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Assessment of the potential of biotic regulation by Brassica cover-crops used as biofumigants. Case of *Verticillium dahliae* affecting Sunflower crop in southwestern France.

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LIST OF ABBREVIATIONS

MS: Microsclerotia

GSL: Glucosinolate

ITC: Isothiocyanate

BS: Bare soil

BM: Brown mustard

TR: Turnip rape

FR: Fodder radish

SB: Shoot biomass

RB: Root biomass

SBS: Sunflowers preceded by an uncropped fallow period (Bare soil)

SBM: Sunflowers preceded by brown mustard as cover crop during the fallow period

STR: Sunflowers preceded by turnip rape as cover crop during the fallow period

SFR: Sunflowers preceded by fodder radish as cover crop during the fallow period

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1. INTRODUCTION

Verticillium dahliae Kleb. is the causal agent of an economically important soil-borne plant disease in the world affecting more than 400 plant species including cultivated crops and weeds, with a continuously expanding host range (EFSA, 2014). In Occitanie, the France's leading sunflower producing region in terms of acreage, Sunflower Verticillium Wilt (SVW) spread across the region since 2013 because of a depletion of spatial and temporal biodiversity and related provision of ecosystem services, e.g., pest and disease control, caused by the high prevalence of the durum wheat-sunflower rotation (Altieri, 1999; Duru et al., 2015; Kremen et al., 2012), resulting in approximately 42% of sunflower fields contaminated in the region in 2017, with 9% of plants severely affected (Chambre d'agriculture Occitanie, 2017). Symptoms of the disease appear first when a sunflower crop approaches the bud stage. In the soil, the germination of fungal resting structures, the microsclerotia (MS), stimulated by host root exudates, results in hyphae penetrating the sunflower roots and reaching the xylem vessels. The fungal elements move up acropetally through the xylem, which causes vascular discoloration and the progressive apparition of limb interveinal chlorosis and necrosis. As the vessel elements become obstructed, water and nutrients flows are hindered and sunflowers display a characteristic wilted appearance. At physiological maturity, black longitudinal stripes can appear on sunflower stems. Subsequent premature death and reduction in head size, thousand-kernel-weight and oil content may lead to yield losses up to 2 Mg ha^{-1} (Lecompte et Mestries, 2012).

As a consequence of the use of resistant cultivars over large areas in the world as the main disease management strategy, the genetic resistance provided by the dominant *Ve1* gene, the most effective way to control SVW (Fradin et al., 2009; Quiroz et al., 2008; Yadeta et Thomma, 2013) starts being circumvented as this is generally the case when disease management strategies lack diversification (Finckh, 2008). Also due to its high genetic plasticity, *Verticillium dahliae* has been reported to have overcame the *Ve1*-mediated genetic resistance in sunflowers in the USA (Gulya, 2004), in Argentina (Radi et Gulya, 2006), Canada (Gulya, 2007) and Europe (García-Ruiz et al., 2014). Although new sources of resistance have been found for sunflower crop (Missonnier et al., 2017; Radi et Gulya, 2006),

few alternative and efficient management strategies are available, especially since the interdiction of chemical fumigants, e.g., methyl bromide prohibited in 1993 by the Montreal Protocol on Substances that Deplete the Ozone Layer. In Occitanie, sunflower crop is grown as cash crops every two years in most of the farms and cultural control strategies, e.g., crop rotation and removal of crop residues, are not effective in this short term since the fungus produces numerous dark MS that ensure the survival of the pathogen for up to 14 years without the presence of any sensitive host (Wilhelm, 1955; Hu et al., 2013). The pathogen is also able to persist and produce inoculum on roots of common crops and weeds in an endophytic and asymptomatic manner which makes it even more difficult to control (Malcolm et al., 2013; Wheeler et Johnson, 2016). Physical control strategies, e.g., soil solarization, amending the soil with high carbon (C), nitrogen (N) or volatile fatty acids-containing materials, can be effective to control the disease by decreasing soil inoculum density but are hardly applicable as large-scale strategies (Berbegal et al., 2008; Goicoechea, 2009; Tenuta et Lazarovits, 2004, 2002a). Biological control techniques that include the use of fungal antagonists (Jabnoun-Khiareddine et al., 2009; Narisawa et al., 2002; Yuan et al., 2017), mycorrhizal fungi (Karagiannidis et al., 2002; Liu, 1995), and antagonist rhizobacteria (Berg et al., 2001, 1994) are currently being developed but few are available for field application (Fradin et Thomma, 2006; Klosterman et al., 2009). Therefore, sustainable strategies aiming at enriching and complementing the current panel of management practices for controlling verticillium wilt are needed (Finckh, 2008; Korthals et al., 2014). Among them, biofumigation, performed by the subsequent cultivation, grinding and incorporation of brassica cover crops, is a technique aiming at mitigating soil-borne pests and pathogens by brassica rotation or green manure crops (Kirkegaard et al., 1993) as already demonstrated for several diseases (Arthy et al., 2005; Larkin et al., 2011; Motisi et al., 2009; Rosa et Rodrigues, 1999), pests (Matthiessen et Kirkegaard, 2006) and weeds (Haramoto et Gallandt, 2005; Petersen et al., 2001). Based on the valorization of ecological processes and ecosystem services, this technique is also viewed as an agroecological pest and disease management practice that has the potential to foster transition towards sustainable agriculture by substituting agricultural inputs, e.g. chemical soil fumigants, and redesigning

agroecosystems (Debaeke et al., 2017a; Hill et MacRae, 1996; Reddy, 2017; Wezel et al., 2014). Biofumigation is particularly suited for rotations with long fallow periods, up to nine months for the durum wheat-sunflower rotation, during which brassica cover crops also supply several ecosystem services including carbon sequestration, enhanced nutrient cycling, soil stabilization, and biodiversity conservation (Altieri, 1999; Constantin et al., 2011; Eriksen et Thorup-Kristensen, 2002; Hartwig et al., 2002). As sunflower crop requires low amounts of agricultural inputs, supplies important sources of pollen and nectar for pollinators (Debaeke et al., 2017a; Delaplane et Mayer, 2000), performs well when managed under certain agroecological practices, e.g., intercropping with soybean (Lande et al., 2012; Tribouillois et al., 2012), and is potentially adaptable to climate change (Debaeke et al., 2017b), the previous cultivation of brassica biofumigant crops could contribute to further increasing the resilience of the durum wheat-sunflower rotation by enhancing its spatial and functional biodiversity and resource use efficiency (Altieri et al., 2015), and potentially contributing to SVW mitigation. The disinfection service provided by biofumigation is thought to be related to secondary metabolites inherent to the brassica family, the glucosinolates (GSLs), a cluster of 132 molecules usually classified as aliphatic, aromatic or indole depending on the structure of their side chain (Agerbirk et Olsen, 2012). Both exuded in the rhizosphere during cover-crop cultivation and released at their destruction in larger quantities (Motisi et al., 2009), GSLs are degraded by myrosinase enzyme into various biocide compounds, e.g., Isothiocyanates (ITCs), thiocyanates, nitriles, oxazolidine-thione and epithionitriles, whose proportion and type is determined by the GSL type from which they are synthesized (Radojčić Redovnikovic et al., 2008; Vaughn et Berhow, 2005).

The types of GSLs and their concentration in brassica tissues vary upon genetic (species and cultivar), biotic (pathogen pressure) and abiotic (photoperiod, soil type, pH) environmental and crop management factors (date of sowing and destruction, fertilization, and irrigation) (Björkman et al., 2011). After incorporation, the success of biofumigation mainly depends on environmental conditions (soil organic matter and water content and pH) and management practices (incorporation depth and grinding size) as they will determine the proportion of biocide compounds reaching the targeted soil

79 borne pathogen (Couëdel et al., 2017; Gimsing et Kirkegaard, 2009; Michel, 2008). Although results
80 from in vitro studies demonstrated a biocidal activity of ITCs on *V. dahliae* (Neubauer et al., 2015, 2014;
81 Olivier et al., 1999; Zurera et al., 2009), their relationship with disinfection is however difficult to
82 confirm because in-field studies that investigated the biofumigation potential of brassica to control
83 *V.dahliae* provide little information on the type or concentration of GSLs and yielded inconsistent
84 results (Berbegal et al., 2008; Blok et al., 2000; Davis et al., 1996; Korthals et al., 2014; Subbarao et al.,
85 1999). In addition, the potential of biofumigation to control SVW in field conditions has never been
86 documented.

87 Therefore, the aim of this research was to assess the potential of biotic regulation of *Verticillium*
88 *dahliae* affecting sunflower by implementing biofumigation with brown mustard, turnip rape and
89 fodder radish cover crops compared to uncropped fields during the interculture period of a durum
90 wheat-sunflower rotation. To do so, the effect of biofumigation performed with each brassica cover
91 crop on (1) primary inoculum of *Verticillium dahliae*, (2) SVW incidence and severity over the growing
92 season, and (3) sunflower yield was evaluated in two field trials in southwestern France during three
93 consecutive years. Additionally, a bioassay was performed under semi-controlled conditions to
94 measure the survival of *Verticillium dahliae* inoculum in biofumigated soil. The hypothesis was that
95 biofumigation reduces inoculum, SVW incidence and severity and increases sunflower yield.

96 ## 2. MATERIAL AND METHODS

97 ### *2.1. Experimental design and crop management*

98 From 2015 to 2017, two field trials were set up at INRA's experimental station in Auzeville
99 (southwestern France) according to the following rotation: durum wheat, brassica cover crop,
100 sunflower. Both fields had a history of severe SVW. The field experiments conducted from 2015 to
101 2016 and from 2016 to 2017 are referred as AUZ16 and AUZ17, respectively. AUZ16 was on a loamy
102 soil with pH 7,0 and 1,6% organic matter whereas AUZ17 was on a clay-loamy soil with pH 8,2 and 1,4%
103 organic matter.

Three species of brassica cover crops were selected based on their ability to grow rapidly in the short autumn fallow period and on their contrasted profile in GSLs with brown mustard (*Brassica juncea (L.) Czern cv. Etamine*) being rich in aliphatic and aromatic GSLs, fodder radish (*Raphanus sativus (L.) cv. Anaconda*) rich in aliphatic GSLs and turnip rape (*Brassica rapa (L.) subsp Oleifera cv. Chicon*) containing all three types (Seassau et al., 2016). AUZ16 and AUZ17 were, respectively, arranged in one and three blocks composed of four treatments including bare soil (BS), brown mustard (BM), turnip rape (TR) and fodder radish (FR). The size of each plot was 6 m x 120 m for AUZ16 and 6 m x 50 m for AUZ17.

Cover crops were sown on September 8th and 5th in AUZ16 and AUZ17, respectively. Sowing densities for fodder radish were 10 kg ha^{-1} for both fields, and they were 4 and 5.3 kg ha^{-1} for BM, 5.3 and 4 kg ha^{-1} for TR for AUZ16 and 17, respectively. In AUZ16, a molluscicide (©Sluxx) was applied twice at a rate of 8 kg ha^{-1} and an insecticide was applied once (©Mavrik Flo) at a rate of 0.3 l ha^{-1}. No pesticides were applied in AUZ17. Cover crops were not irrigated in AUZ16 due to sufficient rainfall whereas one irrigation was performed in September in AUZ17 (33 mm m^{-2}) to ensure homogeneous establishment. Near flowering stage, cover crops were ground and incorporated, and the soil was subsequently compacted on December 7th and 8th for AUZ16 and AUZ17 respectively.

A sunflower cultivar susceptible to Verticillium wilt (*Helianthus annuus cv. Kapllan*) was sown on April 28th and April 20th for AUZ16 and AUZ17 respectively with a density of 7 plants m^{-2} (50 cm row width). Herbicides were sprayed twice in AUZ16 (©Mercantor Gold at 30 l ha^{-1} and ©Racer ME at 2 l ha^{-1}) and four times in AUZ17 (©Mercantor Gold at 1,2 l ha^{-1}, ©Racer ME at 1,2 l ha^{-1}, ©Roundup Innov at 1 l ha^{-1} and ©Stratos Ultra at 2 l ha−1). Fertilization was adjusted according to the amount of soil mineral N measured before sowing of sunflower and was carried out twice in AUZ17 at 60 and 50 kg N ha^{-1}. Mean air temperature (°C) and precipitation were recorded daily during cover crops and sunflower growing season (Table 1). The weather station was located at INRA's experimental station in Auzeville.

Table 1: Monthly mean temperature (°C) and rainfall (mm) through the cover-crop and the sunflower growing season in 2015-2016 and 2016-2017.

Soil cover	Month	Monthly mean temperature (°C)		Monthly total rainfall (mm)	
		AUZ16	**AUZ17**	**AUZ16**	**AUZ17**
Cover-crop	September	17.1	19.7	22	9
	October	14.0	14.4	23.5	50
	November	11.3	10.4	51.5	60
	December	10.0	6.9	6.5	11
Total (cover-crops)				**103.5**	**130.0**
Sunflower	April	12.3	12.8	51.5	28
	May	15.3	17.2	76	103
	June	19.3	22.2	66.5	54
	July	22.0	21.9	41	64
	August	22.4	22.4	20	21
	September	20.3	18.9	9	4
Total (Sunflower)				**273.0**	**274.0**

2.2. Characterization of cover crops and sunflower

Cover crops were sampled three months after sowing (at flowering stage) to assess production of shoot and root biomass on four 0,5 m² (AUZ16) and two 0,45 m² (AUZ17) squares per plot. Plants were rinsed with tap water, roots were cut from shoots and both were separately weighed to measure fresh biomass. Sub-samples were dried at 80°C for 48 h, weighed and subsequently ground (1 mm particle) to determine N, C and sulfur content with a CHN-2000 analyzer (LECO) according to Dumas (1831). In addition, plants were homogeneously sampled in each plot, rinsed and ground using an ELIET primo mill. The mixtures were immediately stored in sealed bags, frozen and stored at −80°C for GSLs content analysis and for future laboratory experiments.

For sunflower N status, two indicators were measured at flowering stage: the shoot N content (Nm) and the Nitrogen Nutrition Index (NNI). At flowering, five plants were cut at the soil surface on each plot. After drying at 80°C for 48 h, plants were ground (1 mm particle) and both C and N contents in sub-samples were determined with a CHN-2000 analyzer (LECO). NNI was calculated as follows:

141 $$NNI = \frac{Nm}{Nc}$$

142 where Nm is the total N concentration for all the aerial parts, and Nc is the critical total N
143 concentration, i.e., the minimum N concentration needed to obtain the maximum dry matter
144 production by the crop, calculated for the weight of aerial dry matter (ADM) measured for each plot.
145 Nm and Nc are expressed as % of ADM. Debaeke et al. (2012) proposed the following critical dilution
146 curve for sunflower:

147 $$Nc = 4.53 \times ADM^{-0,42}$$

148 A value of NNI ≥ 1 indicates non- limiting N nutrition for the crop; NNI = 1 is optimal N nutrition and
149 NNI < 1 emphasizes N deficiency.

150 ### *2.3. GSL extraction and analysis*

151 GSL extraction and analysis was performed in a specialized laboratory (Institute for Water and Wetland
152 Research, Rabboud University, The Netherlands) using a method from de Graaf et al., (2015). Frozen
153 plant materials were freeze dried and ground as fine as possible with a Tetsch MM 300 mixer mill at
154 30 Hz for 1 min. Aliquots of 50 mg were weighed in 2.0 ml Eppendorf tubes, 1 ml 70% Methanol (MeOH)
155 was added, and the samples were boiled for 10 min at 90°C. After boiling, samples were placed for 15
156 min in an ultrasonic bath and centrifuged at 6500 rpm for 10 min. The supernatant was added to a 0,5
157 ml DEAE Sephadex A-25 column. The pellet was kept, washed twice with 1 ml 70% MeOH, vortexed
158 and placed in the ultrasonic bath for 15 min. After centrifugation at 6500 rpm for 10 min, the
159 supernatant was added to the same column. The column was washed twice with 1 ml 70% MeOH,
160 once with 1 ml MilliQ water and twice with 1 ml 20 mM NaOAC buffer (pH 5.5). Thereafter, 20 µl of
161 aryl sulfatase (Sigma type H-1 of Helix pomatia) was added to the columns and flushed down with 50
162 µL NaOAc buffer (pH 5.5). The columns were covered with aluminium foil and incubated over night at
163 room temperature. The next day, the resulting desulphoglucosinolates were eluted from the column
164 with 2 times 0,75ml MilliQ water. The elution was frozen with liquid N, freeze dried, dissolved in 1 ml

MilliQ water and measured on the HPLC. Quantities of aliphatic, aromatic and indole GSLs produced in brassicas shoot and root biomass in AUZ16 and AUZ17 were subsequently calculated by combining GSLs concentration data and cover crop shoot and root dry biomass production.

2.4. Assessment of soil Inoculum density after biofumigation

With the aim of assessing the quantity of MS in the soil after biofumigation, the following experiment was conducted. In AUZ17, two 30 cm soil columns per plot were collected with a soil auger at sunflower flowering stage. Within each block, soil was sampled along two horizontal transects on the 6th row of sunflower crop of each plot to minimize soil heterogeneity. To kill conidia and pieces of mycelium of *V. dahliae*, samples were air dried at ambient temperature for at least two weeks without being exposed to direct sunlight. Afterwards, soil samples contained *Verticillium dahliae* only as MS. Then, dried samples were sieved through a 0.2 mm sieve and five sub-samples of 12.5 mg were taken from the fraction retrieved at the bottom. Sub-samples were manually spread over petri dishes containing an EPAA selective medium (Mansoori, 2011). A sheet of paper was placed below each petri dish to retrieve soil particles that might have fallen outside, as devised by Goud and Termorshuizen (2003). Petri dishes were then incubated for two weeks at 24 ± 1 °C in the dark. Colony Forming Units (CFU) on 120 petri dishes (5 dishes × 2 soil samples × 4 treatments × 3 blocks) were then counted by the same person with a binocular loupe (©Leica L2, GX15).

2.5. Bioassay on the survival of V.dahliae inoculum in biofumigated soil

One strain of *Verticillium dahliae* was selected among eight for its aggressiveness, the earliness of symptom appearance on sunflower and for high inoculum production. It was isolated from a single MS collected on an infected sunflower stem in a field located in Verfeil (Haute Garonne, France) near the trial site. Inoculum was cultured for two weeks on potato dextrose agar medium (PDA, Difco) (39 g/l, 150 mg of streptomycin, pH 6) and grown at 25 ± 1 °C in the dark for MS production. The petri dishes were opened and dried at 24 ± 1 °C in the dark for 5 days. The dry MS-containing upper layer was gently scratched with a scalpel to collect the MS. Pure inoculum was then sift in nested sieves and the

80–50 µm fraction was retrieved and vigorously rinsed with demineralized water. MS were stored at 24° ± 1 °C in the dark until use.

A pot experiment, adapted from Tenuta and Lazarovits (2002), was then conducted to assess the biocide effect of biofumigation on *Verticillium dahliae* inoculum in semi-controlled conditions. Firstly, soil from AUZ17 was sampled, sieved at 2 mm size particle and air-dried for at least 2 weeks. Plastic pots were pierced at the bottom to ensure drainage, and a filter paper was placed at the bottom to prevent soil flowing out of the pots. Then, frozen biomass of BM, TR and FR, previously collected in AUZ17 at the flowering stage, was individually ground and mixed homogeneously with 180 g of dry soil at a rate of 2 Mg ha^{-1} of dry biomass (corresponding to 1.8 g of fresh frozen biomass for BM and TR and 2.2 g for FR). The mixtures were used to fill five pots per cover crop and five additional pots were filed only with soil, as control treatment (CT). Subsequently, MS-containing mesh bags were prepared by (1) mixing homogeneously 15 mg of pure MS with 5 g of rinsed and inert sand (160–100 µm size particle), (2) adding 25 mg of the mixture to a mesh bag (3 x 3 cm) prepared from polyamide screening (Diatex©, 50 µm pore size) and (3) sealing the bag with iron wires coated with inert plastic. Two mesh bags were buried in each pot. Pots were then slightly compacted, placed at 24° ± 1 °C in the dark and kept moist with demineralized water. Two weeks later, mesh bags were retrieved from the pots, gently rinsed with tap water and their content was spread over EPAA petri dishes, which were subsequently incubated at 24° ± 1 °C in the dark. After two weeks, the petri dishes were gently rinsed to remove sand particles, and CFUs on each petri dish were counted with a binocular loupe (©Leica L2, GX15).

2.6. Disease and yield assessment

Disease assessment: Sunflower wilt symptoms were assessed weekly from 52 to 118 days after sowing (DAS) in AUZ16 and from 57 to 127 DAS in AUZ17, on three zones of 25 (AUZ16) and 15 plants (AUZ17) per treatment. A total of 10 disease recordings were done up to full maturity of sunflower on 300 plants (25 plants × 3 zones × 4 treatments) and 540 plants (15 plants × 3 zones × 4 treatments × 3 blocks) in AUZ16 and AUZ17, respectively. The disease severity was scored using a 0–4 scale:

0 = healthy plant, 1 = [0–20%], 2 =]20–50%], 3 =]50–80%] and 4 = >80% of the plant displaying wilt symptoms. From severity scores, the incidence of SVW, the area under the disease progress curve (AUDPC) and the disease severity index (DSI) were calculated. The AUDPC was calculated based on recordings done until 96 DAS for both field as symptoms of sunflower natural senescence might have distorted the last disease assessments. AUDPC was calculated as follows (Madden et Campbell, 1990):

$$AUDPC = \sum_{i}^{n-1} \left[\frac{y_i + y_{i+1}}{2}\right] \times (t_{i+1} - t_i)$$

where n is the number of evaluations, y the score of Verticillium wilt and t the DAS of the i^{th} recording. AUDPC was calculated for each plant assessed in both fields.

The disease severity index (DSI) was calculated up to 96 DAS for each of the 12 (AUZ16) and 36 zones (AUZ17) according to Li et al. (2017) :

$$DSI = \frac{100 * number\ of\ diseased\ plants\ in\ each\ score * value\ of\ the\ corresponding\ score}{Total\ number\ of\ plants\ scored\ * Value\ of\ the\ maximum\ score}$$

Yield: Firstly, achene production was assessed on each zone by sampling the heads of the 25 (AUZ16) and 15 (AUZ17) sunflowers selected for disease assessment. Achenes were separated from sunflower heads with a threshing machine, cleaned with a densimetric column and weighed. They were subsequently dried at 80 °C for 48 h and weighed. The thousand-kernel weights were measured and additional subsamples were taken for oil content analysis. To estimate fresh achene production by avoiding buffer areas, 4.2 m wide strips were harvested at the center of each treatment (6 m width) with an experimental combine harvester.

2.7. Data analysis

Statistical analysis was conducted on nine measured variables: soil trial and pot experiment CFU counts, AUDPC, DSI, disease incidence, thousand-kernel weight, kernel oil content and fresh and dry achene weight. The means and standard errors of each variable were calculated for each treatment. Prior to statistical analyses, homoscedasticity by Levene's test (confidence level of 0.95) and the

normality of residuals by the Shapiro-Wilk's test (confidence level of 0.95) were tested. Analysis of variance (ANOVA) was performed to evaluate the effect of cover-crop type on each measured variable and means were compared with a Tukey test. Non-parametric data were analyzed with Kruskal-Wallis test and means were compared with Nemenyi test. All analyses were done using R software

3. RESULTS

3.1. Weather conditions

Weather conditions indicators, especially monthly mean temperature (°C) and monthly total rainfall (mm) are presented in Table 1. While mean temperature throughout the growing season was similar for both years, cover-crops benefited from a greater rainfall in AUZ17 (130 mm) than in AUZ16 (103.5 mm). Rainfall was particularly low in AUZ17 in September (9 mm), at cover-crop sowing, as compared to AUZ16 (22 mm), which might have hindered cover-crop establishment and development. In both fields, the weather was favorable to sunflower development. Optimal climatic conditions for SVW development were met in both years as temperatures ranged between 21°C and 27°C in July and August for AUZ16 (22°C and 22.4°C) and in June, July and August for AUZ17 (22.2 °C, 21.9°C and 22.4°C). Rainfall was also propitious to disease development especially from June to August (127,5 mm for AUZ16 and 139 mm for AUZ17).

3.2. Cover crops yield and composition

Ranging from 6.06 to 4.16 Mg ha^{-1}, total dry biomass production was higher in AUZ16 than in AUZ17 (from 1.38 to 2.11 Mg ha^{-1}) and FR cover crops produced the largest amount of dry biomass for both years, followed by TR (4.96 and 1.50 Mg ha^{-1} in AUZ16 and AUZ17) and BM (4.16 and 1.38 Mg ha^{-1}). Carbon content ranged from 38.85 to 44.14 % of dry biomass in AUZ16 and from 39.69 to 40.74 % of dry biomass in AUZ17 and was higher in BM biomass for both years. Nitrogen composition was generally higher in AUZ16 (2.94-3.04 % of dry biomass) compared to AUZ17 (1.95–2.13 % of dry biomass) and was also higher in BM cover crops for both fields. Sulfur concentrations were lower in

AUZ16 (0.56-0.61 % of dry biomass) than in AUZ17 (0.64-0.76 % of dry biomass) and were the highest in TR biomass, and the lowest in fodder radish cover crop for both fields.

Table 2: Total fresh and dry biomass production (Mg ha^{-1}) and carbon, nitrogen and sulfur content (% of dry biomass) of B. juncea, R. sativus an B. rapa subsp. oleifera at cover-crop destruction.

	Total biomass				**Biomass composition**					
	Fresh (Mg ha^{-1})		Dry (Mg ha^{-1})		Carbon (%)		Nitrogen (%)		Sulfur (%)	
	AUZ16	AUZ17	AUZ16	AUZ17	AUZ16	AUZ17	AUZ16	AUZ17	AUZ16	AUZ17
Brown Mustard	34.43 ± 4.86	8.87 ± 3.31	4.16 ± 0.58	1.38 ± 0.47	44.14 ± 1.78	40.74 ± 1.03	3.04 ± 0.28	2.13 ± 0.31	0.59 ± 0.05	0.73 ± 0.11
Turnip rape	35.07 ± 4.25	9.64 ± 2.93	4.96 ± 0.39	1.50 ± 0.46	39.84 ± 1.56	39.69 ± 0.66	2.81 ± 0.40	2.01 ± 0.11	0.61 ± 0.10	0.76 ± 0.08
Fodder radish	64.87 ± 3.55	16.00 ± 5.99	6.06 ± 0.59	2.11 ± 0.71	38.85 ± 1.51	40.06 ± 1.00	2.94 ± 0.51	1.95 ± 0.28	0.56 ± 0.11	0.64 ± 0.04

3.3. Glucosinolates in Brassica juncea, Raphanus sativus and Brassica rapa subsp. oleifera cover-crops.

The GSLs profiles and concentrations in shoot (SB) and root biomass (RB) of BM, FR and TR cover-crops grown in AUZ16 and AUZ17 are presented in Table 3.

Generally, brassica cover-crops grown in AUZ16 had higher total GSLs concentration than plants from AUZ17. The most striking differences concern RB of BM (30.56 µmol.g^{-1} in AUZ16 and 0.12 µmol.g^{-1} for AUZ17), RB of FR (13.69 µmol.g^{-1} in AUZ16 and 0.47 µmol.g^{-1} for AUZ17) and RB of TR (25.60 µmol.g^{-1} in AUZ16 and 1.21 µmol.g^{-1} for AUZ17). Some cultivars produced certain GSLs in only one experiment as it is noticeably the case for gluconasturtiin in RB of BM (12.72 µmol.g^{-1} in AUZ16 to 0.02 µmol.g^{-1} in AUZ17), and for gluconapin in RB of TR (2.13 µmol.g^{-1} in AUZ16 to 0.00 µmol.g^{-1} in AUZ17).

Table 3: Glucosinolates profiles and concentrations in shoot (SB) and root (RB) biomass of brown mustard, fodder radish, and turnip rape grown in AUZ16 and AUZ17 (µmol g^{-1}dry tissue). (-) = 0.

	Glucosinolate concentration (µmol.g-1 dry tissue)											
	Brown mustard (BM)				Fodder radish (FR)				Turnip rape (TR)			
	AUZ16		AUZ17		AUZ16		AUZ17		AUZ16		AUZ17	
	SB	RB	SB	RB	SB	RB	SB	RB	SB	RB	SB	RB
Aliphatic												
Sinigrin	41.07	16.27	25.72	0.10	(-)	(-)	(-)	(-)	(-)	(-)	0.17	(-)
Glucoerucin	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.32	(-)	(-)
Glucoraphanin	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.30	(-)	(-)
Glucoraphasatin	(-)	(-)	(-)	(-)	0.46	13.09	(-)	0.47	(-)	(-)	(-)	(-)
Gluconapin	(-)	(-)	0.18	(-)	(-)	(-)	(-)	(-)	3.32	2.13	0.87	(-)
Progoitrin	(-)	(-)	0.30	(-)	(-)	(-)	(-)	(-)	6.62	8.79	3.95	0.40
Glucobrassicanapin	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.31	(-)	(-)
Unknown aliphatic 6,37	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sum Aliphatic	**41.07**	**16.27**	**26.20**	**0.10**	**0.46**	**13.09**	**0.00**	**0.47**	**9.94**	**11.85**	**4.99**	**0.40**
Aromatic												
Gluconapoleiferin	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.09	(-)
Gluconasturtiin	(-)	12.72	0.31	0.02	(-)	(-)	(-)	(-)	(-)	10.48	0.20	0.50
Glucotropaeolin	(-)	(-)	0.14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sinalbin	(-)	(-)	0.04	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sum Aromatic	**0.00**	**12.72**	**0.49**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**10.48**	**0.29**	**0.50**
Indole												
4-hydroxyglucobrassicin	(-)	0.16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Glucobrassicin	0.18	0.16	0.16	(-)	2.06	0.39	0.62	(-)	1.22	0.71	1.09	0.03
4-methoxyglucobrassicin	(-)	0.26	(-)	(-)	0.14	0.12	0.02	(-)	(-)	(-)	(-)	(-)
Neoglucobrassicin	0.39	0.99	0.54	(-)	(-)	0.09	0.11	(-)	5.71	2.55	9.25	0.28
Unknown 18.503	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sum Indole	**0.56**	**1.57**	**0.70**	**0.00**	**2.20**	**0.60**	**0.75**	**0.00**	**6.93**	**3.27**	**10.34**	**0.31**
Sum Total	**41.64**	**30.56**	**27.39**	**0.12**	**2.66**	**13.69**	**0.75**	**0.47**	**16.87**	**25.60**	**15.62**	**1.21**

In BM plants, six (AUZ16) and eight (AUZ17) GSLs from the three main types were detected in SB and RB. In AUZ16, BM SB (41.64 µmol.g^{-1}) and RB (30.56 µmol.g^{-1}) had the highest total GSLs concentrations of all brassicas tissues analyzed and it was also true for SB of BM (27.39 µmol.g^{-1}) in AUZ17. GSL richness in BM tissues is mainly due to high sinigrin (41.07 µmol.g^{-1} SB and 16.27 µmol.g^{-1} RB in AUZ16 and 25.72 µmol.g^{-1} SB in AUZ17) and gluconasturtiin contents (12.72 µmol.g^{-1} RB in AUZ16).

In both field trials, SB and RB of FR contained four GSLs and the proportion of aliphatic GSLs was the highest, followed by indole GSLs. While glucoraphasatin (13.09 µmol.g^{-1} RB) and glucobrassicin (2.06 µmol.g^{-1} SB) were predominant in plants from AUZ16, concentrations of all GSLs analyzed in SB and RB of FR grown in AUZ17 were very low, ranging from 0.16 µmol.g^{-1} dry tissue of SB to 0.71 µmol.g^{-1} dry tissue of RB.

TR plants contained eight GSLs in AUZ16 and seven in AUZ17. Aliphatic GSLs concentration were dominant in SB and RB from AUZ16 (9.94 and 11.85 µmol.g^{-1} respectively) whereas SB from AUZ17

288 had a higher indole GSL content (10.34 µmol.g^{-1} dry tissue). TR SB and RB in AUZ16 and SB from AUZ17
289 were rich in progoitrin and neoglucobrassicin and gluconasturtiin was the major GSLs in RB of TR from
290 AUZ16. GSL concentration in RB of TR from AUZ17 was low.

291 Quantities of aliphatic, aromatic and indole glucosinolates were calculated for both field trials by
292 combining dry SB and RB production with their corresponding GSLs concentrations (Fig. 1). Total GSL
293 yield at flowering stage was tremendously higher in AUZ16 and quantities of BM GSLs, FR GSLs and TR
294 GSLs in AUZ17 were respectively lowered of 84.5%, 81.9% and 97.6%, compared to AUZ16. In both
295 fields, BM produced the largest amount of total GSLs (16535.3 and 2555.4 µmol.m^{-2} for AUZ16 and
296 AUZ17 respectively), followed by TR (9244.05 and 1669.8 µmol.m^{-2}) and FR (3869.6 and 90.8 µmol.m^{-2}).
297 Shoot biomass was generally the major source of GSLs as it contained 86.6% and 72.1% of MB and
298 TR total GSL production in AUZ16 and 99.8%, 96.9% and 80.6% of MB, TR and FR total GSL production
299 in AUZ17.

300 All brassica yielded predominantly aliphatic GSLs in AUZ16 (15306.5 for BM, 5120.8 for TR, and
301 2865.24 µmol.m^{-2} for FR) while, in AUZ17, it was only the case for BM in AUZ17 (2442.5 µmol.m^{-2}) as
302 TR and FR produced mainly indole GSLs (1086.1 and 73.2 µmol.m^{-2} respectively). Overall, TR produced
303 the highest amounts of indole GSLs over both growing seasons (3067.9 and 1086.1 µmol.m^{-2} in AUZ16
304 and AUZ17, respectively) followed by FR (1004.4 and 73.2 µmol.m^{-2} in AUZ16 and AUZ17, respectively)
305 and BM (308.0 and 66.7 µmol.m^{-2} in AUZ16 and AUZ17, respectively). Overall, aromatic GSLs were
306 produced in the smallest quantities and were only found in TR (1055.3 and 49.6 µmol.m^{-2} for AUZ16
307 and AUZ17) and BM (920.8 and 46.2 µmol.m^{-2} for AUZ16 and AUZ17).

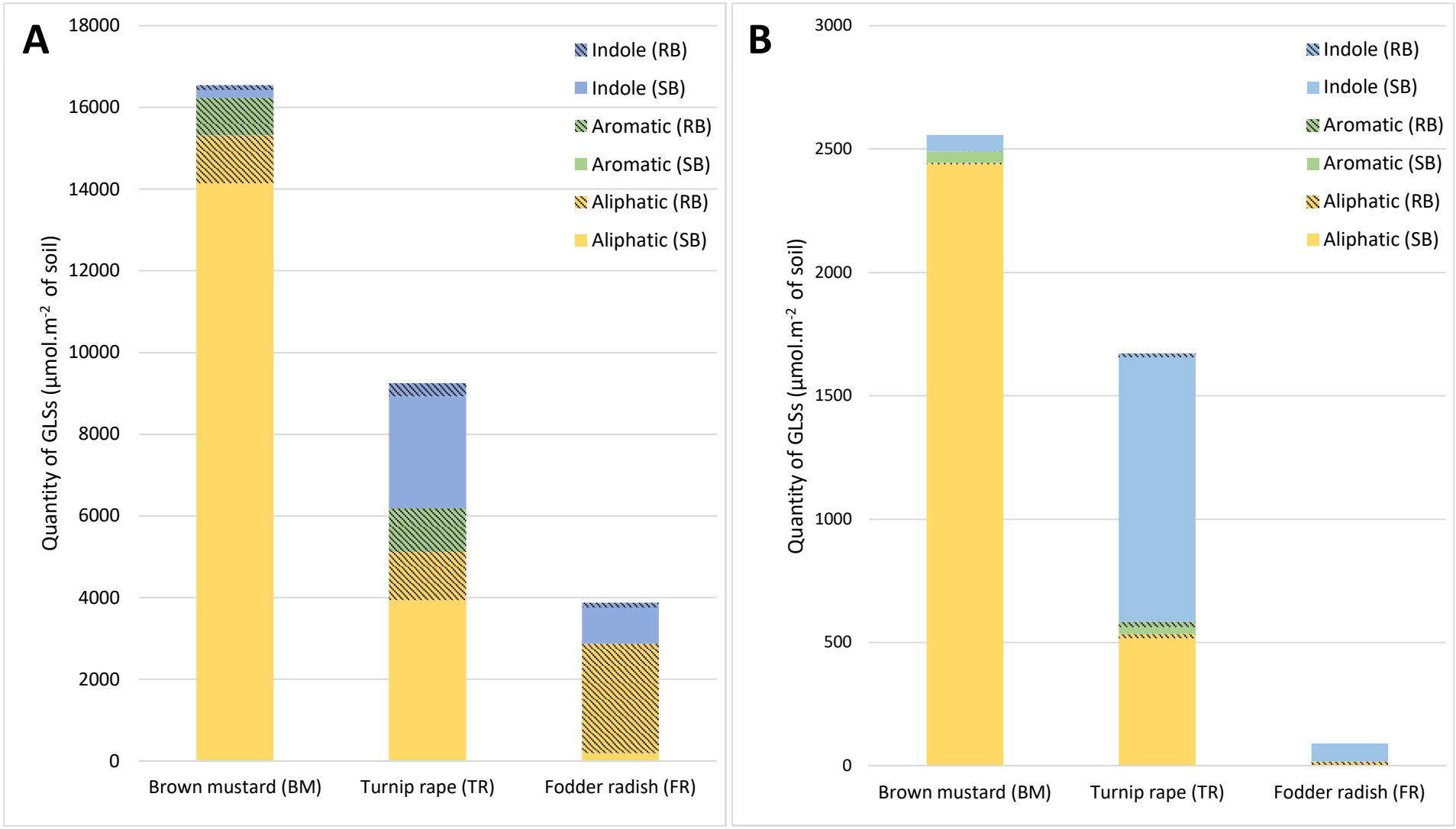

Fig. 1: Quantity of aliphatic, aromatic and indole glucosinolates (µmol.m^{-2} of soil) produced in the shoot (SB) and root biomass (RB) of Brown mustard (BM), Turnip rape (TR) and Fodder Radish (FR) at flowering stage in AUZ16 (A) and AUZ17 (B).

3.4. *Characterization of sunflower crop at flowering stage*

The aerial dry biomass of Sunflowers preceded by a Bare Soil (SBS), by Brown Mustard (SBM), Turnip Rape (STR) and preceded by Fodder Radish (SFR) were measured at flowering stage and their corresponding nitrogen nutrition index (NNI) was calculated (Table 4). The aerial dry biomasses of sunflowers were higher in AUZ16 than in AUZ17 for all treatments. The aerial dry biomasses in AUZ16 were ranging from 8.1 Mg ha^{-1} for SBS to 11.88 Mg ha^{-1} for SBM. In AUZ17, STR yielded the highest aerial dry biomass (8.52 Mg ha^{-1}) while both SBS and SBM had the lowest (7.37 Mg ha^{-1}).

In AUZ16, NNI varied from 0.71 for SFR to 1.08 for SBM and, in AUZ17, from 0.84 for SBM to 1.13 for SFR. For both experiments, SBS tended to have lower NNI compared to sunflowers preceded by a brassica. NNI of STR in AUZ16 and of SBM in AUZ17 were the lowest among all treatments and inferior to 1 which denotes N deficiency although it was not visible in the fields. Generally, sunflowers preceded by a brassica had non- limiting N nutrition (NNI>1). This was true for SFR from both trials.

Table 4: Production of aerial dry matter (ADM, Mg ha^{-1}) and nitrogen nutrition index (NNI) at sunflower at flowering stage as a function of previous crop (BS: Bare Soil; BM: Brown Mustard; TR: Turnip Rape; FR: Fodder Radish) in AUZ16 and AUZ17. [a]: standard deviation not presented as there was only one value for each treatment.

	Aerial dry biomass (Mg ha^{-1})		Nitrogen Nutrition Index (NNI)	
	AUZ16[a]	AUZ17	AUZ16	AUZ17
Sunflower - BS	8.13	7.37 ± 1.13	0.78	0.99 ± 0.09
Sunflower - BM	11.88	7.37 ± 1.77	1.08	0.84 ± 0.19
Sunflower - TR	9.12	8.52 ± 0.94	0.71	1.13 ± 0.17
Sunflower - FR	11.73	8.36 ± 1.67	1.02	1.01 ± 0.23

3.5. *Soil inoculum of Verticillium dahliae in the field trial and survival in the pot experiment*

Soil inoculum densities were evaluated from soil sampled in AUZ17 at sunflower flowering stage (Fig. 2) and are expressed as CFU g^{-1}, which corresponds to the number of microsclerotia per gram of soil. Soil inoculum densities were the highest for BS plots (229.3 CFU g^{-1}) compared to plots biofumigated with a brassica before planting sunflower crop. Among those, plots biofumigated with TR (136 CFU g^{-1}) had the highest soil inoculum density, followed by plots biofumigated with BM (130.7 CFU g^{-1}) and with FR (80 CFU g^{-1}). The treatment had no significant ($p = 0.223$) effect on soil inoculum density.

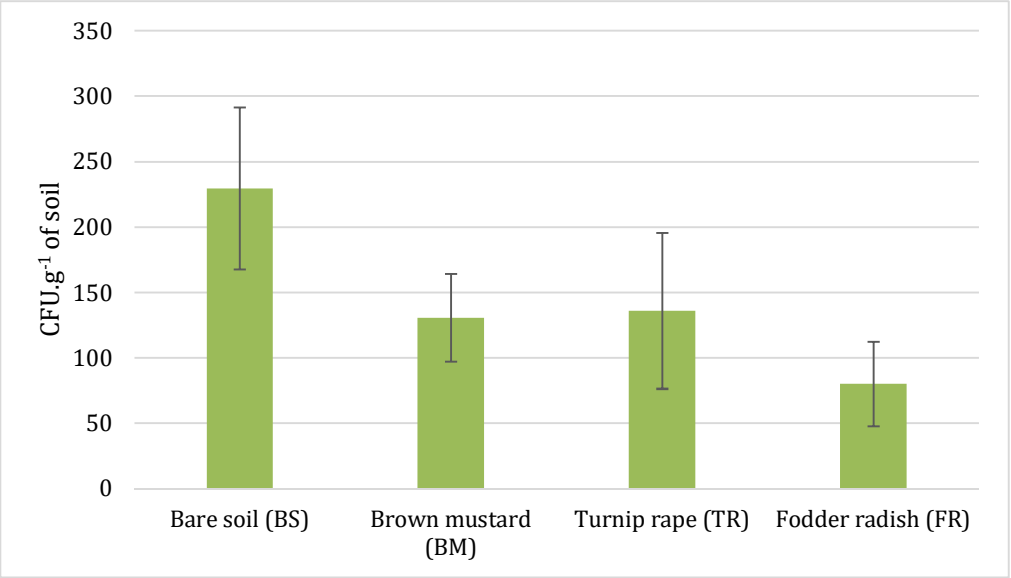

Fig. 2: Soil Inoculum density (CFU g^{-1} of soil) at sunflower flowering stage (76 DAS) as a function of treatment prior to sunflower sowing (BS: Bare soil; BM: Brown mustard; TR: Turnip rape and FR: Fodder radish) in AUZ17.

In addition to measuring MS survival after biofumigation in the field, a bioassay was conducted to measure inoculum survival in soil biofumigated with fresh brassica biomass (Fig. 3). Results showed that number of MS was the highest in mesh bags from unamended control treatment (1944 CFU.g^{-1}) compared to pots amended with brassica tissues. Among those, mesh bags retrieved from pots with BM tissues (1228 CFU.g^{-1}) had the highest inoculum density, followed by FR

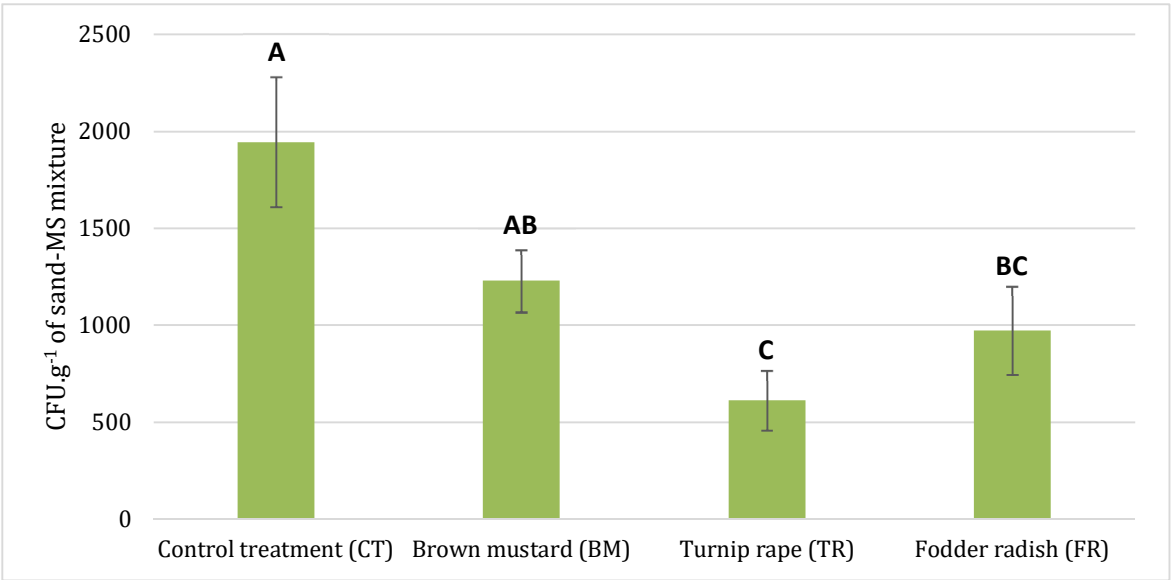

Fig. 3: Inoculum density (CFU.g^{-1} of mixture) from mesh bags biofumigated with brassica biomass incorporated in soil from AUZ17 in a pot experiment. (CT: Control treatment; BM: Brown mustard; TR: Turnip rape; FR: Fodder radish).

biofumigated mesh bags (972 CFU.g^{-1}). Inoculum density was the lowest in pots biofumigated with TR tissues (612 CFU.g^{-1}). Statistical analysis of inoculum densities measured in the bioassay highlighted a significant (p = 7.155e-05) effect of the treatments on inoculum quantities. While inoculum from BM pots was lower than inoculum from CT pots (—36.8%; not significant at p<0.05), biomass of FR (—50.0%; p = 0.014) and TR (—68.5%; p = 3.9e-05) significantly decreased inoculum compared to CT. Among the biofumigated pots, only the differences between inoculum densities from TR pots and from BM pots were statistically significant (p = 0.036).

3.6. Incidence and severity of sunflower verticillium wilt in the field trials

In AUZ16, the first recording of verticillium wilt incidence (52 DAS) highlighted a more precocious apparition of symptoms on SBS (49.3%) compared to SFR (5.3%), SBM (1.33%) and STR (0%) (Fig. 4A), and the difference between incidence of SBS and STR is statistically significative (p = 0.033). At 59 and 68 DAS, SBS incidence (73,3% and 81,3%, respectively) still stood out from incidence of STR (2.7% and 8%), SFR (8% and 12%) and SBM (10.7% and 13.3%). While SBS incidence reached a plateau from 75 DAS (89.3%) to 96 DAS (93.3%), incidence of sunflowers preceded by a cover crop were still forming a distinct group with lower incidence (75 DAS: SBS ≠ SFR, p = 0.033; 81 DAS: SBS ≠ SMB, SFR, and STR, all at p<0.05), although they slowly increased, from 36.0% (75 DAS) to 69.3% (96 DAS) for SBM, from 17,3% to 67.3% for SFR and from 28% to 61.3% for STR.

In AUZ17, symptoms appearance occurred earlier on both SBS (21.5%) and SBM (20.7%) at 57 DAS, while the incidence was lower for SFR (14.8%) and STR (14.1%) (Fig. 4B). Distinction between treatments started being clearer from 71 DAS where SBS (48.9%) and SBM (45.2%) had the highest incidence, followed by SFR (38,5%) and STR (37%). At 77, incidence of SBM (53.3%) started to distinguish from incidence of SBS (63.7%) whereas incidence of SFR (43.7%) and STR (42.2%) had slightly risen. At 84 DAS, incidence of STR (63%) increased more rapidly than incidence of SFR (56.3%), which was significantly lower than incidence of SBM (76.3%, p = 0.0337) and of SBS (80.7%, p = 0.0074).

356 At 96 DAS, SBM (85. 9%) and SBS (85.2%) incidence converged again, and 74.8% of STR and 68.1% of
357 SFR displayed disease symptoms.

358 In AUZ16, DSI levels followed a similar evolution than incidence from 52 to 96 DAS (Fig. 4C). At 52DAS,
359 SVW severity was the highest for SBS (22.0%) compared with SFR (2.0%), SBM (0.3%) and STR (0%)
360 (SBS ≠ STR; $p = 0.033$). DSI levels of SFR (5.0%), SBM (3.3%) and STR (2.7%) remained quite low until
361 68 DAS whereas DSI of SBS slightly increased (35%). It was only from 75 DAS that DSI levels of SBM
362 (14.7%), STR (10.7%) and SFR (10.7%) started to extend progressively while SBS still had a much higher
363 DSI (50%). At 81 and 89 DAS, differences in DSI between SBS and all other treatments were statistically
364 significative ($p<0.05$). Final DSI levels reached 80.0% for SBS while all sunflowers preceded by a cover
365 crop had lower levels as DSI was of 46.0% for SBM, 38.7% for STR and of 37.0% for SFR. In AUZ17, DSI
366 levels slowly increased and differences between treatments became visible from 71 DAS where SBS
367 had the highest DSI (28.3%), followed by SBM (23.9%), STR (18.9%) and SFR (15.6%) (Fig. 4D). While
368 DSI of SBS had a sustained and consistent growth until flowering stage (48 % at 96 DAS), DSI
369 progression of SBM softly decreased to reach 43.5% at 96 DAS but was still faster than DSI progression
370 of STR (38.0%) and SFR (28.0%) until 96 DAS.

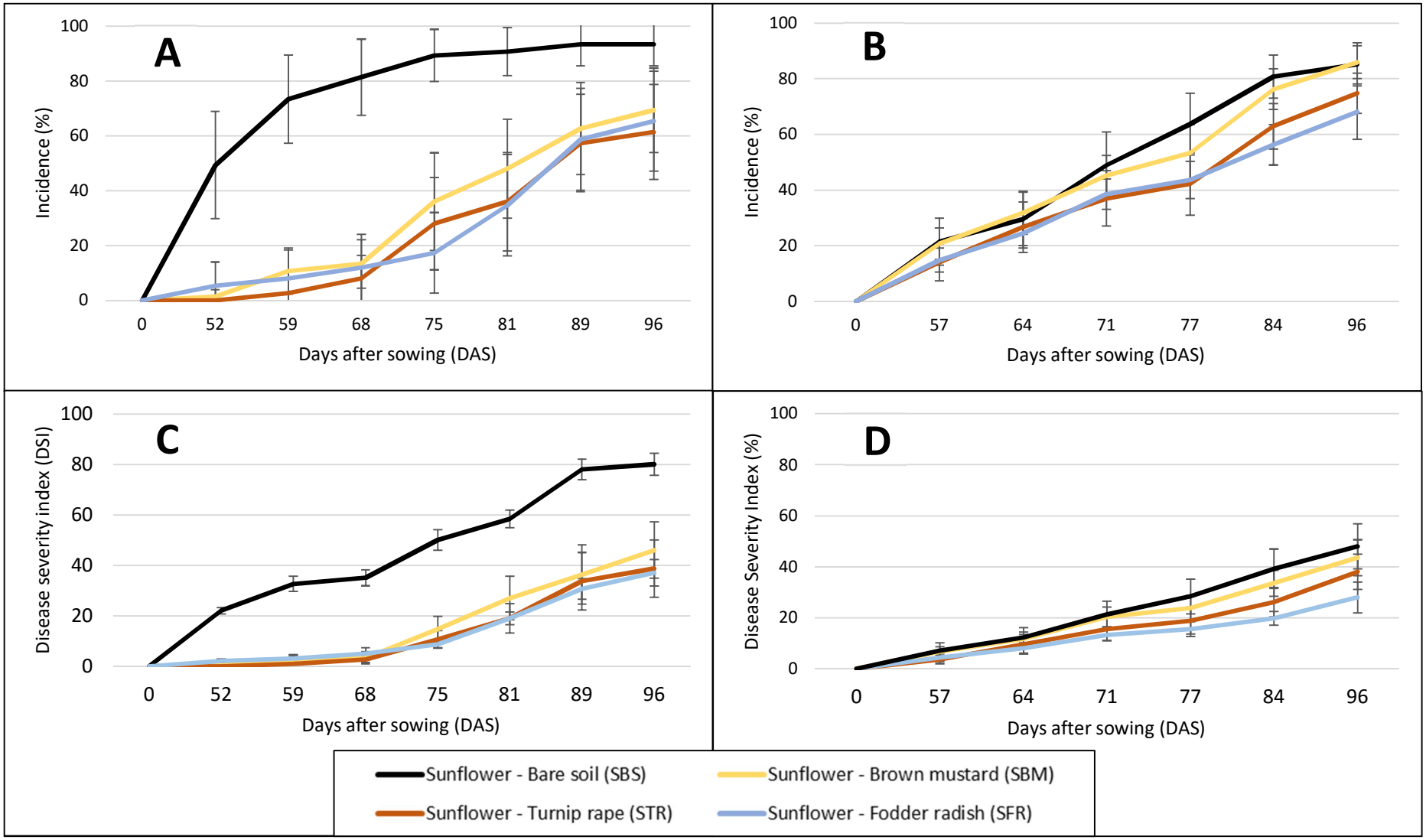

Fig. 4: Evolution of SVW incidence from sowing to flowering stage (96DAS) in AUZ16 (A) and AUZ17 (B) and evolution of SVW disease severity index (DSI) from sowing to flowering stage (96DAS) in AUZ16 (C) and AUZ17 (D).

In AUZ16, SBS had the highest AUDPC value (91.5), followed by SBM (30.7), SFR (25.1) and STR (24.8) (Fig. 5). AUDPC was significantly ($p < 2.2e-16$) influenced by the treatments and statistical analysis revealed differences between SBS and all other treatments ($p<0.05$) but not among sunflowers preceded by a brassica. In AUZ17, AUDPC was also higher for SBS (44.7) while SBM (39.8), STR (31.7) and SFR (25.6) had lower values. Treatment ($p = 5.226e-07$) and block effects (p-value = 0.00555) were also statistically significant and differences among treatments were revealed by statistical analysis (Fig. 5). AUDPC of STR and SFR were both significantly ($p<0.05$) lower than AUDPC of SBS. Among sunflowers preceded by a brassica, only AUDPC of SFR was significantly ($p<0.05$) lower than AUDPC of STR.

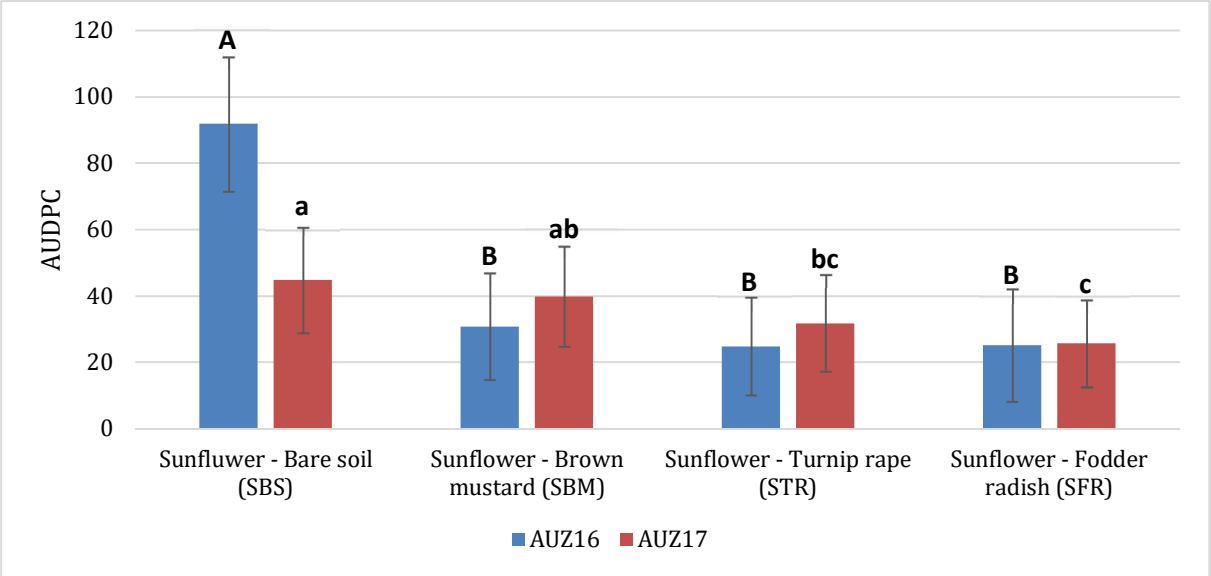

Fig. 5: Area Under the Disease Progress Curve (AUDPC) of sunflower verticillium wilt from 0 to 96 DAS for AUZ16 and AUZ17. Capital letters show differences among treatments in AUZ16 and lower-case letters show differences among treatments in AUZ17 ($p>0.05$).

3.7. Yield and quality of sunflower

In both experiments, all measured yield parameters were the lowest for SBS except that SBM had the lowest achene oil content in AUZ16 (Table 5). On the contrary, SFR often had the best quantitative and qualitative yield in both experiments although STR also seems to have performed well. Statistical analysis did not reveal significant effect of the treatments on all sunflower yield parameters in both experimental plots.

Table 5: Fresh and dry achene weight (Mg ha^{-1}), thousand-kernel weight (g) and achene oil content (% dry achene weight of sunflower crops from AUZ16 and AUZ17. a: standard deviation not presented as there was only one value for each treatment.

	Fresh achene weight (Mg ha^{-1})		Dry achene weight (Mg ha^{-1})		Thousand-kernel weight (g)		Oil content (% of dry achene weight)	
	AUZ16[a]	AUZ17	AUZ16	AUZ17	AUZ16	AUZ17	AUZ16	AUZ17
SBS	2.06	2.51 ± 0.45	2.84 ± 0.54	3.32 ± 0.97	48.49 ± 1.15	48.21 ± 5.59	50.31 ± 1.53	52.81 ± 2.17
SBM	2.60	2.69 ± 0.14	3.62 ± 0.21	3.32 ± 0.90	51.08 ± 5.67	50.62 ± 5.51	48.83 ± 1.73	53.50 ± 1.07
STR	2.73	2.78 ± 0.43	3.48 ± 0.60	3.74 ± 1.46	50.99 ± 6.05	49.39 ± 11.50	50.59 ± 2.08	53.59 ± 1.62
SFR	2.44	3.01 ± 0.30	3.83 ± 0.41	3.58 ± 0.69	54.77 ± 1.43	50.75 ± 7.48	49.90 ± 1.65	54.43 ± 1.13

4. DISCUSSIONS

4.1. Overall effect of biofumigation on Verticillium dahliae affecting sunflower

Understanding progression of SVW in response to biofumigation in a given field is a complex challenge as pedoclimatic conditions, crop management practices, quantity of soil primary inoculum, *V. dahliae* strain and cultivar tolerance all influence disease development (Berlanger et Powelson, 2005; Gulya et al., 1997; Pegg et Brady, 2002; Quiroz et al., 2008). In both trials, cover crops yielded significant amounts of biomass and GSLs, although both were lower in AUZ17 likely because warm and dry climatic conditions hindered cover crop establishment. In both experiments, sunflower crop benefited from favorable weather conditions and had a vigorous growth as shown by ADM and NNI measured at flowering stage (Table 4) and confirmed by visual observations. In AUZ17, soil inoculum densities were lower for sunflower plots preceded by a brassica, and, in both experiments, biofumigant cover crop reduced progression of SVW incidence, DSI and AUDPC and seemed to have increased yields compared to uncropped plots. This tends to confirm the biofumigation potential of brown mustard, fodder radish and turnip rape on *V.dahliae* already emphasized by other workers (Michel et al., 2007; Seassau et al., 2016; Yohalem et Hall, 2009).

4.2. Underlying mechanisms explaining inoculum reduction after biofumigation

Results from the pot experiment highlight a significant ($p = 7.155e-05$) biocide effect of biomass decomposition of all brassica tissues tested on inoculum of *V.dahliae*. As both the soil and brassica biomass used in the bioassay were sampled in AUZ17, the significative biocide effect of brassica tissue decomposition observed in semi-controlled conditions are comparable with the effect that

biofumigation performed in AUZ17 might have had on soil inoculum. Therefore, results from the bioassay suggest that lower soil inoculum densities observed in plots preceded by a brassica in AUZ17, as compared to uncropped plots (Fig. 2), could indeed be the result of biofumigation despite the non-significant effect of the treatment ($p = 0.223$). This biocide effect of brassica tissue decomposition is highly likely to have decreased soil inoculum densities since biofumigation reduced soil primary inoculum of *V.dahliae* in several field studies (Davis et al., 1996; Koike et Subbarao, 2000; Shetty et al., 2000; Steffek et al., 2006; Xiao et al., 1998). Results from the pot experiment are also in line with previous research. For instance, Neubauer et al., (2014) and Seassau et al. (2016) reported that biomass decomposition of brown mustard and fodder radish cultivars reduced inoculum density of *V.dahliae* in bioassays. While Neubauer et al., (2014) found that biomass of brown mustard was the most toxic to MS in sterilized sand, mesh bags biofumigated in soil amended with brown mustard in the present study had the highest inoculum survival among brassica tested. This difference could be explained by the incorporation of lower quantities of biomass of brown mustard in this experiment and by the use of soil as substrate, whose organic material and microbial activity might have contributed to lowering the proportion of ITCs reaching the MS (Gimsing et Kirkegaard, 2009). In this study, the light disinfection brought by biomass of brown mustard might indeed be due to its high concentration in sinigrin (Table 3), which degrades in ITCs that are toxic for *V.dahliae* (Neubauer et al., 2015; Witzel et al., 2013). The greatest biocidal effect brought by decomposition of TR tissues in semi-controlled conditions could be due to their higher concentration in progoitrin (aliphatic) that degrades in oxazolidine-2-thione, also toxic to *V.dahliae* (Karapapa et al., 1997). In TR biomass, neoglucobrassicin and glucobrassicin (both indole GSLs) might also have decreased viable MS as they form toxic thiocyanates upon enzymatic degradation (Couëdel, 2017). However, the reductions of inoculum levels observed in both the pots and fields biofumigated with fodder radish in AUZ17 are unlikely the result of a biocide effect of GSL degradation products since both the concentration (Table 3) and the quantity of GSL (Fig. 1B) provided by fodder radish biomass in AUZ17 was low. Therefore, these results tends to confirm that GSL degradation products may not be the only chemicals

contributing to the disinfection when biofumigation is performed (Kirkegaard, 2009; Shetty et al., 2000). For instance, N-rich materials were shown to reduce inoculum of *V. dahliae* when incorporated in soils because they produce toxic nitrous acid (HNO_2) and ammonium (NH_3) (Conn et al., 2004; Tenuta et Lazarovits, 2004, 2002a, 2002b). Following cover crop incorporation, NH_3 is more likely to be produced in soils with low organic matter content and pH above 8, as it was the case in AUZ17. The decomposition of organic material brought by cover crops also directly and indirectly contributes to decreasing primary inoculum as it can release fungitoxic compounds and fosters microbial activity that parasitizes, inhibits the germination and competes with *V. dahliae* (Bonanomi et al., 2007; Goicoechea, 2009). In particular, lignin from crop residues have been shown to reduce inoculum *of Verticillium longisporum* by increasing production of ligninolytic-enzymes in the soil, which are also able to degrade melanin (Butler et Day, 1998), the major component of MS outer layer. Thus, it is thought that melanin degradation by these enzymes alters MS protective layer which are therefore more susceptible to parasitism and inhibition in soils (Shetty et al., 2000), although this degradation could also increase MS susceptibility to GSL degradation products during biofumigation.

With the same mechanisms being involved, biofumigation can also negatively affect beneficial microorganism in soils. While some beneficial fungi (*Trichoderma spp.*) and antagonistic nematodes thrive after the incorporation of brassica tissues in the soil, the effects of biofumigation on arbuscular mycorrhizal fungi and on nitrogen fixing bacteria seems contrasting (Couëdel et al., 2017). Generally, the potential disservices caused by biofumigation on soil communities are not well understood and need to be further investigated.

4.3. Biofumigation reduces incidence and severity of sunflowers

In both experiments, sunflowers preceded by an uncropped soil had the highest final incidence (93.3% and 85.2% in AUZ16 and AUZ17, respectively), final DSI (80.0% and 48%) and AUDPC (91.5 and 44.7), which demonstrates a biotic regulation of verticillium wilt symptoms by the incorporation of brassica tissues, confirmed by results from other field studies (Davis et al., 1996; Michel et al., 2007; Subbarao

et al., 1999; Xiao et al., 1998). In the experimental conditions described in this study, biofumigation with turnip rape and fodder radish seems to have better regulated SVW than biofumigation performed with brown mustard. For instance, in AUZ16, sunflowers preceded by turnip rape had the lowest final incidence (61.3%), and AUDPC (24.8) whereas sunflowers preceded by fodder radish had the lowest final DSI (37%). In AUZ2017, sunflowers with fodder radish as previous crop had the lowest final incidence (68.1%) and DSI (28%, $p<0.05$). Interestingly, low inoculum densities appear to be related to low disease indicators. For instance, sunflower plots preceded by fodder radish had the lowest soil inoculum densities in AUZ17 (80 CFU.g^{-1}) and the lowest disease indicators. In addition, intermediate soil inoculum densities in sunflower crops biofumigated with brown mustard and turnip rape (130.7 and 136 CFU.g^{-1}) coincided with intermediate AUDPC (39.8 and 31.7, respectively). These observations suggest that primary inoculum level in the field correlates to incidence and severity of verticillium wilt symptoms, as emphasized by previous research on olive and artichoke (Berbegal et al., 2007; López-Escudero et Blanco-López, 2007).

4.4. Biofumigation tends to increase sunflower yield and quality

Sunflowers preceded by an uncropped interculture period had the lowest yield parameters for both experiments except that oil content was the lowest for sunflowers biofumigated with brown mustard in AUZ16 (Table 5). In both experiments, biofumigation performed with brassica tended to increase yield quantity and quality, which is in accordance with results from studies on the effect of broccoli residue incorporation on cauliflower affected by verticillium wilt (Subbarao et al., 1999; Subbarao et Hubbard, 1996). Among brassica, turnip rape and fodder radish seem to have increased yield parameters the most. For instance, in AUZ16, sunflower plots biofumigated with turnip rape had the best fresh achene weight (2.73 Mg ha^{-1}) and oil content (50.6% dry achene weight) while plots with fodder radish as previous crops had the highest dry achene (3.83 Mg ha^{-1}) and thousand kernel weights (54.77 g). Data from AUZ17 tend to confirm this trend as sunflowers on plots biofumigated with turnip rape had the highest dry achene weight (3.74 Mg ha^{-1}) and sunflowers preceded by fodder radish had the highest fresh achene (3.01 Mg ha^{-1}) and thousand-kernel weights (50.75 g) and achene

oil content (54.43%). In both experiments, a relation between disease indicators and yield quantity and quality appears to emerge. Sunflowers preceded by turnip rape in AUZ16 had the lowest final incidence and AUDPC while their fresh achene weight and achene oil content was the highest among all treatments. Sunflower plots biofumigated with fodder radish had the lowest final DSI and the highest dry achene and thousand kernel weights. IN AUZ17, the relationship was more marked for sunflowers with fodder radish as previous crop since all disease indicators were the lowest and all yield parameters were the highest, except that dry achene weight was the best for plots biofumigated with turnip rape. Since such relationships have already been documented in studies on verticillium wilt affecting cotton (Bejerano-Alcazar et al., 1997; Erdogan et al., 2006) and potato (Davis et al., 2001), it is likely that it also exists for *Verticillium dahliae* affecting sunflower, although it has to be confirmed by empirical data.

Based on the likely relations between (1) soil inoculum density and wilt incidence and severity and (2) wilt incidence and severity and sunflower yield, the following assumption can be made: In both experiments, biofumigation decreased soil inoculum density that in turn, induced a lowered SVW incidence and severity. Then, sunflowers succeeding to biofumigant crops were less affected by the disease and benefited from a lowered pathogen pressure that allowed them to produce a better quantitative and qualitative yield as compared to sunflowers from control plots. Such relationships seem plausible since soil inoculum density was highly correlated with both verticillium wilt incidence and cotton yield during three successive years in a field study (Paplomatas et al., 1992), although they have to be validated for the *Verticillium dahliae* – Sunflower pathosystem specifically.

5. CONCLUSION

In this study, biofumigation was performed with brassica cover crops grown during the long fallow period of the durum wheat – sunflower rotation and its potential to regulate *Verticillium dahliae* affecting sunflower was evaluated in two field trials and in a pot experiment in semi-controlled conditions. Biofumigated sunflower plots had lower inoculum densities, a lower progression of

symptoms and tended to have better qualitative and quantitative yields compared to plots remained uncropped during the fallow period preceding sunflower planting. Among brassica tested, fodder radish seems to have provided the highest biotic regulation of the pathogen, although turnip rape was also efficient. Therefore, these results tend to confirm that biofumigation with brassica, as an agroecological practice performed in the frame of a diversified disease management strategy, has the potential to mitigate *Verticillium dahliae* affecting sunflower crop while enhancing the resilience and the sustainability of durum wheat – sunflower rotation by providing numerous ecosystem services (Debaeke et al., 2017a; Finckh, 2008; Wezel et al., 2014). Future research should focus on (1) understanding the underlying mechanisms explaining the decrease of primary inoculum of *Verticilliulm dahliae*, (2) investigating the complex relationships between the quantity of soil borne inoculum, the expression of sunflower verticillium wilt symptoms and yield losses. Additionally, (3) the characterization of disservices caused by biofumigation with brassica species on soil beneficial organisms seems crucial to foster its integration in sustainable farming systems.

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REFERENCES

Agerbirk, N., Olsen, C.E., 2012. Glucosinolate structures in evolution. Phytochemistry 77, 16–45. doi:10.1016/j.phytochem.2012.02.005

Altieri, M.A., Nicholls, C.I., Henao, A., Lana, M.A., 2015. Agroecology and the design of climate change-resilient farming systems. Agronomy for Sustainable Development 35, 869–890. doi:10.1007/s13593-015-0285-2

Altieri, M. a., 1999. The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems and Environment 74, 19–31. doi:10.1016/S0167-8809(99)00028-6

Arthy, J.R., Akiew, E.B., Kirkegaard, J.A., Trevorrow, P.R., 2005. Using Brassica spp. as biofumigants to reduce the population of Ralstonia solanacearum., in: Bacterial wilt disease and the Ralstonia solanacearum species complex. p. 159–165.

Bejerano-Alcazar, J., Blanco-Lopez, M.A., Melero-Vara, J.M., Jimenez-Diaz, R.M., 1997. The influence of verticillium wilt epidemics on cotton yield in southern Spain. Plant Pathology 46, 168–178. doi:10.1046/j.1365-3059.1997.d01-221.x

Berbegal, M., García-Jiménez, J., Armengol, J., 2008. Effect of Cauliflower Residue Amendments and Soil Solarization on Verticillium Wilt Control in Artichoke. Plant Disease 92, 595–600. doi:10.1094/PDIS-92-4-0595

Berbegal, M., Ortega, A., García-Jiménez, J., Armengol, J., 2007. Inoculum Density-Disease Development Relationship in Verticillium Wilt of Artichoke Caused by *Verticillium dahliae*. Plant Disease 91, 1131–1136. doi:10.1094/PDIS-91-9-1131

Berg, G., Fritze, A., Roskot, N., Smalla, K., 2001. Evaluation of potential biocontrol rhizobacteria from different host plants of Verticillium dahliae Kleb. Journal of Applied Microbiology 91, 963–971. doi:10.1046/j.1365-2672.2001.01462.x

Berg, G., Knaape, C., Ballin, G., Seidel, D., 1994. Biological control of Verticillium dahliae kleb. by natural occurring rhizosphere bacteria. Archives Of Phytopathology And Plant Protection 29, 249–262. doi:10.1080/03235409409383116

Berlanger, I., Powelson, M.L., 2005. Verticillium wilt [WWW Document]. The Plant Health Instructor. doi:10.1094/PHI-I-2000-0801-01

Björkman, M., Klingen, I., Birch, A.N.E., Bones, A.M., Bruce, T.J.A., Johansen, T.J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L.E., Stewart, D., 2011. Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. Phytochemistry 72, 538–556. doi:10.1016/J.PHYTOCHEM.2011.01.014

Blok, W.J., Lamers, J.G., Termorshuizen, A.J., Bollen, G.J., 2000. Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping. Phytopathology 90, 253–259. doi:10.1094/PHYTO.2000.90.3.253

Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology.

Butler, M.J., Day, A.W., 1998. Destruction of Fungal Melanins by Ligninases of *Phanerochaete chrysosporium* and Other White Rot Fungi. International Journal of Plant Sciences 159, 989–995. doi:10.1086/314093

Chambre d'agriculture Occitanie, 2017. Bilan Tounresol 2017. Bulletin de santé du végétal grandes cultures. Hors-Série. Edition Ouest Occitanie 1–17.

Conn, K.L., Tenuta, M., Lazarovits, G., 2004. Liquid Swine Manure Can Kill Verticillium dahliae Microsclerotia in Soil by Volatile Fatty Acid, Nitrous Acid, and Ammonia Toxicity. Phytopathology 95, 28–35. doi:10.1094/PHYTO-95-0028

Constantin, J., Beaudoin, N., Laurent, F., Cohan, J.-P., Duyme, F., Mary, B., 2011. Cumulative effects of catch crops on nitrogen uptake, leaching and net mineralization. Plant and Soil 341, 137–154. doi:10.1007/s11104-

010-0630-9

Couëdel, A., 2017. Cultures intermédiaires en mélanges biscpécifiques crucifères - légumineuses pour produire des services conjoints de gestion de l'azote, du soufre et de bio-contrôle. Réunion comité technique CRUCIAL.

Couëdel, A., Seassau, C., Wirth, J., Alletto, L., 2017. Services et dis-services de régulation biotique par allélopathie et biofumigation produits par les cultures intermédiaires multiservices de crucifères. Innovations agronomiques 62, 71–85.

Davis, J.R., Huisman, O.C., Everson, D.O., Schneider, A.T., 2001. Verticillium wilt of potato: A model of key factors related to disease severity and tuber yield in southeastern Idaho. American Journal of Potato Research 78, 291–300. doi:10.1007/BF02875694

Davis, J.R., Huisman, O.C., Westermann, D.T., Hafez, S.L., Everson, D.O., Sorensen, L.H., Schneider, A.T., 1996. Effects of green manures on verticillium wilt of potato. Phytopathology. doi:10.1094/Phyto-86-444

de Graaf, R.M., Krosse, S., Swolfs, A.E.M., te Brinke, E., Prill, N., Leimu, R., van Galen, P.M., Wang, Y., Aarts, M.G.M., van Dam, N.M., 2015. Isolation and identification of 4-α-rhamnosyloxy benzyl glucosinolate in Noccaea caerulescens showing intraspecific variation. Phytochemistry 110, 166–171. doi:10.1016/j.phytochem.2014.11.016

Debaeke, P., Bedoussac, L., Bonnet, C., Bret-Mestries, E., Seassau, C., Gavaland, A., Raffaillac, D., Tribouillois, H., Véricel, G., Justes, E., 2017a. Sunflower crop: environmental-friendly and agroecological. Oilseed and fats Crops and Lipids 1–12. doi:10.1051/ocl/2017020

Debaeke, P., Casadebaig, P., Flenet, F., Langlade, N., 2017b. Sunflower crop and climate change: vulnerability, adaptation, and mitigation potential from case-studies in Europe. Oilseed and fats Crops and Lipids 24, 1–15. doi:10.1051/ocl/2016052

Debaeke, P., van Oosterom, E.J., Justes, E., Champolivier, L., Merrien, A., Aguirrezabal, L.A.N., González-Dugo, V., Massignam, A.M., Montemurro, F., 2012. A species-specific critical nitrogen dilution curve for sunflower (Helianthus annuus L.). Field Crops Research 136, 76–84. doi:10.1016/j.fcr.2012.07.024

Delaplane, K.S., Mayer, D.F., 2000. Crop pollination by bees. CABI, Wallingford. doi:10.1079/9780851994482.0000

Dumas, J.B.A., 1831. Procedes de l'analyse organic. Ann. Chim. Phys. 247, 198–213.

Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M.-A., Justes, E., Journet, E.-P., Aubertot, J.-N., Savary, S., Bergez, J.-E., Sarthou, J.P., 2015. How to implement biodiversity-based agriculture to enhance ecosystem services: a review. Agronomy for Sustainable Development. doi:10.1007/s13593-015-0306-1

EFSA, 2014. Scientific opinion on the pest categorisation of Verticillium dahliae Kleb, EFSA Journal. Parma, Italy. doi:10.2903/j.efsa.2014.3928

Erdogan, O., Sezener, V., Ozbek, N., Bozbek, T., 2006. The Effects of Verticillium Wilt (Verticilli1m dahliae Kleb.) on Cotton Yield and Fiber Quality. Asian Journal of Plant Sciences.

Eriksen, J., Thorup-Kristensen, K., 2002. The effect of catch crops on sulphate leaching and availability of S in the succeeding crop on sandy loam soil in Denmark. Agriculture, Ecosystems & Environment 90, 247–254. doi:10.1016/S0167-8809(01)00214-6

Finckh, M.R., 2008. Integration of breeding and technology into diversification strategies for disease control in modern agriculture. European Journal of Plant Pathology 121, 399–409. doi:10.1007/s10658-008-9273-6

Fradin, E.F., Thomma, B.P.H.J., 2006. Physiology and molecular aspects of Verticillium wilt diseases caused by V. dahliae and V. albo-atrum. Molecular Plant Pathology 7, 71–86. doi:10.1111/j.1364-3703.2006.00323.x

Fradin, E.F., Zhang, Z., Ayala, J.C.J., Castroverde, C.D.M., Nazar, R.N., Robb, J., Liu, C.-M., Thomma, B.P.H.J., 2009. Genetic Dissection of Verticillium Wilt Resistance Mediated by Tomato Ve1. Plant physiology 150, 320–332. doi:10.1104/pp.109.136762

García-Ruiz, R., García-Carneros, A.B., Molinero-Ruiz, L., 2014. A New Race of Verticillium dahliae Causing Leaf Mottle of Sunflower in Europe. Plant Disease 98, 1435–1435. doi:10.1094/PDIS-04-14-0360-PDN

Gimsing, A.L., Kirkegaard, J.A., 2009. Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil. Phytochemistry Reviews 8, 299–310. doi:10.1007/s11101-008-9105-5

Goicoechea, N., 2009. To what extent are soil amendments useful to control Verticillium wilt? Pest Management Science 65, 831–839. doi:10.1002/ps.1774

Goud, J.C., Termorshuizen, A.J., 2003. Quality of Methods to Quantify Microsclerotia of Verticillium dahliae in Soil. European Journal of Plant Pathology 109, 523-534. doi:10.1023/A:1024745006876

Gulya, T., 2007. New strain of Verticillium dahliae in North America. HELIA 30, 115-120. doi:10.2298/HEL0747115G

Gulya, T., 2004. Two New « Verticillium » Threats to Sunflower in North America, in: Proceedings Sunflower Research Workshop. p. 1-5.

Gulya, T., Rashid, K.Y., Masirevic, S.M., 1997. Sunflower Diseases, in: Sunflower Technology and Production. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, p. 263-379. doi:10.2134/agronmonogr35.c6

Haramoto, E.R., Gallandt, E.R., 2005. Brassica cover cropping: I. Effects on weed and crop establishment. Weed Science 53, 695-701. doi:10.1614/WS-04-162R.1

Hartwig, N., Science, H.A.-W., 2002, U., 2002. Cover crops and living mulches. Weed Science 50, 688-699.

Hill, S.B., MacRae, R.J., 1996. Conceptual Framework for the Transition from Conventional to Sustainable Agriculture. Journal of Sustainable Agriculture 7, 81-87. doi:10.1300/J064v07n01_07

Hu, X., Bai, Y., Chen, T., Hu, D., Yang, J., Xu, X., 2013. An optimized method for in vitro production of Verticillium dahliae microsclerotia. European Journal of Plant Pathology 136, 225-229. doi:10.1007/s10658-013-0170-2

Jabnoun-Khiareddine, H., Mejda Daami-Remadi, B., Fakher Ayed, B., Mohamed El Mahjoub, B., 2009. Biological Control of Tomato Verticillium Wilt by Using Indigenous Trichoderma spp. The African Journal of Plant Science and Biotechnology 3, 26-36.

Karagiannidis, N., Bletsos, F., Stavropoulos, N., 2002. Effect of Verticillium wilt (Verticillium dahliae Kleb.) and mycorrhiza (Glomus mosseae) on root colonization, growth and nutrient uptake in tomato and eggplant. Scientia Horticulturae.

Karapapa, V.K., Baig, M.A., Heale, J.B., Rossiter, J.., 1997. Glucosinolate response in winter oilseed rape Brassica napus ssp. oleifera to Verticillium dahliae (non-pathogenic) and V. longisporum comb. nov. [Karapapa, Bainbridge & Heale, 1997] (pathogenic)., in: Proceedings of the Seventh International Verticillium Symposium. Cape Sounion, Athens, Greece. p. 50.

Kirkegaard, J.A., 2009. Biofumigation for plant disease control - from fundamentals to the farming system, in: Walters, D. (Éd.), Disease Control in Crops: Biological and Environmentally Friendly Approaches. Blackwell Publishing Ltd, p. 1-266. doi:10.1002/9781444312157

Kirkegaard, J.A., Gardner, P.A., Desmarchelier, J.M., Angus, J.F., 1993. Biofumigation - using Brassica species to control pests and diseases in horticulture and agriculture, in: N. Wratten and R.J. Mailer (Éd.), Proceedings 9th Australian Research Assembly on Brassica. Wagga Wagga, NSW, p. 77-82.

Klosterman, S.J., Atallah, Z.K., Vallad, G.E., Subbarao, K. V, 2009. Diversity, Pathogenicity, and Management of Verticillium Species. Annual review of phytopathology 39-62. doi:10.1146/annurev-phyto-080508-081748

Koike, S., Subbarao, K., 2000. Broccoli residues can control Verticillium wilt of cauliflower. California Agriculture.

Korthals, G.W., Thoden, T.C., van den Berg, W., Visser, J.H.M., 2014. Long-term effects of eight soil health treatments to control plant-parasitic nematodes and Verticillium dahliae in agro-ecosystems. Applied Soil Ecology 76, 112-123. doi:10.1016/j.apsoil.2013.12.016

Kremen, C., Iles, A., Bacon, C., 2012. Diversified farming systems: An agroecological, systems-based alternative to modern industrial agriculture. Ecology and Society 17. doi:10.5751/ES-05103-170444

Lande, N., Jouffret, P., Tribouillois, H., Cristante, P., Lecomte, V., Bedoussac, L., Justes, E., 2012. Evaluating economic and technical performances of sunflower-soybean intercrop in French farming systems, in: Proceedings of the 18th International Sunflower Conference, Mar del Plata (Argentina).

Larkin, R.P., Honeycutt, C.W., Olanya, O.M., 2011. Management of Verticillium wilt of potato with disease-suppressive green manures and as affected by previous cropping history. Plant Disease 95, 568-576. doi:10.1094/PDIS-09-10-0670

Lecompte, V., Mestries, E., 2012. Tournesol et Verticillium dahliae. Etat des lieux et moyens de lutte pour 2013., in: Rencontres Techniques Regionales du CETIOM - Sud-Ouest. p. 1-14.

Li, X., Zhang, Y., Ding, C., Xu, W., Wang, X., 2017. Temporal patterns of cotton Fusarium and Verticillium wilt in Jiangsu coastal areas of China. Scientific Reports 7, 12581. doi:10.1038/s41598-017-12985-1

Liu, R.-J., 1995. Effect of vesicular-arbuscular mycorrhizal fungi on verticillium wilt of cotton. Mycorrhiza 5, 293-297. doi:10.1007/BF00204965

López-Escudero, F.J., Blanco-López, M.A., 2007. Relationship Between the Inoculum Density of *Verticillium dahliae* and the Progress of Verticillium Wilt of Olive. Plant Disease 91, 1372-1378. doi:10.1094/PDIS-91-11-1372

Madden, L. V., Campbell, C.L., 1990. Nonlinear Disease Progress Curves, in: Epidemics of Plant Diseases. Springer, Berlin, Heidelberg, p. 181-229. doi:10.1007/978-3-642-75398-5_6

Malcolm, G.M., Kuldau, G.A., Gugino, B.K., Del Mar Jiménez-Gasco, M., 2013. Hidden Host Plant Associations of Soilborne Fungal Pathogens: An Ecological Perspective. Phytopathology 103, 538-544.

Mansoori, B., 2011. An improved ethanol medium for efficient recovery and estimation of *Verticillium dahliae* populations in soil. Canadian Journal of Plant Pathology 33, 88-93. doi:10.1080/07060661.2010.534894

Matthiessen, J., Kirkegaard, J., 2006. Biofumigation and Enhanced Biodegradation: Opportunity and Challenge in Soilborne Pest and Disease Management. Critical Reviews in Plant Sciences 25, 235-265. doi:10.1080/07352680600611543

Michel, V., 2008. Biofumigation – principe et application. Station de recherche Agroscope Changins-Wädenswil ACW, Centre de recherche Conthey, 1964 Conthey 1-6.

Michel, V., Ahmed, H., Dutheil, A., 2007. La biofumigation, une méthode de lutte contre les maladies du sol. Revue suisse Vitic. Arboric. Hortic 39, 145-150.

Missonnier, H., Jacques, A., Bang, J.S., Daydé, J., Mirleau-Thebaud, V., 2017. Accounting for biotic spatial variability in fields: Case of resistance screening against sunflower Verticillium wilt. PLoS ONE 12, 1-14. doi:10.1371/journal.pone.0181050

Motisi, N., Montfort, F., Faloya, V., Lucas, P., Doré, T., 2009. Growing Brassica juncea as a cover crop, then incorporating its residues provide complementary control of Rhizoctonia root rot of sugar beet. Field Crops Research 113, 238-245. doi:10.1016/j.fcr.2009.05.011

Narisawa, K., Kawamata, H., Currah, R., 2002. Suppression of Verticillium wilt in eggplant by some fungal root endophytes. European Journal of Plant.

Neubauer, C., Heitmann, B., Müller, C., 2014. Biofumigation potential of Brassicaceae cultivars to Verticillium dahliae. European Journal of Plant Pathology 140, 341-352. doi:10.1007/s10658-014-0467-9

Neubauer, C., Hüntemann, K., Heitmann, B., Müller, C., 2015. Suppression of Verticillium dahliae by glucosinolate-containing seed meal amendments. European Journal of Plant Pathology 142, 239-249. doi:10.1007/s10658-015-0607-x

Olivier, C., Vaughn, S.F., Mizubuti, E.S.G., Loria1, R., 1999. Variation in Allyl Isothiocyanate production within Brassica species and correlation with fungicidal activity. Journal of Chemical Ecology 25.

Paplomatas, E., Bassett, D., Broome, J., DeVay, J., 1992. Incidence of Verticillium wilt and yield losses of cotton cultivars (Gossypium hirsutum) based on soil inoculum density of Verticillium dahliae. Phytopathology. doi:10.1094/Phyto-82-1417

Pegg, G.F., Brady, B.L., 2002. Verticillium wilts. CABI, Wallingford. doi:10.1079/9780851995298.0000

Petersen, J., Belz, R., Walker, F., Journal, K.H.-A., 2001, U., 2001. Weed suppression by release of isothiocyanates from turnip-rape mulch. Agron. J 93, 37-43.

Quiroz, F., Corro Molas, A., Rojo, R., Pérez Fernández, J., Escande, A., 2008. Effects of no tillage and genetic resistance on sunflower wilt by Verticillium dahliae. Soil and Tillage Research 99, 66-75. doi:10.1016/j.still.2007.12.007

Radi, S., Gulya, T., 2006. Sources of Resistance to a New Strain of Verticillium dahliae on Sunflower in North America-2006, in: 29th Sunflower Research Workshop, 10–11 January. Fargo, p. 7.

Radojčić Redovnikovic, I., Glivetić, T., Delonga, K., Vorkapić-Furač, J., 2008. Glucosinolates and their potential role in plant. Periodicum Biologorum 110, 297-309.

Reddy, P.P., 2017. Agro-ecological Approaches to Pest Management for Sustainable Agriculture. doi:DOI 10.1007/978-981-10-4325-3

Rosa, E.A.S., Rodrigues, P.M.F., 1999. Towards a more sustainable agriculture system: The effect of glucosinolates on the control of soil-borne diseases. Journal of Horticultural Science and Biotechnology 74, 667-674. doi:10.1080/14620316.1999.11511170

Seassau, C., Desserre, D., Desplanques, J., Mestries, E., Dechamp-Guillaume, G., Alletto, L., 2016. Control of Verticillium dahliae causing sunflower wilt using Brassica cover crops, in: Proceedings of th 19th International Sunflower Conference, 29 May - 3 June 2016, Edirne, Turkey. p. 717-725.

Shetty, K.G., Subbarao, K.V., Huisman, O.C., Hubbard, J.C., 2000. Mechanism of Broccoli-Mediated Verticillium Wilt Reduction in Cauliflower. Phytopathology 90, 305-310. doi:10.1094/PHYTO.2000.90.3.305

Steffek, R., Spornberger, A., Altenburger, J., 2006. Detection of Microsclerotia of Verticillium dahliae in Soil Samples and Prospects to Reduce the Inoculum Potential of the Fungus in the Soil. Agriculturae Conspectus Scientifi cus Agric. conspec. sci 71, 145-148.

Subbarao, K. V, Hubbard, J.C., 1996. Interactive Effects of Broccoli Residue and Temperature on Verticillium dahliae Microsclerotia in Soil and on Wilt in Cauliflower. Phytopathology 86, 1303-1310.

Subbarao, K. V, Hubbard, J.C., Koike, S.T., 1999. Evaluation of Broccoli Residue Incorporation into Field Soil for Verticillium Wilt Control in Cauliflower. Plant Disease 83, 124-129. doi:10.1094/PDIS.1999.83.2.124

Tenuta, M., Lazarovits, G., 2004. Soil properties associated with the variable effectiveness of meat and bone meal to kill microsclerotia of Verticillium dahliae. Applied Soil Ecology 25, 219-236. doi:10.1016/j.apsoil.2003.09.007

Tenuta, M., Lazarovits, G., 2002a. Ammonia and Nitrous Acid from Nitrogenous Amendments Kill the Microsclerotia of Verticillium dahliae. Phytopathology 92, 255-64. doi:10.1094/PHYTO.2002.92.3.255.

Tenuta, M., Lazarovits, G., 2002b. Identification of specific soil properties that affect the accumulation and toxicity of ammonia to *Verticillium dahliae*. Canadian Journal of Plant Pathology 24, 219-229. doi:10.1080/07060660309506999

Tribouillois, H., Cristante, P., Estragnat, A., Champclou, D., Vericel, G., Lande, N., Bedousssac, L., Justes, E., 2012. Is sunflower-soybean intercropping an efficient solution for increasing natural resources use efficiency and yield production ?, in: Proceedings of the 18th International Sunflower Conference, Mar del Plata (Argentina).

Vaughn, S.F., Berhow, M.A., 2005. Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification. Industrial Crops and Products 21, 193-202. doi:10.1016/j.indcrop.2004.03.004

Wezel, A., Casagrande, M., Celette, F., Vian, J.-F., Ferrer, A., Peigné, J., 2014. Agroecological practices for

sustainable agriculture. A review. Agronomy for Sustainable Development 34, 1-20. doi:10.1007/s13593-013-0180-7

Wheeler, D.L., Johnson, D.A., 2016. Verticillium dahliae Infects, Alters Plant Biomass, and Produces Inoculum on Rotation Crops. Phytopathology 106, 602-613. doi:10.1094/PHYTO-07-15-0174-R

Wilhelm, S., 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. Phytopathology 45, 180-181. doi:10.1155/2012/212075

Witzel, K., Hanschen, F.S., Schreiner, M., Krumbein, A., Ruppel, S., Grosch, R., 2013. Verticillium suppression is associated with the glucosinolate composition of Arabidopsis thaliana leaves. PloS one 8, e71877. doi:10.1371/journal.pone.0071877

Xiao, C.L., Subbarao, K. V., Schulbach, K.F., Koike, S.T., 1998. Effects of Crop Rotation and Irrigation on *Verticillium dahliae* Microsclerotia in Soil and Wilt in Cauliflower. Phytopathology 88, 1046-1055. doi:10.1094/PHYTO.1998.88.10.1046

Yadeta, K.A., Thomma, B.P.H.J., 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. Frontiers in plant science 4, 1-12. doi:10.3389/fpls.2013.00097

Yohalem, D., Hall, D., 2009. Selection and partial characterisation of biofumigants for management of Verticillium wilt in strawberries, in: Multitrophic Interactions in Soil, June 24-27, 2007. IOBC/wprs Bulletin. Dijon, p. 201-206.

Yuan, Y., Feng, H., Wang, L., Li, Z., Shi, Y., Zhao, L., Feng, Z., Zhu, H., 2017. Potential of Endophytic Fungi Isolated from Cotton Roots for Biological Control against Verticillium Wilt Disease. PloS one 12, e0170557. doi:10.1371/journal.pone.0170557

Zurera, C., Romero, E., Porras, M., Barrau, C., Romero, F., 2009. in Vitro Suppression of Phytophthora Cactorum and Verticillium Dahliae Potential Strawberry Pathogens By Brassica Tissues. Acta Horticulturae 267270. doi:10.17660/ActaHortic.2009.842.45

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Assessment of the potential of biotic regulation by Brassica cover-crops used as biofumigants. Case of *Verticillium dahliae* affecting Sunflower crop in southwestern France.

Key-words: Sunflower crop, *Verticillium dahliae*, Biofumigation, Brassica, Cover-crop, Glucosinolate, Agroecology

Mots clés : Tournesol, *Verticillium dahliae*, Biofumigation, Brassicacées, Culture intermédiaire, Glucosinolate, Agroecology

Résumé : Le Verticillium dahliae du tournesol est un champignon pathogène vasculaire dont les dégâts et les dommages sur les cultures de tournesol sont en constante augmentation dans le Sud-Ouest de la France. L'implantation de cultures intermédiaires de brassicacées dans la longue interculture de la rotation blé dur – tournesol, très fréquente dans le Sud – Ouest de la France, peut fournir de nombreux services écosystémiques mais leur potentiel de régulation biotique de ce pathogène est mal caractérisé. Cette étude visait à évaluer le potentiel de régulation biotique des cultures intermédiaires de brassicacées par biofumigation sur le *V.dahliae* du tournesol. La moutarde brune (*Brassica juncea (L.) Czern cv. Etamine*), la navette (*Brassica rapa (L.) subsp Oleifera cv. Chicon*), et le radis fourrager (*Raphanus sativus (L.) cv. Anaconda*) ont été évalués pour leur potentiel de biocontrôle de la verticilliose du tournesol dans deux expérimentations au champ et leur potentiel biofumigant sur *V.dahliae* a été évalué laboratoire. La biofumigation réalisée avec des couverts de brassicacées semble efficace pour diminuer l'inoculum primaire, réduire la progression des symptômes et améliorer les rendements des tournesols affectées par *Vertcillium dahliae*. Des efforts de caractérisation des mécanismes mis en jeux lors de la biofumigation, et la compréhension des dis-services apportés semblent nécessaires avant d'envisager l'adoption de cette technique dans des agroécosystèmes.

Abstract: *Verticillium dahliae* causing Sunflower Verticillium Wilt (SVW) is a vascular pathogenic fungus of increasing importance in southwestern France. Biofumigation performed with brassica cover-crops grown during the long fallow period of the durum wheat – sunflower rotation is not only able to provide numerous ecosystem services but might also be efficient in regulating SVW. This study aimed at assessing the potential biotic regulation provided by brown mustard (*Brassica juncea (L.) Czern cv. Etamine*), rape (Brassica rapa (L.) subsp Oleifera cv. Chicon), and fodder radish (Raphanus sativus (L.) cv. Anaconda) on *Verticillium dahliae* affecting sunflower in two field trials and in a bioassay. Results suggest that biofumigation performed with those cultivars seems to decrease soil inoculum densities, the progression of sunflower wilt symptoms and increase quantitative and qualitative yields of sunflower. The underlying mechanisms involved and especially the potential disservices caused by biofumigation need to be further investigated before this technique can be integrated in diversified and holistic disease management strategies.

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