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Biofumigation to protect oilseed crops: focus on management of soilborne fungi of sunflower☆

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Abstract – Sunflower (*Helianthus annuus* L.) is one of the three most productive oilseed crops worldwide. Soilborne diseases limit yields and are challenging to manage. The fungi *Verticillium dahliae*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina* can survive in the soil for many years and spread. Following the ban on fumigants, biofumigation, which consists of growing, chopping and incorporating a Brassicaceae cover crop to allow biocidal compounds production in the soil, may be an alternative. Biocidal effects of the hydrolysis of glucosinolate into active compounds, such as isothiocyanates, have been shown in laboratory studies, but the effectiveness of biofumigation varies more in the field. The present study reviews the main factors that determine effective biofumigation to protect sunflower. Since the toxicity of isothiocyanates to pathogens varies widely among the latter, we reviewed studies that assessed the suppressive effect of products of glucosinolate hydrolysis on *V. dahliae*, *S. sclerotiorum* and *M. phaseolina*. Farmers can use many mechanisms to increase isothiocyanate production, which may protect sunflower crop effectively. Increasing biomass production and chopping the cover crop during mild temperatures and before rainy periods could increase biofumigation effectiveness. Further field experiments are needed to confirm the potential of biofumigation to control soilborne diseases of sunflower and assess potential disservices to beneficial soil communities, given their potential key role in the control of soilborne pathogens.

Keywords: *Helianthus annuus* / cover crops / Brassicaceae / glucosinolates / agroecological crop protection

Résumé – Protéger les cultures oléagineuses par la biofumigation : le cas de la gestion des champignons telluriques du tournesol. Le tournesol (*Helianthus annuus* L.) est l'une des trois cultures oléagineuses les plus productives dans le monde. Les pathogènes telluriques limitent sa productivité et leur contrôle est difficile. Les champignons telluriques *Verticillium dahliae*, *Sclerotinia sclerotiorum* et *Macrophomina phaseolina* peuvent survivre plusieurs années dans le sol et sont en recrudescence. Suite à l'interdiction de plusieurs fumigants, la biofumigation, qui consiste en la mise en place, la destruction et l'incorporation de culture intermédiaire de Brassicacées permettant la production de composés biocides dans le sol, pourrait être une alternative. L'effet biocide des produits de l'hydrolyse des glucosinolates, tels que les isothiocyanates, a été démontré au laboratoire, mais l'efficacité de la biofumigation est variable en plein champ. Cette revue a pour objectif de recenser les déterminants majeurs de l'efficacité de la biofumigation pour la protection du tournesol. La toxicité des isothiocyanates étant variable selon les bioagresseurs visés, le second objectif est de recenser les études ayant évalué les effets suppressifs des produits de la dégradation des glucosinolates, contre les champignons telluriques *V. dahliae*, *S. sclerotiorum* et *M. phaseolina*. Les agriculteurs peuvent mettre en place plusieurs leviers afin d'améliorer la production d'isothiocyanates, permettant potentiellement une protection efficace de la culture du tournesol. Maximiser la production de biomasse puis détruire le couvert lors de températures douces et avant une période pluvieuse pourraient améliorer l'efficacité de la biofumigation. Des expérimentations en plein champ

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supplémentaires sont nécessaires pour confirmer le potentiel de la biofumigation pour contrôler les pathogènes telluriques du tournesol et évaluer ses potentiels disservices contre les communautés microbiennes du sol, au regard de leur importance potentielle dans le contrôle des pathogènes telluriques.

Mots clés : *Helianthus annuus* / cultures intermédiaires multi-services / Brassicaceae / glucosinolates / protection agroécologique des cultures

1 Introduction

1.1 Oilseed crop production and protection

1.1.1 Factors that limit crop yield

Since 2015, soybean (*Glycine max*), rapeseed (*Brassica napus* subsp. *napus*) and sunflower (*Helianthus annuus* L.) have been the three main oilseed crops produced worldwide (FAOSTAT, 2020). In 2018, their worldwide production was ca. 345, 75 and 50 million t/annum, respectively (FAO, 2020). While the global area of these crops is expanding, unfavorable weather conditions threaten their production (FAO, 2018). Despite the moderate water requirements of sunflower, drought is the main environmental factor that limits its growth (Debaeke *et al.*, 2017a), and high temperature can decrease its final production of seeds and oil (Harris *et al.*, 1978). In most European countries that produce sunflower (Romania, Spain, France, Bulgaria, and Hungary), yield gaps of 1.1–2.4 t/ha have been reported, and climate change could be partly responsible for them (Debaeke *et al.*, 2017a). Biotic stress also limits oilseed crop production worldwide. At least 30 sunflower diseases are known. The most damaging and widespread fungal diseases are downy mildew (*Plasmopara halstedii*), phoma black stem (*Phoma macdonaldii*), phomopsis stem canker (*Phomopsis helianthi*), white mold (*Sclerotinia sclerotiorum*) and *Verticillium* wilt (*Verticillium dahliae*) (Seassau, 2010; Vear, 2016; Debaeke *et al.*, 2017b), most of which are soilborne pathogens (*P. halstedii*, *S. sclerotiorum*, *V. dahliae*). More recently, *Cadophora malorum* has been reported as a new soilborne fungus of sunflower (Martín-Sanz *et al.*, 2018; Molinero-Ruiz, 2019). In the context of climate change, *Macrophomina phaseolina* could be favored by ground dryness and temperatures of 28–30 °C (Šárová *et al.*, 2003). *S. sclerotiorum* and *V. dahliae* could tolerate unfavorable periods better (Wilhem, 1955; Debaeke *et al.*, 2017a) *via* their long-term structures – sclerotia and microsclerotia (MS), respectively –, which remain viable in the soil for many years (Mol *et al.*, 1995; Ćosić *et al.*, 2012).

1.1.2 The challenge of managing soilborne fungi

Protecting crops from soilborne organisms is more challenging than protecting them from foliar pests (Matthiessen and Kirkegaard, 2006). Soilborne fungi such as *V. dahliae* and *M. phaseolina* can survive as MS up to 14 years (Wilhem, 1955) and 4 years (Watanabe, 1973), respectively. *S. sclerotiorum* produces sclerotia that may survive for 3 years (Ćosić *et al.*, 2012). Soilborne pathogens can coexist in the soil (Raaijmakers *et al.*, 2009), and their heterogeneous distribution makes monitoring them costly and usually ineffective (Matthiessen and Kirkegaard, 2006). For many oilseed diseases, genetic resistance is one of the most effective protection methods, but it breaks down frequently due to the appearance of new virulent strains, as observed for sunflower diseases (Vear, 2016; Debaeke *et al.*, 2017b; Molinero-Ruiz, 2019). To reduce the pressure of soilborne pathogens, farmers used to fumigate vegetable and ornamental crops intensively with methyl bromide (Hoffmann and Malkomes, 1974; Duniway, 2002; Martin, 2003). However, methyl bromide was phased out under the Montreal Protocol in 2005 due to its depleting effects on the ozone layer (Laegdsmand *et al.*, 2007; Gimsing and Kirkegaard, 2009). Other synthetic compounds were subsequently used to control soilborne pathogens, such as 1,3-dichloropropene (phased out in the European Union [EU] in 2007), chloropicrin (phased out in the EU in 2012) and methyl-isothiocyanate (MITC), the primary breakdown product of metam-sodium (Ibekwe, 2004). MITC has a broad biocidal activity but alters important soil functions such as nutrient cycling (Macalady *et al.*, 1998). It is also highly volatile, with much of it transferred to the atmosphere after application (Dungan *et al.*, 2003).

Like for genetic resistance, maintaining the efficacy of pesticides after repeated use is difficult (Matthiessen and Kirkegaard, 2006). Synthetic fumigants may become less toxic due to soilborne pathogens developing resistance (Goldman *et al.*, 1994) and/or increased biodegradation of their chemicals (Warton *et al.*, 2003). This latter misunderstood phenomenon comes from the ability of microorganisms, mainly bacteria, to catabolize xenobiotics in the soil after repeated exposures with a short interval between applications (Warton *et al.*, 2003; Matthiessen and Kirkegaard, 2006). Microorganisms can accelerate the degradation, which decreases their persistence and effectiveness for soilborne pathogens (Warton *et al.*, 2003; Di Primo *et al.*, 2003). This phenomenon has been observed with metam sodium used for potato (*Solanum tuberosum*) *Verticillium* wilt (VW) (Di Primo *et al.*, 2003). When a soil develops increased biodegradation, fumigation requires several years before it can recover an effective biocidal effect (Warton *et al.*, 2003). In the meantime, the use of fumigants seems ineffective and wasteful (Matthiessen and Kirkegaard, 2006).

1.1.3 Alternatives for managing soilborne diseases

The breakdown of resistance and the current context of agroecological transition have decreased the use of broad-spectrum fumigants (Warmington and Clarkson, 2016) and increased interest in alternative methods of crop protection (Martin, 2003). Reliance on combined and natural mechanisms to protect crops has been encouraged by Integrated Pest Management (IPM), as described in the EU Framework Directive 2009/128/EC. IPM is implemented through eight principles, and the first one is based on preventing and/or suppressing harmful organisms using a variety of methods, such as crop rotations. IPM favors the use of sustainable biological methods (Barzman *et al.*, 2015). Since isothiocyanates (ITCs) are biologically active compounds, and MITC is

widely used as a fumigant, there is interest in transposing this biocidal activity of biological sources of ITCs to suppress soilborne pathogens and diseases (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Gimsing and Kirkegaard, 2006). This natural alternative to fumigation, called "biofumigation" (Kirkegaard *et al.*, 1993), involves growing, chopping and incorporating crops that produce ITCs. Brassicaceae (crucifers) are widely used for this technique (see part 2).

The utility of biofumigation has been observed for protecting vegetable crops (Michel, 2014; Morris *et al.*, 2020) and, to a lesser extent, wheat (*Triticum aestivum*, Kirkegaard *et al.*, 2000) and beetroot (*Beta vulgaris* ssp. *vulgaris*, Motisi *et al.*, 2009). Many studies of *in vitro* approaches have shown promising results of biofumigation for soilborne diseases. In the field, however, the effectiveness of biofumigation has varied more (Motisi *et al.*, 2010; Morris *et al.*, 2020). Nonetheless, mechanisms for suppressing pathogens effectively in the field are increasingly understood (Kirkegaard and Matthiessen, 2004; Matthiessen and Kirkegaard, 2006; Morris *et al.*, 2020), and biofumigation appears to be an environmentally friendly defense strategy (Lazzeri *et al.*, 2004) considered as a part of IPM (Gimsing and Kirkegaard, 2009; Kruger *et al.*, 2013). Among oilseed crops, sunflower seems to be particularly suitable for protection using biofumigation. It is sown in spring, after a long fallow period when soils are usually left bare. A Brassicaceae cover crop introduced during this period would fit into the rotation easily, thus diversifying it. It would also improve:

- soil structure and reduce erosion (Thorup-Kristensen *et al.*, 2003; Justes *et al.*, 2012);
- nutrient management, through catch crop and green manure effects for nitrates and sulfates (Constantin *et al.*, 2011; Couëdel *et al.*, 2018a; Couëdel *et al.*, 2018b);
- soil organic matter (Kirkegaard and Matthiessen, 2004).

To follow the fundamental agroecological principle of diversifying crop rotations (Altieri, 1999), this review does not discuss rapeseed protection using Brassicaceae cover crops and biofumigation. However, it does present studies that used Brassicaceae as a biofumigant crop. Biotic stresses are not still a major issue for soybean in France (Lamichhane *et al.*, 2020) or in Europe. This is in part because soybean is currently grown on small areas and in diversified rotations (Lamichhane *et al.*, 2020). The interest in biofumigation to protect soybean remains low and studies rare. Thus, this review excludes soybean protection using biofumigation, although some studies showed promising results. Fayzalla *et al.* (2009) showed that soybean root rot and soybean wilt, caused by *Fusarium oxysporum*, *Rhizoctonia solani*, *M. phaseolina* and *Sclerotium rolfsii*, could be reduces with mustard in field conditions.

With a focus on sunflower, the objectives of this review are to:

- highlight the main factors that determine effective biofumigation;
- review studies on laboratory or field experiments performed to evaluate suppressive effects of synthetic GSLs/ITCs or Brassicaceae incorporation on *V. dahliae*, *S. sclerotiorum* and *M. phaseolina*.

Since studies of sunflower protection using biofumigation are rare (to our knowledge), most studies concerned other plant hosts. Thus, after describing the biofumigation concept and process briefly, factors that drive ITC production are detailed to provide a set of mechanisms that results in effective biofumigation. Suppressive effects of glucosinolate (GSL) products on sunflower soilborne diseases are reviewed based on studies of a variety of host crops. Finally, non-GSL-related suppressive effects of biofumigation and the utility of including Fabaceae with Brassicaceae to protect sunflower against soilborne disease are also discussed.

2 The biofumigation process

2.1 Biofumigation concept and the use of Brassicaceae

Biofumigation is defined as the suppressive effect of GSL-containing species on soilborne pathogens through the liberation of volatile compounds, mainly ITCs, released after hydrolysis of GSLs by the enzyme myrosinase during tissue disruption and incorporation into the soil (Kirkegaard *et al.*, 1993; Kirkegaard and Matthiessen, 2004). GSLs occur naturally in families of the order Capparales: Tovariaceae, Resedaceae, Cappareaceae, Moringaceae and mainly Brassicaceae (Fenwick *et al.*, 1983; Brown and Morra, 1997; Van Dam *et al.*, 2009). They are widely cultivated as vegetables (cabbage [*B. oleracea* var. *capitata*], radish [*Raphanus raphanistrum* subsp. *sativus*], and rocket [*Eruca vesicaria* ssp. *sativa*]), condiments (mustard [*Brassica juncea*]), forage (fodder radish [*Raphanus sativus* var. *longipinnatus*] and turnip rape [*Brassica rapa* subsp. *rapa*]), oilseed crops and cover crops during fallow periods. However, plants that contain GSLs can be used to control soilborne pathogens through biofumigation (Kirkegaard *et al.*, 1993; Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006) and are considered to be a biological alternative to conventional soil fumigation (Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Laegdsmand *et al.*, 2007; Clarkson *et al.*, 2015). Bactericidal activity of ITCs has been reported (Brown and Morra, 1997; Smith and Kirkegaard, 2002; Bending and Lincoln, 2000), as have fungicidal (Angus *et al.*, 1994; Manici *et al.*, 2000; Smith and Kirkegaard, 2002), nematicidal (Lazzeri *et al.*, 1993; Riga, 2011; Ntalli and Caboni, 2017), insecticidal (Borek *et al.*, 1995a; Borek *et al.*, 1998) and herbicidal activities (Haramoto and Gallandt, 2004). Biofumigation can reduce pest abundance and disease incidence (Morris *et al.*, 2020), but its degree of pest suppression can vary significantly. Some studies concluded that biofumigation did not suppress soilborne pathogens (reviewed by Kirkegaard and Matthiessen, 2004; Motisi *et al.*, 2010). After rape incorporation, Davis *et al.* (1996) observed no significant differences in *V. dahliae* population in the soil compared to that without residue incorporation, while the incidence of VW on potato was reduced significantly compared to that on potato grown after a fallow period. VW can be caused by an interaction between *V. dahliae* and nematodes like *Pratylenchus penetrans* (Martin *et al.*, 1982; Rowe and Powelson 2002) or *Pratylenchus neglectus* (Scholte and s'Jacob, 1990) which may facilitate the penetration of *V. dahliae* in roots, but no information is available about the direct effect of residue

incorporation on *P. neglectus* in this study. However, no significant correlation has been found between VW symptoms or yield and the nematode. Hartz *et al.* (2005) also reported that biofumigation (with mustard) did not significantly reduce *V. dahliae* population in the soil or VW on tomato (*Solanum lycopersicum*). A review of Motisi *et al.* (2010) noted an increase in disease intensity after biofumigation for some pathogens. Moreover, some studies may not be published because they unexpectedly observe no significant effects of biofumigation (Morris *et al.*, 2020). This variability is due to the many biological and physical factors that influence the effectiveness of biofumigation (Motisi *et al.*, 2010). Thus, knowledge about GSL and ITC production, and a systematic approach to field research through analytical studies are needed (Kirkegaard and Matthiessen, 2004).

2.2 The GSL-myrosinase system

GSLs are organic anions characterized by a common b-thioglucose, a sulfonated oxime moiety and a side-chain group (Fenwick *et al.*, 1983). This side chain determines the type of GSL: aromatic, aliphatic or indolyl (Fenwick *et al.*, 1983; Brown and Morra, 1997; Mithen, 2001). To date, 132 GSLs have been identified in Brassicaceae tissues (Couëdel *et al.*, 2019). Native GSLs have little or no biocidal activity or toxicity (Manici *et al.*, 1997). Species that contain GSL produce myrosinase, a group of similar-acting enzymes (Brown and Morra, 1997) that are also produced by some microorganisms in soils (Gimsing and Kirkegaard, 2009). In intact plant tissues, GSLs and myrosinase are physically separated (Gimsing and Kirkegaard, 2009). The isolation seems to be intercellular (Brown and Morra, 1997), with GSLs in the vacuoles and myrosinase in specialized myrosin cells (Höglund *et al.*, 1992). Both compounds are distributed throughout Brassicaceae tissues (Wittstock and Gershenzon, 2002), and cells must be disrupted physically for them to contact each other (Brown and Morra, 1997). The result is rapid hydrolysis into biologically active products such as ITCs and other products of GSL degradation, such as nitriles, organic cyanides, oxazolidinethiones and ionic thiocyanates (Brown and Morra, 1997; Gardiner *et al.*, 1999). Mature tissues have less myrosinase activity (Iversen and Baggerud, 1980).

2.3 GSL-hydrolysis products and non-GSL products

The biocidal effect of the products of GSL hydrolysis is function of the chemical composition of the GSL side chain, their concentration, environmental conditions and the exposure time of the target organism (Fenwick *et al.*, 1983; Lazzeri *et al.*, 1993; Laegdsmand *et al.*, 2007; Gimsing and Kirkegaard, 2009). Each compound differs in its persistence in the soil, stability and toxicity (Borek *et al.*, 1995b; Manici *et al.*, 2000).

ITCs are produced rapidly after Brassicaceae tissues are disrupted (Morra and Kirkegaard, 2002). Their concentration in the soil peaks 30 min after incorporation and can be detected for up to 12 days (Gimsing and Kirkegaard, 2006). ITCs are highly volatile, and the shorter their side chain is, the more volatile they are (Brown and Morra, 1997). Due to their high volatility, their toxicity is assumed to spread around the point of chopping (Angus *et al.*, 1994). Only aliphatic and aromatic GSLs produce ITCs (Matthiessen and Kirkegaard, 2006), and they are recognized as the most biologically active products of GSL hydrolysis, with broad-spectrum activity (Fenwick *et al.*, 1983; Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006). ITCs are toxic because of their irreversible interaction with proteins, mainly nucleophilic reagents (Brown and Morra, 1997; Borek *et al.*, 1995a). The reaction damages the protein structure and functions of pest cells (Dufour *et al.*, 2015).

Despite the lower toxicity of the other products of GSL hydrolysis, they may also help control soilborne organisms and work synergistically with ITCs (Brown and Morra, 1997). Other non-GSL secondary metabolites, such as sulfur-containing organic compounds (*e.g.* sulfoxides, amino acids such as methionine and cysteine, sulfonium compounds) may also have toxic effects on soil organisms (Bending and Lincoln, 1999).

3 Increasing biofumigation effectiveness for sunflower production

With more than 350 genera (Beilstein *et al.*, 2006; Abideen *et al.*, 2013) and 3200 species (Abideen *et al.*, 2013), Brassicaceae present a wide scope for farmers to choose the most promising crops for effective biofumigation, based on their GSL concentrations and profiles, and biomass production (Sarwar *et al.*, 1998). Farmers can act at multiple levels to improve the biofumigation potential (Borek *et al.*, 1995b; Brown and Morra, 1997; Matthiessen and Kirkegaard, 2006; Gimsing and Kirkegaard, 2009):

- choice of Brassicaceae species;
- amount and profile of GSLs produced by the crop;
- rate of GSL conversion into ITCs;
- persistence of biocidal compounds in the soil.

3.1 The choice of the biofumigant Brassicaceae species

Morris *et al.* (2020) emphasized that species in the genus *Eruca* and *Raphanus* had the highest biofumigation effectiveness. However, most studies about biofumigation concern brown, white or Ethiopian mustard and rape (rapeseed and forage rape) (Sarwar *et al.*, 1998; Kirkegaard and Matthiessen, 2004; Reau *et al.*, 2005; Clarkson *et al.*, 2015). Brown mustard has high concentrations of sinigrin GSL, which hydrolyzes into 2-propenyl-ITCs. Considered as a highly toxic ITC (Motisi, 2009), it may explain brown mustard's promising results for crop protection (see part 4). The utility of choosing forage rape cultivars as a biofumigant crop was demonstrated by Gardiner *et al.* (1999), who studied products of hydrolysis after incorporation of cv. Dwarf Essex. Plants were incorporated using a rototiller at the bud-to-early-flowering stage. The most abundant product of hydrolysis measured in the soil was the 2-phenylethyl-ITC (2-PE-ITC), obtained from the aromatic 2-phenylethyl-GSL (2-PE-GSL), the main GSL in the roots of both cultivars. Smith and Kirkegaard (2002) demonstrated the toxicity of this ITC to pests. Moreover, Larkin *et al.* (2010)

measured a lower VW incidence on potato after forage rape (cv. Dwarf Essex) incorporation as green manure compared to a continuous potato (non-rotation) control. However, farmers harvest rapeseed crops to produce oil, so destroying them at the flowering stage and/or incorporating them as a green manure seems unrealistic in the context of oilseed crop production. The advantage of rapeseed would rely more on an allelopathic effect during development, with continuous production of ITCs by its living roots (Rumberger and Marschner, 2003) or after harvest, during roots decomposition (Reau *et al.*, 2005), both of which would provide a source of biocidal compounds (mainly ITCs) against soilborne fungi. Rumberger and Marschner (2003) demonstrated this phenomenon, observing that live roots of canola cv. Monty (low root GSL) and cv. Rainbow (high root GSL) released 2-PE-ITC continuously into the rhizosphere, which affected soil microbial communities (bacteria and eukaryotes) without accumulating in the soil. Despite the interest in rape for its allelopathic and, to a lesser extent, biofumigant effects, the trend since the 1960s has been to select and breed varieties with lower GSL concentrations. Thus, "double-low" varieties (*i.e.* low in erucic acid and GSLs) have been introduced (Boag *et al.*, 1990). GSLs may be undesirable or even toxic to mammals (rats and roe deer) when GSL concentrations increase in rape tissues (Fenwick *et al.*, 1983; Boag *et al.*, 1990). It is possible, however, to breed canola with higher 2-PE-ITC concentration without affecting shoot or seed GSL concentrations (Potter *et al.*, 2000). Since the GSL concentration necessary to have a toxic effect on soilborne pathogens remains unknown, low-GSL cultivars may still have biocidal effects (Couëdel *et al.*, 2019). For example, Kirkegaard *et al.* (2000) found no significant difference in the decrease in inoculum survival of the fungus *G. graminis* var. *tritici* between canola with high (cv. Tamara and cv. Karoo) and low (cv. Oscar and cv. Monty) root GSL concentrations, even though the pairs of varieties produced different 2-PE-ITC concentrations. In a pot experiment, Michel *et al.* (2008) showed that the number of live MS of *V. dahliae* in soils after the low GSL canola (cv. Talent) were approximatively 60 MS/g of soil, compared to that in an unamended control (approximatively 90 MS/g of soil), but the differences were not significant. To our knowledge, no study has examined the potential of rapeseed to control soilborne diseases of sunflower in field (through biofumigation and/or allelopathic effects). Seassau *et al.* (2016) observed, *in vitro*, a significant reduction in the germination or the development of *V. dahliae* (strains from sunflower) exposed to rapeseed (cv. Mosa), selected for its low GSL concentration compared to the unamended control.

Although most studies have focused on Brassicaceae green manures for biofumigation, seed meals could be used as an alternative strategy (Mazzola *et al.*, 2001) since they have more biological activity than green manures. GSLs are concentrated in the seeds and retained in the meal after crushing (Borek and Morra 2005). Thus, seed meals can be a source of GSLs (Brown and Morra, 1997; Morra and Borek, 2010) that stimulate soil microbial communities and suppress soilborne pathogens (Mazzola *et al.*, 2017). This alternative, however, would be better suited for small areas of crops with high commercial value than large areas of sunflower because of the high cost of seed meals.

3.2 Increasing GSL concentrations and profiles

A positive relation exists between GSL concentrations in Brassicaceae tissues and their ability to suppress pests and diseases during biofumigation (Morris *et al.*, 2020). The concentration and the profiles of GSLs (aliphatic, aromatic and indolyl) vary among Brassicaceae species (Kirkegaard and Sarwar, 1998; Bellostas *et al.*, 2004; Bhandari *et al.*, 2015) and between their shoots and roots (Kirkegaard and Sarwar, 1998; Van Dam *et al.*, 2009; Bhandari *et al.*, 2015). Roots usually have higher GSL concentrations than shoots, even though roots have lower biomass than shoots (Gimsing and Kirkegaard, 2006; Van Dam *et al.*, 2009; Bhandari *et al.*, 2015). This difference may be explained by a higher pathogen pressure belowground than aboveground (Van Dam *et al.*, 2009; Bhandari *et al.*, 2015). Biotic stress, such as herbivore damage and pathogen infection, increases GSL concentrations in Brassicaceae tissues (Van Dam *et al.*, 2009). It is important that biotic stress does not decrease biomass production too much, however, because a positive relation exists between Brassicaceae biomass and its GSL concentrations (Kirkegaard and Sarwar, 1998). A large amount of biomass is thus required for effective biofumigation (Clarkson *et al.*, 2015). Morris *et al.* (2020) predicted that less than 0.53 t dry matter of biomass/ha would result in ineffective biofumigation. Thus, it is important that cover crops be established well to maximize their biomass. While application of fertilizers (nitrogen and sulfur) increases GSL concentrations (Booth *et al.*, 1991; Li *et al.*, 2007), applying them to cover crops is neither recommended nor profitable.

The effectiveness of biofumigation also depends on the growth stage of the plant. During development of Brassicaceae, GSLs turn over or redistribute within its organs (Booth *et al.*, 1991). GSL concentration peaks at the early flowering stage in the whole plant, then it starts to decrease in shoots and roots and increase in the seeds, whose GSL concentration peaks at maturity (Booth *et al.*, 1991; Sarwar and Kirkegaard, 1998; Michel, 2008). Because seeds have much less biomass than shoots and roots, which decreases the amount of biomass available for biofumigation (Morris *et al.*, 2020), the optimal timing for biofumigation is at the maximum value of biomass × GSL concentration (Matthiessen and Kirkegaard, 2006). The recommended stage at which to destroy crops is thus flowering (Michel, 2008), which also has the advantage of avoiding seed-set.

3.3 Improving the conversion of GSLs into ITCs

For effective biofumigation, maximizing the hydrolysis reaction that converts GSLs into ITCs is crucial to generate high ITC concentration in the soil (Borek *et al.*, 1995b; Brown and Morra, 1997; Gimsing and Kirkegaard, 2009). Under laboratory conditions, Brassicaceae sometimes released only 19% of the total potential ITCs produced (Brown *et al.*, 1991). This conversion efficiency reached 62.5–100% for Brassicaceae seed meals in sterile sand (Neubauer *et al.*, 2015). In the field, the efficiency was estimated at 60% (Gimsing and Kirkegaard, 2006). The efficiency depends mainly on agronomic practices and soil and climate conditions. The stage of development of the Brassicaceae for biofumigation

must be considered, due to the decrease in myrosinase activity in mature tissues (Iversen and Baggerud, 1980). Brassicaceae tissues must be chopped finely to maximize contact between myrosinase and GSLs (Matthiessen and Kirkegaard, 2006). Thus, chopping at high speed and using hammers instead of blades is recommended (Matthiessen *et al.*, 2004; Michel, 2008). Dilution with large amounts of water is then crucial to ensure tissue maceration and soil moisture to hydrolyze GSLs into ITCs and other products (Matthiessen *et al.*, 2004; Michel, 2008; Gimsing and Kirkegaard, 2009). ITC concentration increased by up to 7–10-fold when 42 mm of water was added to a soil after biofumigation (Matthiessen *et al.*, 2004). However, Gimsing and Kirkegaard (2006) observed no difference after irrigating with 18 mm over 3 hours after biofumigation. Warmer temperatures also increase hydrolysis (Matthiessen and Kirkegaard, 2006; Michel, 2008; Gimsing and Kirkegaard, 2009). Matthiessen and Shackleton (2005) observed that the biological activity of 2-PE-ITC was significantly lower at 5 °C than at 10–20 °C. Consequently, farmers should carefully choose the day on which to perform biofumigation. Days with temperatures above 10 °C and with rain forecast to fall within a few days could improve the conversion of GSLs into ITCs, which would favor effective biofumigation. In the soil, a pH around neutral results in ITC production, while acid pH favors nitrile production (Brown and Morra, 1997).

3.4 Maximize persistence of ITCs in the soil

Un-hydrolyzed GSLs and the ITCs produced persist in soils from a few days to a few weeks (Brown and Morra, 1997), with the concentrations of GSL and ITC peaking 30 min after Brassicaceae incorporation (Gimsing and Kirkegaard, 2006) to 30 hours (Gardiner *et al.*, 1999). Maximizing the persistence of ITCs is crucial to increase the duration of exposure of soilborne pathogens, which increases biofumigation effectiveness (Borek *et al.*, 1995b; Brown and Morra, 1997).

The main pathway of ITC losses is volatility (Brown and Morra, 1997). To decrease these losses, solarization is used with vegetable crops to trap volatile ITCs (Morris *et al.*, 2020). This technique consists of covering the soil with transparent polyethylene sheets (Katan, 1981), but it is impractical over larger areas, such as those of oilseed crops. Thus, rapid incorporation of the chopped Brassicaceae is highly recommended (Gimsing and Kirkegaard, 2006; Michel, 2008). Sorption on soil components is another pathway of ITC loss. For example, ITCs had lower toxicity in soils with high organic matter content (>1%) (Gimsing and Kirkegaard, 2009; Neubauer *et al.*, 2014), which suggests that ITCs reacted with organic matter's nucleophilic reagents. Soil pH and texture had little influence on ITC persistence in the soil (Brown and Morra, 1997), unlike heavy rainfall (70–90 mm), which could cause ITCs to leach, thus reducing their persistence (Laegdsmand *et al.*, 2007).

Microbial degradation is a key factor that influences ITC losses in the soils (Brown and Morra, 1997). Using an autoclaved soil in biofumigation experiments increased the stability of ITCs (Rumberger and Marschner, 2003). Farmers have little influence on this factor, but soils that have never been fumigated may not experience increased biodegradation (Warton *et al.*, 2003). Because fumigation is used less often with oilseed crops than with vegetable crops, mainly because of the high cost of protecting large areas, soils of oilseed crops may not experience this increased biodegradation.

4 Suppressive effects of GSL products on the soilborne diseases of sunflower targeted

Under optimal conditions that maximize GSL concentrations, their conversion into ITCs and persistence in the soil, the effectiveness of biofumigation will depend greatly on the target species, since pathogens vary greatly in their sensitivity to ITCs (Brown and Morra, 1997; Smith and Kirkegaard, 2002). To assess the sensitivity of sunflower pathogens to biofumigation, this review focuses on laboratory or field experiments performed to evaluate suppressive effects of synthetic GSLs/ITCs or Brassicaceae incorporation on *V. dahliae*, *S. sclerotiorum* and *M. phaseolina* (Tab. 1). Since studies of sunflower protection using biofumigation are rare (to our knowledge), most studies concerned other plant hosts of these pathogens, mainly vegetable.

4.1 Experiment using synthetic ITCs/GSLs

In vitro studies of synthetic ITCs or synthetic GSL + myrosinase tested the sensitivity of pathogens and screened the most effective GSL profiles (Tab. 1, part a). Neubauer *et al.* (2014) tested five ITCs, all of which were lethal to *V. dahliae* MS. Aromatic ITCs (benzyl-ITC and phenylethyl-ITC obtained by Glucotropaeolin and Gluconasturtiin hydrolysis) were much more toxic than aliphatic ITCs. Among the same profiles of ITCs (aromatic or aliphatic), ITCs with lower molecular weight tended to be more effective than ITCs with higher molecular weight. To suppress *S. sclerotiorum*, aromatic ITCs were also more effective than aliphatic ITCs. Overall, benzyl-ITC was the most effective ITC against *S. sclerotiorum* mycelial development and sclerotia (Kurt *et al.*, 2011), while methyl-ITC and allyl-ITC were among the most effective ITCs at reducing mycelial growth (Kurt *et al.*, 2011; Ojaghian *et al.*, 2012). For *M. phaseolina*, mycelial development was also reduced by allyl-ITC (Mazzola *et al.*, 2017).

4.2 Experiments using Brassicaceae (*in vitro* or in pots)

To screen the potentially most effective varieties and/or species of Brassicaceae, and to assess effects of hydrolysis products of GSLs to manage soilborne fungi, experiments were performed using Brassicaceae biomass (*e.g.* crushed, ground, macerated) instead of synthetic compounds (Tab. 1, part b). To control *V. dahliae*, *S. sclerotiorum* and *M. phaseolina*, mustard varieties, especially *Brassica juncea* (brown/Indian mustard), were used mainly as a source of GSLs and ITCs in biofumigation studies. Mustard species often showed significant suppression of *V. dahliae* (Olivier *et al.*, 1999; Neubauer *et al.*, 2015; Seassau *et al.*, 2016), *S. sclerotiorum* (Ojaghian *et al.*, 2012; Rahimi *et al.*, 2014; Warmington and Clarkson, 2016) and *M. phaseolina* (Mazzola *et al.*, 2017). Some cultivars of turnip rape (*Brassica rapa*), forage radish

Table 1. Summary of the suppressive effects of GSLs/ITCs or Brassicaceae against three soilborne fungi of sunflower: *Verticillium dahliae*, *Sclerotinia slcerotiorum* and *Macrophomina phaseolina* using synthetic GSLs-/-ITCs *in vitro* or in pot (a), Brassicaceae *in vitro* or in pot (b), Brassicaceae in greenhouse and in the field (c), and at the rotation scale (d).
Target pathogen/plant: fungus studied and plant from which it was isolated (when mentioned); methods: GSLs/ITCs used or destruction/incorporation mechanisms of the Brassicaceae; Brassica species (cv./var.): the Brassicaceae and the cultivar or variety (when mentioned) used for biofumigation; crop to protect: the host plant; GSL/ITC measured: compounds in the Brassicaceae; 2-PE-ITC: 2-phenylethyl-isothiocyanate; a.o.t: among other treatments; Br: Brassicaceae; M.p: *Macrophomina phaseolina*; MS: microsclerotia; NA: not available; NS: not significant; S.s: *Sclerotinia sclerotiorum*; UC: unamended/untreated control; V.d: *Verticillium dahlia*; VW: *Verticillium* wilt.

(a) *In vitro* or in pot experiments using synthetic ITCs/GSLs

Target pathogen/plant	Methods	Main results	Reference
V. dahliae/strawberry	Soil infested with MS exposed to 3 aliphatic (methyl ITC, 2-propenyl ITC, 4-methylsulfinyl-3-butenyl-ITC) and 2 aromatics (benzyl-ITC, 2-PE ITC) *versus* UC	All ITCs suppressed MS Aromatic ITC were more toxic than aliphatic ITC	Neubauer *et al.* (2014)
V. dahliae/strawberry	22 natural soil and sterile quartz sand infested with MS exposed to 150 nmol/g of 2-propenyl-ITC *versus* UC	In sterilized soil: 100% of MS suppressed In natural soil: 9% to 92% of MS suppressed	Neubauer *et al.* (2014)
S. sclerotiorum/ mustard and lupin	S.s and other pathogens exposed to different concentrations of 2-PE-ITC *versus* UC	S.s had among the lowest tolerance to 2-PE-ITC than other pathogens	Smith and Kirkegaard (2002)
S. sclerotiorum/potato	Mycelium exposed to different concentrations of pure-ITC (methyl, allyl and butyl-ITC) *versus* UC	Reduction of the mycelial growth 100% of inhibition at the highest concentration of methyl and allyl ITCs	Ojaghian *et al.* (2012)
S. sclerotiorum/ various crops	S.s exposed to different concentrations of pure aliphatic ITC (methyl, allyl, butyl and ethyl) and aromatic (ethyl, phenyl, benzyl and 2-PE) *versus* UC	Methyl and benzyl-ITC reduced mycelial growth Benzyl-ITC reduced sclerotia viability All ITCs (except low concentration of phenyl and 2-PE) reduced the production of apothecial	Kurt *et al.* (2011)(*)
S. sclerotiorum/ various crops	Infested soils transplanted with pepper seedlings exposed to synthetic ITCs (Kurt *et al.*, 2011*)	Allyl and 2-PE ITCs reduced the incidence of S.s on pepper by 76.7% and 70% at low concentration, respectively	Kurt *et al.* (2011)
S. sclerotiorum/NA	Sclerotia of S.c or other pathogens exposed to different concentrations of synthetics GSLs (2-propenyl, 2-hydro-3-butenyl, benzyl, and methylsulfinylalkyl)	GSLs inhibited S.s growth Methylsulfinylalkyl was the most effective	Manici *et al.* (1997)

(b) In vitro and pot experiment studies using Brassicaceae

Target pathogen	Methods	Brassica species (cv./var.)	GSL/ITC measured	Main results	Reference
V. dahliae/sunflower	Mycelium or MS exposed to shoots and roots of Br sampled at mid-flowering and grinded separately *versus* UC	*B. juncea* (Etamine), *S. alba* (Abraham), *B. rapa* (Avalon), *B. napus* (Mosa), *R. sativus* (Anaconda)	Main GSL measured in shoots and roots: *S. alba*:4-hydroxybenzyl, *B. napus*: 2-PE, *B. juncea*: 2-propenyl, *B. rapa*:4-pentyl, 2-PE and 1-methoxy-3-indolylmethyl	Br reduced mycelial growth (*B. juncea*, the most effective) and MS germination (*B. rapa*, the most effective)	Seassau *et al.* (2016)
V. dahliae/eggplant and cotton	Mycelium exposed to powdered tissues of Br	*B. oleracea* (caulorapa)	NA	1 g of *B. oleracea* reduced mycelial growth by 68.7%	Fan *et al.* (2008)
V. dahliae/NA	Mycelium exposed to macerated leaf and stem of Br sampled at flowering *versus* UC	28 cv. of *B. nigra* and 35 cv. of *B. juncea*	Main ITC measured in shoots: allyl, 2-PE, benzyl, 3-butenyl	Br reduced the radial growth of V.d and reached 100% for 19 cv. of *B. nigra* and 20 cv. of *B. juncea*	Olivier *et al.* (1999)
V. dahliae/strawberry		19 cv. of *B. juncea*, *R. sativus* and *S. alba*	Main GSLs measured in shoots: *B. juncea*:		Neubauer *et al.* (2014)

Table 1. (continued).

	Sterile quartz sand infested with MS amended with freeze-dried ground Br sampled at mid-flowering or non-Br species *versus* UC		2-propenyl, *S. alba*: benzyl, *R. sativus*: 4-methylthio-3-butenyl	Shoot of *B. juncea* was the most efficient to reduce viable MS (69.3 to 81.3%) than other Br or UC	
V. dahliae/strawberry	MS exposed to seed meals of Br or autoclaved seed meals *versus* UC	16 cv. of *S. alba, B. carinata*, *B. juncea*, *B. napus*	Main GSL/ITC measured in seeds: *S. alba*: 4-hydroxybenzyl, *B. napus*: 3-butenyl, 4-pentenyl, 2-PE *B. juncea* and *B. carinata*: 2-propenyl-GSL	Seed meals of *B. juncea* and *B. carinata* reduced viable MS by 92.4 to 100%. NS effects of *S. alba* and *B. napus*	Neubauer *et al.* (2015)
V. dahliae/soils naturally infested	Infested soil amended with grinded shoots of Br sampled at flowering and soils heavily watered *versus* UC (a.o.t)	*B. juncea* (ISCI-99, ISCI-20– high GSL); *B. napus* (Talent – low GSL)	NA	Living MS were reduced by 66% with ISCI-99, 55% with ISCI-20. NS effect of Talent	Michel *et al.* (2008)
S. sclerotiorum	S.s exposed to powder of Br (shoot, root, seeds) dried, hydrolysed and freeze *versus* UC	*B. juncea* (Cutlass), *B. rapa* (Parkland, Echo), *B. napus* (Hyola401, RGS003)	Main ITC measured in shoots: *B. juncea* (Cutlass): allyl	*B. juncea* (Cutlass) was the most effective to inhibit radial growth	Rahimi *et al.* (2014)
S. sclerotiorum/potato	Mycelium and sclerotia exposed to macerated or irradiated dried tissues of Br (shoots and roots sampled at the 10-leaf stage) *versus* non-Br or UC	*B. napus* (Mettah), *B. juncea* (Bresska), *B. campestris* (Orrega)	Main ITC measured: methyl, allyl, butyl	All Br reduced mycelial growth and sclerotia formation *B. juncea* was the most effective	Ojaghian *et al.* (2012)
S. sclerotiorum/oil rape	Mycelium exposed to powdered tissues of Br	*B. oleracea* (Caulorapa)	NA	1 g of *B. oleracea* reduced mycelial growth by ~20%	Fan *et al.* (2008)
S. sclerotiorum/lettuce	Mycelium and sclerotia exposed to a dry powder of Br sampled at flowering *versus* UC (a.o.t)	*B. juncea* (Vittasso, Pacific Gold, Caliente 99), *B. napus* (Temple – low GSL control), *E. sativa* (Nemat), *R. sativus* (Terranova), *S. alba* (Brisant), Biofence	Main GSLs measured: *B. juncea*: 2-propenyl, *S. alba*: 4-hydroxybenzyl, *R. sativus*: 4-methylsulfinyl-3-butenyl, *E. sativa*: 4-methylthiobutyl	All Br (especially *R. sativus*) reduced germination of S.s *B. juncea* were the most effective to inhibit mycelial growth	Warmington and Clarkson (2016)
M. phaseolina/NA	Infested soil amended with mustard cake *versus* UC (a.o.t)	*B. juncea* (NA)	NA	Reduction of M.p by 100% after 30 days	Sharma et al., 1995
M. phaseolina	Infested soil pasteurized or non-pasteurized amended with seed meals of Br *versus* UC	*B. juncea* (Pacific Gold), *B. napus* (Athena), *S. alba* (NA)	Main GSLs measured: *B. juncea*: 2-propenyl, *B. napus*: 3-butenyl, *S. alba*: 4-OH-benzyl	Non-pasteurized soils: inconsistent reduction of M.p density, reduction of roots infection of strawberry Pasteurized soils: M.p density increased	Mazzola *et al.* (2017)

(c) Field and greenhouse studies

Target pathogen	Methods	Brassica species (cv./var.)	Crop to protect (cv./var.)	GSL/ITC measured	Main results	Reference
V. dahliae	1 field/2 years: Br chopped at early flowering, incorporated and the soil was compacted *versus* bare soil (UC)	*B. juncea* (Etamine), *R. sativus* (Anaconda), *B. rapa* (Chicon)	Sunflower (Kapllan)	Measured in shoots and roots: overall during the 2 years: *B. juncea*: 2-propenyl, *R. sativus*: 4-methylthio-3-butenyl and 1-methoxy-3-indolylmethyl, *B. rapa*: 2-hydro-3-butenyl and 1-methoxy-3-indolylmethyl	Br reduced VW severity both years *R. sativus* was the most effective (DSI = 37% and 28%) *versus* UC (DSI = 80% and 48%)	Galaup *et al.* (pers. comm.)
V. dahliae	1 greenhouse/1 year: soils infested with MS sampled from fields exposed to biofumigation (Pinkerton *et al.*, 2000*) *versus* sterile soils (UC)	*B. napus* (Dwarf Essex)	Eggplants (Imperial Black Beauty)	NA	Br reduced eggplants biomass compared to UC	Pinkerton *et al.* (2000)

Table 1. (continued).

V. dahliae	1 field/2 years: Br cut at ground level, chopped, spread and rotovated below 25 cm depth, irrigated (field capacity) compared to non-Br species and bare soil, all treatments were solarized or non-solarized (a.o.t)	*B. napus* (Dwarf Essex)	Norway Maple trees	NA	Br combined with solarization reduce VW severity compare to Br sole crop	Pinkerton *et al.* (2000)*
V. dahliae	1 field/2 years: broccoli residue chopped, incorporated, and disked *versus* UC (a.o.t)	*B. oleracea* (italica)	Cauliflower (White Rock)	NA	MS densities decreased after Br compared to initial densities (50 to 75% reduction) VW severity was lower after Br compared to UC The plant height, the number of harvestable heads and the weight of total harvest increased compared to UC	Subbarao *et al.* (1999)
V. dahliae	6 field trials/2 years: Br flail-mowed, incorporated both years and rolled; sprinkler-irrigated the second year compared to non-Br species and bare soil (UC) (a.o.t)	*B. napus* (Humus), *B. juncea* (Pacific Gold), *S. alba* (Ida Gold, ISCI 20), Caliente (*B. juncea* × *S. alba*)	Tomato (Halley)	For above ground biomass during one year: *B. juncea*: 2-propenyl, *S. alba*: benzyl 4-hydroxybenzyl	NS suppressive effect on V.d in the soil Overall, no effect on tomato fruit productivity in the six field trials compared to bare soil	Hartz *et al.* (2005)
V. dahliae	1 field/3 years: Br chopped at flowering and incorporated into the soil *versus* UC (a.o.t)	*B. juncea* (ISCI20)	Grafted eggplants (Prosperosa)	NA	Partial results of biofumigation Biofumigation combined with grafting was more efficient	Garibaldi *et al.* (2009)
V. dahliae	2 fields/1 year: fresh cauliflower residues disk-incorporated twice below 25–30 cm depth and irrigated *versus* UC (a.o.t)	*B. oleracea* (Marine)	Artichoke (Blanca de Tudela)	NA	MS densities remained low compared to UC (NS) Inconsistent effects of Br residue on disease incidence, severity, and yield	Berbegal *et al.* (2008)
V. dahliae	2 fields/2 years: Br compared to non-Br species and UC	*B. oleracea* (Excelsior)	Potato (Russet Burbank)	NA	Br reduced V.d inoculum by 50% and VW by 69% at highest rate NS effect on root infection and yield compared with UC	Ochiai *et al.* (2007)
V. dahliae	1 field/1 year: Br incorporated at flowering with a rototiller (twice) compared to non-Br species and UC	*B. juncea* (ISCI-20)	Strawberry (Elsanta)	NA	Reduction of MS by 19% compared with UC	Michel *et al.* (2008)
V. dahliae	2 farms/1 year: Br finely mulched at flowering and incorporated with a rototiller *versus* UC (a.o.t)	*B. juncea* (ISCI-20)	Sweet pepper (Red beefhorn, Somborka)	NA	Overall, reduction of MS in both farms (48% to 74%)	Michel *et al.* (2008)
V. dahliae	1 Greenhouse/1 year: dried Br sampled at full flowering, incorporated below 20 cm depth, irrigated (35 mm), compared to non-Br and UC (a.o.t)	*B. juncea* (ISCI-99 and Etamine)	Tomato (Admiro)	Methylsulfinylalkyl, benzyl, 2-propenyl, and 2-hydro-3-butenyl	Short-term: NS effect on MS reduction Long-term: MS reduced by 80%	Michel (2014)
V. dahliae	1 greenhouse/1 year: biofence expanded on soil surface (250 g/m^2), incorporated below 20 cm depth, irrigation (20 mm water + biofence flowable) 6 times, compared to non-Br and UC (a.o.t)	Biofence	Tomato (Admiro)	NA	NS effect of biofence and biofence FL	Michel (2014)

Table 1. (continued).

M. phaseolina	1 field/2 years: mustard oil cake amendment or mustard residues mixed (hand spade), incorporated below 30 cm depth, irrigated or not *versus* UC (a.o.t)	*B. juncea* (Pusa bold)	Cluster bean	NA	Reduction of M.p and dry root rot Mustard oil cake was more effective by 38% than mustard residues	Mawar and Lodha (2002)
M. phaseolina	1 field/2 years: Seed meal incorporated, and plots irrigated (surface saturation)	*B. juncea* (Pacific Gold), *B. alba* (Ida Gold)	Strawberry (Camarosa)	NA	NS effect on strawberry plant biomass, total number of fruit produced and total fruit biomass	Mazzola *et al.* (2017)

(d) Rotation scale studies

Target pathogen	Methods	Brassica species (cv./var.)	Crop to protect	GSL/ITC measured	Main results	Reference
V. dahliae	10 years of 2-year rotation with potato−Br (1 × Br−1 × P) Br was either incorporated as green manure (Dwarf Essex) or harvested without incorporation (canola) compared to non-Br Crops and continuous potato (1 × P−1 × P)	*B. napus* (canola), *B. napus* (Dwarf Essex)	Potato (Russet Burbank)	NA	Overall, rapeseed reduced VW and canola had inconsistent effects Higher tuber yields after Canola (+6.8%) compared to continuous potato, and inconsistent effects of rapeseed	Larkin *et al.* (2010)
V. dahliae	7 years with potato−Br rotation (3 × Br−2 × P−1 × Br−1 × P) Br was incorporated into the soil by disking or rotovating compared to non-Br species and barre soil	*B. napus* (Dwarf Essex and Bridger)	Potato (Russet Burbank)	NA	Inconsistent effects of Br on V.d population in the soil Reduction in VW NS differences of the yield compared to bare soil (see Davis *et al.*, 1996)	Davis *et al.* (2010)
V. dahliae	5 years with potato−Br rotation (3 × Br−2 × P) Br was incorporated into the soil by disking or rotovating compared to non-Br species and barre soil (UC)	*B. napus* (Dwarf Essex and Bridger)	Potato (Russet Burbank)	NA	Overall, NS effects on V. d and yield Reduction in VW	Davis *et al.* (1996)
V. dahliae	2 fields with strawberry−Br rotation compared to non-Br rotation (a.o.t) Br was harvested and residues flailed shredded, air dried on the soil surface for 48 h and incorporated into the soil below 15–20 cm depth with a rototiller	*B. oleracea* (Marathon), *B. oleracea* (Oliver)	Strawberry (Selva)	NA	Reduction of MS density (up to 83%), and VW severity in the rotation with Br Increase of strawberry growth	Subbarao *et al.* (2007)

(*Raphanus sativus*), Kohlrabi (*Brassica oleracea* cv. caulorapa) and *B. napus* were among the most effective species, but were more variable than *B. juncea*. In these studies, anti-fungal effects of ITCs and other products of GSL hydrolysis were assessed on mycelial growth and/or the long-term survival structures of the pathogens. Effectiveness of Brassicaceae varied among the forms of the pathogens. Seassau *et al.* (2016) showed that mycelial growth of *V. dahliae* isolated from sunflower was suppressed mainly by *B. juncea*, while MS germination was suppressed mainly by *B. rapa*. Since biofumigation occurs a few months before sunflower sowing, its suppressive effects would affect long-term survival structures of pathogens because of the low persistence of GSLs and ITCs.

4.3 Field approaches to biofumigation

In vitro and pot studies have shown promising biocidal effects on *V. dahliae*, *S. sclerotiorum* and *M. phaseolina*. In field conditions, however, results varied more among studies (Tab. 1, part c), due to the many factors that influence the effectiveness of biofumigation. The only study of sunflower crop protection reported a significant reduction in VW incidence and severity following three Brassicaceae cover crops and biofumigation compared to that with a bare soil (Galaup *et al.*, pers. comm.). In both years of its field experiment, *R. sativus* was the Brassicaceae that reduced VW incidence the most, followed by *B. rapa* and *B. juncea*. The ability of biofumigation with a given species to reduce VW varied between years due to differences in the biomass incorporated into the soil each year. The largest reduction in VW was associated with the largest biomass produced. In strawberry (*Fragaria* × *ananassa*) field experiments, Michel *et al.* (2008) observed a significant reduction of MS in soils after biofumigation with *B. juncea.* Conversely, Hartz *et al.* (2005) considered *B. juncea* an ineffective biofumigant: it did not decrease the density of *V. dahliae* in the soil and had no effect on tomato productivity compared to a fallow control. Michel (2014) observed no significant effects of *B. juncea* on *V. dahliae* density in the soil, in the short-term, but a reduction of 80% was observed a few months after biofumigation. Because of the low persistence of ITCs, they could not have caused this suppressive effect. Instead, the reduction in MS may have been caused by stimulation of specific groups of microbial communities during mustard decomposition and organic matter addition, as supported by other studies (Mazzola *et al.*, 2007; Ochiai *et al.*, 2008; Mazzola *et al.*, 2017). Thus, organic inputs could improve soil biological status by increasing both the diversity and size of populations of beneficial species through physico-chemical changes (Ochiai *et al.*, 2008; Davis *et al.*, 2010; Omirou *et al.*, 2011).

5 Non-GSL-related suppressive effects on pathogens, and the multifunctionality of Brassicaceae

Pathogen suppression by green manure addition has been attributed to indirect effects of higher microbial competition rather than a direct effect on pathogen inoculum (Davis *et al.*, 1996; Davis *et al.*, 2010). This involvement of microbial communities was supported by long-term studies at the rotation scale when Brassicaceae and non-Brassicaceae species were incorporated (Tab. 1, part d). Davis *et al.* (2010) observed that cover crops reduced VW on potatoes more than fallow did. Sudangrass (*Sorghum vulgare* var. sudanense cv. Monarch) was a more effective cover crop than *B. napus* cv. Dwarf Essex and cv. Bridger. The authors also suggested that another beneficial effect of sudangrass was the potential control of root knot nematodes. Larkin *et al.* (2010) also observed a significant reduction in VW on potato after a canola cover crop and to a lesser extent after a rapeseed cover crop. Davis *et al.* (2010) and Larkin *et al.* (2010) concluded that, beside the direct toxic effects of products of GSL hydrolysis, VW may have been suppressed due to a change in microbial communities that increased microbial competition after cover crop incorporation. The reduction in VW may be explained by the increase in *Fusarium equiseti* in the soil observed by Davis *et al.* (2010), which suggests a potential antagonism between the two fungi.

Increasingly, biofumigation benefits are considered along with other green manure benefits, such as addition of organic matter to soils (Kirkegaard and Matthiessen, 2004). This non-GSL-related pathway of suppression may be involved in reducing pathogens and disease severity in the studies that used low-GSL cultivars observed by Kirkegaard *et al.* (2000) and Michel *et al.* (2008). The potential key role of microbial communities in suppressing pathogens emphasizes the need to assess potential disservices of products of GSL hydrolysis on these beneficial communities. To date, these disservices and their influence have been rarely studied, and the review of Couëdel *et al.* (2019) reported inconsistent impacts of Brassicaceae on non-target species. No effect of Brassicaceae incorporation was observed on nitrifying bacteria in field studies (Omirou *et al.*, 2011). Conversely, Bending and Lincoln (2000) observed that application of ITCs disrupted microbial communities, reducing the growth of nitrifying bacteria in clay-loam soils. One mechanism to avoid these potential disservices could be cover crop mixtures (Couëdel *et al.*, 2019), which would provide more nutrients, and thus increase microbial diversity and activity, while preserving GSL production. Couëdel *et al.* (2018c) showed that, compared to Brassicaceae sole crops, 50/50 bi-species mixtures of Brassicaceae and Fabaceae reduced GSL production/ha by an average of only 19%. Mixtures would maintain most of the potential of Brassicaceae to suppress pathogens and could mutualize other benefits provided by either Brassicaceae or Fabaceae. Couëdel *et al.* (2018a) showed that this mixture captured the same amount of nitrate as Brassicaceae alone and had a larger nitrogen-green manure effect. This mixture also provided the same sulphate-catch crop and sulfur-green manure effects as Brassicaceae sole crops (Couëdel *et al.*, 2018b). This result could be due to increased biomass production and abiotic-resource-use efficiency (Jensen, 1996). Besides protecting against pathogens and nutrient enrichment, mixtures provide a bundle of ecosystem services. They reduce soil disturbance and erosion, maximize water-use efficiency, increase long-term carbon sequestration and support pollinators and other beneficial insects (Therond *et al.*, 2017; Justes and Richard, 2017; Chapagain *et al.*, 2020). Thus, mixtures of Brassicaceae and

Fabaceae could increase sunflower productivity. Combining cover cropping of mixtures with biofumigation represents a holistic multi-pest protection approach that relies on several ecological mechanisms, which is in line with the principles of IPM. Besides diversifying rotations (which may provide break-crop effects), encouraged by IPM Principle 1 (P1), it is a major environmentally friendly protection method (Lazzeri *et al.*, 2004). It may prevent reliance on the synthetic compounds used in fumigation (Clarkson *et al.*, 2015), and thus fulfill the principles of giving preference to non-chemical methods (P4), selecting pesticides to avoid undesired impacts of broad-spectrum fumigants on non-target beneficial communities (P5) and reducing pesticide use (P6). Some of biofumigation's ability to protect of sunflower would be due to the ITCs produced by Brassicaceae, and developing resistance to them is highly improbable because of the complex cluster of chemically different components involved (Ntalli and Caboni, 2017). Moreover, ITC toxicity varies among pests (Smith and Kirkegaard, 2002), which could allow for specific actions on targeted pests (P5). The potential increase in some antagonist fungi (*e.g. Fusarium* spp., as reported by Davis *et al.* (2010)) after incorporating cover crops represents another ecological mechanism to suppress soilborne pathogens (Médiène *et al.*, 2011), which could be enhanced by including Fabaceae in mixtures, because it could diversifies the tissues incorporated (Couëdel *et al.*, 2019).

Some principles of IPM, however, could be difficult to implement for soilborne fungi of sunflower. Monitoring microscopic and heterogeneous pathogens such as *V. dahliae*, as recommended by P2, would be too expensive. Thus, it is challenging to determine thresholds for intervention (P3). Nevertheless, biofumigation could still help farmers reach the underlying objectives of IPM: minimize use of broad-spectrum biocides, environmental contamination, disruption of beneficial communities and development of resistance (Matthiessen and Kirkegaard, 2006; Barzman *et al.*, 2015).

6 Conclusion

Soilborne diseases threaten sunflower productivity. VW, sclerotinia head and stalk rots, and charcoal rot have been expanding worldwide in the past several years or could be in the future. They are challenging to manage because of their ability to survive in the soil and the lack of sustainable effective control methods. Thus, biofumigation could be an interesting agroecological alternative for protecting sunflower, especially as a part of IPM. This review showed that multiple factors must be considered for effective biofumigation. For sunflower production, a biofumigant crop can be grown during the fallow period just before sunflower. Ideally, the Brassicaceae should be chopped at early flowering, temperatures of ca. 10 °C minimum and just before a rainy period, since high temperatures and soil water content increase the hydrolysis of GSLs into ITCs. Brassicaceae should be incorporated quickly into the soil after pulverization to reduce volatile losses.

For effective suppression by biofumigation, Brassicaceae with high GSL concentrations are recommended. The types of GSLs/ITCs produced by Brassicaceae are also important to consider, since the biocidal effect of GSLs depend on the target pathogen. According to the ITCs tested and the Brassicaceae incorporated, long-term survival structures and mycelia of *V. dahliae*, *S. sclerotiorum* and *M. phaseolina* were susceptible most of the time.

While aromatic ITCs and mustards seem to be the most effective, an increasing number of studies emphasize non-GSL-related effects of Brassicaceae and non-Brassicaceae cover crops. Nutrient enrichment after incorporating cover crops has strong effects on microbial communities that may stimulate antagonist species of pathogens in the soil. These effects are supported by studies that show negative correlations between microbial activity/diversity and the incidence of symptoms. The potential key role of microbial communities in the suppressive effect of Brassicaceae incorporation could explain the positive results obtained with Brassicaceae with low GSL concentration, such as canola. This highlights the need to assess effects of Brassicaceae incorporation on beneficial communities precisely, since the results to date are scarce and inconsistent. Nonetheless, cover crop mixtures that include Fabaceae could be an interesting mechanism to avoid potential disservices to beneficial communities, while maintaining suppressive effects on target pathogens. Further research, including field experiments, are needed to confirm the benefits of these mixtures.

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