in Table 5.7. Abbreviations: M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weed, no cover crop in spring nor fall.

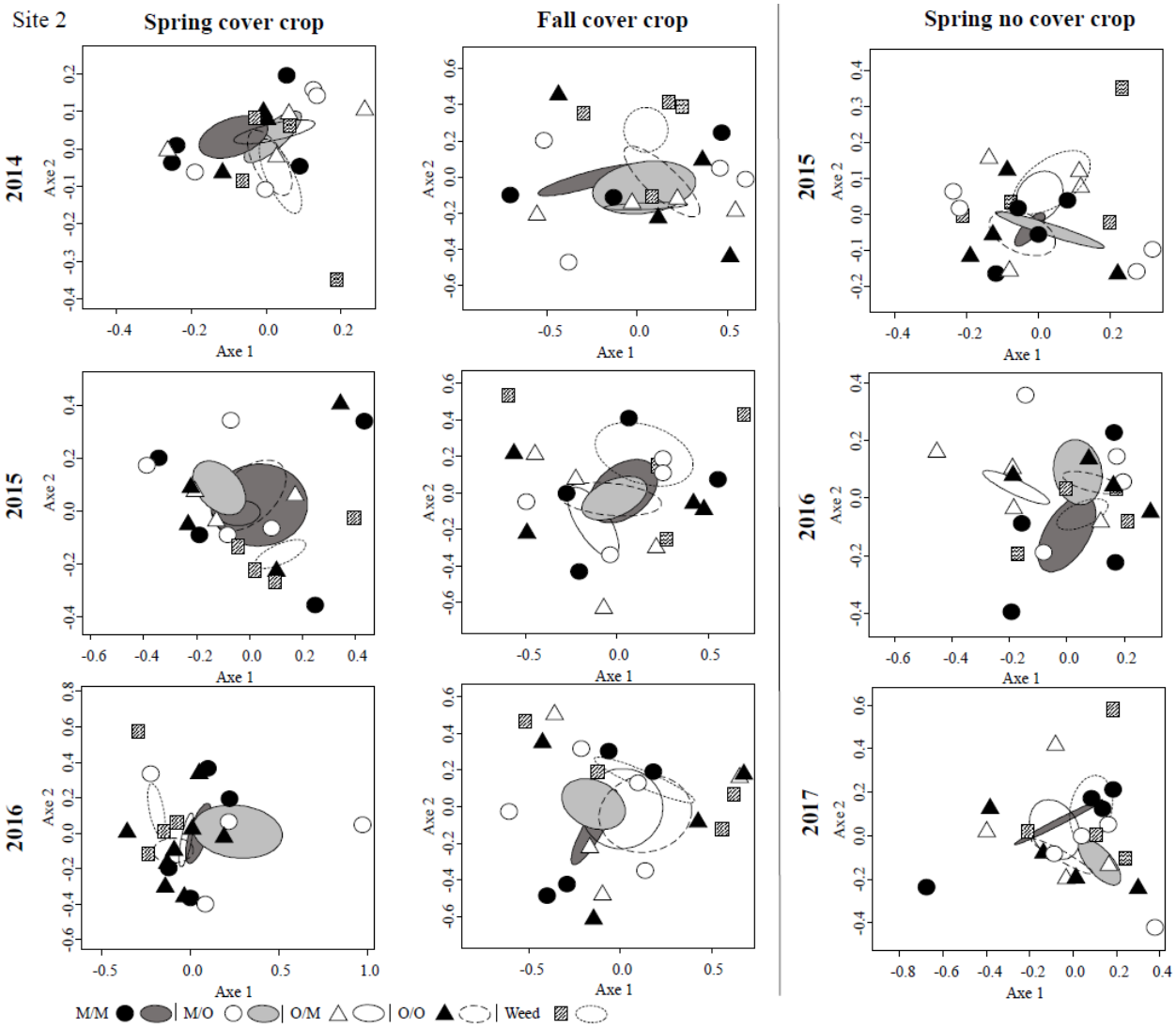

Figure 5.7. Principal coordinates analysis (PCoA) showing the weed communities according to treatments at site 2. Calculations of ellipses based on SE. Species are not represented in the graph. Spring and fall PCoA represent weed communities established in cover crops. Spring no cover crop (right) shows weed communities that established in undisturbed soil. The variance explained by axes, R^2 and significance of ordinations are described in Table 5.7. Abbreviations: M/M, spring Indian mustard cover crop/fall Indian mustard cover crop; M/O, spring Indian mustard cover crop/fall oat cover crop; O/M, spring oat cover crop/fall Indian mustard cover crop; O/O, spring oat cover crop/fall oat cover crop; Weed, no cover crop in spring nor fall.

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CHAPTER 6. FINAL CONCLUSION

6.1 Summary

Biofumigation is an agronomic technique that caught the attention of agronomists since a decade as an alternative of chemical soil fumigants. The influence of this practice on soil pathogens and nematodes has been well documented. The main outcomes from weed management studies reveal biofumigation in the field could reduce seed germination, establishment and weed biomass. However, highly variable and contrasting results from literature underlined a lack of understanding of weed response to the technique.

The thesis focused on weed species susceptibility, responses of surviving weeds, and impacts on key processes of population dynamics after exposure to allelochemicals generated during the biofumigation technique. Three studies were carried out, where the main objective was to assess and understand the impacts of biofumigation on annual weed populations defining factors by a deeper comprehension of seed and individual weed responses as driving mechanisms. An overall conceptual diagram of major outcomes for each weed responses investigated is shown in Figure 6.1.

The objectives of the study in chapter 3 were to assess the dose response of weed seeds to Indian mustard biofumigation and associate responses to seed dormancy state, initial dormancy, and seed morphological characteristics. A laboratory methodology was developed to expose seeds of *C. album*, *V. cracca*, *D. carota*, *A. artemisiifolia*, *S. viridis*, *A. theophrasti*, *G. quadriradiata*, and *T. pratense* to allelochemicals produced after rehydrating dried mustard tissues. *Galinsoga quadriradiata* and *D. carota* were the most affected by the treatment, where maximal mortalities were 97 and 95%, ED_{50} values for germination were 2.30 and 3.23 mg cm^{-2}

and LD_{50} were 3.99 and 4.44 mg cm^{-2} of dried mustard tissues, respectively for each species. Linear regression analyses revealed that initial dormancy was positively associated to ED_{50} and LD_{50} values with a significant interaction with seed surface and seed width, respectively. Exposure to allelochemicals generated from the rate 7.00 mg cm^{-2} of dried mustard tissues increased *A. artemisiifolia* seed mortality for after-ripened seeds by 293% and by 58% for primary dormant seeds, compared to untreated seeds. *Chenopodium album* secondary and primary dormant seed mortality increased by 730% and 106%, respectively. For *D. carota*, secondary and primary dormant seed mortality increased by 1,193% and 156%, respectively. Results of the first study underlined differential susceptibility of weed species and susceptibility of seeds in secondary dormancy. Furthermore, seed responses may be related to the number of dormant seeds in a seed lot interacting with the seed size and testa thickness.

Laboratory and greenhouse experiments in chapter 4 were conducted to assess the impact of biofumigation on *A. artemisiifolia* and *A. theophrasti* fitness components (survival, growth, reproduction) of surviving plants across three generations. Biofumigation treatment decreased weed fitness by reducing seed germination and survival, increasing seedling mortality, deferring emergence and flowering, and decreasing number of seeds produced for *A. artemisiifolia*. However, second and third generations may improve their tolerance to biofumigation by the induction of dormant seeds as for *A. artemisiifolia*, by superior seedling survival for *A. artemisiifolia* and *A. theophrasti*, by a higher seed production for *A. theophrasti*, and by an enlarged relative weight of the embryo, and testa thickness for *A. artemisiifolia*.

In chapter 5, pot and field experiments were conducted to evaluate the impact of biofumigation on seed bank persistence, seedling establishment, and to observe in the field the impact on population dynamic processes and changes in weed community structure. *Ambrosia*

artemisiifolia and *G. quadriradiata* seeds were exposed in pots at different biofumigation rates (1,667; 2,500; 5,000; 7,500, and 10,000 kg ha^{-1} of Indian mustard biomass incorporated in soil). The biofumigation rate 2,500 kg ha^{-1} increased significantly *A. artemisiifolia* seed mortality by 75.9%. *Galinsoga quadriradiata* emergence was reduced significantly by 32.4% at 7,500 kg ha^{-1}. Seasonal variation and long-term impact of biofumigation on weed community and population dynamics were assessed in a three-year field experiment. In 2014, the biofumigant potential was low and consequently Indian mustard cover crops had no impact on weed emergence. As the amount of ITC measured increased in 2015 and 2016, Indian mustard decreased weed establishment within the cover crop growth. Moreover, post incorporation weed spring emergence decreased in 2015. Allelopathic interference of Indian mustard contributed significantly to reduce weed biomass when plants tissues produced more than 600 µg of allyl-ITC g^{-1} of plant in our experiment. Dicotyledonous species were more vulnerable to biofumigation than monocotyledon species communities. There was no benefit to repeat biofumigation for three years, nor to perform biofumigation twice a year. This last study provided further information about *Brassica* cover crops negative impact on key defining processes of weed population dynamics. Whenever the impact of the biofumigation technique on key processes are significant, notable changes in weed diversity and community structure may occur. However, high variability from weed responses, intrinsic technique considerations, intraspecific response and intergenerational changes in weed responses may complicate our ability to elucidate and clearly understand changes in field populations and communities.

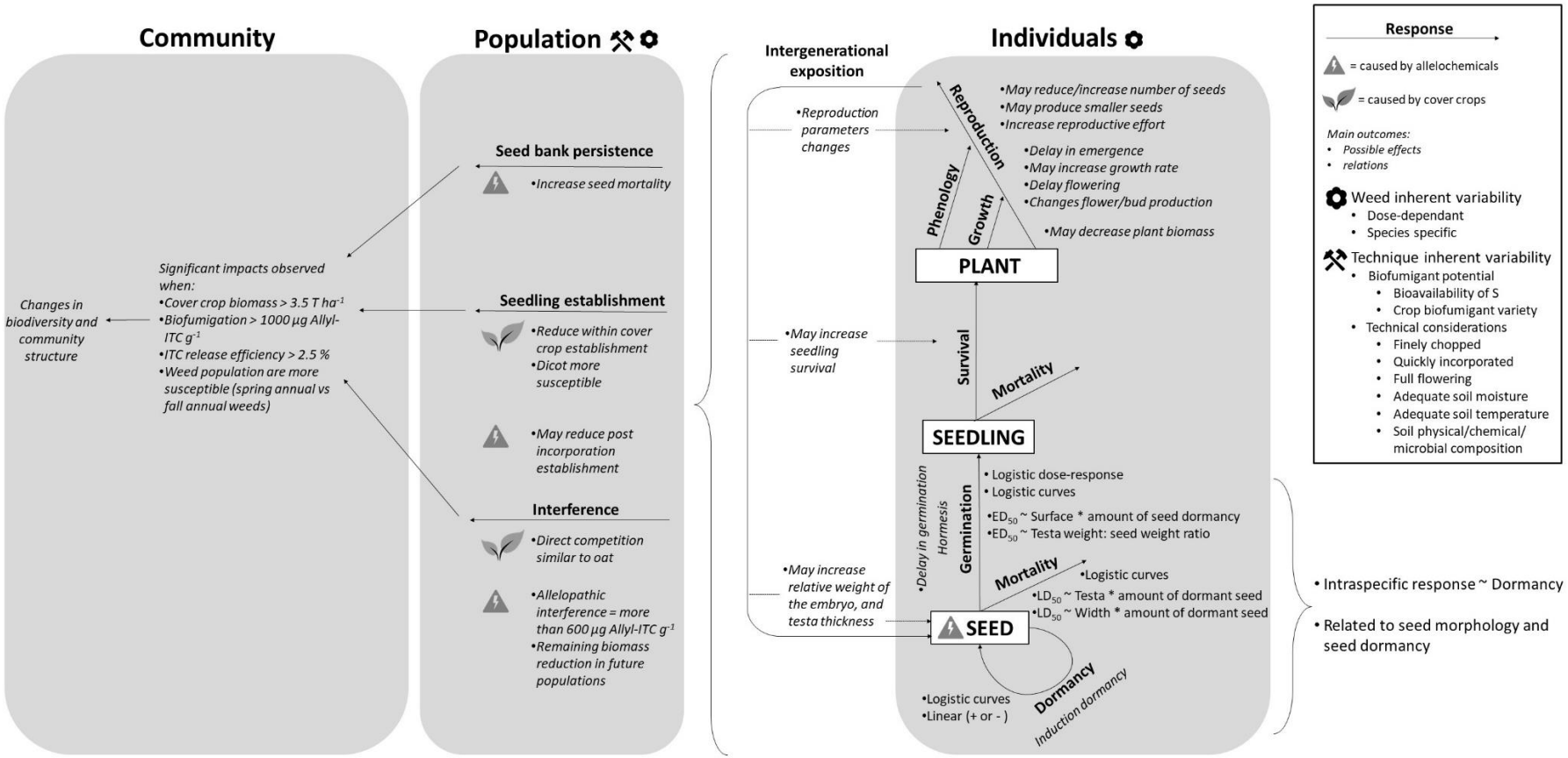

Figure 6.1. Conceptual diagram of the weed responses investigated in the thesis. Main outcomes, results and observations made in the studies in chapters 3, 4 and 5 are represented. The logical starting point is the seed exposed to allelochemicals. For several individuals and population levels, source of variability from weeds, or biofumigation technique are specified. Responses from several individuals provide information and understanding of the impact of biofumigation on key weed population dynamics processes.

6.2 Conclusion

The thesis has filled several gaps in the literature concerning the response of weeds to biofumigation practices. The work described in chapter 3 successfully categorized weed seed susceptibility to biofumigation allelochemicals according to seed dormancy, seed size and testa thickness. The study also revealed detailed interspecific and intraspecific seed responses to biofumigation. Chapter 4 provided exhaustive quantification of weed fitness and phenological modelling of weeds exposed to sublethal biofumigant allelochemicals. Quantification of target weed fitness from *Brassica* cover crop biofumigation significantly contributed to our understanding of consequences of biofumigation on weed population performance in the field. Furthermore, evidence of modifications in seed and plant intergenerational responses are unique, but also underlined the importance to use the biofumigation technique rationally and advisedly. Finally, results in chapter 5 revealed that weed population responses under field experiments were related to the intraspecific variation of biofumigant potential of the *Brassica* cover crop. Furthermore, when *Brassica* cover crop biofumigation altered significantly key processes of population dynamic and was performed at the right timing, the technique led in some cases to changes in weed diversity and in-community structure.

From a weed ecophysiological perspective, results enhanced the recognition of allelopathy as a defining factor of population dynamics. The dissertation also provided important information about fitness of surviving plants to allelochemical pressure in agroecosystems. Increased knowledge of weed responses are now available and should be considered when biofumigation practices are part of weed management strategies. Agronomic communities may rationalize the utilization of *Brassica* cover crops and biofumigation for weed control with an

increased comprehension of the impact of biofumigation on key processes of weed population dynamics.

6.3 Future work

The following research should be undertaken for further understanding of the impacts of biofumigation and biofumigant cover crops on weeds.

1. As different types of ITC may have specific effect on seeds responses, experiments conducted in chapters 3 and 4 should be redone with other biofumigation species. Those experiments may lead to additional field study assessing different species or varieties of biofumigant cover crops.
2. More weed species should be tested according to the methodology in chapter 3, especially monocotyledons, to expand the results. Furthermore, more species with different types of seed dormancy may provide sufficient statistical power to rank the different susceptibilities to physiological and physical seed dormancies.
3. Seedling survival and seed morphological characteristics change across generations of weeds exposed to sublethal concentrations of biofumigation allelochemicals. Mechanisms involved in the transgenerational inheritance of allelochemical tolerance require deeper investigation.
4. Research should investigate the physiological mechanisms of seed dormancy induction from biofumigant allelochemicals.
5. Our study suggested a physiological mechanism of seed dormancy of *D. carota,* and thus necessitates future investigation.

6. Chapter 5 revealed inconsistent evidence for the hypothesis about the relation between weed life history and susceptibility to biofumigation. An analysis based on species emergence periodicity, seed traits (morphology and dormancy), and other traits related to phytotoxic tolerance should be investigated in the field and could lead to better predictability of weed susceptibility to biofumigation.

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